THE DEVELOPMENT OF AUTOMATED SYSTEMS FOR CLINICAL AND RESEARCH USE

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SUMMARY

Basic research necessary for the development of advanced methods for clinical chemistry are reviewed. It is concluded that a new class of fast analyzers are required which are inherently adapted to computer data reduction. A new analytical system using centrifugal force to transfer and mix fluids in a multiple-cuvet rotor yields data signals at intervals of 3.3 milliseconds. It is applicable to a variety of analyses, but is especially suited to the determination of enzymes by measuring reaction rates.

Before undertaking the development of new bioanalytical systems it is advisable to ask two questions. The first is: "Do we need new and more sophisticated analytical systems?", and the second is: "What are the requirements for such systems?" We refrain from asking whether we, or anyone else, could develop them.

The reasons why mechanized and automated analytical systems are required for both clinical and research use need not be extensively documented here. The case for clinical chemistry has been well made in recent studies sponspored by the National Institute of General Medical Sciences[1]. One has only to think of inborn errors of metabolism[2] to realize the large number of biochemical analyses which should be generally available, and the larger number which present and future research will make desirable. The clinical laboratory is one of the narrow passages through which the prodigious outpouring of research in biochemistry reaches the patient and, clearly, this passage should be as wide as possible.

This paper outlines the basic problems in the analysis of clinical samples, and presents directions in which the solutions may be found. It is evident that progress is made by coming to conclusions that produce action, even if the conclusions may prove to be partially wrong; rather than following the much safer course of presenting obvious generalizations that do not lead to, and cannot be easily affirmed or negated by experiment. The question of what systems and concepts are best will be solved

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by casual inspection of any clinical laboratory in the year 1980. With this premeditated bias clearly understood, let us proceed to a discussion of the following areas.

I. Reagents
II. Analysis
III. Microbiology

I. REAGENTS

By reagents we mean all those substances, chemical standards, enzymes, cofactors, antisera, and standard antigens required in any aspect of the practice of medicine, excluding drugs used exclusively as therapeutic agents. For convenience we will also include vaccines, which are used as reference antigens, and for the production of antisera. Most reagents in present use present few problems either for local or commercial production. The problem of the large variety of simple solutions required may be partially solved by clever modifications of analyses so that a maximum number of common denominator solutions are used. The possibility of devising machines which continuously make solutions from dry reagents, titrate or otherwise analyze them, and which then feed them continuously into analyzers has hardly been considered to date.

For expensive substrates or other reagents, the path of progress appears to lie in the direction of attempting to decrease production costs, and at the same time decrease reagent volume requirements by miniaturizing the analytical system, and by divising methods for insuring that only the amount of reagent actually required is prepared. Enzymes are increasingly used as reagents, and rather little research has gone into the problem of substantially reducing the cost of enzyme production while also increasing purity. The general experience in chemical technology has been that continuous processes are better than batch processes. The tendency in preparative biochemistry, however, has been to scale up batch-type operations rather than to explore continuous processes. It must be admitted that many of the techniques and much of the instrumentation required for continuous enzyme isolation do not as yet exist. This will only change when the requirements are widely understood and the work on biochemical engineering more amply supported. Continuous combined fermentation–fractionation systems are now under development in Oak Ridge by Dr. G. David Novelli and co-workers, and at University College London by Dr. M. D. Lilly and associates. Studies on the economics of enzyme production carried out by Dr. Lilly clearly indicate the advantage of continuous isolation techniques tailored to each enzyme. This is one area of chemical technology in which few engineers are being trained, and where few laboratories devoted specifically to research on technique and systems development exist.

With respect to antisera and antigens, many of the same problems occur. Specific antisera require that pure antigens be available. The production of these requires that large-scale, high-resolution methods for particle separation be developed. A program specifically aimed at this problem has been in existence at Oak Ridge for a number of years under joint NIH-AEC sponsorship. Out of this program have come a spectrum of new centrifuges, termed zonal centrifuges, now totalling over fifty in number[3,4]. Nine of these types are now commercially available. For convenience we have divided them into groups given letter designations. The A-series

rotors operate at low speed and are useful for separating large particles such as whole cells, nuclei, mitochondria, and chloroplasts. B-series rotors operate at speeds ranging up to 50000 rev./min and are useful for the separation of particles down to the size of large proteins such as macroglobulins and nucleic acids. Several reviews of work done, largely with B-series rotors, have been published[5,6].

The most widely used zonal rotors are the B-XIV and B-XV[7], now constructed almost exclusively of titanium. These, however, will be superseded by the B-XXIX and B-XXX rotors recently developed at Oak Ridge[8]. These allow gradients to be recovered either from the rotor center or the rotor edge, and are extremely useful for concentrating and purifying virus particles from liter volumes of solution. The particles may be banded in a very small gradient at the rotor edge which is then recovered from the edge without using large amounts of expensive reagents for displacement purposes. Details of the operation of B-series rotors are presented elsewhere[7]. Centrifuges of the K-series (Fig. 1) are specifically designed for large-

Fig. 1. K-II-C large-scale zonal centrifuge developed at Oak Ridge for vaccine purification.

scale vaccine purification[9]. Currently, highly purified influenza vaccine is made commercially with these centrifuges, and considerable progress has been made on the purification of rabies virus for vaccine purposes. Purification of many other vaccines is now possible. Modifications of the K centrifuge will probably be useful in large-scale protein fractionation generally.

As pure viral and bacterial proteins and polysaccharides become more widely available, better antisera and more specific and reliable tests will be possible. These can be no better than the reagents, however, and every effort must be made to develop methods for producing pure reagents inexpensively. Many problems must be solved before this can be done. These include development of methods for doing the following on a *continuous* basis: (1) medium sterilization, (2) cell culture, (3) cell harvest, (4) cell breakage, (5) isolation of specific cell components, (6) concentration of macromolecules, (7) fractional precipitation, (8) precipitate collection and resolubilization, (9) chromatography, (10) electrophoresis, (11) gel filtration, and (12) assay. The problems arise from the requirements that these be continuous and that they be optimized to the limits allowed by present technology.

II. ANALYSIS

The distinctive thing about automation is that it involves feed-back control, *i.e.*, the results may change the process. While considerable *mechanization* has been introduced into clinical chemistry, there is very little automation. The question of where and of how much automation will be required remains to be considered.

Many different mechanized or partially automated systems have been, or are about to be, introduced into clinical chemistry. (The pioneering, far-sighted, and original work of Dr. Leonard Skeggs must be acknowledged, however.) A classification of analyzers presently available is of interest in the light of the requirements which this equipment should meet. Whether or not these requirements can be met at present is not at issue. Technical requirements, once properly stated and widely understood, have a way of being met. The expectation of a new development often creates the environment in which it can occur.

Analyzers have been divided into two classes[10]: Class I systems analyze a large number of samples for a single component or activity and depend on specific chemical reactions. They can be run in parallel to increase the number of different tests done, or designed to change rapidly from one test to another.

Systems of Class II depend not on specific chemical reactions, but on high-resolution separations to achieve specificity. The separations may be monitored by using group-specific reactions, absorbance, conductivity, heat of sorption, refractive index, or liquid density. Class II systems include amino acid analyzers[11], nucleotide analyzers[12], carbohydrate analyzers[13], and analyzers for separating Krebs cycle intermediates[14]. These systems are useful for research; however, their cost, complexity, size, and slow data output make them of limited usefulness clinically. It is important not to lose sight of the fact that these limitations may not always hold. If every effort is made to miniaturize and accelerate them to the limits of available technology, it may be possible to use them routinely. Some of the results of work at Oak Ridge on the miniaturization of the amino acid analyzer have been recently published[15].

Even when not useful for routine work, the contributions made by analyzers of class II should not be underestimated. They offer the possibility of quantitatively determining the levels of large numbers of different substances in blood, urine, and tissues. As changes in specific substances are correlated with disease states, systems may be designed to analyze many samples rapidly for the few key substances. In a real sense, work with class II analyzers is the "basic research" of clinical chemistry.

Using the nucleotide analyzer developed at Oak Ridge[12] on the foundation of nucleotide chromatographic analysis laid by Dr. Waldo Cohn and collaborators[16], Dr. C. D. Scott and collaborators have found that over 140 constituents could be separated from human urine[17,18] (Fig. 2). Using the carbohydrate analyzer developed by Dr. J. G. Green[13], Scott has found over 20 carbohydrates routinely in human urine[18]. The Body Fluids Analyses Group, working in the Chemical Technology Division of the Oak Ridge National Laboratory, is now systematically improving these systems and identifying the constituents separated. This work will have considerable impact on clinical chemistry in the future.

Returning to analytical systems of class I, these may be subdivided into two groups depending on whether reactions are run in parallel (class IA) or in sequence (class IB). Class IB systems produce data after a delay equal to the total procedure time, at intervals determined by the time interval necessary to discriminate between consecutive samples. To say that systems of class IB have revolutionized clinical chemistry is not an understatement.

We are concerned here, however, with future requirements. These are set by the nature of computers and certain basic concepts of automation. This is made clear by classifying all analyzers in a different way, depending on how they may be tied to computers, into "slow" and "fast" analyzers. The data from slow analyzers must be stored in an intermediate form because it is produced at a rate very slow indeed compared with the rate at which computers can absorb and process it. Hence the use of punched tape, strip cart recorders, magnetic tape, or punched cards. With slow analyzers, use of computers is a luxury for most laboratories. (As computer costs drop, this may not always be true). A large fraction of the equipment cost is in interface equipment.

We define "fast" analyzers as analyzers which produce data at such a rapid rate that the information can be processed in "real time," *i.e.*, fed directly into the computer. How fast is fast? Slow systems produce data at intervals measured in minutes or tens of seconds. *Fast analyzers must produce it at intervals measured in milliseconds.* The computations in the computer are usually measured in microseconds, individual events occurring in nanoseconds. If the statistical analysis applied to one data point takes 900 microseconds, and an analyzer produces one data point per minute, an "on-line" computer is active $\frac{1}{6{,}666}$ of the time. Efficiency can be increased by time sharing, but it would clearly be best to match the analyzer's speed to the computer if possible. The driving reason for this, however, is not merely to use the computer efficiently; rather it is to make possible true automation with feedback control of the analysis. If the computer is to do this, it must have results to work with as soon as possible, *i.e.*, *during* the analysis. Speed is a dividend of the basic requirements of computerized control which include on-line quality control

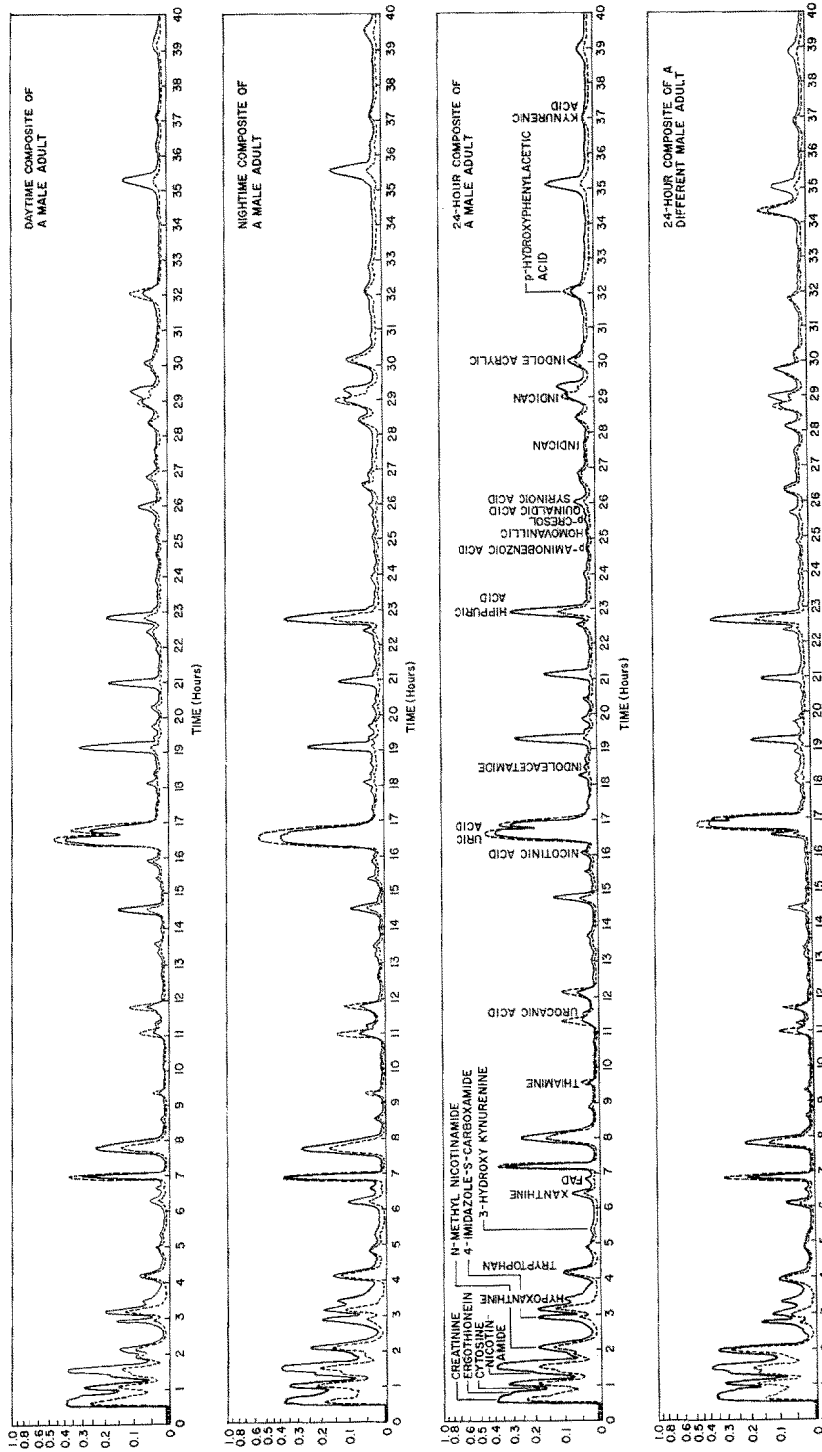

Fig. 2. Typical chromatograms for the UV analyzer showing reproducibility, difference in diurnal cycle, and similarity between different urine samples. Run conditions: column, 0.45 cm. I.D. X 316 stainless steel with 10 μ diameter. Biorad AGI-x8; urine sample, 2 ml each; temperature, 25° increasing to 60° after 15 h; pressure 1500–2300 psig; elution, sodium acetate–acetic acid buffer at pH 4.4 varying in concentration from 0.015 to 6 M at an average flow rate of 28 ml/h. Solid lines, at 260 mμ; broken lines, at 280 mμ. (From: Scott, 1968[18], with permission.)

which can only be achieved by statistical analysis. The point is not merely to have a statistical evaluation of the results; rather it is to state at the outset what standard error is acceptable, and then require the system to keep working until the results are in the required range. This means duplicate analyses, reruns when required, statistical evaluation of errors in standards, etc., *i.e.*, the system must be automated.

Fast analyzers, if they can be built, will have one very important additional dividend. They would function at a rate which more closely matches human attention span than do present systems. It requires only a very few seconds to decide from looking at a series of results expressed as a calibration curve whether an analytical system is operating correctly. Similarly, a rapid glance can tell an analyst whether any of the samples were over or under-diluted, and whether duplicates agree. The possibility of doing a large number of analyses during the time required to evaluate the results visually is important. During this period the system can occupy one's full attention. The *man–machine–computer* match is essential for the optimum use of each. Far from being replaced, the operator should be in command of a system producing data as rapidly as he can assimilate it and decide whether it is necessary to make adjustments and repairs. However, it is hoped these will not be necessary. I believe every effort should be made to supply fast information readout which will remove the "black box syndrome" and put the operator in constant communication with the system.

The Fast Analyzer Project at Oak Ridge is concerned with the basic theoretical, experimental, and engineering studies required to evaluate this problem and, it is hoped, to solve it. We have no illusions concerning the difficulties involved in conceiving, testing, developing, and evaluating such systems. However, we see no insurmountable problems at the moment. The initial work has been concerned with the measurement and transfer of small fluid volumes independent of solution viscosity, density, surface tension, or air bubbles. This is a classical problem in microanalysis, and many other attempts have been made to solve it.

One solution is to use centrifugal force to fill and empty small tubes fabricated from a plastic having a very low affinity for water[10]. A schematic diagram of the way in which such tubes may be filled and emptied is shown in Fig. 3. The problem with small volumetric devices has been the shape of the meniscus. In a centrifugal force field, this is flattened to the point where the error in measuring and transferring volumes in the 35–125 λ range is less than $\pm$ 1%, and can probably be improved. This is an "unhandy" way to measure and transfer liquids; however, it is amenable to mass mechanized measurement and transfer.

The problem of carrying out a large number of exactly parallel reactions and of making all measurements over a very short time period is not easily solved, especially if those basic elements which form in varying sequence analytical methods are to be included. These elements are: measurement, transfer, incubation, timed reagent addition, heating, precipitation, filtration or centrifugation, extraction with organic solvents, and measurement of absorbance at one or more wavelengths. We have, however, made some progress. Our experimental prototype analyzers are designated G-series systems, and we have completed two of them, G-I and G-II. These use centrifugal force for fluid transfer, a dynamic mixing procedure, and a rapid method for determining the absorbancies of a series of samples. The time interval between readings for the G-II system is 3.3 milliseconds for 15 reactions

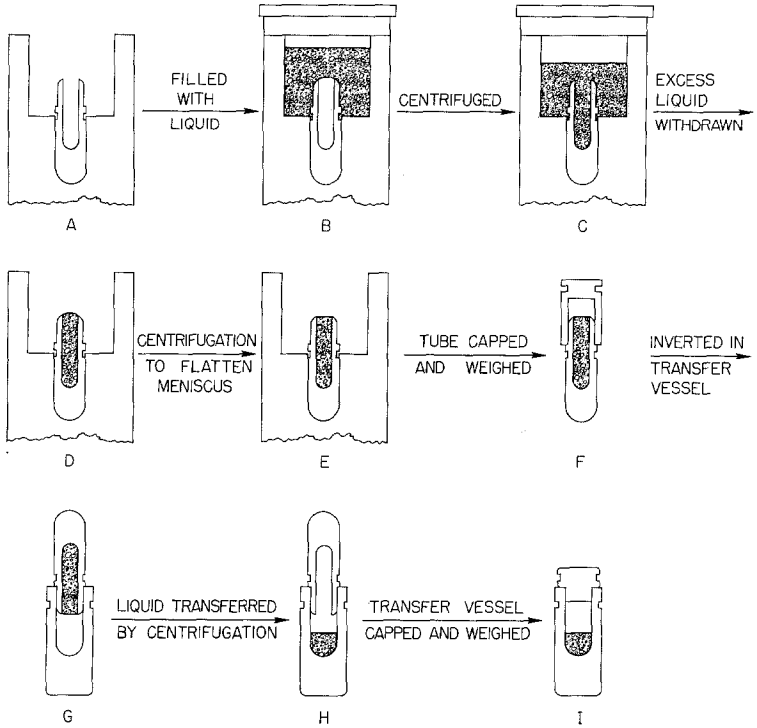

Fig. 3. Schematic diagram of procedures used to determine reproducibility of filling and transfer method using small plastic tubes. (From Anderson, 1968[10], with permission.)

carried in parallel, and 1.5 milliseconds for the 40 parallel reactions in G-I. Details on the operation of these systems are recorded elsewhere[20]. At these speeds, the results must be monitored on an oscilloscope and are easily recorded photographically.

Analysis of a series of standards for total protein by using the biuret reaction is shown in Fig. 4. The photograph was taken 30 seconds after the system was started, and the time between peaks in the scan is 3.3 milliseconds.

Between proof-of-principle and the development of devices which may become generally useful lie much effort and expense indeed. However, it now appears that fast analyzers may do for so-called "wet chemistry" and for enzyme assay what computers have done for numerical computation. Factors which have been limiting in the past cease to be so, and the comprehension and evaluation of the final results becomes limiting. It may take several years before fast analyzer–computer combinations become commonplace; however, they will probably be standard by 1980. Once *fast analysis* is accepted as a possible and desirable goal, the technical problems will be essentially solved.

III. MICROBIOLOGICAL STUDIES

The identification of infectious agents, both bacterial and viral, by using present techniques is time consuming and requires many special skills. Efforts in a number of laboratories are now aimed at reducing the time involved and at yielding

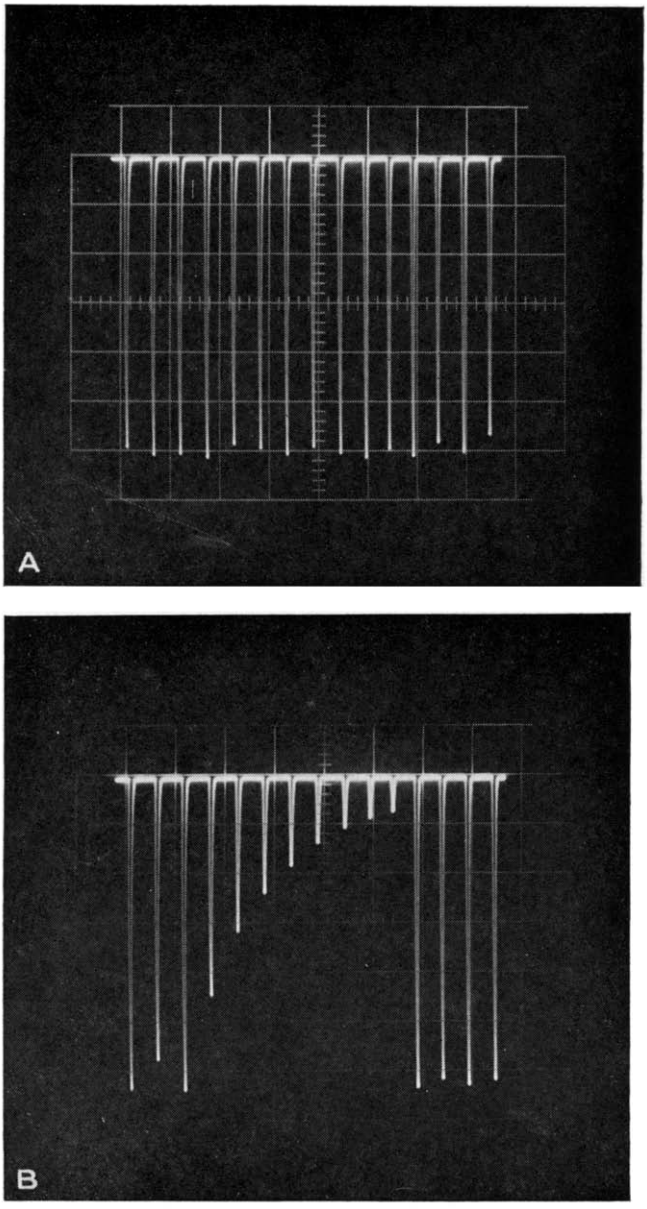

Fig. 4. Protein analysis with General Medical Sciences-AEC G-II analyzer using biuret reaction. A—air blanks; B—fifteen parallel readings as follows: Peaks 1, 3, 12–15, water blanks; peak 2, reagent blank; peaks 4–11 standard curve ranging from 0.1–0.8% protein in 0.1% increments, Time between peaks: 3.3 mseconds. Read at 550 nm. Note that upper "baseline" represents zero percent transmission or infinite absorbancy.

more quantitative results. Dr. Donald Glaser at Berkeley has been developing large-scale methods for conducting studies in bacterial genetics which are also adaptable to the problem of identifying bacteria and of screening them for resistance to antibiotics.

At the AEC Los Alamos Scientific Laboratory, Dr. M. A. Van Dilla has been exploring the use of violet laser beam to produce fluorescence in nuclei stained with a modified Feulgen reaction[21]. The number of nuclei and their DNA content may

be quantitatively measured. We are exploring the possibility of extending this approach to the detection and identification of bacterial cells and virus particles. In parallel studies, methods for counting virus particles when present in very low concentrations have been developed[22]. Whether these approaches will make possible rapid identification and counting of infectious agents remains to be seen. The point is that the possibilities of modern technology should be thoroughly explored in the interests of the clinical sciences. Certainly a nation which can afford to expend a large fraction of its budget to explore space, to develop supersonic transports and ever more complex instruments of war can afford an occasional review of its technologies to see if new ways to improve human health can be found[23].

Note added in proof: In the short interval since the preparation of this manuscript GeMSAEC Fast Analyzers have become commercially available from Electro-Nucleonics, Inc., Caldwell, N.J.; Tennecomp, Inc., Oak Ridge, Tennessee (computerized); and Union Carbide Corporation, Development Department, Tarrytown, New York.

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