Meetings

Application of Automation to Urinary Steroid Assays

On 18 and 19 September 1961 the American Cancer Society sponsored a conference on the application of automation to urinary steroid assays. The meeting was attended by a small group of university, hospital, and industrial representatives concerned with problems of automation of analytical procedures, studies of steroid hormone metabolism, and the role of steroid hormones in physiological and pathological processes. Leo T. Samuels (University of Utah) served as chairman.

The meeting was organized because of growing recognition that the problem of analyzing the large number of steroid hormone metabolites in the urine of normal individuals and of patients has led to several paradoxical situations. First, physiological correlations have of necessity been made with compounds most readily accessible to quantitative estimation, not necessarily with compounds whose excretion levels yield the most meaningful data in terms of interpretation of physiologic and pathologic phenomena. Second, as more and more detailed analyses of urinary excretion products were made and as methods became more complex, fewer patients could be studied in detail in a single laboratory. Since urinary steroid assays have already been instrumental in detecting biochemical errors resulting in overt disease, it seemed desirable to explore the possibility of applying automated procedures for these analyses. One approach is automation of the separation and analysis of multicomponent mixtures. The other is automation of repetitive analyses of single components.

Ralph H. Müller (Los Alamos Laboratories) reviewed some of the general problems of automation of chemical analysis, discussed some of his own pioneer work on the automation of titrimetric and polarographic methods, and considered some of the many devices now in use for the performance of multiple analyses in a repetitive fashion. In discussing this most illuminating paper, the members of the conference considered the potential application of automated analytical methods in this field in sequential analyses of excretion patterns in diseases such as cancer. It was emphasized that the interpretation of data obtained in this way would probably require automatic computers for the multifactorial analyses that would be required. In the past, the correlation of biochemical differences between normal individuals and patients with disease has often been based on only a few factors. It was emphasized, however, that a limiting factor in such methods might be the input of clinical data, because of difficulties in the collection of specimens and of obtaining material from suitable control subjects. Comparison of an individual with a population or with himself at different times was discussed at considerable length, and it was pointed out that these constitute two valid and appropriate means of reducing the signal-to-noise ratio.

T. F. Gallagher (Sloan-Kettering Institute) discussed the studies in his laboratory directed toward rapid quantitative analysis of multicomponent mixtures utilizing infrared spectral data. This has been the approach in two situations—(i) for mixtures of known compounds in unknown amounts, plus background material (noise), and (ii) in situations in which some of the components of a mixture are known but others cannot be specified (unspecified components then become part of the background). The methods consist of converting the reference spectra to a digital form by an analog converter. The punched paper tapes thus obtained contain the information on the spectra. The infrared spectrum of a mixture to be analyzed is also converted to a digital form. A biorthogonal function is calculated that is the error spectrum between each of the pure compounds and a combination of all the others. When the area of this error spectrum is reduced to a minimum, a specific coefficient is obtained for that particular biorthogonal function. Once these biorthogonal functions have been calculated, they remain the same for all subsequent mixtures of the same components. From these functions a computer derives the proportions of the individual compounds in the mixture. The remainder is the background. A comparison of this procedure with conventional chromatographic methods for separation and quantitation of steroid mixtures gave a high order of agreement. Discussion of this important advance in technique centered about the problem of differences in amount and kind of background introduced in biological preparations from different sources. It was pointed out that differences in diet and secretory activity of endocrine glands could make interpretation difficult. It was agreed that methods such as this have great potential for rapid screening of urinary extracts in order to select those that may require or deserve more intensive investigation.

The application of paper chromatography to the analysis of urinary steroid mixtures was discussed by I. E. Bush (University of Birmingham, England). He first considered errors in the estimation of steroids by elution from paper and pointed out that quantitative data for single steroids can be obtained if it is recognized that the steroid is spread over a larger area of the paper than is apparent from the color reactions customarily employed. He pointed out that since the development of color in a paper chromatogram is a heterogeneous reaction, high concentrations of reagents must be employed and conditions for color development must be rigidly standardized if satisfactory quantitative data are to be obtained. He described a device which he has recently constructed in which the paper strips, after chromatographic development, can be inserted, passed through troughs containing the color developing reagents, dried under rigidly standardized conditions, and then scanned spectrophotometrically. The data are presented in digital form. An important feature of this apparatus is the scanning of the paper at two wavelengths: (i) the wavelength of maximum absorption of a colored complex and (ii) a wavelength somewhat removed from this. The second scan, which is subtracted from the first electronically, serves to correct for inequalities in light transmission of the paper. This feature of the instrument,

together with improved optics, makes it possible to obtain linear results for a number of colorimetric analyses over quite a wide range and permits the estimation of steroids with an accuracy of about ±4 percent.

In the discussion of this paper, D. F. Johnson (National Institutes of Health) described an automated apparatus for performing gradient elution column chromatography. This apparatus consists of an automatically regulated gradient and a stream splitter that separates the column effluent into two portions. The solvent is evaporated, and the samples are redissolved in solvents appropriate either for ultraviolet absorption measurement or for a color reaction such as blue tetrazolium reduction. It is also possible to obtain samples for measuring radioactivity.

The subsequent discussion concerned a number of commercially available devices for scanning paper chromatographic strips for radioactivity.

E. C. Horning (Baylor University) discussed the application of vapor-phase chromatography to the separation and identification of steroid mixtures. He considered selection of liquid phases and thermal programming and reviewed his own important contributions to the separation of various groups of steroid hormone metabolites. H. H. Wotiz (Boston University) described his work on the separation of synthetic mixtures of the three major estrogens—estrone, estradiol, and estriol—as well as analyses of urinary extracts for these three substances. There was general agreement that vapor-phase chromatography is potentially an extremely powerful tool for repetitive analysis of multicomponent mixtures.

In the closing session A. M. Bongiovanni (Children's Hospital of Philadelphia) discussed various clinically observed disorders of adrenal function and their exploration by means of urinary steroid assays. It was evident from his remarks that more rapid and efficient analytical methods would help the clinician to reach important decisions quickly and to explore abnormalities of steroid metabolism that result in less dramatic changes in the excretion pattern.

Other participants at the conference were Jack Anderson (Technicon Instruments Corporation), Saul Aronow (Massachusetts General Hospital), W. Averill (Perkin-Elmer Corporation), E. Beaulieu (Columbia), R. Borth (Geneva, Switzerland), Michael Brennan (Henry Ford Hospital), Ralph I. Dorf-

man (Worcester Foundation), E. L. Durrum (Beckman Instruments), Kristen Eik-Nes (University of Utah), Leon Hellman (Sloan-Kettering Institute), Raymond Jonnard (Prudential Insurance Company), C. F. Kossack (International Business Machines), Seymour Lieberman (Columbia), Avery A. Sandberg (Roswell Park Memorial Institute), and J. F. Tait (Worcester Foundation).

A limited number of transcripts of this conference is available from the American Cancer Society, Inc., 521 West 57th Street, New York 19.

LEWIS L. ENGEL

Massachusetts General Hospital and *Department of Biological Chemistry, Harvard Medical School, Boston*

Forthcoming Events

April

14–18. Federation of American Societies for Experimental Biology, Atlantic City, N.J. (M. O. Lee, 9650 Wisconsin Ave., Washington 14)

14–19. American Inst. of Nutrition, Atlantic City, N.J. (A. E. Schaefer, Bldg. 16-A, Natl. Institutes of Health, Bethesda 14, Md.)

14–19. American Soc. of Biological Chemists, Inc., Atlantic City, N.J. (F. W. Putnam, Dept. of Biochemistry, Univ. of Florida College of Medicine, Gainesville)

15–18. American College Personnel Assoc., Chicago, Ill. (B. A. Kirk, Counseling Center, Univ. of California, Berkeley)

15–18. National Education Assoc., Council of Mathematics Teachers, San Francisco, Calif. (Chief of Information, Dept. of the Army, Washington 25)

16–18. Flight Test Instrument Symp., intern., Cranfield, England. (College of Aeronautics, Cranfield)

16–18. Spins and Phonons, conf., Bristol, England. (P. M. Llewellyn, H. H. Sills Physics Laboratory, Royal Fort, Bristol 8)

16–19. American Personnel and Guidance Assoc., annual, Chicago, Ill. (J. Fishbein, Science Research Associates, 259 E. Erie St., Chicago 11)

16–19. Interactions between Mathematical Research and High-Speed Computing, symp., American Mathematical Soc.–Assoc. for Computing Machinery, Atlantic City, N.J. (E. Pitcher, AMS, 190 Hope St., Providence 6, R.I.)

16–19. Paleoclimatology and Paleopedology, symp., International Soc. for Plant Geography and Ecology, Stolzenau, Germany. [R. Tüxen, Intern. Vereinigung für Vegetationskunde, Stolzenau (Weser)]

16–19. Vacuum Ultraviolet Radiation Physics, intern. conf., Los Angeles, Calif. (G. L. Weissler, Univ. of Southern California, Los Angeles 7)

16–20. American Physiological Soc., Atlantic City, N.J. (R. G. Daggs, APS, 9650 Wisconsin Ave., Washington 14)

16–20. American Soc. for Pharmacology and Experimental Therapeutics, Atlantic