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REVIEW ARTICLE

Automated Techniques in Pharmaceutical Analysis

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Automation is undoubtedly one of the most loosely used terms in scientific literature. It may mean the partial mechanization of a hand-operated pipet or refer to a 500,000-dollar computer-linked mass spectrograph. With this range of interpretation it becomes readily apparent that from the over 5,000 papers dealing with automation, a great amount of selectivity would have to be used by anyone attempting a review of this field.

Automation has been used in almost all scientific areas and many extensive reviews exist in the literature. As an example, Kingsley has published a series of biennial reviews on clinical chemistry. These have appeared in *Anal. Chem.*, "Analytical Reviews—Applications," and contain a large section on automation (1–6). *Anal. Chem.*, in its "Fundamental Reviews," also appearing biennially, covers many other specialized areas such as chromatography, spectrophotometry, titrations, polarography, and instrumentation, many of which contain sections on automation (7–13). Other journals also contain specialized reviews, but there do not appear to be any reviews that deal with automation as it applies to pharmaceutical analysis. In addition, there do not appear to be any reviews that emphasize the array of techniques that have been used or may be applicable to pharmaceutical analysis, since most reviews deal with only one technique or with a certain class of compounds. In view of this situation, the authors feel that a review is needed of the techniques available to an analyst faced with problems in pharmaceutical automation, rather than a tabulation of the compounds to which these techniques have been applied. The authors have attempted such a review. For those whose interest is primarily in how a specific analysis has been automated, reference, whenever possible, is made to existing bibliographies. In making the selections for the review, it is certain that the authors have inadvertently omitted important work in certain fields and it is hoped that what is presented will significantly outweigh the omissions.

Historical—Traditionally, pharmaceutical manufacturing has been a batch operation. Samples were removed from the batch at various manufacturing steps according to a well-defined protocol. These samples were composited and subsamples of the composite samples were analyzed. These composite assays were supplemented by additional tests such as identity and weight variation. This approach, coupled with extensive pilot plant investigation, and supplemented by rigid manufacturing process control, gave reasonable assurance that each individual dose contained the proper amounts of active ingredient.

As the percentage of active ingredient decreased due to more potent drugs, as manufacturing operations changed, and in general, as the science of quality control became more sophisticated, it became apparent that content uniformity could not be assumed but required testing in certain formulations. This concern was implemented by a 1963 "National Formulary" proposal for a *content uniformity* test. This test was later made official for a limited number of tablet monographs (14). "The United States Pharmacopeia" took similar action

and made the *content uniformity* test official in 1965 (15). It soon became apparent that this increased level of testing would require many more highly trained analysts, more laboratory facilities, and would significantly increase the cost of manufacturing drugs. This increase in analytical workload expedited, more than anything else, the rapid proliferation of automated analytical techniques in pharmaceutical quality control. Even though equipment for automated analysis is generally expensive and the effort to convert manual methods to automated procedures is also costly and time-consuming, the potential savings in salaries, laboratory space, and analysis time usually make the conversion worthwhile (16).

Another, and perhaps even more significant trend, has become evident. Previously, all manual methods were designed to accommodate the limitations or circumvent problems associated with manual operation. Initially, automation was designed to simply mechanize the manual method even though some of the reasons for proceeding in a certain way were no longer necessary or even desirable. It soon became evident that, in certain cases, automated methods using new principles, including methods that were sometimes difficult or impossible to conduct manually, could now be used to great advantage. In many cases, old problems of interference could be eliminated and nonstoichiometric or time- and temperature-dependent steps could be accommodated with the precision required for good pharmaceutical quality control. Semiautomated or fully automated instruments are now on the market which readily provide data that were difficult or impossible to obtain previously by manual operations.

Quantities of data have become so easy to get that it is not difficult for an analyst to be deluged with data, mostly pertinent, from the relatively few production lots which he used to analyze with little effort a few years ago. It might appear that he has come full circle, except that now he has become a calculator or recorder rather than an analyst. This condition has hastened the advent of the automated readout systems and the use of digital computers. With these new tools, he may reinstate himself as an objective interpreter of his now fully automated analytical system. *Provided* his system has been properly designed and is being properly applied, he can now assure his management of a level of quality control and quality assurance never previously attainable.

The word "provided" is a keyword and the uncertainty surrounding it is the major reason for a review of this type. Automated analysis has necessitated an extensive reeducation of all analysts attempting to convert to these techniques. The field of automation has been characterized by a great amount of new research. The task is so extensive that, only by reviewing his own efforts in the light of the experiences of others, can the automation analyst progress at the pace that will be necessary in the years ahead.

ORGANIZATION

For the purpose of this review, *automated methods* have been divided into two general classifications: *Continuous-flow Analysis* and *Discontinuous Analysis.* In addition, a section on *Chromatography* and a section on *Readout Devices and Computers* have been included. The review closes with a *miscellaneous* section and a discussion of the applicability of automated methods to pharmaceutical analysis.

CONTINUOUS-FLOW ANALYSIS

Continuous-flow analysis was introduced by Skeggs in 1957 (17). This technique, more than any other single advancement, has made possible the great impact of automation on the modern analytical laboratory. Continuous analysis, utilizing a flowing stream, results in the use of fairly simple equipment. The equipment, as a result of the ingenuity of many workers and the manufacturer, has become remarkably versatile and most procedures can be adapted to this technique. It is relatively easy to control many of the variables in the assays by simple adjustment of the equipment. Reactions need not go to completion nor does the sample have to reach equilibrium at any point in the system. Difficult separations can often be achieved by means of convenient dialysis modules, chromatography, liquid-liquid extractions, or distillation. The vast number of applications of the equipment and published papers on these techniques gives an analyst a ready resource on which to base his research and development efforts.

Continuous-flow analysis has certain inherent problems associated with it. It does not strictly parallel previous manual operations since discrete sample integrity may not always be maintained throughout the apparatus. Often the basic chemistry of a method must be modified to allow its automation by continuous-flow analysis. Careful attention must be paid to the elimination of sample interaction, tailing of peaks, assay drift problems, and systematic bias. In general, it is relatively simple to set up a new analysis on a continuous-flow system and achieve a precision of about $\pm 5\%$. After that may come weeks or months of work to optimize all analysis conditions such as bubble patterns, the tilt of glass joints, the elimination of surging in the system, *etc.*, so that the precision necessary for acceptable pharmaceutical quality control can be achieved. Unfortunately, at this stage, the procedure appears deceptively simple and the tendency is to transfer the analysis to a neophyte technician with little or no training in continuous-flow methods. This is when disaster may strike suddenly. As with any automated system, the continuous-flow analyzer can turn out prolific incorrect data with little regard for the intent of the analyst. A wise management recognizes this problem and rewards the operators for the special skills required to successfully operate the equipment. This is especially true in the case of pharmaceutical quality control since the operator is expected to produce results consistent with the traditional high level of precision required in this field.

The primary proponent of continuous-flow analysis is the Technicon Corporation, manufacturer of an automatic analyzer.[1] This equipment has been ac-

[1] This superscript will indicate a Technicon instrument throughout this article; when it is used with automatic analyzer, the specific instrument is the AutoAnalyzer, Technicon Corporation, Tarrytown, N. Y.

cepted almost universally and a vast number of papers have been written describing its specific applications. Technicon Corporation has recently made available an extensive bibliography containing 1825 literature references to uses of their automatic analyzer (18).

In their system, samples and reagents, segmented by air bubbles, are aspirated by means of a multi-tube proportioning pump which delivers the predetermined volume ratios required for the specific analysis. The samples continuously follow one another through the flow system and are combined at various locations with reagents under controlled conditions to carry out chemical reactions or produce physical changes in the sample. As the sample streams are directed from module to module, they can be heated, incubated, dialyzed, filtered, extracted, distilled, digested, or treated in any of a number of other ways prior to the end result being detected. The stream may be evaluated colorimetrically, spectrophotometrically, fluorometrically, or can be monitored by electrodes, scintillation counters, *etc.* Samples can be interspersed automatically with wash solutions so that response peaks are obtained or they can be continuously monitored to obtain a steady-state condition which represents the maximum signal obtainable under the operating conditions established for that particular determination. Standards are run occasionally in place of the samples and the equipment is thus calibrated for the specific method being utilized.

Theory of Operation—A number of papers have been published on the theory of operation of continuous-flow analysis, especially as it applies to the automatic analyzer.[1] Thiers has published a number of excellent articles on continuous-flow methods and his work is widely quoted in the literature. In 1964 he published an in-depth discussion of the precision, accuracy, and inherent errors in automatic continuous-flow analysis (19). His discussions of the influence of the height of sample in the sample cup, the errors due to sample interaction, and the special problems of dynamic equilibrium involved in continuous-flow methods have led to more rigorous operational procedures. He distinguished between estimates of sample repeatability obtained by one technician making multiple measurements on one sample and estimates of reproducibility which come only from long-term quality control data. He also distinguished accuracy from repeatability and reproducibility by defining accuracy as the average bias. In 1967 Thiers further elaborated on continuous-flow theory (20, 21). He determined that continuous-flow systems follow first-order reaction kinetics and that two parameters, half wash time "$W^1/_2$" and lag phase time "L," can be determined and used in the design and evaluation of continuous-flow instruments and methodologies. These two parameters can be utilized to evaluate effects of such factors as sequence of standards, sampling rates, proportion of sampling and wash time, interaction between samples, percentage of steady state reached in a given sampling time, and other key considerations in methodology development. Chaney used the principles of fluid mechanics to study certain characteristics of continuous flow in glass, Teflon, and polyethylene tubing as it relates to segmented flow (22). Agren *et al.* proposed a mathematical approach to provide equations for the relative concentrations of solutes in co- and counterflow dialysis (23). The estimation of linear and nonlinear cross-contamination in flow analysis was presented in mathematical form by Wallace (24). A computer has been utilized by Blaivas and Mencz for on-line standardization, correction for interaction and drift, and automatic performance control of individual automatic analyzer[1] channels (25, 26). A very thorough discussion of several unique maintenance steps and operating procedures, which would apply to any automatic analyzer[1] system, have been presented by Zajac (27). Glick evaluated several factors such as sunlight, room temperature, *etc.*, which he found could affect results of certain determinations (28). The use of gradient elution was illustrated by Lindquist in his technique for the determination of the optimum concentration of reagents and the evaluation of interferences in continuous-flow analysis (29). Smythe *et al.* presented the results of a 4-year study of the fundamental theories of continuous-flow analysis (30). This is an excellent paper and should be required reading for every automation analyst doing work in continuous-flow analysis. These authors discuss steady-state, phasing, wash characteristics, proportioning, air segmentation, dialysis, and flow cell characteristics.

New Concepts in Flow Analysis—Since the introduction of the continuous-flow analysis concept, specifically the automatic analyzer,[1] researchers have continually modified and extended applications to encompass nearly the whole spectrum of current analytical techniques. This review will cover some of the more unique contributions to this ever-expanding field of research. While many of the techniques covered are not specifically utilized in pharmaceutical analysis, those presented might be adaptable to this field.

Blaedel and Laessig have presented a comprehensive review entitled "Automation of the Analytical Process Through Continuous Analysis" (31). This review covered the literature through 1963 with 617 references. In table form they presented their literature search under the following headings: Analyzed for substance, Sample matrix, Mode of measurement, Remarks, References. A paper entitled "Automated Analysis in Nutritional Research" has been presented by Evans and Thomas (32). These authors cited the literature, through 1966, with 31 references. While their review is not exhaustive, they explore the development and theory of automated analysis. These authors discuss the advantages offered by continuous-stream analysis systems for both sequential analysis of a series of samples and for monitoring changes in a single sample presented to the system continuously.

Lento first introduced an automated continuous-stream polarographic method providing a new and versatile system for electrochemical analysis (33). Without referring to Lento's work, Fleet *et al.* reported on their continuous-flow polarographic system (34). A special flow-through polarographic cell and a solution-controlling cam assembly for the manifold proportioning pump were described. Sawyer introduced the use of a redox detector in an automatic analyzer[1] system to determine reducing sugars (35). He fashioned

a small-volume electrode assembly from conventional glass connectors (Technicon) fitted with a fused platinum wire and a rigid Teflon tube, providing a KCl bridge to a calomel reference electrode. The detector, coupled to a suitable pH meter and recorder, measured the redox potential for a ferricyanide/ferrocyanide system during the oxidation of glucose. A system which employed ion-specific electrodes was described by Jacobson (36). He gave the precautions that must be taken when attempting to use these electrodes for direct potentiometric analysis.

Continuous monitoring of a polymerization reaction by infrared scanning was accomplished by Hirsh and Bridgland (37). These authors used a 0.1-mm. KBr flow cell, an infrared spectrophotometer, and a pump to circulate reactants through the flow cell for infrared scanning. Sorin and Vargues (38) presented a summary of their elegant theoretical and mathematical approach to the acquisition of concentration gradients using an automatic analyzer proportioning pump.[1] The paper included references indicating the use of this type of system by these and other authors. Khoury and Cali described an automatic analyzer[1] system that performed repetative ultraviolet scanning for drug-stability studies and for pharmaceutical quality control (39). In a technique to study the role of water balance in an animal, Manston (40) used an automatic analyzer[1] system. The unique feature of this method involved his use of a continuous dialyzer to filter and transfer ^{24}Na and tritium compounds from a turbid sample to an aqueous recipient stream. The dialysate was collected in a fraction collector for scintillation counting (using a Beckman DPM-100 instrument). The authors proposed that other isotopes may be similarly determined in turbid samples using the continuous dialyzer assembly. Jenner (41) used an automatic sample introduction and fraction-collection system for determining the molecular weight distribution of dextran using a Sephadex column and an automatic analyzer.[1] The fraction collector turntable was transferred directly to an automatic sampler for dextran determination by a modified anthrone method.

An automated vitamin assay system capable of determining four separate vitamins on a two-channel automatic analyzer system[1] was presented by Albright and Degner (42). Two vitamins (pyridoxine and riboflavin) were simultaneously determined, then, employing a stopcock system and reagent changes, two other vitamins (niacinamide and thiamine) were assayed using the same automatic analyzer manifold and reaction system. In order to use a single ultraviolet spectrophotometer and a single recorder to determine the potency of an antibiotic sample and its associated blank, Kuzel and Coffey designed a unique cell positioner (43). The positioner, controlled by the automatic analyzer sampler (Sampler II),[1] accurately allowed the positioning of microaperture flow cells. This attachment made possible alternate monitoring and recording of streams flowing through two separate flow cells in a single spectrophotometer and recorded both results on a single pen recorder.

A system was reported by Roodyn which he designated Multiple Enzyme Analysis (M.E.A.) (44). He placed a series of substrates in an automatic sampler.[1] These substrates were aspirated into a continuous gradient stream of buffered enzyme mixture. Thus he estimated a gradient dilution profile of the enzyme mixture using a single analytical system. The concept of continuous aspiration of a sample and the discrete sampling of reagents could be useful for other applications. Ruzicka and Lamm discussed principles of substoichiometric isotope dilution analysis for the determination of micro amounts of materials (45). In this method a limited amount of a reagent, such as dithizone, was added, and competition for the reagent occurred. If, in a continuous-flow system, a radioactive substance is reacted with the limited reagent and then extracted, a certain activity will be obtained in a flow scintillation counter. If an equal quantity of the radioactive substance is added to an unknown quantity, a competition occurs for the reagent. Decrease in activity after subsequent extraction and counting is proportional to the amount of unknown substance in the sample. Briscoe *et al.* also described experiments which optimize continuous-flow substoichiometric analysis employing isotope dilution (46).

Stein (47) presented a new automated dialysis method using an automatic analyzer[1] dialyzer. He studied the binding of small ionic or uncharged species to macromolecules. He showed that continuous-flow, short-term, dialysis yields the same information as the classical equilibrium technique. Binding profiles were determined in a matter of hours rather than days as in the case of the classical equilibrium method. A new concept for drug screening for antiparasitic compounds by an *in vitro* automated bioanalysis system was presented by Cenedella and Saxe (48). Ferrari *et al.* presented additional data at a later date (49). Several parameters were studied simultaneously and structure-activity relationships could be evaluated by an examination of the various effects on parasite metabolism.

In their review of quality control of pharmaceuticals by automated spectrophotometric analysis, Kuzel and Roudebush presented a technique for the study of the characteristics of commercially available spectrophotometer flow cells (50). Included in this paper was a description of a fast-flow technique for measuring turbidity of microbiological organisms, and its utility in developing a new automated microbiological assay system. The fast-flow technique (1 ml./sec.) overcomes the serious flow birefringence problems traditionally encountered when attempting to measure the turbidity of rod-shaped organisms in microbiological testing. Dowd *et al.* described the use of a continuous digestor[1] to carry out photolysis in their determination of cyanocobalamin in pharmaceutical dosage forms (51). Pollard *et al.* (52) devised a technique in which a continuous-flow scintillation counter measured radioactive material in the dialysate stream of an automatic analyzer.[1] Later Pollard and Waldron presented their work on the automatic radioimmunoassay for insulin (53). These authors designed an elaborate filtration/lamination and radioactive scanner device for their radioimmunoassay of insulin by the double-antibody precipitation-filtration method.

Bartley and Poulik developed a rapid screening technique for the determination of optimum conditions for ion exchange and gel filtration of proteins or their degraded subunits (54). To monitor column eluates, these authors used an automatic analyzer[1] Lowry technique. Their unique system collected and introduced individual drops into the analytical system. The resultant "shadow-gram" produced an effective picture of column operations as related to column height and/or flow rates, and buffers. They stated the major advantages of this technique were speed (2–6 hr.) and economy of materials. Glenn used an automatic analyzer system[1] for the turbidimetric analysis of serum globulin (55). He used a 6-mm. flow cell and interference filters (600 mμ in text reference and 660 mμ in schematic) in a colorimeter to measure turbidimetric response. A linear response (0 to 4 g./100 ml.) was obtained when serum or plasma was continuously diluted and mixed with aqueous sodium sulfate.

Continuous-Flow Equipment Modifications—The many divergent problems encountered by investigators attempting to use conventional continuous-flow analysis systems has led to the development of many special devices or techniques. A few of the more unique innovations that are directly related to pharmaceutical analysis, or could have possible application to this field, are presented. Dowd *et al.* incorporated a double-bed ion-exchange resin adsorbant column between the automatic analyzer[1] sampler and the chemical reaction systems (56). This integral part of the flow system was utilized to preferentially remove interfering substances prior to the automatic chemical determination of calcium pantothenate in multivitamin preparations. The analysis rate for this chemical method was reported to be 10 samples per hour with a 45-min. analysis time, compared to a microbiological method producing 20 samples per day with a 24-hr. analysis time. Voser discussed the use of a liquid ion-exchange treatment of samples from biological media, such as fermentation broths, for the removal of interfering substances prior to analysis (57). This method was used for those materials not removable by dialysis or liquid-liquid extraction techniques. Skinner and Docherty incorporated a magnetic stirrer in a flowing stream just prior to the colorimeter to smooth recorder response after using a continuous filter (58). These workers also placed mixing coils in an ultrasonic bath. A timer periodically activated the bath, thereby freeing the coils of accumulated deposits.

Several types of flow cells have been designed for automatic analysis systems. These flow cells are described in several publications (59–62). Hopkinson and Lewis made a simple modification of a Sampler II[1] so that substrate only flows through the manifold system while enzyme samples are being withdrawn (63). This is useful where expensive or scarce reagents are used. Taylor and Northmore redesigned a Sampler I[1] for use with samples dissolved in a volatile organic solvent (64). To insure sample homogeneity during aspiration, Nelson and Lamont installed a rapid-spinning, nonwettable Teflon paddle on a Sampler I sampling crook (65). Kuzel designed a modified Sampler I to increase its versatility, provide better wash between samples, and to negate the influence of sample cup solution levels (66). Tappel and Beck constructed an ice bath for the Sampler II to prevent denaturation of enzyme samples during their residence in the sampler plate (67).

Prior to the introduction of proportioning-pump tubing compatible with concentrated mineral acids and with certain organic solvents, Taylor and Marsh introduced the concept of solution displacement (68). In a stoppered container was placed either a water-immiscible organic solvent or a flexible container of a water-miscible solution. Controlled pumping of water through a proportioning pump then displaced an equivalent volume into the reaction stream. Terranova *et al.* pumped mercury metal to displace a pyridine-acetone solution and thus circumvented leaching of pump tubing by this solvent mixture (69). Roudebush described a displacement and pumping method to transport a chloroform extract through only glass and Teflon tubing for ultraviolet measurement (70). This prevented ultraviolet-absorbing materials from being extracted from proportioning-pump tubing. Anderson *et al.* (71) encountered a schlieren effect during the flow of a chloroform extract-ethanol mixture through a 1-cm. flow cell (A. H. Thomas). They corrected the resultant erratic recorder response by attaching a single thickness of glassine weighing paper over the flow cell window on the phototube side of the cell. A stable constant-voltage source was designed by White and Guager to make a single-beam spectrophotometer compatible with a ratio recorder[1] (72). G. K. Turner Associates also have a device for coupling their fluorometer (Model 111) to a ratio recorder.[2]

Thomas designed an automatic sampling device, using a recycling cam timer and a set of solenoid valves, to introduce standards and samples from several reaction vessels to an automatic analyzer[1] system (73). To facilitate the handling of dialyzer membranes, Zajac described a simple holder and soaking tank that helps prevent accidental puncturing of the membranes (74). Wrightman *et al.* designed a unique sample solution attachment for a solid sampler[3] for the simultaneous determination of aluminum hydroxide, magnesium hydroxide, and acetylsalicylic acid (75). Because of solubility differences in the three compounds, a two stage dissolution technique in the solid sampler[1] homogenizer was used. The aspirin and $Al(OH)_3$ were solubilized using aqueous sodium hydroxide through the conventional system of the unit. Then through a specially designed, solenoid-operated, constant-level volumetric device, acetic acid was added to the mixture to adjust the sample to pH 4. All three compounds were solubilized under these conditions and were available for simultaneous analysis. Mercaldo and Pizzi incorporated an ultrasonic probe attached to a solid sampler[1] for sample preparation and for catalyzing the hydrolysis of phenobarbital in an automatic analysis system (76).

Irvine and Marwick redesigned a Sampler II[1] by adding a water jacket to provide a 37° constant-temperature sampler (77). Using a special manifold they injected the complement and antigen into sera samples

[2] G. K. Turner Associates, Palo Alto, Calif., Prod. Bull. No. 20M568.
[3] SOLIDprep, Technicon Corporation, Tarrytown, N. Y.

in the cups. The cups in the sampler-incubator were used to carry out the antigen-antibody-complement reaction, after which the samples were drawn into the automatic analyzer system for measurement of the degree of hemolysis. In order to obtain shorter sampling times with a Sampler I[1], Reid and Wise modified this unit by mounting the sample crook timing cam on the opposite side of the drive wheel (78). This modification reversed the sampling cycle characteristics of the sampler. They evaluated the system under nonsteady-state conditions. Due to the low concentration of a steroid in the coating of a tablet formulation, Stevenson and Comer used a novel device for removal and dissolution of the active ingredients in a minimum volume of solution (79). They placed one tablet along with a 13-mm. Teflon-coated stirring bar and 20 ml. of solution in each of twelve 25 × 100-mm. round-bottom centrifuge tubes. The 12 tubes were placed on a special stirring device for dissolution of the tablet coatings. The solubilized ingredients were then determined simultaneously in an automatic analyzer[1] system. Tappel and Beck designed a continuous-monitoring system for multiple enzymes in column chromatography effluents (80). They used a Sampler II as a fraction collector and as a sampler for subsequent colorimetric analysis. A second Sampler II was used for substrate introduction into the analysis stream. These two samplers were controlled by an on-off timing device and a remote relay for synchronization. Three different substrate samples were injected into each enzyme sample stream by the second sampler. Two groups of three substrate samples each were employed alternately to monitor two successive fractions of the effluent stream in the first sampler for six different enzymes. Owen presented a circuit diagram and operating instructions for a unit to evaluate recorder amplifiers and to assess deterioration of reference and sample photocells in colorimeters and flame photometers (Technicon) (81). Such a device should be helpful to those who do not have spare modules available for trouble shooting.

Specialized Continuous-Flow Modules—Since the introduction of the automatic analyzer concept in 1957, the Technicon Corporation has, through its own research, development, and adaptation of new techniques of others, introduced many new specialized modules compatible with continuous-flow analysis. In 1966 Levine and Larrabee described the Flame III photometer for the simultaneous determination of sodium and potassium in sera (82). This modified unit followed the introduction of Flame I in 1960 and the Flame II in 1964. Advantages of the new flame photometer were claimed to be quiet operation, capability of analyzing 60 samples per hour, and the use of natural gas and compressed air as fuel.

A new automated amino acid analysis system (T.S.M.—Technicon Sequential Multisample Amino Acid Analyzer) was first introduced by Eveleigh *et al.* (83) in 1967. Whitehead (84), in 1966, announced a new automatic sampler (Sampler T-40).[1] This sampler can accommodate 40 cups or tubes. Each sample container has an attached sample identification card. While the sample is being withdrawn into the analysis system, the accompanying identity code is automatically read from the identification card. The identification code is then presented with each assay result produced from multiple analysis of the sample. The result is positive sample identification.

In 1964, Skeggs and Hochstrasser first introduced the concept of multiple automatic sequential analysis (85). This system performed and recorded eight separate tests from a single sample. In 1965, Whitehead reported on a new automatic analyzer system (SMA 12[1]) enlarged to perform 12 simultaneous tests per sample (86). This unit produced a total of 314 analyses per hour. A completely new sequential multiple-analysis instrument (SMA 12/60[1]) was first described by Smythe *et al.* in 1967 (30). This completely redesigned instrument will provide 12 separate analyses simultaneously at the rate of 60 samples per hour or a total of 720 analyses per hour. This analyzer incorporated a newly designed sampler, proportioning pumps, dialyzers, flow cells, heating baths, multichannel colorimeters, and automatic data retrieval. While this system was designed for use in clinical chemistries, adaptation of the new "micro-modules" to present pharmaceutical control procedures could result in more rapid techniques along with a drastic reduction in sample and reagent requirements. Van Belle has used one of the systems (SMA 12) in a pharmaceutical research laboratory for performing chronic and acute toxicity studies (87).

Gochman first announced the cell counter system[1] (88). This module provides for an automated determination of white cell count, red cell count, and hemoglobin at the rate of 60 samples per hour. In the past year, several new modules have been introduced commercially[1] but, to date, no publications have appeared utilizing these modules.

Solid Sampling Methods—Soon after the introduction of the automated continuous-stream analysis concept, many analysts in the pharmaceutical industry realized the potential of such a technique for quality control. At the time of its introduction, the automatic analyzer[1] offered great potential as a versatile technique for rapid repetitive assays. However, the system was designed to accept only liquid samples. Since a large segment of pharmaceutical quality control is concerned with unit dose analysis, an automated method for converting individual solid samples to individual liquid samples was necessary. Holl and Walton first described the development of such a device (89). A sample plate capable of holding 50 individual tablets was designed. This sampler was able to introduce tablets, one at a time, into a blender type of container (Waring). An automatic syringe pipet introduced a preset volume of solvent and the blender was activated to disintegrate and dissolve the samples. An automatic sample probe introduced a portion of the prepared solution to an automatic analyzer[1] system for chemical analysis. The blender was then automatically emptied, washed, and readied for the dissolution of the next solid sample. During 1964, Technicon Corporation made such an apparatus commercially available as a solid sampler. A description of the commercial unit was given by Ferrari *et al.* (90).

Wrightman and Holl first described an automatic analyzer system[1] coupled to a solid sampler for produc-

tion line control of tablet compression operations (91). By installing two extra pens on the strip-chart recorder, the upper and lower product limits were drawn as continuous lines. Production operators, within minutes after tablet compression, could have a visual presentation of individual tablet content and could thus determine whether the current operation was within acceptable control limits. Holl *et al.* described automated pharmaceutical spectrophotometric assays as applied to 12 drug compounds covering 30 products or dosage forms (92). They described the substitution of a continuous filter for a dialyzer to clarify dissolved tablet samples and the use of an ultraviolet spectrophotometer as a read-out device. Beyer and Houtman used the solid sampler-automatic analyzer system[1] to determine tolbutamide in tablets (93). Both colorimetric and ultraviolet methods were used. These authors also described an automatic system for tablet dispensing, weighing, and weight recording for individual tablets. These individual tablets (25 at a time) were collected in a tube. This tube was transferred to an automatic dissolution and titration apparatus for potentiometric titration of tolbutamide.

An automatic analyzer[1] with a solid sampler was used by Wachtel and Peterson to determine the acid-consuming power of antacid tablets by a colorimetric method at the rate of 20 tablets per hour (94). Beyer presented an automated steroid tablet assay which utilized an automated extraction procedure and a colorimetric reaction (95). Wrightman *et al.* described the simultaneous determination of aluminum hydroxide, magnesium hydroxide, and acetylsalicylic acid in single tablets (75). The special solid sampler technique is described elsewhere in the review. Smith *et al.* presented an automated method using a solid sampler and a flame photometer to determine potassium in various food products (96). A solid sampler was used by Siriwardene *et al.* to prepare biological material for automated Kjeldahl determination of nitrogen (97). Burns described a new bead-chain solid sampler for use in continuous monitoring of a flowing solid (98).

Dowd *et al.* used the solid sampler[1] for single tablet assays by their "common handle" method described under continuous digestion in this review (99). The determination of cardiac glycosides by acid-induced fluorescence using the solid sampler for single tablets was given by Khoury (100). Cali and Khoury developed an automated fluorometric procedure for determining ethinyl estradiol and its methyl ether in the presence of other steroids in antifertility tablet formulations (101). Mercaldo and Gallo determined phenothiazine by automated colorimetric and ultraviolet spectrophotometric methods in various pharmaceutical formulations (102). Kuzel gave an automated general method for the analysis of tertiary amines in multicomponent tablets or capsules (103).

Wolski discussed the general concepts of the application of a solid sampler and an automatic analyzer[1] to a quality assurance program (104). Ahuja *et al.* used the solid sampler and an automatic analyzer[1] system for single capsule assays (105). They developed special solid sampler techniques to prevent loss of powder during dissolution of capsule powders containing imipramine hydrochloride and desipramine hydrochloride in various pharmaceutical formulations. These same authors also gave automated techniques for stability analysis and quality control of individual tablet and capsules containing phenylbutazone and oxyphenbutazone (106). The use of the automatic analyzer[1] for quality control in the manufacture of a wide variety of complex pharmaceutical items was outlined by Fernandez *et al.* (107). Bryant *et al.* assayed single tablets of isoxsuprine hydrochloride by dissolution in a solid sampler followed by dialysis and direct measurement of ultraviolet absorbance at 220 mμ (108). Weber *et al.* automated the differential spectrophotometric method for assay of pyridoxine in multivitamin tablets (109).

Continuous Digestion—Less than 2 years after the introduction of the automatic analyzer system,[1] Ferrari announced the development of a continuous digestion system to automate the classical Kjeldahl method for nitrogen (110). This module was described as a glass tube with helical indentations rotating continuously within a split-combustion tubular-type furnace. Samples and reactants were introduced continuously at one end of the tube by a proportioning pump. Continuous rotation of the tube transported the liquids as a thin film. At the exit end of the tube, the pyrolysis products were withdrawn continuously for analysis. This digester could perform both reductive and oxidative types of digestion. Later Ferrari *et al.* presented a detailed discussion of the principles of Kjeldahl digestion techniques and their application to continuous digestion (111). These authors presented automated digestion results for nitrogen determinations of animal feeds, dried yeast cells, insulin compounds, and antibiotic fermentation samples. The method has found wide acceptance as an automated technique.

Several researchers have devised new uses for and important modifications of the continuous-digestor technique. Dowd *et al.* encircled a rotating helix with a series of 300-w. photoflood lamps to photolytically release cyanide from cyanocobalamin (51). The quantitatively released cyanide was then used for the automatic determination of this vitamin. Mandl *et al.* devised a new apparatus for continuous digestion of biological materials in a flowing stream (112). In a rigidly controlled temperature chamber they vertically mounted an 8-mm. × 5 × 60.9 cm. (2 × 24 in.) glass coil. Air and acidified sample were introduced concurrently through a Y tube at the top of the column. Thus, the sample was swept through the heated glass coil. Automatic sampling of a portion of the digested material was accomplished at the bottom of the coil. Grasshoff built a continuous ultraviolet digestor for the determination of organic phosphate in seawater nutrients (113). He used an air-cooled quartz coil encircling a 22-cm. 900-w. high-pressure mercury lamp having a maximum emission at 252 mμ. Dowd *et al.* developed a nitrogen determination for automation of single tablet assays of a large number of different pharmaceuticals (99). They used this technique as a "common handle" method incorporating a solid sampler, an autodigestor, and a single automatic analyzer[1] manifold to assay tablets of varied formulations containing 1.0–30.0 mg. of nitrogen per tablet. The method can be used for tablet manufacturing con-

trol when the active ingredient is the only nitrogen-containing compound.

Feller *et al.* utilized continuous digestor[1] for solvent evaporation (114). A flow system continuously extracted ethopabate into chloroform. The solvent was evaporated in the heated helix. The resultant residue was then continuously dissolved in acidic methanol for subsequent colorimetric analysis. Mislan and Elchuk used the continuous-digestor unit to concentrate an aqueous solution for automatic injection into a conventional atomic absorption spectrophotometer (115). Their unit produced a 10-fold concentration of samples resulting in analytical sensitivity in the low parts per billion range. UaConaill and Muir modified this digestor for the evaporation of diethyl ether (116). They designed special fittings to direct an airstream through the rotating helix in a direction opposite to the liquid flow for the safe removal of ether vapors. An automated digestion technique for organophosphorous compounds which can analyze thin-layer or paper chromatographically separated zones as samples for rapid quantitative data was reported by Ott and Gunther (117).

Continuous Extraction—While some investigators have used the automatic digestor[1] for liquid-liquid extraction in a continuous-flow system, other workers have constructed less complex extraction devices. Greely *et al.* performed a chloroform extraction of aqueous alcohol steroid samples by passing the air-segmented mixture through a single mixing coil (118). The chloroform extract was resampled for automated chemical analysis. Strickler *et al.* used glass bead-filled coils for automation of butanol-extractable and butanol-insoluble serum iodine determinations (119). Browett and Moss designed a new horizontal "pancake" spiral extractor for carrying out the extraction of lead from urine with dithizone in chloroform (120). They gave details of a new phase separator and a new flow cell for use in their system. In the determination of lead in biological fluids, Hadley applied the same reaction as Browett and Moss but used a different system (121). He gave details of his phase-separator design. Mercaldo and Gallo reported an automated colorimetric extraction procedure for the determination of phenothiazine derivatives using glass-jacketed, controlled-temperature, extraction coils (102).

Fournier *et al.* used a 3.04 m. polyethylene coil for the continuous ethylene dichloride extraction of estrogens from the urine of pregnant mares (122). Reextraction of the ethylene dichloride extract with sulfuric acid-phenol reagent was subsequently performed. The polyethylene was found to overcome problems of the tendency of urine to adhere to glass. In his method for the automated determination of tertiary amines, Kuzel used a 4.478 m. Teflon coil for the extraction of an aqueous dye complex into chloroform (103). He described a nonmixing, nonair-segmented general extraction technique that resulted in good sample separation without emulsion formation. For the automated determination of ergotamine in fermentation products, Valentini used a two-solvent extraction (benzene and isopropanol) and regular glass mixing coils (123). He separated the aqueous-solvent phases in small-volume special glassware. Voser used liquid ion exchangers (such as Amberlite LA2) in an automatic analyzer[1] procedure for the determination of desferrioxamine B in fermentation broths (57). He used a special nonhelical device for liquid ion-exchange removal of interfering colored material from the broth samples. This technique is applicable where dialysis or solvent extraction is not possible. Viktora and Baukal used an automated double-extraction procedure for the estimation of lipophilic drugs (124). Untreated serum or urine samples were extracted into ethyl acetate and reextracted from the solvent with sodium carbonate (4%) or 0.1 *N* NaOH followed by ultraviolet measurement at 280 mμ.

Ek *et al.* developed an automatic analyzer[1] method to control the manufacture of time-release pellets in capsule dosage forms containing methapyrilene fumarate, pyrilamine maleate, and phenylpropanolamine hydrochloride (125). The active ingredients were extracted into chloroform under alkaline conditions. A portion of the extract was collected for gas chromatographic determination of the individual antihistamines while the remainder of the flowing stream was back-extracted with acid, filtered on a continuous filter unit, and the absorbance measured at 314 mμ. The solution passing through the spectrophotometer flow cell was then used for colorimetric determination of phenylpropanolamine. Sehgal and Vezina used ethanol extraction and fluorometry for the determination of the antibiotic antimycin A (126). The method was applicable to fermentation broths, and extraction concentrates of the antibiotic. Eriksson and Nyberg reported single-tablet assays of amines and quaternary compounds by an automated extraction procedure (127). They utilized the ability of methyl orange to form ion pairs with amines in the cationic state and with quaternary ammonium ions. These were extracted with chloroform and determined colorimetrically at 420 mμ. Avanzini *et al.* described a unique phase separator and colorimeter flow-cell attachment in an automatic analyzer[1] system used to determine bacterial respiration (128). They used methyl ethyl ketone extraction of acidified formazan. The latter was formed reductively from tetrazolium red in the presence of certain microorganisms. Pinnegar described a new liquid-liquid extractor using porous polyethylene disks stacked in a 3-mm. × 10-cm. glass tube through which aqueous and solvent phases were pumped (129). His technique lessened emulsion problems when analyzing fermentation broths.

A rapid automated liquid-liquid extraction method (100 samples per hour) was presented by Wallace (130). He described a technique for the determination of atropine by chloroform extraction of its narceinate reaction product. His extraction was accomplished in a 1.2 m. (4-ft.) 3-mm. o.d. glass coil without bubble segmentation. For rapid uniform extraction, a specially designed phase separator was described along with a special flow cell hook-up for the colorimeter.

A novel extraction apparatus was revealed by James and Townsend (131). To an approximately 1.2-m. length of 32 mm. (0.125 in.) i.d. Teflon tubing, mounted horizontally under tension, was attached an eccentric ball race. The vibrator was driven by an electric motor at 100 c.p.s. They claimed that this form of "assisted extraction"

gave reproducible transfer of corticosteroid from dichloromethane into a sulfuric acid reagent during the relatively short residence time in the extractor tube. The authors also describe a new type of phase separator.

Continuous Distillation—Mandl *et al.* described a new microdistillation apparatus for the determination of microgram quantities of fluoride at the rate of 20 samples per hour (132). The sample was flash-distilled in a Teflon coil at 170° under vacuum. The fluoride and water vapor were swept through a microdistillation column to a microcondenser. The distillate was then colorimetrically analyzed. This microdistillation device should have other potential uses in automated chemistry because of its small sample volume requirement and applicability to the determination of volatile substances in liquid media. Hanawalt and Steckel (133) developed a continuous vacuum still incorporated in an automatic analyzer[1] system. They gave details of the still using two jacketed mixing coils maintained at 95° under 38 cm. of Hg vacuum. Manly *et al.* used a 135° heating bath[1] to volatilize fluoride in their study of fluoride availability of dentifrice (134). A distillation head separated the liquid and vapor phases. Duncombe and Shaw were interested in determining lower aldehydes and ketones in experimental cultures of microorganisms (135). They designed special glassware for incorporation into an automatic analyzer[1] system. The sample stream was volatilized using either air or nitrogen as a carrier gas. The gas and liquid phases were separated for colorimetric assay. They gave details of a number of special glass fittings that could be useful in other applications. Sawyer and Dixon used the same type of distillation apparatus as described by Duncombe and Shaw for the automatic determination of alcohol in beer (136).

Ayers used a steam-distillation method for an ultraviolet measurement of nicotine alkaloids (137). The special distillation apparatus that she used is available from the Technicon Corporation. A special flow cell was used in a spectrophotometer (Beckman DB). Davies *et al.* gave details of a continuous distillation head used for quality control of alcohol in an aqueous flavor base (138).

General Applications—The value of continuous-flow analysis in the pharmaceutical control laboratory has been enhanced by the utilization of class or "common-handle" methodologies. By this approach, a majority of the great variety of organic compounds encountered today may be assayed by a relatively few basic flow systems. Primary aromatic amines can be determined by the Bratton-Marshall reaction (91), while tertiary amines and quaternary ammonium compounds are conveniently handled by the various dye-complex extraction procedures (79, 103, 127, 130, 139, 140). The automated Kjeldahl method offers a procedure for most pharmaceutical compounds which contain nitrogen (97, 99, 110, 111). Ultraviolet measurement permits the direct analysis of diluted samples (43, 50, 70, 92, 93, 102, 108, 125, 137), while repetitive UV sample scanning allows multiple wavelength measurements (39). The measurement of natural or induced fluorescence offers another general technique for the automation analyst (100, 101).

Several review articles have been published which discuss automated continuous-flow procedures. Automated antibiotic analysis is covered as a special section of this review. Shibazaki and Yamamoto have written a series of articles entitled "Analysis of Pharmaceuticals by an AutoAnalyzer" (141). Reference must again be given to the review of Blaedel and Laessig which covered the automation literature through 1963 (31). Guilbault reviewed automated enzyme procedures in *Anal. Chem.* "Fundamental Reviews" (12, 13). Schwartz and Bodansky authored a review "Automated Methods for Determination of Enzyme Activity" (142). This paper included 78 literature references through 1962. These same authors have recently published an updated review through early 1968 entitled "Utilization of Automation for Studies of Enzyme Kinetics" (143). Most notable in the latter review was their inclusion of computer techniques in automated enzyme studies. A general discussion related to enzyme automation was given by Schwartz (144). Problems associated with conversion of manual methods to automated methods and factors related to calculation of enzyme activity in automated enzyme systems were noted. Posen *et al.* gave a general discussion of automatic analyzer[1] technology as applied to investigation of enzyme inhibitors, study of time-dependent reactions, study of time-course of enzymatic reactions, and the use of gradient techniques (145). A review of automated steroid analyses through mid-1967 has been compiled by Russo-Alesi and Khoury (146). Twenty-eight references were cited. Khoury reviewed the field of automated vitamin analysis (147). Specific procedures for the water-soluble vitamins were recommended. The review contained 29 references. The "Technicon Bibliography" (18) must again be cited at this point as an excellent source for reference to automated flow procedures.

DISCONTINUOUS ANALYSIS

This technique essentially mechanizes manual analysis steps while maintaining discrete sample separation throughout the analysis. In theory, it should be easy to automate a manual method by this technique. All the necessary and well understood mechanical steps can be planned in advance, and if all of them can be accommodated by the equipment, automated analysis development should be straightforward. This is true to a great extent; however, certain key steps used routinely in manual techniques such as solvent-solvent extraction, filtration, digestion, *etc.*, generally remain difficult or impossible to perform on most commercially available batch analyzers. Many batch analyzers can be adapted to use very small amounts of sample and can usually be made to conserve expensive reagents. Sample interaction should not be a problem unless reusable reaction containers, which may be a part of the system, are not adequately cleaned between samples. This condition can result in serious sample interaction and drift of the entire assay. Generally, batch analyzers are more complex mechanically than continuous-flow equipment and trouble-free operation has been a problem. Experience with any one piece of commercial equipment is not very extensive and relatively little

published information is available on specific applications of these analyzers.

It must be stated that in the authors' opinion, neither continuous-flow nor batch analysis is inherently superior and the choice of technique should be made on the nature of the problem, the equipment available, the amount of pertinent information already available in the literature, and the objectives of the analyst.

Batch Analyzers—A large number of discontinuous or batch analyzers are now commercially available. One of the best known batch analyzers is marketed by the American Optical Instrument Company.[4] The current model is a completely redesigned version of the original instrument and details of this design were presented by Crawford (148). The unit withdraws a measured sample from a programmed test tube sampler, dilutes it with a diluent or reagent into a large turntable equipped with reaction tubes. Additional reagents can be added at various locations and the samples can be mixed and incubated. Samples are withdrawn and read in a spectrophotometer with the results being printed in digital form. The reaction vessels are washed and dried in the turntable and reused. Relatively few papers have been published on the use of this instrument (149–151). Rehm *et al.* (152) discussed the design and utility of another automatic analyzer.[5] This new instrument has been designed to run content uniformity assays on tableted pharmaceuticals. This automatic analyzer can be programmed to dispense a tablet, add solvent, sonically dissolve the tablet, filter the solution, dilute it to a preselected volume, and measure its absorbance either in the visible or ultraviolet range. The instrument can assay up to 400 tablets at rates up to 120 per hour in unattended operation. It can perform up to 20 different programmed analyses with each set-up. Variables are: type of solvent, sonifying time for dissolution, dilution, and wavelength of measurement. Typical precision is claimed to $\pm 1\%$.

Several automated clinical systems may have application in the pharmaceutical field. One system[6] consists of a compact module which withdraws very small samples from the inner row of a process turntable and transfers these samples, plus a diluent or reagent, into cells in the outer row. Additional reagents may be added at various times and the resultant solutions are measured by a colorimeter. The process turntable containing 90 cells and samples is removable for extended incubation and for thorough washing in an automated wash module. Up to 300 assays per hour may be performed (153). Another system[7] contains some unique features not found in most other instrument systems (154). The processing is done in units of 15 samples. Each batch of 15 samples is transferred from one module to another manually since experiments have indicated that mechanized transfer did not speed up the process but significantly reduced flexibility and increased cost. The sampler module measures and transfers as little as 15 μl. of sample to an inner mixture transfer carrier. The sample is washed out with diluent and reagents are added. The reaction vessels can then be centrifuged and the supernatant automatically transferred to another unit for further reagent addition. The solutions are read in a flow-through colorimeter and measurements are tabulated on a digital printer. The readout system employs an integrator and the printed value is an average obtained over 1 or more sec. Each unit of 15 samples requires 3 min. for processing in each module.

An automated analytical system is also available from Norelco-Unicam.[8] This system picks up a selected volume of sample for dilution, adds reagents to the diluted sample in a thermostated chamber, and prints out the result in a digital form from a spectrophotometer, fluorometer, flame emission unit, or atomic absorption analyzer. Programs are set up on a preformed keyplate. Bausch & Lomb have recently introduced an instrument[9] for enzyme analysis which consists of a single module. A sample is withdrawn from an inner row of sample cups, diluted with reagent into an outer row of optical quality cells, and is brought up to reaction temperature in about 12 min. The substrate is added, mixed, and the reaction rate is monitored by a self-contained filter photometer. The concentration of the sample is printed in International Enzyme Units together with a serial number identification. The system uses disposable sample cups and cells, and has an analysis rate of 30 samples per hour. An automated system for enzyme analysis has been made available by LKB.[10] It also pipets a sample from a sample rack, brings it to temperature, adds preheated reagents, and monitors the change in absorbance with time. The angle between the recorded reaction curve and the upper time axis is directly proportional to enzyme concentration (155). A system has recently been introduced by Hycel[11] which samples in a unique way using a water-air-sample-air-extra aliquot-desired sample-extra aliquot sequence from a 60-position tray. Samples and reagents are added to tubes in 1 to 10 channels of a moving train. After an incubation period, the reaction mixtures are drawn into a 10-station colorimeter. The tubes are emptied, washed, and reused. One to ten methods can be individually programmed for each sample solution on a program board. The entire unit is controlled by an integral computer. Results appear as steady-state responses on preprinted chart paper.

Two new systems have just been announced (156). Beckman Instruments, Inc., has introduced an analyzer[12] that is a completely self-contained system which samples as little as 5–15 μl. of sample, produces a protein-free filtrate by automatically filtering the suspension, and transfers the filtrate to reaction vessels made of disposable plastic. The filtrate can be diluted and provision is made for addition of up to 14 reagents. The solution is read in a dual-beam filter photometer and the output is presented as a digital printout, as a strip-chart record, or is fed directly to a computer. The dual-channel system is designed to carry out most

[4] Robot Chemist, AO Inst. Co., Analytical Inst. Div., Richmond, Calif.

[5] Ciba-Aminco Assayomat, American Inst. Co., Inc., Silver Springs, Md. Preprints No. 2.2-1-66; 17-4-A1D-67.

[6] Clino-Mak Mark II, Lab-Line Biomedical Prod. Inc., Melrose Park, Ill.

[7] Mecolab, National Instrument Laboratories, Inc., Rockville, Md.

[8] Series AC Automatic Analytical Systems, Philips Electronics Instruments, Mount Vernon, N. Y.

[9] Zymat 340, Bausch & Lomb, Rochester, N. Y., Prod. Bull. 34-6016.

[10] Reaction Rate Analyzer, LKB Instruments, Inc., Rockville, Md.

[11] Mark X, Hycel, Inc., Houston, Tex.

[12] Discrete Sample Analyzer DSA-560, Beckman Instruments, Inc., Fullerton, Calif., Prod. Bull. M-11

standard blood chemistries at rates up to 160 per hour. This instrument should have special appeal to those who have very limited samples as in the case of small animal blood studies.

DuPont Instruments has introduced a prototype of their clinical analyzer[13] which offers a new concept in laboratory test automation. In the DuPont system, the reagents for each test are packaged in a special kit or pack which also serves as the reaction chamber and optical cell for photometric analysis. Certain packs contain an individual disposable chromatographic column for either ion-exchange or gel-filtration methodology. A separate pack is used for each test performed on a sample and each pack is coded for operator and computer identification. The operator programs the system by placing the appropriate individual packs behind each sample cup in an input tray. The analyzer inputs the sample cup, reads the succeeding packs for instructions, measures the sample, and adds diluent according to the code on the pack. It then proceeds to chromatograph the sample or injects the sample and diluent directly into the plastic reaction chamber. Reagents, contained within individual compartments in the pack, are added by breaking their seals hydrostatically. Two different stations are used for addition of reagents and mixing of the sample. At the end of the reaction time, the flexible sides of the reaction chamber, made from ionomer resin[14] films, are formed into an optical cell and filters of the proper wavelength are set. The readout is computed and printed on a report form which also contains a photocopy, automatically produced in the instrument, of the submission card which accompanies the sample. Results are available in about 7 min. without need of any start up or base line adjustment time. Samples are processed with essentially no interaction since the only component in common between samples is the sample probe and this is washed between samples. Analysis rate is from 50–100 samples per hour. The entire system is operated, controlled, monitored, and interpreted by a specially designed integral computer. It would appear that instruments designed along these lines may have a great potential in related fields of analysis, such as pharmaceutical quality control.

One automatic chemical analyzer[15] that provides an almost unbelievable capacity can analyze 135 samples per hour for up to 24 tests simultaneously yielding a total of over 3,000 determinations per hour. This work is said to be equivalent to 100 qualified technicians using conventional methods. Liquid samples are measured and diluted, reagents added, and measurements are made by a batch process (157).

In Vitro Dissolution Measurements—The term automation, when used in the literature with reference to *in vitro* drug-dissolution studies, has either described a system in which the amount of a compound dissolved in solution is recorded *versus* time, or it refers to an automatic sampling device which collects solutions for subsequent analysis. No systems have been reported which include, in a single package unit, automatic sample preparation, readout or collection, and cleanup between samples.

In 1962, Schroeter and Wagner introduced a system in which direct measurements of capsule and tablet dissolutions were recorded by either a continuous flow or an intermittent sampling technique (158). A filtering sample tube was inserted into a USP disintegration basket assembly, and the filtered sample was pumped through a flow cell before returning to the original solution. Absorbance measurements were continuously recorded at any one wavelength between 220 and 800 mμ. This continuous-loop system was used when final absorbance values were within the spectrophotometer range. If dilutions were required, the sample solution was pumped to a timer-controlled three-way valve. When the valve was in the normal nonsampling mode, the pumped solution was returned to the basket assembly. During the sampling cycle, the solution was fed into a diluent stream, mixed in a special dilution-mixing chamber, passed through the flow cell, and then drained into a waste container. Niebergall and Goyan later described a continuous technique wherein the rapid dissolution rates of certain multiparticulate systems were measured (159). Solvent was mixed in a baffled beaker to which the powdered sample was added. A portion of the liquid was pumped through a specially designed flow cell within a recording spectrophotometer and then returned to the beaker. Total time for most studies was less than 3 min.

In 1963, Schroeter and Hamlin reported a system which utilized intermittent sampling of the dissolution media and very short light path flow cells for monitoring the *in vitro* dissolution process of tablets and capsules (160). Another system in which the dissolution liquid was circulated through a continuous flow cell was described for sustained-release tablets by Sjogren and Ervik (161).

Michaels *et al.*, in 1965, described a system which withdrew a portion of the dissolution fluid from a USP disintegration apparatus for analysis by an automatic analyzer[1] flow system (162). Colorimetric analysis by the Bratton-Marshall method or the blue tetrazolium procedure was performed continuously and simultaneously as the dissolution process was carried out. McClintock *et al.* reported the use of a nuclear *in vitro* method for the continuous measurement of dissolution rates (163). A special stirring apparatus was designed which circulated the dissolution liquid past a dry-well containing a detector (Geiger-Müller). Measurement of the radiation from a ^{59}Fe tracer incorporated into a standard tablet formulation was continuously recorded on a strip-chart recorder. The system was compared to other *in vitro* procedures. Also in 1965, Steinberg *et al.* introduced an automatic instrument for evaluating antacids *in vitro* (164). A titration assembly was described which automatically added and recorded the amount of acid required by the sample solution to maintain a constant preset pH. The recorded result represented the function, pH *versus* volume of titrant. A technique of continuous dissolution measurement was reported by Lapidus and Lordi in 1966 (165).

[13] Automatic Clinical Analyzer, E. I. du Pont de Nemours & Co., Inc., Instrument Products Division, Wilmington, Del., Prod. Bull. A-61659.

[14] Surlyn is DuPont's registered trademark for its series of ionomer resins.

[15] Auto Chemist, AGA Corp., Lidingo, Sweden.

A tablet was affixed to a cylinder (Kel-F) which was then placed in a sample flow cell (Rutgers). Dissolution media were circulated past the exposed tablet and through a spectrophotometer flow cell in a closed loop system, while the output of the spectrophotometer was continuously recorded.

In 1967, Ferrari and Khoury disclosed a new concept for automated drug dissolution studies which they said would permit a more realistic assay of the availability of drugs under simulated gastrointestinal conditions (166). A slowly rotating flask which had deep indentations in its side was used as the dissolution vessel to provide a closer analogy to the sloshing action created within the gastrointestinal tract. Filtered solution was withdrawn from the sample vessel continuously during the dissolution process, segmented with air, sent through the sample side of a continuous dialyzer, and then returned to the sample vessel *via* a proportioning pump. Fresh dissolution solvent was pumped as the recipient stream in the dialyzer unit, and transfer of a portion of the solute from sample to recipient stream occurred by diffusion across the membrane. Analysis of the recipient stream was then carried out by a conventional automatic analyzer.[1] A major advantage of this system was that sample dissolution was measured without changes in the original volume of the dissolution liquid, even if dilutions or chemical reactions had to be made or carried out for analysis measurements. The authors also observed that the use of the dialysis membrane allowed the evaluation of the transport of the dissolved medications under conditions more analogous to that of the cell membrane process.

A sampling apparatus capable of performing multiple dissolution tests was described by Castello *et al.* in early 1968 (167). Twenty individual tests could be performed simultaneously with samples being collected at any predetermined time intervals for subsequent analysis. Once the dissolution vessels were filled with solvent and samples were added, the system performed automatically throughout the sampling cycles. Withdrawal of the solution was achieved by a novel suction sampling bulb and sample solutions were collected in individual cups. Cleanup and preparations for the following samples still remained a manual operation.

Lastly, Pernarowski *et al.* described a continuous system for *in vitro* dissolution measurements (168). A tablet or capsule was placed in a basket container which was affixed beneath a stirring impeller. This was then inserted into the dissolution fluid contained in a 1-l. flask. During the dissolution process, filtered solution was drawn from the flask at the rate of from 50 to 70 ml./min., while fresh solvent continuously replaced the withdrawn solution. The filtered solution was pumped through a recording spectrophotometer. At any point during the dissolution process, the type of solvent used to replace the withdrawn solution could be changed by turning a two-way stopcock valve. An automatic gradient change from gastric to intestinal fluid was provided by this arrangement.

Antibiotics—Manual microbiological antibiotic assay methods are very tedious because of the great care that must be exercised in performing the many steps required to obtain an assay result. Many of the operations are so critical that, traditionally, the method is subject to a much greater error than that encountered in other pharmaceutical assays. A high level of repetition is necessary to reduce the error to a practical value. As a result, a concerted effort is usually made to convert microbiological assays into chemical assays whenever possible. Because of the inherent advantages of microbiological assays such as specificity, sensitivity, and measurement of the antibacterial activity, this conversion is not always desirable. Automation of both the microbiological and chemical methodologies has led to much greater efficiency and, in many cases, dramatic increases in repeatability and accuracy. Gerke and Ferrari have published an excellent review of these automation efforts (169). They list 37 references. A few of these papers deserve special mention since they illustrate new techniques. Gerke *et al.* presented the first paper on the use of continuous-flow bioassays for both respirometric and turbidimetric analysis (170). They used *Klebsiella pneumoniae*, *Escherichia coli*, and *Saccharomyces mellis* as the test organisms. Haney *et al.* (171) introduced several improvements to the continuous-flow methods of Gerke *et al.* (170). These authors used formaldehyde between samples to prevent the accumulation of cells on the internal walls of the flow system, thereby reducing drift. Drift problems were further reduced by an improved assay design. They also used a second pump to allow a greater range of dilution. *Candida tropicalis* and *Escherichia coli* were used as the test organisms. A detailed description of the critical parameters of the experiments outlined in the previous paper was presented by Gerke *et al.* (172).

Shaw and Duncombe first reported the use of a biostat in continuous-flow analysis (173). In this system, the inoculum was continuously grown in a small glass vessel at 37° and fed with nutrient medium. A constant volume was maintained by an overflow arrangement. When an equilibrium was established, this device became a continuous source of freshly growing cells for the assay. Not only did this provide a convenient source of fresh inoculum, but the inoculum grown in this way became much more sensitive to certain antibiotics. These authors were also the first to utilize *Staphylococcus aureus* for continuous-flow assays and designed a system which could increase the rate of responses from 20 to 40 per hour (174). Platt *et al.* further developed the turbidimetric approach by using *Streptococcus faecalis* for the analysis of antibiotics such as tetracyclines, bacitracin, ampicillin, penicillins, and vernamycin A and B; some in mixtures with either polymyxin or neomycin (175). Pagano *et al.* first introduced the concept of continuous dilution for antibiotic analysis (176). This principle was based on the work of Menzies, who originally described the technique for another application (177). In this technique a continuous sample stream was withdrawn from a container of sample while an equal amount of diluent was continuously added to the sample container. A log dilution was obtained which, when recorded against microbiological response, produced both a qualitative and a quantitative record of the antibiotic response. This system was used for the analysis of fungichromin and amphotericin A and B by measuring the respirometric response of *Candida*

tropicalis. Platt *et al.* measured a turbidimetric response while using the concept of continuous dilution as an automated assay method (178). Dilution time was found to be directly related to the log of antibiotic concentration and a printout of the dilution time in seconds was used for calculation purposes. Several workers have used microbiological continuous-flow analysis for study of bacterial growth and metabolism (179–183). Avanzini *et al.* described a technique for the determination of bacterial respiratory activity by reduction of tetrazolium red to formazan (128). The formazan was subsequently extracted with methyl ethyl ketone for colorimetric measurement. This technique might be practical for use in the analysis of antibiotics. Other parameters that might be used for measurement are metabolite, enzyme, protein, DNA, and RNA production. A number of workers have done basic work from which these techniques might develop (179–181, 184, 185). Continuous-flow analysis may also be useful in confirmatory tests for coliform bacteria according to Herschdoerfer (186). Problems connected with the quantitative determination of antibiotics in continuous-flow systems were reviewed by Kauppinen who listed 17 references (187). A review (with 32 references) of the application of the automatic analyzer[1] to fermentation problems was made by Hatano (188). A large number of continuous-flow methods have been developed for the chemical analysis of antibiotics. In addition to those listed in the review by Gerke and Ferrari (169), there are three other papers that should be mentioned. Avanzini *et al.* modified the hydroxylamine assay for penicillin (189). Blanks were run by substitution of a reagent inversion technique for the conventional penicillinase reaction. Lane and Weiss recently reported on the FDA evaluation of an automated hydroxylamine method for a variety of penicillins by comparison with the manual iodometric method on a very large number of samples (190). They concluded that the automated method was reliable and precise and that the analyst could use it with complete confidence. Kuzel and Coffey reported on the use of a dual-channel, modified automatic analyzer[1] system for the simultaneous analysis of samples and blanks of erythromycin (43). Differential spectrophotometry could not be used because of the high blanks obtained on fermentation samples.

Several batch and semiautomated systems have been described for the analysis of antibiotics. Patient reviewed the Glaxo approach to partial automation of their penicillin assay facility (191). Complete automation was considered too costly, difficult, and time-consuming. Their approach was to automate only those steps where precision was a major necessity, or where operations were repetitive and highly tedious. Specially designed apparatus was described for automating these steps. The remaining steps, such as transportation of tubes, *etc.*, were left to unskilled technicians. Instances were also given where complete automation was found to be necessary and such apparatus was also described. DiCuollo *et al.* reported on the partial automation of a large plate method for microbiological assays (192). They used an automatic diluter (Aminco) for serial dilutions. A pronged applicator board was lowered into the diluted solution tray and the diluted samples were simultaneously transferred to the seeded agar plate. After manual incubation, the zones were photographed and measured using an optical comparator. Trotman described a diluter for making serial dilutions of samples for the determination of minimal inhibitory concentration of antibiotics (193). Tsuji *et al.* used an automated readout for their turbidimetric assays (194). Samples were presented in groups of three tubes to a reader by a modified linear fraction collector. The tube contents were withdrawn into three flow cells and the turbidities were sequentially measured on a spectrophotometer (Hitachi-Perkin-Elmer model 139), using a cell positioner (A. H. Thomas Co.). The turbidity readings were recorded on a card punch (IBM 526) for further data processing. An elaborate automated turbidimetric assay system was described by McMahan (195). The system automatically diluted assay solutions and standards, simultaneously, into moving racks of empty tubes. Each rack contained a waterproof IBM card which directed all subsequent analysis steps. One of several inoculated media was added, and the tubes were incubated in a continuously moving train in a water bath. The incubated tubes were automatically read in a colorimeter and the results were punched on IBM cards. The analysis rate was 50 samples per hour. Burns and Hansen also described a completely automated system for turbidimetric analysis (196). Their system started with unfiltered fermentation broths, automatically filtered them, diluted the samples, added inoculum, incubated the samples in a hot air oven, read the incubated samples, and calculated the results by means of an on-line computer. Analysis rate was 40 samples per hour with about 25% standards needed. A unique self-cleaning filter arrangement was used in this system.

A progress report on a novel device for isolating bacterial colonies was presented by Falch and Hedén (197). This system coated a moving belt with nutrient agar, inoculated the agar with a vibrating needle before gelation occurred, and then added a very dilute solution of the sample as a band on the moving agar belt. The moving belt was incubated in an oven and the individual colonies could then be sampled manually or automatically. Versions of this system could have numerous related applications for research, development, or control. Mansberg (198) reviewed the design of an automatic colony counter and Malligo (199) proposed certain correction factors to increase the precision of this instrument. The design of an unusual colony counter was reported by Ingels and Daughters (200). This device utilized the principle that bacterial colonies formed spherical lenses as they grew radially from the parent bacterium. These colonies had a focal length of about 2 cm. A sensor array was located at this focal plane. While colonies formed bright spots at this focal plane, scratches on the plate, bubbles in the agar, and other imperfections were out of focus. An electronic scanner swept the plate in 1 sec. and the colonies were counted by an electronic counter. A very clever "nearest neighbor" inhibit system was used to prevent coincidence counting. Even fused colonies could be detected.

Bowman *et al.* described a photoelectric scanner which measured bacterial growth in nutrient agar-filled

capillaries (201). Growing microcolonies of bacteria could be detected and counted by drawing the capillary through a narrow beam of light and measuring the resultant light scattering. This technique may have application in antibiotic assay, testing of bacterial contamination, and in other biological systems where swelling, growth, or lysis of cells or particulates in a semisolid gel may occur. A commercial version of this instrument[16] is now available. A computer-controlled flying spot scanner for counting colonies of microorganisms and identification of the organism by observation of the colony morphology was the subject of a paper by Glaser and Wattenburg (202). The paper is covered in more detail in the computer section.

Atomic Absorption—Since 1960, after years of encouragement by Dr. Walsh (203), atomic absorption has become one of the fastest growing areas of spectroscopy. This technique has gained wide acceptance because of its specificity, sensitivity, broad application, and its relatively simple equipment. Usually large numbers of samples are processed at one time and it is not unusual to have 200 or more samples for analysis. With this large number of samples, it was only a matter of time before the technique was automated. Kahn published two review papers on the principles of atomic absorption and its associated equipment (204, 205). Lacy coupled an automatic sampler to an atomic absorption spectrometer for the determination of magnesium, and to a flame photometer for measurement of potassium and calcium (206). Klein *et al.* first utilized an atomic absorption spectrometer as part of a continuous-flow system for the analysis of calcium (207). In a later paper two of the authors combined this method with an improved method for phosphate (208). Mislan and Elchuk have added a modified continuous digester[1] for preconcentration of samples to increase sensitivity (115). A modified Sampler II[1] was used to present 100-ml. samples to the preconcentrator. The concentrated samples were collected in a special vessel and introduced into the atomic absorption spectrometer in a batch mode. A multichannel spectrometer for simultaneous atomic absorption and flame-emission analysis was described by Mavrodineanu and Hughes (209). Boling reported the use of an integrating analog computer to eliminate the effect of noise and increase the speed and precision of atomic absorption (210). Readout was presented in digital form.

Several manufacturers offer automated sample presentation for atomic absorption. A 24-position automatic sampler[17] in which the cycle and aspiration times are variable is available (Beckman). This can be coupled to the manufacturer's digital display system for automated readout. An atomic absorption-flame emission spectrophotometer[18] with a 32-position changer which samples and automatically rinses the sample line has been announced by Norelco-Unican. An automated atomic absorption system was reported by Gaumer *et al.* (211). Samples were diluted semiautomatically and then presented to the spectrometer from a 240-sample turntable. Readout was obtained by use of Perkin-Elmer modules (DCR-1 and DDR) and a printer (Hewlett-Packard). A newer version of this unit has been announced recently.[19] It allows 200 samples to be automatically presented to the atomic absorption module, or, if required, samples can be automatically diluted prior to analysis. The dilution step reduces the sample capacity to 100 samples. The unit can average up to 16 separate measurements on each sample and can analyze up to 65 different metallic and semimetallic elements. The readout has been modified but is still printed in digital form on paper tape. A new instrument (Jarrell-Ash) was discussed by Müller (212). This is a fully compensated ratio atomic absorption/flame-emission robot instrument. It samples from a vial, dilutes the sample, and directs it to the spectrometer. The results are automatically calculated and printed on an output typewriter.

CHROMATOGRAPHY

Advances in the field of chromatography have progressed at a rapid pace within the last decade. Thin-layer chromatography (TLC) has, in many instances, replaced the more time-consuming process of paper chromatography. The wide acceptance of the TLC technique in the pharmaceutical industry has been borne out in a recent review by Comer and Comer which contained over 250 references (213). Zweig reviewed the literature of 1966 and 1967 covering TLC and other chromatography techniques in the latest "Fundamental Reviews" issue of *Anal. Chem.* (13). Recently, the analyst time required by the TLC technique has been reduced significantly by the utilization of precoated plates. Quantitation in TLC and paper chromatography has been greatly improved by the use of automatic scanning instruments. The latest issues of the various annual buyers guides provide an excellent source of information on the commercial availability of these devices (214–216). Automation in the field of chromatography has evolved mainly in the areas of column and gas chromatography. These areas, therefore, comprise the bulk of the review in this field.

Column Chromatography—Column chromatography has become an indispensable tool and a routine analytical procedure in many analytical development and pharmaceutical quality control laboratories. The many amino acid analyzers and the variety of automatic column chromatographic equipment commercially available today attest to the wide acceptance of this technique in the pharmaceutical and medical fields (214–216). The origin of automation in this technique began in 1948 when Stein and Moore attached an automatic fraction collector to a starch column (217). Due to the great interest in amino acid separation and quantitation, ion-exchange column chromatography has been responsible for the bulk of the application of automation to this technique. Spackman *et al.* published the first of their well-known works in this field in 1958 (218). A detailed account of this and the many other

[16] Aminco Micro Automatic Scanning Bacteria Analyzer, Am. Inst. Co., Inc., Silver Springs, Md.

[17] Beckman Instruments, Inc., Fullerton, Calif., Prod. Bull. 7123.

[18] Norelco-Unicam SP90 Atomic Absorption-Flame Emission Spectrophotometer, Philips Electronic Instruments, Mount Vernon, N. Y.

[19] Perkin-Elmer, Instrument Div., Norwalk, Conn., Prod. Bull. L-303.

innovations through 1965 has been compiled by Hamilton (219). *Anal. Chem.* offers excellent reviews in column chromatography in the biennial "Fundamental Reviews" editions; the latest being that of Walton (13) and Zweig (13). Each of these reviews covers the literature of 1966 and 1967. The "Technicon Bibliography" also covers many automated column chromatographic systems (18).

As a brief representation of automated column chromatography in the pharmaceutical control and development laboratory, several papers have been selected as examples of the different techniques in use today. An automatic method for the separation and estimation of neomycin A, B, and C has been published by Kaptionak *et al.*, who used a carbon column while continuously monitoring the effluent with ninhydrin reagent (220). Conca and Pazdera assayed streptidine in the presence of streptomycin by elution from an ion-exchange column using the automatic analyzer[1] for quantitation by an oxidized nitroprusside procedure (221). As little as 0.1% streptidine could be detected. Harmeyer *et al.* described the use of 2,4,6-trinitrobenzene sulfonic acid in amino acid analyzers in place of the usual ninhydrin reagent for the quantitation of amino acids (222). Edmundson *et al.* discussed the use of an ultraviolet readout system for the detection and quantitation of proteins, peptides, and amino acids (223). The advantages and disadvantages of this system were discussed and the use of computerized calculations was noted. A system for the simultaneous analysis of column effluent by ninhydrin reaction and fluorescence measurement was described for peptides and carbohydrates by Armstrong (224). Hunt (225) gave details of another simultaneous procedure which monitored column effluents of enzymatic digests of labeled RNA (^{3}H and ^{14}C). A specially constructed polyethylene liquid-scintillation flow cell and a dual-channel liquid-scintillation flow monitor were used. Dual-wavelength (260 and 280 mμ) spectrophotometric measurements were also obtained using a modified spectrophotometer (Beckman DB).

The automatic introduction of sample solutions to a chromatographic column was first described by Murdock *et al.* in 1966 (226). Sample solutions were placed in eight 1-ml. Teflon capillary coils mounted on a rotating center section of an automatic sampler. Seals between the rotating section and the fixed ends of the sampler were provided by O-rings. The sampler timer energized a motor which rotated the center section one-eighth of a turn bringing fresh sample coils sequentially into a buffer stream. The sample injector was placed at either the inlet or outlet side of a high-pressure pump. When column pressures of less than 120 p.s.i. were generated, the injection system performed well on the outlet side of the pump. However, when pressures above this level developed, leaks occurred during the rotation of the center section and reliable performance was obtained only when the sampler was placed before the pump. Provision for a water-jacketed temperature control of the sample coils was described. A further discussion of this system was given by Hirs (227). Independent development led Dus *et al.* to an automatic sequential sample injector of a very similar design (228). The system described by Dus *et al.* offered several advantages over that of Murdock *et al.* Twenty-four samples were contained in nylon tubes connected between two 24-port rotary valves working in synchrony. Automatic sample introduction into the column was achieved by rotation of both center valve core sections. These were connected to the outlet side of the high-pressure pump, and the column head, respectively. The system was successfully tested under pressures up to 500 p.s.i. and was routinely operated at pressures of around 350 p.s.i. An additional rotary-valve mechanism, which had been previously described for a stepwise 4-buffer elution program was utilized (229). A sequential rotary sample injection valve of similar design is now commercially available.[20] Another system, based on this type of sequential sample introduction, was described by Alonzo and Hirs (230). This apparatus employed nylon sample tubes and a series of check valves to inject the sample solution into either of two columns. A low-pressure pump was used to displace the sample solutions into the column heads while a separate high-pressure pump was used for elution.

In 1966 Eveleigh and Thomson introduced a new concept of automatic sample injection for ion-exchange columns (231). Samples were adsorbed on an ion-exchange resin contained within nylon cartridges in an indexing block. This block was moved automatically at preset intervals and the sample cartridges were inserted, in turn, between the buffer pump and the analytical column. The sample cartridge then essentially became an extention of the analytical column. A multichannel programming valve was also described by Eveleigh and Thomson for use in this or other elution systems (232). A detailed description of a sophisticated version of this injector design was given by Eveleigh *et al.* (83). A film-loop timer was described which controlled the index positions of the multichannel valve, giving the system almost unlimited versatility. The sample introduction device consisted of a turntable, capable of holding two rows of 40 sample containers. Two sample containers were used for each sample, one being connected to the basic amino acid column, while the other was connected to the neutral and acidic amino acid column. Thus, up to 40 samples could be loaded on the turntable at any time for automatic unattended analysis. This instrument is now manufactured commercially and product literature is available.[21]

One additional system for automatic column sample injection has been described by Dymond (233). Like the system of Alonzo and Hirs (230), Dymond utilized a special pump to transfer the sample solution to the column head, and a separate pump for buffer elution. Dymond, however, stored the sample solutions in tubes above which a wash cup was attached. These tubes were then placed in a turntable and a probe sequentially sampled each tube. Quantitative sample transfer was obtained by the rinsing of the sample tube with the wash solution.

[20] Chromatronix, Inc., Berkeley, Calif., Prod. Bull. ROV568.
[21] Technicon Corporation, Tarrytown, N. Y., Prod. Bull. 595-6-8-10-C.

An interesting insight into the future of column chromatographic analysis was given by Hamilton (234) in a panel discussion at the 1967 Technicon Symposium. High pressure, turbulent flow, and microbore column chromatography may reduce analysis time and increase detection sensitivity to a level which, up to now, has been unobtainable. It is unquestionably clear that important developments in column chromatographic systems will proceed at a rapid pace in the near future.

Gas Chromatography—The value of gas chromatographic (GC) analysis as a technique in the pharmaceutical and medical laboratories is universally recognized. Theivagt *et al.* (6) lists over 100 applications of this technique in the 1967 "Analytical Reviews—Applications" issue of *Anal. Chem.*, Pharmaceuticals section, while Juvet and Dal Nogare (13) list 667 references in the Gas Chromatography section of the 1968 "Fundamental Reviews" issue. *Gas Chromatography Abstracts* also provides an up-to-date categorized account of the literature in this field (235).

Total automation of the GC technique used in the pharmaceutical laboratory can be divided into five parts: (*a*) Sample preparation; (*b*) Sample injection; (*c*) Generation of the analog signal (chromatography); (*d*) Analog to digital conversion; and (*e*) Relation of digital data to concentration (calculation). Parts *c*, *d*, and *e* have been subdivided and outlined by Gill and Habgood in an editorial article which appeared in the December 1967 issue of *Gas Chromatography* (236). This entire issue was devoted to "Quantitative Gas Chromatography—Fundamentals to Automation." Parts *a* and *b* are reviewed in this section, and further references to Parts *d* and *e* may be found in the computer section of this review.

The only reference to automatic sample preparation for GC analysis found was that of Ek *et al.* (125). An automatic analyzer[1] was used to perform automatic chloroform extractions on aqueous solutions of antihistamines, while a portion of these extracts was collected for manual injection into a gas chromatograph. The internal standard technique was used for quantitation of results. Internal standard was added to the aqueous solution of the samples before they were diluted to their final volumes for sampling. In this manner, the effect of extraction variations within the automatic analyzer system was not a problem and the volumes collected for injection were not critical.

Automatic GC sample injection, as it applies to pharmaceutical applications (a high-boiling solute dissolved in a relatively low-boiling solvent), has been achieved by four basic approaches. Jarrel and Allison in 1964 reported a system in which 20 liquid samples, manually loaded in μl. syringes, were sequentially and automatically injected into a gas chromatograph (237). The 20 syringes were mounted on a turntable unit above the column injection port. Upon indexing of the turntable, the syringe directly over the injection septum was operated by plungers powered by compressed air. Evaporation of the solution within the syringe before injection proved to be negligible.

An automatic device for the application of evaporated samples (solid residues) to a gas chromatograph was described by Podmore in 1965 (238). Sample solutions were pipeted into small ferrous metal cylinders that had been previously siliconized and conditioned at 300°. After the solvent had evaporated, leaving the solute residue, the cylinders were loaded into a glass side-arm of the injection device which, when sealed, was a part of the carrier gas stream system. Sampling was achieved by feeding the cylinders into the flash heater section by means of a solenoid-controlled dropping device. To remove the previous sample cylinder before introduction of a fresh cylinder to the flash heater zone, a magnetic field was developed around the heater area which drew the previous cylinder out and automatically dropped it into a waste arm. In this manner, only one cylinder was allowed to reside in the flash heater zone during sampling.

Several other workers have since published their designs of injection systems based on the residue principle. Tinti evaporated samples in small glass capillary tubes which were then placed in a vertically mounted turntable (239). The turntable was loaded, sealed, purged with carrier gas, and as the turntable indexed, the sample capillaries were dropped into the flash heater. No convenient means was provided to remove the capillaries from the column head. Ruchelman designed a system in which stainless steel gauzes, resting on a Teflon plate, were loaded with sample solution (240). This system made use of the differences in surface tension between Teflon and stainless steel to help insure quantitative deposition of the sample solution to the gauze. After solvent evaporation, the gauzes were loaded into a vertical turntable and sample introduction into the flash heater zone was again achieved by the indexing of the sealed and gas-purged turntable. These gauzes were dropped into a sample basket which facilitated removal of the spent gauzes at the end of a run. By a simple connection, the turntable could be removed from the column and provisions were made for manual syringe injection into the same column. Within a year after the appearance of Ruchelman's work, Lantz and Morgart gave details of a commercially available gas chromatograph based on this design (241). A horizontal turntable and an automatic temperature programmer were adapted to the production model.

Two very similar sample introduction devices were published almost simultaneously by Borth *et al.* (242), and by Harkness and Torrance (243). In each system, residue sample containers were placed in a sealed tube with a spacing device separating one container from another. The attachment to the chromatographic column was provided at one end of the tube and sample positioning was achieved by placing a paramagnetic metal section behind the samples. Movement of a magnetic device along the outside of the tube produced a similar movement by the paramagnetic section, and in turn, caused sample injection. Appleqvist and Melin described another system using the residue technique (244). Sample solutions were evaporated inside small metal tubes which were loaded into a vertical turntable. The turntable, as in the other systems, was sealed, purged, and indexed to drop each tube sequentially into the flash heater. This system, however, had a mechanical provision within the heater unit to remove

and store each spent sample tube before a fresh tube was sampled.

The latest and most sophisticated design based on the residue sample injection method was described by McGregor (245). Samples were evaporated on porous platinum balls and were placed in a turntable which was not a part of the carrier gas system. Upon indexing of the turntable, the sample ball fell into a Teflon-cavity stopcock valve which was then automatically rotated to introduce the ball into the carrier gas stream and flash heater zone. Before the next sample was introduced, a pneumatically controlled rake swept the spent ball into a second Teflon cavity stopcock valve which, when rotated automatically, removed the ball from the carrier gas stream. The advantage of this system is that samples may be added to the turntable unit at any time while the sealed column system is left undisturbed.

A new automatic sampling technique was introduced by McCarty (246) in early 1968 which utilized the automatic injection device previously described by Lantz and Morgart (241). In this technique, a small Teflon capsule was loaded with from 2 to 8 μl. of a low-boiling solvent containing the dissolved sample. A Teflon plug was then inserted into the opening of the capsule resulting in a sealed system containing the sample solution. When the sealed capsule was dropped into the flash heater zone of the gas chromatograph by the automatic sample injector system, pressures from 15 to 25 Atm. were produced within the container. This caused the plug to be blown from the capsule, thereby releasing the sample and solvent as a vapor. A special dimpled collection tube was used in place of the original collection tube described by Ruchelman. This prevented the capsule and/or plug from being blown out of the flash heater area. A further description of this system was given by Lantz and Morgart (247).

The fourth approach to automatic GC sample injection was described by Evrard and Couvreur in 1967 (248). This system consisted of a turntable and holding tubes that contained liquid samples. A probe sequentially sampled the solution, drawing the liquid into a 20-μl. valve. By the automatic rotation of the valve, the sample aliquot was transferred into a solvent rinse stream which washed the solution into a cool precolumn. The solvent was evaporated by the flow of the carrier gas and vented from the system at the base of the precolumn while the solute remained as a residue at the head of the precolumn. Heating coils were then energized around the precolumn and the high-boiling solutes were eluted into the flash heater section of the chromatographic column. From this point the normal GC elution process continued.

As a summary of the methods which have been devised for the automatic injection of samples into gas chromatographs, the residue-injection technique offers a distinct advantage over liquid-injection methods in that it eliminates the appearance of the solvent peak. An exception to this, however, seems to be the liquid-injection system of Evrard and Couvreur (248), which employed the vented precolumn to expell solvent vapors. An additional feature found in this system was that exact premeasured volumes were not required for quantitative sampling. Only volumes in excess of that required to fill the sample valve were needed. The design of this system is, however, more complex when compared to the relatively straightforward approaches employed in the other systems. Sampling in the residue technique systems involves the pipeting of sample solutions onto the carrier containers prior to injection. This step is time-consuming and provides opportunities for manual errors. This is also true for the sealed-capsule technique. In addition, the residue and sealed-capsule systems, when loaded with samples and purged, cannot be disturbed once the sampling run has begun. The exception here was the novel approach of McGregor (245). Lastly, the technique of Jarrell and Allison (237), while involving the manual loading of μl. syringes, is the only system which totally duplicates a manual injection. This offers a definite advantage in that it adheres to all previously written and established analytical GC procedures and involves no changes in normal methodology.

READOUT DEVICES AND COMPUTERS

One of the most rapidly changing phases of automation is the method of readout. With speeds of analysis being increased tremendously, and with multiple-channel systems becoming ever more popular, a great amount of raw data must be processed in some manner. This, unfortunately, is also likely to be the most boring and unchallenging part of the entire analysis. Many hours of watching a readout device, transcribing data from a strip chart, or reading off digital values from a printed report form often lead to boredom of the operator and result in errors of calculation and/or reporting.

Different degrees of complexity are used in readout methods. The simplest, of course, is watching a meter or counter and recording the values for later calculation. Next comes the use of Heyrowsky's invention, the moving pen recorder. In this case, the equipment can collect the data unattended; and, whenever it is convenient, the operator can interpret the data. The operator must read and record a peak value or a steady-state response line and assure himself that he has correctly interpreted the chart scale. A further refinement is a digital printout from either a steady-state timed value or a peak value that has in some way been sensed by a peak-picking circuit. Almost all the readings discussed so far consist of raw data and must be compared to standards run under the same conditions at approximately the same time, *i.e.*, within the stability limits of the system. Once the readings are available, mathematical manipulations are required and a report must be written by an operator. A few systems use precalibrated charts and the results can be read off the charts directly. A further refinement is known as direct concentration readout. This may be an integral part of the system or it may be an accessory attachment. The necessary electronics must be provided and some means for adjusting the response slope is required. Most direct concentration readouts depend on the system obeying Beer's law. Results are usually printed out in digital form on a paper tape.

A major step forward has been the application of

some form of computer logic to automated system readouts to provide a multiplicity of output formats. The simplest of these is the "off-line" data acquisition system in which some form of computer readable record is made during analysis time. This might be punched card, punched tape, analog magnetic tape, or digital magnetic tape. These records are subsequently calculated on a remote computer and reported as hard copy to the appropriate submitter. The data can also be easily stored in machine-retrievable form for future reference. Recently, a number of automated systems have gone one step further and have been put "on-line." In this case, the computer is fed the raw data directly. With essentially immediate access to calibration data and possibly positive sample identification, the computer can identify and calculate the results as soon as the pertinent data has been generated. Again, a hard copy can be made and results can be stored in machine-retrievable form for future reference. The computer can also monitor critical performance parameters of instrument operation, and sound alarms, and/or print out diagnostic messages from preprogrammed malfunction sensors.

A further extension of the above-mentioned techniques is now being employed by some systems. This is known as "closing the loop." Previously, the automated instrument was the active partner of this combination and the readout or computer was the passive component with only a monitoring function. Computers are now being programmed to control the automated instrument, actuate valves, position components, and adjust electrical quantities. In addition, the computer can monitor and interpret data to maintain a control parameter or carry out a programmed sequence of events. These systems may be responsive to the operator's commands only on initial startup, or may interact with the operator in dialogue form during operation. The latter allows the operator to use his intellect to alter the course of analysis based on the data furnished him by the computer.

The reference to computers so far has implied the use of general-purpose digital computers usually supplied by a manufacturer other than the automated instrument manufacturer. Certain problems have been very evident in the use of this type of system. Computer manufacturers exist in a digital world and instrument manufacturers usually exist in an analog world. Seldom do instrument manufacturers and the computer manufacturers fully understand each others equipment, and problems of linking the two systems together hardware-wise are rather common. Software or programming is even more complex, and many difficult problems are encountered in attempting to adapt general purpose digital computers to laboratory instrumentation. Programming is one of the most underrated professional skills in existence. A successful programmer for laboratory automation must not only have a thorough knowledge of the computer language he is using and an understanding of the detailed requirements of the specific computer, but he must also have a thorough knowledge of the minute details of the analytical problems. People with backgrounds in both these areas are hard to find. Because of the rapid proliferation of computers and the obvious advantages to be gained, most users must more or less "grow their own" programmers by a concentrated reeducation of their experienced laboratory personnel.

One possible solution to the problem is beginning to emerge. Automated instruments are beginning to appear that consist not only of the instrument, but also include an integral special purpose computer. With the dramatic decrease in the cost of manufacturing computers, the ultra compact designs due to integrated circuits and the savings available in nonduplication of components, much greater use of integral special purpose computers will be seen in the future. The advantages are obvious. One manufacturer is responsible for the entire system, he knows his equipment, the components are designed to complement one another, and the user does not have to worry about interfacing, programming, equipment incompatibility, or noise pickup due to long transmission lines.

There will undoubtedly still be situations where it will be more economical to have a number of similar instruments connected to a central computer, rather than having each instrument computerized individually. This would be especially true in the case of slow data-rate instruments such as gas chromatographs and various automatic analyzers. The experience now being gained in such installations will be extremely useful in simplifying future attempts at interfacing these instruments. More extensive knowledge must and will be gained in writing executive monitors that allow essentially independent and random access to a common, time-shared on-line computer. Several presently successful installations attest to the fact that these types of systems can and will continue to be utilized.

In surveying the literature for examples of the various readout systems just discussed, one becomes astounded at the number of papers written and the extensiveness of the effort expended in this area. There are several excellent reviews that should be mentioned at this point. Williams has reviewed the field of computers, process control, and automation for *Ind. Eng. Chem.* since 1956. Because of the rapidly changing state of the art, reviews starting with 1964 were found to be the most pertinent. The numbers of references in this series have increased steadily culminating in over 825 being listed in the 1967 review (249). His reviews are classified according to application, and, within the field of laboratory instrumentation, he lists many of the familiar analytical techniques. Another very useful bibliography, published by the National Library of Medicine, lists 327 papers dealing with computers in Medicine and Biology appearing between mid-1964 and December 1967 (250). Citations are by author's name. Krueger-Thiemer has reviewed the use of digital computers for the analysis of many special chemotherapeutic problems and has discussed the resultant advantages (251). He lists 32 references. The New York Academy of Sciences conducted a symposium in 1964 on the uses of computers in medicine and biology that presented an excellent background in the use of laboratory computers (252). A recent analytical symposium on computers was held at Pennsylvania State University (253). Most of the papers presented there have not been published to

date, but many useful ideas were reported. Many of the *Anal. Chem.* reviews dealing with specific techniques cover automated readouts under various headings such as instrumentation, equipment, or apparatus and methodology. The "Technicon Bibliography" also lists 55 references to the automated data processing of continuous-flow systems (18).

It might be well to list some examples of the various automated readout systems previously described. This list is by no means exhaustive and mention does not imply endorsement of the equipment by the authors, but rather denotes some familiarity with the equipment by at least one of the authors. Bausch and Lomb has introduced a direct concentration readout computer.[22] The readout is linear and can be used for reactions that follow Beer's law. The display is visual and has an output plug for attachment to a recorder. A concentration readout (DCR-1 Perkin-Elmer) can be attached to any spectrophotometer with a 10-mv. full-scale output. It calculates concentration after proper calibration and allows the use of methods which do not obey Beer's law. It is also compatible with a variety of output devices. Gilford Instrument Laboratories (Oberlin, Ohio) offer a digital spectrophotometer, linear in absorbance from 0.000 to 2.000, with a wavelength range of 340–700 mμ. Its output (BCD) allows its use with another system (4006 Data Lister) which prints four digits of results and provides for manual entry of four more digits of identification.

A calculating absorptiometer is available from LKB (254). The unit automatically samples solutions, corrects for reagent or serum blanks, and outputs the data in the appropriate concentration units to either a visual display, a printer, punched cards, punched tape, or a computer. A. H. Thomas (Philadelphia, Pa.) markets an all-electronic direct-concentration readout which displays transmittance, absorbance, or concentration. The unit can be coupled to their digital converter to print out results on paper tape, drive an IBM card punch, or drive a paper tape punch. The system has an automatic sequencer. The concentration readout can also be fitted with a peak detector circuit board to monitor continuous-flow systems. This system can be coupled to their automated reader which can sample from a 20-tube turntable and measure the samples in any suitable spectrophotometer.

Evans Electroselenium Ltd. (Halstead, Essex, England) offers an automatic colorimeter and sampler. The unit samples from a 48-tube, four-segment turntable on an 18-sec. cycle, and reads the samples photometrically. The output is linearized for absorbance measurements or concentration, and the results are printed on a paper tape along with a sequence number. The unit is self-standardizing and self-correcting for drift.

A very elaborate and sophisticated automatic digital spectrophotometer is available from Norelco-Unicam.[23] Its unique design combines the advantages of a null-balance system with the stability of a ratio-measuring system. It will automatically sample up to 50 samples, measure each sample at up to 10 wavelengths, and print the absorbance or transmittance values on an output typewriter. Measurements are made at 30-sec. intervals and samples can be directed to waste or can be automatically returned to the sample cup. The instrument has a built-in cell matching device and provides for automatic slit width and energy source selection. Automatic programmers are available for fast repetitive measurements on a single sample for the study of reaction kinetics.

A number of companies have data-acquisition systems available which are essentially off-line systems intended to record the data for later computer calculation. On-line, time-shared, and control computers are also offered by many of the same manufacturers. Some of the better known manufacturers of smaller laboratory based computers are Control Data, La Jolla, Calif.; Digital Equipment Corp., Maynard, Mass.; Electronic Associates, West Long Branch, N. J.; General Electric, Phoenix, Ariz.; Hewlett-Packard, Palo Alto, Calif.; Honeywell, Framingham, Mass.; IBM, White Plains, N. Y.; Infotronics Corp., Houston, Tex.; Scientific Data Systems, Santa Monica, Calif.; and Varian, Palo Alto, Calif. All of these have proven, tested computer hardware and software that can be utilized for a number of laboratory instrumental applications. A more complete list can be obtained from the "Computers and Automation Annual Buyers Guide" (255) or from a variety of computer buyers guides available through the journals associated with this field.

Several recent papers have been published on laboratory data processing and have not yet appeared in the reviews mentioned previously. Some of these are particularly significant and should be cited here. There are a number of papers that deal with general applications of computers to laboratory instrumentation. Spinrad outlined the steps that are necessary to plan for automation in the laboratory using on-line computers (256). He discussed equipment as well as software, and presented various applications. The preparation of source data for automation was the subject of a paper by McRainey (257). He listed a series of "do's" and "don'ts" for the planning and execution of data input. Some of these are especially noteworthy and should be mentioned here since they apply to automation in general. The list of "do's" includes: (*a*) Study the system in depth before taking final action. Machines are much less flexible than people, and every detail must be worked out in advance. Machines bind you to a system. (*b*) Be sure you have a genuine need for automation and not merely an enchantment. (*c*) Remember to provide adequate controls to assure accuracy of results. (*d*) Collect only the data you need. Some of the "don'ts" are: (*a*) Don't buy equipment first and then determine what to do with it. (*b*) Don't buy a "pig-in-a-poke." Get a valid demonstration of the equipment performing an analysis similar to your own. (*c*) Don't try to do the job alone. Get the cooperation of the people involved in the operation. (*d*) Don't ignore comments and suggestions from operating personnel.

[22] Concentration Computer, Bausch & Lomb, Rochester, N. Y., Prod. Bull. 33-6008 0268

[23] Philips Electronic Instruments, Mount Vernon, N. Y., Prod. Bull. 3-3M/4-68.

Frazer discussed in a very practical, down-to-earth manner the use of digital control computers (258). He surveyed the field of applications of various types of computers and brought in many observations on the hard facts of computer laboratory automation. This is recommended reading for anyone considering laboratory computerization. Frazer discussed the same material in somewhat less technical terms in another recent publication (259). Another very forthright report on the problems of time-sharing on a general purpose digital computer was presented by Secrest (260). This author presented the pros and cons of small dedicated computers *versus* large time-shared computers and revealed, in a frank discussion, the hardware and software problems encountered by this group at the University of Illinois. The general techniques used in multiprogramming of large computers were compared with those used for small computers by Gaylord (261). The design concepts of writing small computer monitors for on-line control systems were discussed in considerable detail and pertinent terminology was defined. Some problems with time-sharing (A Tale of Horror) was the subject of a paper by Wilkinson (262). With a tongue-in-cheek approach, he outlined problems that he has encountered with time-sharing, and information such as this may tend to balance the slightly optimistic descriptions of some of these systems by the various computer manufacturers. Lauer and Osteryoung (263) described a general purpose data acquistion and control system built around a small digital computer. The various interfaces were described, complete with block diagrams of the logic modules. The system was designed so that a majority of analytical instruments could be connected with minimal wiring. Examples were given of the use of this system for several electrochemical techniques.

Several papers deal with specific applications of computers to laboratory automation. The two major areas of application are gas chromatography and clinical chemistry, especially automatic analyzers. Three papers serve as excellent theoretical background for computerization of gas chromatographs. These papers deal with errors in integration of peaks (264), error sources in gas chromatography (265), and the role of quantitation in gas chromatography (266). Simon *et al.* presented a technique for monitoring of gas chromatographs by recording the data on magnetic tape followed by calculation of the results off-line (267). They described a new technique for eliminating the influence of base line drift. A system, using voltage to frequency conversion, plus counting, as a means of area measurement was proposed by Shank and Persinger (268). The signal could be recorded on magnetic tape or fed directly to a computer. Procedures were given for slope detection, drift correction, data normalization, and log compression. Use of fast playback speeds for off-line calculations was compared to normal data rate calculation. Karohl reviewed the various methods of peak detection such as base line tracking, and the use of first, second, and sometimes even third derivatives for differentiation of the signal to determine peak start and stop (269). These higher order derivatives contain base line information useful for more accurate base line extrapolation under a peak. The computer hardware and software which was used to detect peaks and make base line corrections was discussed.

Briggs was one of the first to publish on the use of a general purpose central computer for the simultaneous monitoring of a number of gas chromatographs (270). He described the use of a computer (IBM 1801) for monitoring, controlling, and calculating results from 40 gas chromatographs and one mass spectrometer. As many as 20 of these chromatographs could be in operation simultaneously. McCullough further described this system and emphasized the need for effective monitor and executive programs (271). He also covered peak area calculations and the necessary interface equipment. Sederholm *et al.* presented a further extension of this system by providing for the addition of an NMR instrument and the use of the system in a foreground-background mode (272). Oberholtzer suggested a computer system for gas chromatographs that provided programmed automatic sample injection and timing, as well as data calculation, for high-speed chromatography of gaseous samples (273). The use of computers in clinical chemistry is well covered by the Technicon review. There are also a number of papers in other fields of automation that are of interest. Automated batch fermentation of antibiotics at the Dista plant at Speke, England, was the subject of a brief paper (274). Computers monitored pH, air flow, temperature, and foam formation, and in turn, actuated valves to maintain process control. Sadtler Research Laboratories (Philadelphia, Pa.) are an excellent example of the use of an off-line data acquisition system for the monitoring of a large variety of analytical instruments. They have attached IR, UV, NMR, mass spectrometers, and GC instrumentation to their computer (IBM 360/30) and can provide customer instrumentation service. They also publish extensive collections of spectra in the field of pharmaceuticals and biochemicals.

Several recent publications have described the use of computer techniques for electrochemical methods (275–279). Perone *et al.* interfaced a fast-sweep derivative polarograph to a small digital computer (280). By using repetitive scanning, resolution of closely spaced reduction waves was accomplished and the analytical sensitivity was increased by an order of magnitude over conventional methods. Another series of papers has been published on the use of computers for mass spectrometry. Among the most recent was a paper by Hites and Bieman (281). In their very elaborate system they used a fast-scan spectrometer attached to a gas chromatograph. The output was digitized at 3,000 points per second and recorded on magnetic tape. A computer read the tape, found peak centers, assigned them mass values and intensities, and printed the spectrum in digital form in addition to plotting it graphically. Since data were already available in easily accessible form, many other data manipulations could be performed such as: normalization, comparison with other spectra, correction for background, and library searching. Identification of unknown substances, as well as functional groups, from computerized mass

spectra was the subject of two papers by Crawford and Morrison (282, 283). Glaser and Wattenburg presented a progress report on a very novel use of computers (202). They used a computer-controlled flying spot scanner for counting colonies of bacteria and other microorganisms grown on nutrient agar. They also used the system for identification of the organisms by observation of their colony morphology, growth rate, nutritional requirements, drug resistance, and other observable parameters. Colonies were grown in a "Lazy Susan" environmental chamber, photographed periodically, and the photographs were scanned for pertinent data. Optical density profiles (ODP) of the colonies were shown to possess marked differences between organisms and even between different strains of the same organism. This approach may have an application in a number of biological research and development laboratories.

One of the recent trends has been the increased use of more sophisticated calculators or so-called desk-top programmable computers. Several have been marketed recently by manufacturers such as Hewlett-Packard (Palo Alto, Calif.), Wang (Tewksbury, Mass.), and Olivetti Underwood (New York, N. Y.). These units have facilities for limited programming on magnetic cards and may be a successful alternative to the use of small digital computers in certain cases. Walkenstein *et al.* have adapted one of these calculators for systems use by coupling a solenoid bank to the printer of a scintillation spectrometer. This technique might be applicable to other instruments with a printer output (284).

An application of computers that does not deal directly with laboratory automation, but should be of interest to all automation analysts, was detailed in an article by Tate (285). This article relates the conversion of Chemical Abstracts Service, along with the entire American Chemical Society publications program, to a computer-based operation. This will provide a more timely and efficient information service for all scientists. It will provide a unified search and retrieval system in preparation for the needs of the 1970's.

MISCELLANEOUS APPLICATIONS

Due to the limited scope of this paper, many automated methods which apply to a number of analytical techniques will not be covered. Most of these have been well reviewed in other publications. Automated titrations have been reviewed in *Anal. Chem.*, "Fundamental Reviews" under Amperometric Titrations, Potentiometric Titrations, and Titrations in Nonaqueous Solvents. Other automated techniques covered in these volumes are Ion Exchange Chromatography; Polarography; Inorganic Microchemical and Trace Analysis; Organic Microchemistry; Nucleonics; Infrared, Light Absorption, Nuclear Magnetic Resonance, Mass, X-Ray Absorption and Emission, Mossbauer, Raman, and Ultraviolet Spectrometry (7–13).

Several additional papers of unusual interest to the authors are reviewed. Roth (286) reviewed 10 methods for measurement of moisture in solids. These include both intermittent and continuous methods. An automated osmometer was reported by Forman and Changus (287). An osmometer (Fiske) was modified to automatically process 30 samples at the rate of 15 per hour. The instrument recorded osmolality on a strip-chart recorder and automatically cleaned and dried the sensing device between samples. An interesting instrument was presented by Donaldson and Frommhagen for fluorescent antibody analysis (288). The author states that the equipment could be used for other wet chemistry automation. Two very important papers have been written by Merrifield on the automated synthesis of protein (289, 290). These have formed the basis of an entirely new approach to the stepwise synthesis of peptides and proteins by the solid-phase method. Several applications are reviewed. Bonnafe (291) presented a review of automated *in vivo* applications of continuous-flow analysis which contains 24 references. A review (143 references) of instrumental and automated methods for waste water and water pollution was published by Mancy and Stinson (292).

There are many examples of automated systems leading to new discoveries but one of the most novel must be the work of Kirk (293). During the development of an automated enzyme procedure, he noted that the enzyme was absorbed on glass mixing coils. By fabricating a chromatographic column filled with glass chips, he developed an adsorption and elution sequence for isolation and purification of the enzyme. He then utilized these elution conditions to circumvent his previous adsorption problems in the automated system. It is interesting to speculate about the number of "Recoveries from Serendipity" which may have lurked undetected in the many unsuccessful attempts at automation.

SUMMARY

It can be readily observed from this review that automation in the modern laboratory has been firmly established and extensively applied. This is especially true in clinical laboratories where the major emphasis has been on continuous-flow techniques. The advent of several unique and novel discontinuous systems portends another extensive increase in laboratory automation. If adaptation of these discontinuous systems to pharmaceutical analysis will be even partially as successful as the adaptation of the continuous-flow instrumentation has been, the modern pharmaceutical laboratory will indeed be well-equipped.

With the advent of all this technology, laboratory management must examine their own position carefully and consider how extensively they should or must automate. Some of the reasons for automation which should be considered are the following: (*a*) In many areas a large portion of the analytical work is highly repetitive and a few automated methods could significantly reduce the workload because of automation's great efficiency. (*b*) A laboratory may have a large number of products which require content-uniformity analysis. (*c*) Many assays are time consuming and routine. An analyst would much rather have an instrument perform this work so that he could focus his attention on more challenging problems. (*d*) The number of adequately trained analysts still continues to fall far

short of the demand, and better use can usually be made of these people in more complex assignments. (*e*) Labor and space costs continue to increase significantly. (*f*) As analytical capability increases, more demands are made on the laboratory. (*g*) Automatic analysis usually provides quicker results, enabling faster analytical service to the submitter. (*h*) Costs for additional assays are usually very low and these additional assays can often serve as a key source of information and can provide early warning of production problems or process difficulties. (*i*) Automated methods developed in the research and development phases of a new product significantly ease the transition to full-scale production and production control.

Some of the reasons for not utilizing automation could be: (*a*) A laboratory may have very few assays on any one product and may not be allowed to store up a sufficient number of similar samples to run at one time. (*b*) The variety of products may be so great that the "common handle" approach to automation cannot be effectively utilized. (*c*) The automated methods available may not be acceptable by official compendia. (*d*) Specific techniques now used for analysis may not have been automated and there is no possibility of change in the methodology because of the unique circumstances of the problem. (*e*) The cost of the equipment may be greater than the anticipated savings and the additional analytical capacity that the equipment may offer is of little value.

In addition to the observations just mentioned, there are other factors that need to be considered. In many cases, equipment is not yet available to exactly duplicate the manual methods existing in the official compendia. This has posed a serious problem, but some relief from this situation appears to be emerging. Both the USP XVIII and the NF XIII have proposed, in galley proof, provision for Method I and Method II analysis. Method II is allowed in certain monographs where the content-uniformity test calls for a procedure that differs significantly from the *assay* in the monograph. The NF XIII galley proofs even provide a special technique for calculation of a bias factor, if necessary, to be applied to Method II results. If these proposals become official, the transition to automated methods will be expedited. Some of these methods may still be of such a nature that no commercially available automated equipment will allow compliance with the officially specified assay. There is a genuine need for more instrumentation that closely parallels manual methodology, enabling greater use of this equipment for official products and concurrently making the task easier for those whose responsibility it is to design official procedures.

One characteristic that is common to most automated single-dose assays should be considered further. In traditional manual methods, there is usually an opportunity to reassay a questionable sample, if desired. Since all methods have a certain error associated with them, reassay is a valid means of obtaining a more precise analytical value. With most automated single-dosage form analysis equipment, a single result is obtained on each individual dose and the remainder of the sample is discarded during the course of analysis. Whenever a result is obtained under these conditions that closely approaches the potency limits of the monograph, the analytical error should be considered in making a final disposition. A different, and as yet undefined, set of criteria may be necessary to evaluate such a result, since only one piece of data is available and there is no opportunity for future observations on the dose in question. Aside from possible changes in the interpretation of results, other alternatives suggest themselves. Equipment could be designed that stores the prepared sample until the results of the analysis are available, at which time the sample could be rerun for additional data if desired. All samples could also be saved and run two or more times as a routine practice, or a parallel multichannel system could be utilized. Both of the latter would at least double the workload, and may not be practical in view of the already great demands being made on the analytical laboratory. Equipment could also be designed to be so precise that the associated error need not be considered. All of these approaches may need to be considered before the problem can be resolved.

Much still needs to be done in the field of automation before one can achieve the utopia of a completely automated, computer-controlled system for the conventional analysis of all pharmaceuticals. It is not likely that such a system will be found in the near future, especially one which parallels every step of the time-honored manual procedures. It may not even be desirable to do so. Instead, one must attempt to apply the unique opportunities that automation offers. Specificity, control of analytical variables, dynamic computer control of analysis, resolution of complex spectra, and separation of complex mixtures are but a few examples of the many advantages that automatic instrumentation can provide. Considering the impressive array of equipment already available and in current development, the extensive knowledge of the many capable scientists in the field, and the encouragement offered by the forward-looking editorial policies of the leading scientific journals, the future indeed looks bright for automated techniques in pharmaceutical analysis.

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Note: This review covers the literature through July 1968. Since that time many significant advancements have been made in the field of automation. Most notable of these are laboratory computer technology and automated sample injection in chromatography systems.

RESEARCH ARTICLES

Comparison and Analysis of the Teratogenic Effects of Serotonin, Angiotensin-II, and Bradykinin in Mice

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Abstract □ The incidence of anomalies in untreated and saline-treated controls (both s.c. and i.v.) was 1.5 and 0.26 percent, respectively. Subcutaneous administration of serotonin at 1 mg./mouse and 10 mg./kg. produced respective incidences of 24.4 and 27.0 percent, while i.v. administration caused total litter resorption. Angiotensin, 10 mg./kg., *via* both routes proved to be not significantly teratogenic. Bradykinin, 25 mcg./mouse s.c. produced 0.87 percent malformations, while the i.v. route caused 12.7 percent. The two most teratogenic agents, serotonin and bradykinin, both markedly decreased and increased, respectively, the transfer of radio-^{24}sodium from the maternal blood through the placental barrier to the fetal side of the placenta; angiotensin only slightly decreased this transfer. In addition, the three autocoids were noted to decrease the transfer of isotope from the placenta to the fetus, indicating that vasoconstriction of the fetal placental vessels may have occurred.

Keyphrases □ Teratogenic effects, mice—autocoids □ Serotonin—teratogenic effects, mice □ Angiotensin-II—teratogenic effects, mice □ Bradykinin—teratogenic effects, mice □ ^{24}Sodium, placental transport—autocoids effect □ Placental, uterine vessels—serotonin, angiotensin, bradykinin effect

Serotonin, angiotensin, and bradykinin, all autocoids, have several physiological actions in common. They are potent placental vasoconstrictors (1–4), affect permeability of blood vessels, affect blood pressure, produce some effect on kidney function, and have all been implicated in the production of various pathological states such as carcinoid syndrome, toxemia of burns, pregnancy, and allergy (5–9). Furthermore, they all stimulate various smooth muscle preparations and cause uterine contracture.

It becomes apparent that the administration, endogenous release, or even blockage of the above autocoids' enzymatic destruction could produce untoward effects on normal fetal development. Actually, this has been proven to be the case for serotonin (10–12), reserpine (13), and iproniazid (14). All have been shown to