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17. I thank my fellow astronauts for discussions which have contributed significantly to drafts of this paper, especially the suggestions of J. Allen, J. Kerwin, W. Lenoir, R. Parker, and P. Weitz. Also, I thank our Skylab II crew physician, Dr. P. Buchanan, for his very helpful comments and advice.

High-Pressure Ion Exchange Chromatography

New systems for analyzing large numbers of body fluid components can be used in the clinical laboratory.

Charles D. Scott

There is an increasing interest among medical scientists in new systems being developed for the automatic analysis of large numbers of molecular constituents in various body fluids. The quantities of the constituents of all physiologic fluids represent potentially useful diagnostic information; however, the analysis of urine presents the greatest challenge. In a bibliography on urinary constituents, the literature for a 3-year period has more than 3000 citations to more than 700 molecular constituents, many of which could have pathologic significance (*1*). In this article I describe some of the new high-resolution analytical systems that are based on the use of high-pressure liquid chromatography, and that are at least potentially useful for the analysis of urine and other less complex body fluids. Such systems are used primarily for the identification and quantification of the low molecular weight (less than 1000) components of physiologic fluid.

The term "high-resolution analysis" is used to describe a procedure in which a large number of the constituents of a sample mixture are separated and quantified. Thus, high-resolution analytical techniques provide (i) a means of separating the individual components and (ii) a means of detecting and quantifying the separated components. Several high-resolution liquid chromatography (LC) systems have been developed for these applications, and now a whole family of instruments is available for separating and quantifying various types of biochemical constituents. These include analyzers for ultraviolet absorbing constituents (*2–4*), amino acids and related compounds (*5, 6*), carbohydrates (*3, 7*), and organic acids (*8, 9*); other, more highly specialized analyzers have also been developed. Much of the early work on high-pressure LC was directed toward the development of separation systems for physiologic fluids (*2–4, 10*). Liquid chromatography, as a method for separating complex biochemical mixtures, represents a useful complement to gas chromatography. Although a more rapid separation can be achieved with gas chromatography, sample preparation and volatilization of biochemicals naturally occurring in an aqueous medium can be complicated and time-consuming, and the results are likely to be difficult to quantitate. Conversely, sample preparation for LC systems (many of which use aqueous eluents) is frequently very simple, in

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some cases requiring only a volumetric measurement; however, the separation process in LC can be time-consuming.

The development of LC analytical systems capable of separating large numbers of constituents has involved numerous investigators. Certainly, the early work of Cohn in separating nucleic acid derivatives by ion exchange chromatography (*11*) was important, as was the development of an automated analytical system for amino acids by Moore and Stein (*12*). Hamilton showed that hundreds of ninhydrin-positive compounds in urine could be separated and quantified by a modified amino acid analyzer (*5*), and others followed through on some of Cohn's work to automate the analyses of complex biological fluids in a single system (*13*). Many other contributions in the general area of high-resolution analysis for the clinical laboratory have been published recently (*14*).

Analytical Systems

All of the systems discussed here utilize high-pressure (up to 5000 pounds per square inch; 3.45×10^8 dyne/cm^2) ion exchange chromatography for achieving high-resolution separation, and contain the following major components: (i) a closed tubular column packed with the solid sorbent for separation; (ii) an eluent storage and gradient elution preparation section; (iii) an eluent delivery system equipped to deliver the eluent to, and force it through, the separation column; (iv) a means for introducing the sample into the column; (v) a means for detecting and quantifying the constituents in the column eluate fractions; and (vi) an appropriate data acquisition and processing system (see Fig. 1).

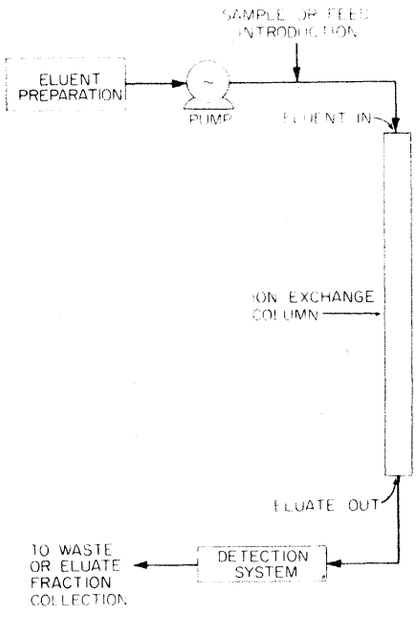

Fig. 1. Components of an automated, high-pressure liquid chromatography system. [From Scott (*10*), courtesy of Academic Press]

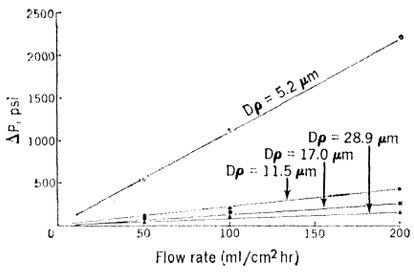

Fig. 2. The effects of particle size, column length, and flow rate on pressure drop in ion exchange columns. The operating conditions were: temperature, 40°C; stainless steel column, size 0.62 by 100 cm; ion exchange resin, Dowex 1-X8. The symbols D_ρ indicate the partial diameter of the ion exchange resin particles.

Variation in the choice of separation media, operating conditions, and eluate monitoring techniques can result in several different analytical systems that, although specific for many different molecular constituents, are basically alike.

Separation systems. The most important component of the high-pressure liquid chromatograph is the separation system. Many new types of sorption media have been developed recently that make possible high-resolution separations, and there are several new systems in which multiple columns are used.

One recent goal has been to produce sorption media in which the solid-phase mass transport resistances are reduced so that the chromatographic system can operate closer to equilibrium conditions; this, in turn, should result in more rapid and more effective separations.

All the systems under consideration here achieve high resolution by using relatively small particles of ion exchange resin (minimum diameter, ~ 10 micrometers) in the stationary sorption phase in chromatographic columns. The columns usually have diameters of 1 to 9 millimeters, and they are 50 to 150 centimeters long. The small particles are used to reduce the solid-phase diffusional effects, and the relatively long columns are necessary to provide a sufficient number of separation stages to achieve the high resolution. A diameter of about 2 or 3 μm probably represents the lower limit of useful particle size since particles smaller than this are almost certain to exist as colloidal suspensions.

Of increasing importance has been the advent of pellicular ion exchange resin in which a thin film of ion exchange material is bonded to the outer surface of an inert solid core. This material has potential for very rapid separations, especially where sorption capacity is not an important consideration.

The major factors that affect the pressure drop across an ion exchange column are the diameter of the particles, flow rate, column length, and fluid properties such as density and viscosity. These effects have not been thoroughly studied for small resin particles; however, preliminary data have shown that the pressure drop across an ion exchange column is inversely dependent on the square of the mean diameter of the ion exchange resin particles and linearly dependent

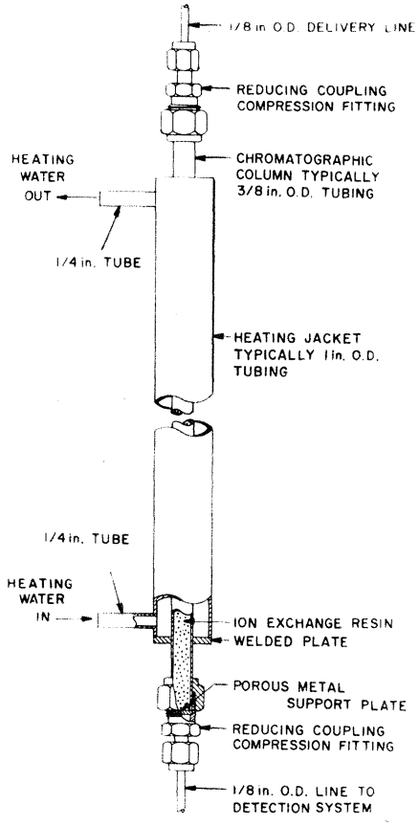

Fig. 3. Typical design of a high-pressure, stainless steel (type 316) LC column with a fluid jacket for temperature control.

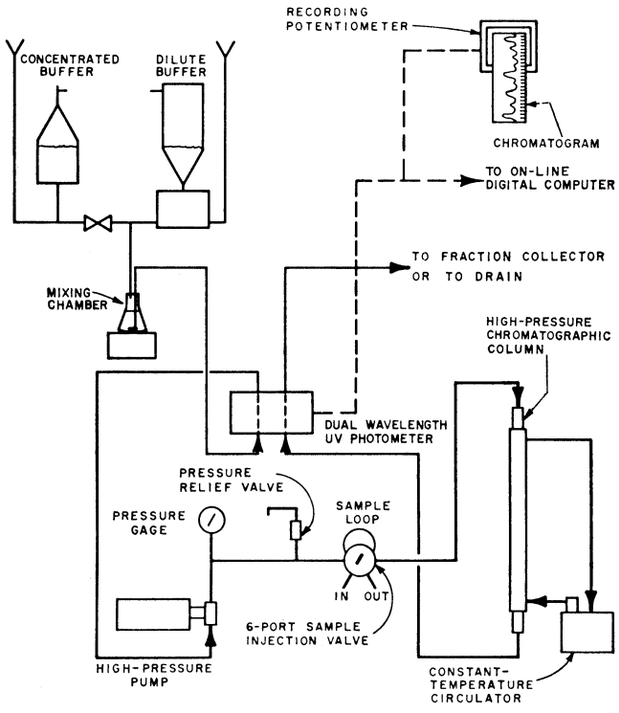

Fig. 4. High-pressure anion exchange chromatograph for the ultraviolet absorbing constituents of body fluids. [From Scott (*10*), courtesy of Academic Press]

on the specific flow rate and the length of the column (Fig. 2).

Metal columns that can be easily fabricated from seamless metal tubing are recommended for high-pressure techniques. Conventional compression tubing fittings can be used for the fluid entrance and exit, and for holding a porous metal support for the resin bed (Fig. 3). Temperature control can be achieved by enclosing the tubing in a heated oven or by enclosing the column in a heating jacket containing a controlled-temperature circulating fluid. The former method is preferred by most of the commercial manufacturers of LC systems, while the latter method is more useful where a large heat load is needed since the circulating liquid has a higher heat capacity.

The geometry of an ion exchange column can have a significant effect on the resolution that is achieved. As the length of a column is increased, the separation of two components becomes more effective; on the other hand, the width of the peaks is also increased. The diameter of the column should not have a significant effect on resolution (provided that comparable flow velocities and a proportionally scaled sample size are used) as long as it is sufficiently small to prevent radial variations in fluid properties but not small enough to require a sample of such limited volume that the separated solutes cannot be detected by the column monitoring system.

The most successful method for packing small-diameter ion exchange resin into a chromatographic column is to force a slurry of the resin particles into the column at a velocity much greater than the settling velocity of the particles (*15*). Such dynamic packing can be achieved either by displacing a thick slurry or by extruding a prepacked bed. Packing by slurry displacement is accomplished by connecting a chamber to the chromatographic column, filling the chamber with a thick slurry of the ion exchange resin, and then displacing the slurry into the column. If the linear velocity of the displacement fluid in the slurry chamber is substantially greater than the settling velocity of the largest particle, size segregation will not occur and the complete task can be completed within a few minutes. During this operation, the flow rate of the liquid should be greater than the anticipated flow rate of the eluent.

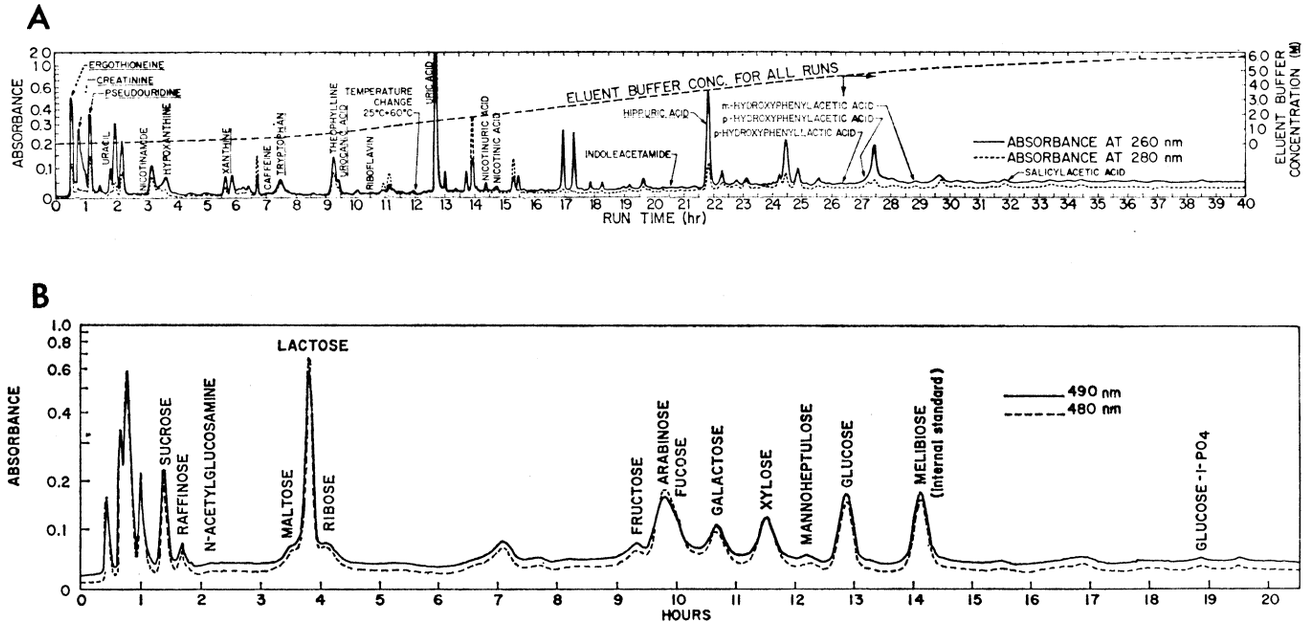

Fig. 5. Typical urine chromatograms from (A) the ultraviolet analyzer and (B) the carbohydrate analyzer. Operating conditions are described in Scott *et al.* (*3*).

When small-diameter columns of considerable length are packed with finely divided ion exchange resin, the rate at which the liquid can be pumped through the slurry chamber and into the column is reduced; this prevents a rapid displacement of the slurry, and size segregation may occur. However, as an alternative, a fixed bed can be packed into a reservoir of larger diameter; then it can be extruded into the small-diameter chromatographic column by displacement with liquid.

Eluent delivery system. Three basic types of eluent delivery systems are used: constant flow devices, constant pressure pumps, and reciprocating piston pumps. In general, the reciprocating piston pumps are less expensive and simpler to use; they are particularly advantageous when gradient elution (that is, an eluent composition that changes with time or elution volume) is used, since the gradient can be developed prior to contact with the high-pressure environment (for example, by interconnection of multiple reservoirs with eluent solutions of varying properties) (*16*). If it becomes necessary to use gradient elution with the constant flow devices or constant pressure pumps, multiple delivery systems with high-pressure coupling are required. A system of flow programming may also be essential in some instances.

Although pulsating pumps cause flow variations, they usually do not have adverse effects on the sorption process in high-pressure systems; flow rates through the column probably do not vary more than ± 10 percent during the pulsation. On the other hand, some on-line detection systems (for example, refractive index monitors) are very vulnerable to variations in flow rate and the pulsations contribute to significant background noise. All of the systems described here use eluate monitoring systems that are not significantly affected by pulsations and they are, therefore, equipped with reciprocating piston pumps.

Sample introduction. The most effective method for introducing a sample into an automated chromatographic system is to feed it through a septum, by means of a syringe and needle, directly into the eluent line just before the eluent contacts the columns. At pressures greater than about 1000 psi (6.9×10^7 dyne/cm^2), however, the syringe technique will usually require that the eluent flow be stopped temporarily. Samples can also be introduced by way of an injection valve. Such a valve usually contains six ports, each pair of which is interconnected. In one orientation of the valve, a sample can be loaded into the sample loop, which becomes a part of the eluent line when the ports are reoriented (by turning the valve handle). Valves that allow automated sample introduction at pressures up to 5000 psi without interrupting the system have been developed (*17*) and are now available commercially.

Eluate monitoring. An eluate detector, which is necessary to complete the automated analytical system, usually monitors continuously a specific eluate property that makes it possible to detect and quantitate the separated constituents. Two detection methods are used in the LC systems described here: (i) flow photometry and (ii) reagent development followed by photometric or fluorometric monitoring. In the latter method, reagents are mixed continuously with the eluate stream and the resulting reaction mixture is monitored continuously.

Continuous flow photometers have been developed specifically for LC (*18*), and now many models are available commercially. In the high-resolution chromatography of physiologic fluids, it is usually desirable for the photometer to provide simultaneous or sequential monitoring at two or more wavelengths. The data thus provided, together with the elution volume, should make it possible to confirm the identification of the separated species.

Reagent development permits the detection of solutes that may be impossible to detect in any other way. It also provides a means for discriminating between various types of compounds, thus partially compensating for an incomplete separation of a complex mixture.

When reagent development is used, the efficiency of monitoring may be affected by the introduction of the metered stream or streams of reagent into the eluate stream, the mixing of the streams, and the chemical reaction occurring between the separated constituent and the reagent.

Accurate metering of the reagent stream is necessary. The reagent can

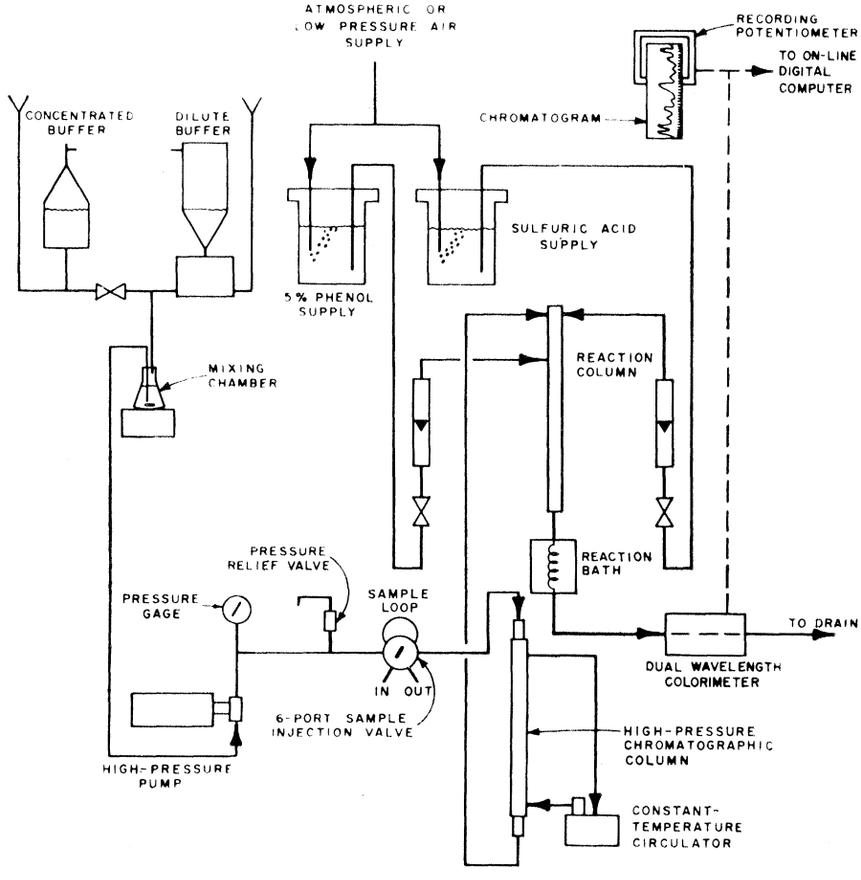

Fig. 6. High-pressure anion exchange chromatograph for analyzing carbohydrates, with colorimetric development for eluate monitoring. [From Scott *et al.* (*3*), courtesy of J. B. Lippincott Company]

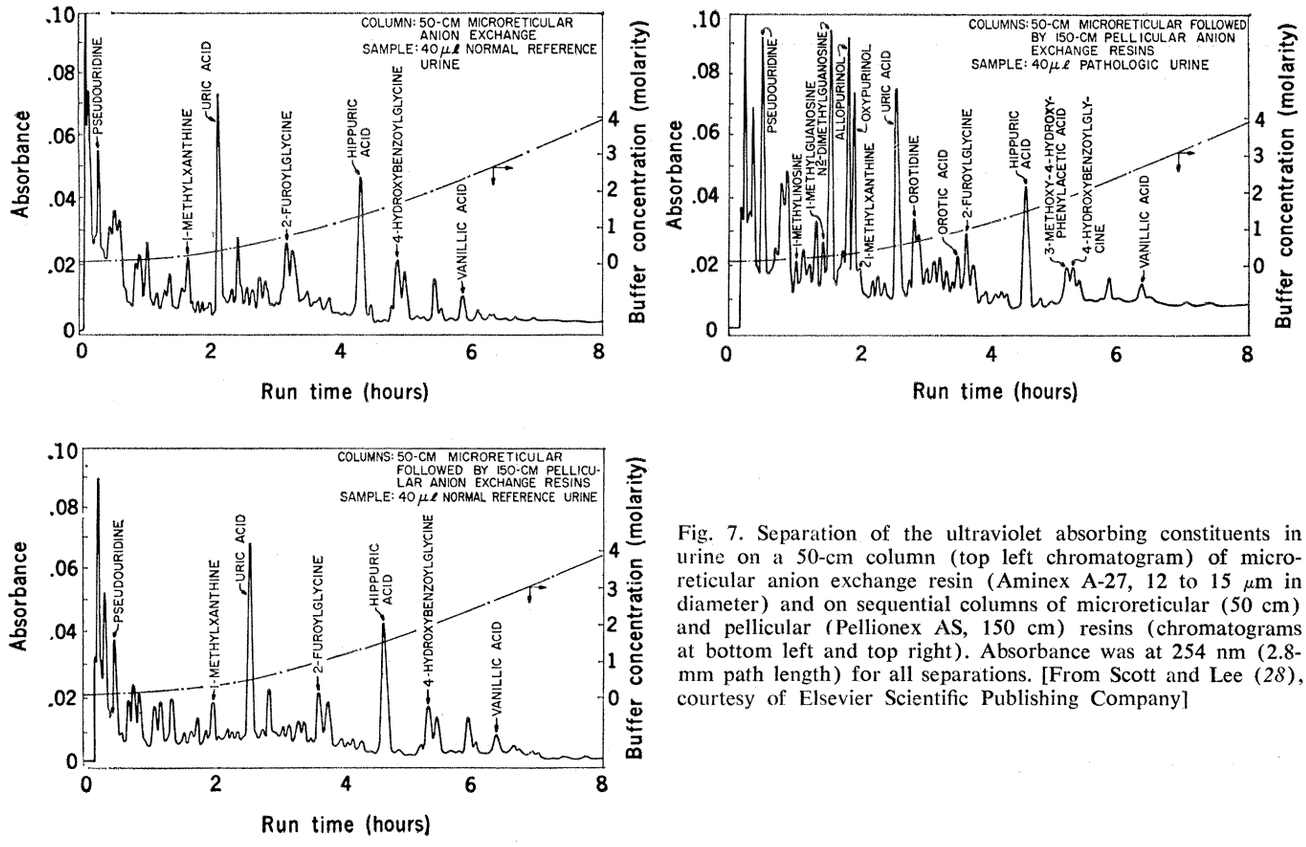

Fig. 7. Separation of the ultraviolet absorbing constituents in urine on a 50-cm column (top left chromatogram) of microreticular anion exchange resin (Aminex A-27, 12 to 15 μm in diameter) and on sequential columns of microreticular (50 cm) and pellicular (Pellionex AS, 150 cm) resins (chromatograms at bottom left and top right). Absorbance was at 254 nm (2.8-mm path length) for all separations. [From Scott and Lee (*28*), courtesy of Elsevier Scientific Publishing Company]

be conveniently contained in a reservoir in which the rate of flow is controlled by applying a near-constant overpressure or hydrostatic head and using narrow-bore tubing or a control valve to provide flow resistance (*19*). If the hydrostatic head or gas overpressure in the reagent reservoir remains essentially constant during the course of a run, the reagent will flow at a relatively constant rate even if only a few milliliters per hour.

Data acquisition and presentation. In most of the systems, a potentiometer (strip-chart recorder) is used for recording the output of the monitor; the information being accumulated in the form of a conventional histogram showing a specific property of the eluate or eluate-reagent reaction mixture as a function of time. Some of the more recent prototype systems include on-line computers for data storage and processing.

The area of each peak present in a chromatogram is directly related to the quantity of material represented by that peak. Quantification of the chromatographic data is achieved by either graphical (strip-chart recorder) or numerical (on-line computer) integration of each chromatographic peak to obtain its area. In cases where mutually interfering peaks occur, the resulting absorbance envelope must be deconvoluted into its individual components. This is most easily done by the on-line computer with conventional spectral stripping techniques (*20*).

Typical analyzers. It is possible to design a whole series of analytical systems simply by varying the separation system or the eluate detector, or both. Two of the more highly developed systems, an analyzer for ultraviolet absorbing constituents (ultraviolet analyzer) and a carbohydrate analyzer, will be described in some detail as typical examples of these high-resolution analyzers. Other systems will be discussed in less detail in the section on recent advances. A notable exclusion will be the amino acid analyzer which has been described many times. For the high-resolution analysis of urine, this analyzer has not been advanced much past the pioneering work of Hamilton (*5*).

The ultraviolet analyzer uses a heated, high-pressure (up to 5000 psi) anion exchange column with concentration gradient elution by an aqueous buffer. A recording dual-wavelength, ultraviolet photometer is used for detection and quantification of the separated constituents (Fig. 4) (*21*). In the advanced models, capillary separation columns are used, which contain Aminex A-27 (an anion exchange resin produced by Bio-Rad Laboratories) in the size range of 10 to 15 μm (*22*). These columns, which are 150 cm long, are fabricated from standard type 316 stainless steel tubing that has an inside diameter of 0.22 cm. A stainless steel heating jacket (outside diameter, 1 inch) surrounds these columns, allowing a circulating heating fluid to maintain temperature control.

The eluent buffer consists of ammonium acetate–acetic acid (*p*H 4.4). Its concentration varies from 0.015*M* to 6.0*M* during the course of the analysis, and the separation column is maintained at ambient (25° ± 1°C) temperature for the first 30 percent of the run and at 60°C thereafter. A miniature, recording, dual-beam ultraviolet flow photometer operating continuously at two different wavelengths, 254 and 280 nanometers, is used as the eluate detector. The dual-beam mode of operation provides a means of referencing the changing properties of the eluent stream by differentially comparing the eluent stream to the eluate stream. Use of a reciprocating piston pump allows reference eluent monitoring at low pressures.

Samples are introduced by a six-port injection valve, and analytical results are presented graphically as a chromatogram showing the ultraviolet ab-

sorbance of the eluate stream versus run time; each molecular constitutent is represented by a chromatographic peak (Fig. 5). Typically, physiologic fluid sample sizes range from 0.1 to 0.5 milliliters, and the separation time varies from 25 to 40 hours for complex mixtures such as urine. The sensitivity varies from a few nanograms to a few micrograms, depending on the photometric properties of the separated solutes.

Carbohydrate analyzer. The carbohydrate analyzer uses the same type of heated high-pressure anion exchange column and ion exchange resin as described for the ultraviolet analyzer. Elution is achieved with an aqueous borate buffer in a concentration gradient, and constituents are detected and quantified by means of a continuous colorimetric system (Fig. 6) (*23*). The borate buffer forms complexes with the neutral carbohydrates to give ionic properties that then allow separation by anion exchange chromatography. A sodium tetraborate–boric acid buffer (*p*H 8.5), in which the borate ion composition varies from 0.169*M* to 0.845*M* is used as the eluent. The column temperature is maintained at a constant 55°C.

Carbohydrates in the eluate are detected colorimetrically by allowing them to react with concentrated sulfuric acid and a 5 percent phenol solution. This reagent development system includes (i) a reaction column into which the eluate and reagents are continuously metered and mixed; (ii) a reaction section maintained at 100°C through which the reaction mixture flows; and (iii) a flow colorimeter that continuously measures the absorbance of the reaction mixture at wavelengths of 480 and 490 nm (Fig. 6). The reagents are metered into the reaction column by using the hydrostatic head in the reagent reservoirs, a fixed pressure drop across a length of capillary tubing, and control valves in the reagent lines.

Typical physiologic fluid samples of 0.5 ml are introduced by a six-port injection valve, and the resulting chromatogram is a measure of the absorbance of the eluate reaction mixture as a function of time (Fig. 5). Separation time for a urine sample is approximately 20 hours.

Identification of separated constituents. Actual identification of separated body fluid constituents requires major experimental effort. Chromatographic peaks can be tentatively identified by comparing their chromatographic properties with those of reference compounds. However, confirmation of the identification requires the isolation of the column eluate fraction represented by a chromatographic peak and the identification of the constituent by chemical as well as spectral methods. In this respect, the gas chromatograph and the mass spectrometer have proved invaluable for identifying solutes that can be volatilized by using high-temperature or derivatization techniques.

To date, the identifications of over 120 ultraviolet absorbing compounds and 18 carbohydrates have been confirmed (see *24, 25*). Many of these have been shown to be important indicators of abnormality or of drug metabolism.

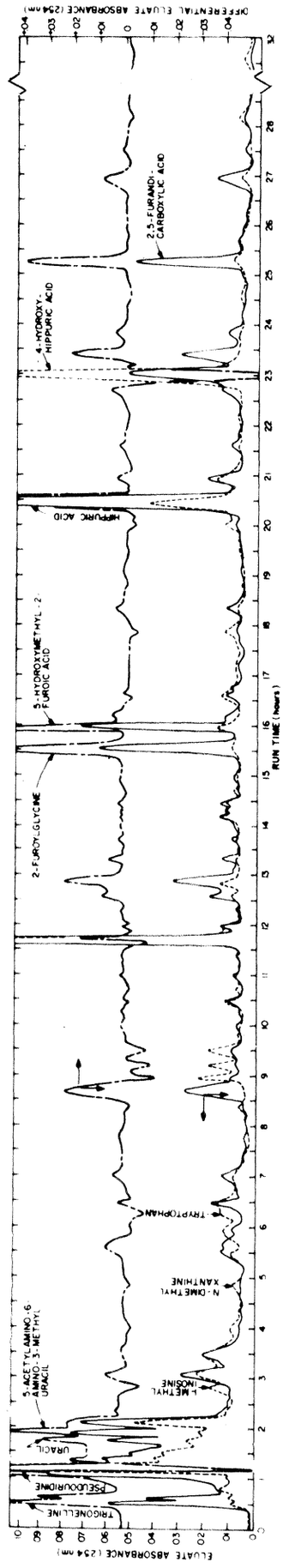

Fig. 8. Differential chromatogram of urine samples showing the comparisons of normal and abnormal subjects. ——, Tracing No. 1 is derived from 200 μl of normal urine, as reference. - - - -, Tracing No. 2 is derived from an equivalent volume of a 24-hour urine composite from a patient with a testicular tumor. — - — -, Differential signal. [From Scott and Pitt (*30*), courtesy of *Journal of Chromatographic Science*]

Recent Advances

Recent efforts to improve high-pressure LC systems have been directed toward increasing sample throughput and increasing the number of physiologic constituents that can be separated and quantified. Systems with multiple sorption columns have been used in attempts to achieve the former objective, and new reagent development systems have been introduced for the purpose of separating additional constituents.

Multiple sorption columns. As new sorption media and reliable chromatographic components become available, additional operating options are possible. For example, multiple ion exchange columns can now be used in series to achieve higher resolution of a complex mixture or to reduce analysis time and thus increase sample throughput.

1) Columns of anion and cation exchange resins can be used in sequence for the determination of the ultraviolet absorbing constituents in physiologic fluids; such constituents are usually anionic, but some are cationic or neutral. Such columns can be operated while the sample mixture is being loaded and the neutral species are being separated (by surface sorption effects). Then, after the loading phase is complete and the remaining components are segregated on either of the columns, they can be eluted separately but simultaneously (*26*).

Operation of the columns in series during the loading phase results in increased resolution in the beginning of the chromatogram. Separate elution of

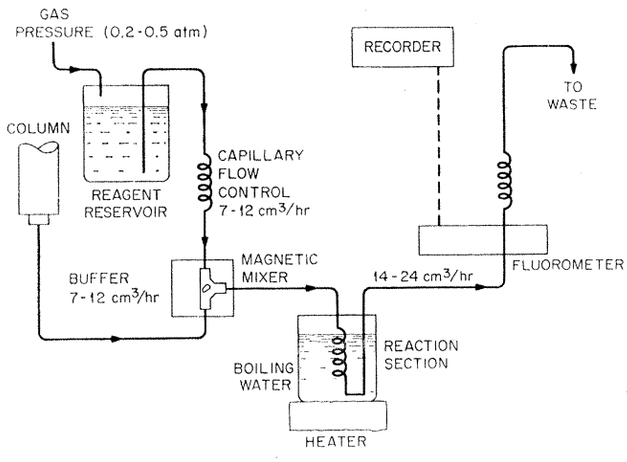

Fig. 9. Cerate oxidimetry for detection of organic acids and other oxidizable compounds after separation by anion exchange chromatography. [From Katz and Pitt (*9*), courtesy of Marcel Dekker, Inc.]

the components in the two columns thereafter greatly improves the resolution in the middle part of the chromatograms and, in fact, results in the resolution of additional chromatographic peaks.

2) Microreticular (micropores throughout the matrix) ion exchange resins contribute to diffusional resistance that results in a relatively slow separation process. Conversely, the recently developed pellicular resins (*27*) in which the inert particles are covered with an active film, achieve a relatively rapid separation, but only at the expense of a severe reduction in capacity that makes high-resolution separations of complex mixtures very difficult. If two columns, each containing one of these ion exchange resins, are used in sequence, it is possible to take advantage of the properties of both resins. Thus, with microreticular resin in the first column, a preliminary separation can be made, and with pellicular resin in the second column, a rapid, final separation can be achieved. Preliminary results indicate that this technique will be useful in the separation of ultraviolet absorbing constituents of physiologic fluids (Fig. 7) (*28*).

Parallel columns. Two or more chromatographic columns can also be operated simultaneously with a single pump and elution system (*29*). Thus two different samples can be analyzed simultaneously and a direct comparison of the chromatographic results can be made either graphically or by way of a true differential mode of operation (*30*).

The use of many parallel columns in a single system permits the analysis of many samples with only a limited increase in expense. By using two parallel columns, quantitative comparisons can be made between the urinary constituents of normal and pathologic subjects, or the urinary constituents of subjects (Fig. 8) prior to and following drug intake. The differential chromatograph yields almost superimposable chromatograms when identical samples are injected into both columns, but striking dissimilarities are noted when different samples are compared.

Reagent development systems. New methods have recently been developed for continuously mixing reagent streams with the column eluate stream so that the mixture can react continuously as it progresses to a flow monitor such as a fluorometer or colorimeter.

1) In one new monitoring technique, which is based on the use of cerate oxidimetry, the reduction of Ce(IV) to fluorescent Ce(III) by separated compounds in the column eluate makes possible the detection of organic acids and other oxidizable compounds (*9*). This fluorometric monitoring of the reduced cerium is highly sensitive (capable of detecting a fraction of a microgram for some biochemicals), and the measurement is made on a developed rather than a consumed species.

Cerium(IV) in 2*N* sulfuric acid is metered and mixed continuously at approximately equal volume with the eluate from a high-resolution LC system, typically one of the anion exchange columns designed for measuring the ultraviolet absorbing constituents in body fluids (Fig. 9). The flow of the reagent is controlled so that it matches the flow of the column eluate (that is, 7 to 12 ml/hour) by flow resistance through a capillary and gas pressure applied over the reagent.

This type of detection system, when connected in series with an ultraviolet photometer, will increase the amount of information obtained from a single separation system (Fig. 10). Obviously as other detection and monitoring systems are developed, they may also be added to existing separation systems to give more definitive data.

2) Another reagent development scheme for a very specific type of compound takes advantage of the interaction of primary amines with the fluorescent reagent, fluorescamine, to detect polyamines that have been separated by a small cation exchange column. Of primary interest are the urinary polyamines: 1,3-diaminopropane, putrescine, spermidine, cadaverine, and spermine. The determination of polyamines in physiologic fluids may be important in clinical diagnosis since some recent studies have suggested that the urine of cancer patients contains increased amounts of these compounds.

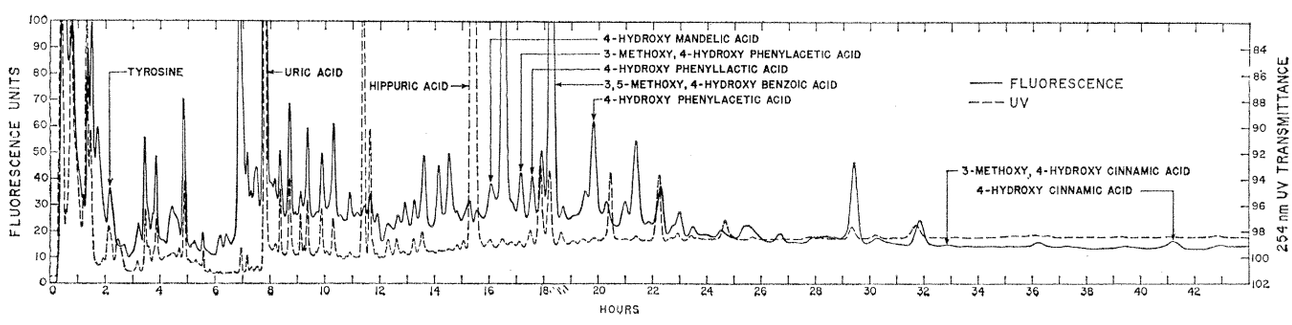

Fig. 10. Chromatogram of normal urine sample (0.27 ml). Urinary constituents were separated by anion exchange chromatography and detected by cerate oxidimetry and ultraviolet photometry. Operating conditions are given in (*9*).

The polyamines can be separated on a 15 by 0.45 cm cation exchange column (70°C) with a combined *p*H-salt gradient. Detection is accomplished fluorimetrically by reagent development, utilizing the reaction between polyamines and fluorescamine which, although nonfluorescent itself, forms a fluorophor with most primary amines (*31*). A typical urine analysis for these compounds requires approximately 90 minutes.

Applications

Most of the experimental data generated by high-resolution LC systems have been used in biomedical research rather than in routine clinical investigations; however, these preliminary results have an important bearing on the applicability of such systems in the clinical laboratory.

For high-resolution techniques to have general utility, it must be established that the body fluids of normal subjects have a definable normal spectrum of chemical constituents and that various pathologic states can be associated with abnormal values of one or more of the constituents.

Ultraviolet and carbohydrate chromatograms from urine and serum samples obtained from clinically normal subjects are similar. For example, about three-fourths of the major peaks are common to all the normal subjects tested, and the concentrations (peak sizes) fall within relatively narrow limits (*32*). The variation during the diurnal cycle is measurable but not prohibitive, and the variation over long periods of time is much less for one individual than that from person to person (*2*).

Some normal values are altered by diet (*33*) and by the ingestion of drugs (*34*). However, there effects are predictable, and nominal control over food and drug intake is sufficient to allow establishment of base-line chromatograms.

Important differences have been noted between chromatograms obtained from samples of urine from normal and pathologic subjects. For example, the urine from a patient with a neuroblastoma had very large homovanillic acid and vanillic acid peaks in the chromatogram from an ultraviolet analyzer, indicating that these compounds may be useful indicators of that pathologic state (*24*). Hippuric acid was absent in the urine of a patient with Lesch-Nyhan syndrome, as indicated by the ultraviolet analyzer (*24*). This, coupled with an increase in benzoic acid excretion, indicates that the glycine conjugation mechanism may have been impaired in that pathologic state. It has also been established that patients with certain malignancies excrete some methylated nucleosides in much larger amounts than do normal subjects (*35*).

The carbohydrate analyzer has also shown considerable differences in the excretion patterns of carbohydrates in subjects with disease. Many carbohydrates are excreted in excess in renal glycosuria and diabetes mellitus (*36*). Patients with pancreatic insufficiency or lactose deficiency also show several carbohydrate excretion abnormalities. The presence of large amounts of xylulose and other sugars in urine after the ingestion of xylose indicates that the xylose tolerance test may not be a true measurement of absorption since that sugar is apparently also metabolized (*36*).

Other useful results have been obtained with high-resolution LC systems, and many of these have been reported in the previously mentioned symposia series (*14*). Once biochemical indicators are found, they can form the bases for future diagnostic tests.

These examples show the utility of the "spectral approach" or of establishing the "chemical profile" of body fluids that can now be achieved with high-resolution analyzers using LC. With such techniques many chemical indicators of various abnormal states can be found without prior knowledge of their existence, and literally hundreds can be screened by a single analyzer system.

It is evident that high-pressure LC systems will be more widely used as the rate at which samples can be analyzed increases, and the identification of additional components of physiologic fluids facilitates the diagnostic process and the monitoring of therapy.

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