

AUTOMATED ANALYSIS IN CLINICAL CHEMISTRY

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Abstract - In only two decades the analytical processes in clinical chemistry laboratories have utterly changed from manual to mechanized. Continuous-flow analysis initiated the change, followed by discrete automation, automatic particle counting, kinetic analysis, chromatography and, most recently, radioimmunoassay. Today's automated processes pour out large amounts of data. In fact, with some modern machines it is easier to discard selected unwanted data on chosen samples than it is to omit the part of the analytical process that produces it. This change has totally inverted the order of difficulty of the various routine tasks in the laboratory. In the old order, performance of the analytical process outweighed the total effort for all other tasks. In the new order, data handling, in its broadest sense, outweighs all other tasks. As a result, significant problems for the laboratory involve extra-laboratory factors to a new and major extent, ranging from exact identification of specimens to proper use of reported data. Automated identification of specimens, from collection to report, will come rapidly as new devices are developed and accepted by clinical technicians. Appropriate end-use of freely available laboratory data poses a much more difficult challenge, as yet unmet. Before the advances brought about by automation are fully utilized major changes may be required in the approach of laboratorian and clinician alike to the proper role of the laboratory in the practice of medicine.

INTRODUCTION

In discussing automated analysis in the laboratories which perform chemical determinations for the medical profession one can choose either of two possible approaches to the subject. Usually one reviews and describes the marvelous new mechanical and electronic devices which have so drastically altered the clinical laboratory in the last two decades. Here this would tend to be a superficial presentation, because the same information could easily be gathered from a variety of commercial concerns. In addition it could not succeed in being useful, because the amount of information has become so great as to prevent one from even summarizing it in a reasonable time. The more interesting, challenging, and controversial choice would be to review, from the vantage point of two decades, the effect that these drastic changes in the clinical laboratory have had on the intellectual approach of the physician to his diagnostic and therapeutic problems. However, this approach presents the opposite problem, in my opinion. The effect has been embarrassingly small. This presentation will, therefore, draw on both approaches. It will start off with a brief before and after picture of the clinical laboratory, showing the change due to automation. Then it will attempt to discuss the effects of laboratory automation, as compared with what they might have been, and what they may be expected to be in the future, given certain changes in approach. It is important to understand that the judgments and conclusions presented here are the personal opinions of a writer who frequently finds himself at variance with the majority.

PRE-AUTOMATION SAMPLE PREPARATION

In the clinical laboratory of a large American hospital, in 1957, one entire

wall was lined with tiers of funnel-racks. Mounted on these tiers were hundreds of standard analytical chemists' conical funnels, each with its fluted filter paper and Erlenmeyer flask below. They looked, from a distance, like bleachers at a soccer match attended by white hooded nuns. The last task each night was for all of the technicians to converge on this area and replace all of the funnels with clean ones, place filter papers in each, and place a clean Erlenmeyer flask under each. The first task in the morning, after the samples had arrived in the laboratory was to dilute an aliquot of each with a protein precipitating solution and to pour the resulting mixtures into these funnels, to prepare protein-free filtrates. This step, of course, preceded any analytical process which was carried out on the protein-free filtrates. Today, in one tenth of the time it took to carry out this filtration, one can place a sample of serum on an automated analyzer which can produce one to twenty or more analytical results without further human attention to that particular sample. This is a tremendous change, even for two decades.

EARLY DISCRETE AUTOMATION

Although continuous-flow analysis seems to have introduced automation in a big way the meticulous historian would observe that discrete automation really tied it for priority. The papers which formed the foundation for both of these quite different approaches to automation appeared in the same volume of the same journal, and only 111 pages apart (Ref. 1,2). Figure 1 shows the so-called Seligson pipet. This extremely simple device elegantly embodies the most important fundamental principles of discrete analysis. It mechanizes the step of picking up, measuring, and diluting liquid samples such as blood serum. The reference is dated exactly twenty years ago.

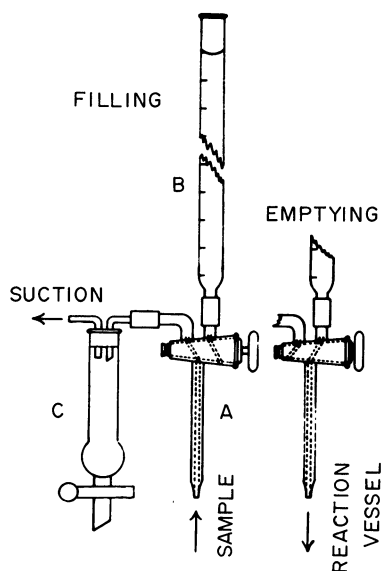


Fig. 1. Seligson pipet. A, Three-way stopcock and sample measuring probe. B, Calibrated buret. C, Waste receiver.

These devices met immediate acceptance in the routine laboratory, because they were dramatically useful. Take, for example, the oft repeated task of measuring 0.200 ml, say, of blood serum into a flask and diluting it to 10.0 ml with a fluid containing, for example, an internal standard for flame photometry. One simply uses a Seligson pipet where portion A in Fig. 1 is calibrated to contain 0.200 ml, and where portion B is marked off to deliver 10.0 ml. Placing the sample container under A one allows the gentle suction to fill portion A with serum and then turns off the stopcock. One then wipes the tip of the pipet and places an uncalibrated flask under the tip and moves the stopcock to the emptying position, thus allowing exactly 10.0 ml to flow out. Since the portion A remains filled with the diluting fluid the net volume in the receiving flask is 10.0 ml.

My first major accomplishment as a fledgling laboratory director in 1957 was to install many of these devices to replace pipets and volumetric flasks. Because the same pipetting volume and diluting volume was used for all samples, precision showed a marked improvement. The budget watchers were very impressed by the decreased bill for broken pipets and flasks. (This was probably the last time they saw automation decrease the overall budget.) Then we learned to make up calibration standards to the same concentration as native serum and dilute them with the same device, and as a result accuracy also improved noticeably.

Close experimental study of this device is an education in discrete automation, and any serious student should make one and test its performance. For example, the volume of portion A is nominally 0.200 ml. But at the time one starts to draw serum sample into it, it is already wet with the diluent from the previous sample. Some of the precious serum must be used to wash this diluent out completely. How much does it take? It is revealing to find the answer to this question by experiment. One learns to redefine the word "completely." One also learns that the speed of sample uptake is involved. Other fundamental concepts of automation which such experiments illustrate include these questions. If incomplete washing prevails, does it really affect accuracy or precision, since standards are diluted like samples and it prevails in both cases? How reproducible is the degree of incompleteness and how much does this depend on how the device is handled? In turning the stopcock to the off position after aspirating the sample how far should one turn, and in which direction? Stopcock barrels may not be perfectly cylindrical, and the exact volume contained when the tip is wiped off is very likely to be a function of stopcock position. Simple experiments will demonstrate that even the way in which the tip is wiped can affect precision and accuracy. Only recently have manufacturers of automated devices recognized the great importance of probe tip wiping, and produced machines which do this. Finally, how much diluent is necessary to remove the last traces of sample from the probe? How fast can the diluent run? Simple dye-dilution experiments designed to measure these factors are eye-openers for most students, and in fact for anyone who has never performed them.

The important feature of these devices, and of discrete instruments which derive from and contain them, is the time they save. With a pipet and volumetric flask a technician might take two or three minutes per sample. With the primitive Seligson pipet twenty or thirty seconds sufficed - a five-fold improvement. Many modern descendants of this device exist. There is no apparent resemblance between any of them and Fig. 1. In many of them one simply places small vials of serum, as removed from the centrifuge, into racks. The machine takes an aliquot, dilutes, mixes, and adds aliquots of other reagents as required. It does so at speeds up to five seconds per sample, and handles samples as low as twenty microliters in volume with the same accuracy as the Seligson pipet handled half a milliliter. Of course, the most striking difference between the old and new is the price.

CONTINUOUS-FLOW ANALYSIS - DILUTION

Dr. Skeggs' first article on continuous-flow analysis also appeared in 1957. Although not taken from his article, Fig. 2 is an attempt to illustrate only the dilution aspect of continuous-flow analysis, for comparison with discrete dilution, leaving momentarily the other powerful innovations of this work. The key difference here is the uninterrupted and continuous nature of all phenomena except the sample presentation. The pump rollers move fluid down all tubes at an unvarying linear rate. Volume rates are set by pump-tube diameters. The composition of the fluid flowing in any tube following the union of two tubes depends only on these volume ratios and the composition of the uniting fluids. The operational equivalent to using the Seligson pipet, or to manipulating a pipet and volumetric flask, is simply to present the sample to the inlet probe.

Continuous-flow analysis gives up the feature of producing a definite volume of a given dilution of sample. Instead it produces a given dilution at a definite rate. Thus it introduces the new dimension, time, as illustrated in Fig. 3. This graph diagrams the temporal relationship of the composition of the flowing stream when the concentration of the sample presented to the probe is abruptly changed from zero to some high value, then to some lower intermediate value.

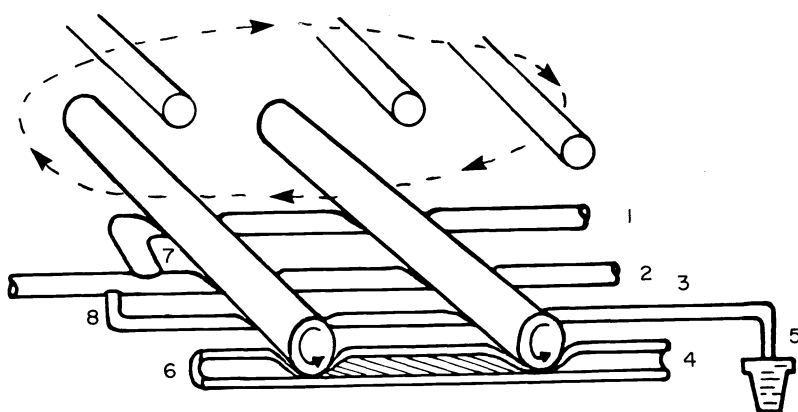


Fig. 2. Continuous-flow dilution. The peristaltic action pump moves fluid down several tubes at different but constant rates, in ml per min. 1-4, Elastic manifold tubes. 5, Sample pick-up tube and sample cup. 6, Downstream end of section of manifold tubing, showing pumping action of rollers. 7, Union-point for reagents from tubes 1 and 2. 8, Addition of sample to combined reagents.

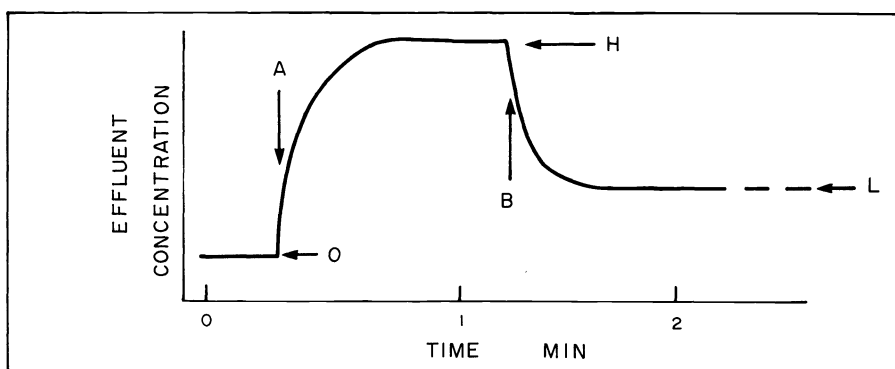


Fig. 3. Changes in composition of effluent stream in continuous-flow analysis. Up until time A the sample probe is in a solution of zero concentration; at time A it is placed in one of high concentration. The effluent concentration changes slowly from zero concentration to a new value H, and remains steady there until the probe is changed to aspirate a sample of lower concentration, at time B. The effluent concentration then moves to a steady state value at concentration L.

Again, very simple dye-dilution experiments with this fundamental portion of any continuous-flow apparatus are of far-reaching value to the student of automation. Studies of the nature and character of the steady state and of the transition state between steady states has been extremely fruitful and has led to almost two orders of magnitude improvement in productivity for continuous-flow analysis.

The modern embodiment of continuous-flow instrumentation is four or five generations removed from the original. It produces data so prolifically that it recognizes the importance of automating the data handling by having its own dedicated digital computer. It can perform twenty different analytical determinations on a fraction of a ml of serum at the rate of better than one sample per min. It has automatic sample identification, and collects information on each patient into an integrated report. This report includes an expression

of the relationship of each result to the accepted Normal Range for the test. There are over 500 of these machines operating in routine laboratories today. The previous generation of continuous-flow devices was controlled by an analog computer, and performed 12 determinations simultaneously on 60 fluids per hour. About 1500 of these are operating today - 20 here in Tokyo.

The true magnitude of the impact of automation on the laboratory can be seen when one realizes that in far less time than the technician of 1957 took to set up the inevitable funnel, filter paper, and flask - in fact in less time than it took to flute the filter paper - a modern device will take the sample and present the final calculated and checked data on a score of determinations. It is even more striking to point out that they use less blood in the process and involve costs for the multiple results which, if corrected for inflation, are no greater than for the single datum of twenty years ago.

OTHER TYPES OF AUTOMATION

Discrete and continuous-flow analysis comprise the two extremes of a spectrum of possible automated devices. Analyzers which handle fluids by exploiting centrifugal force are closer to the former extreme while ion-specific electrodes are being offered in instruments which involve both but come closer to the latter. Particle counters have become a vital part of any routine laboratory. While sophisticated fluid handling and integral automated data manipulation have changed these counters into remarkable analytical automations, the important basic principle involved is the so-called Coulter effect, diagrammed in Fig. 4. If an electric field is maintained through a tiny orifice then particles sucked through this orifice interrupt the field and cause electrical pulses which can be counted and measured. Thus the number and size distribution of red cells, white cells, or platelets in blood can be assayed. The modern version of this instrument carries out six simultaneous counts, three for red and three for white cells. It also measures the average red cell size and the hemoglobin concentration. It takes this instrument 15 seconds to do the triplicate counts and determinations, average

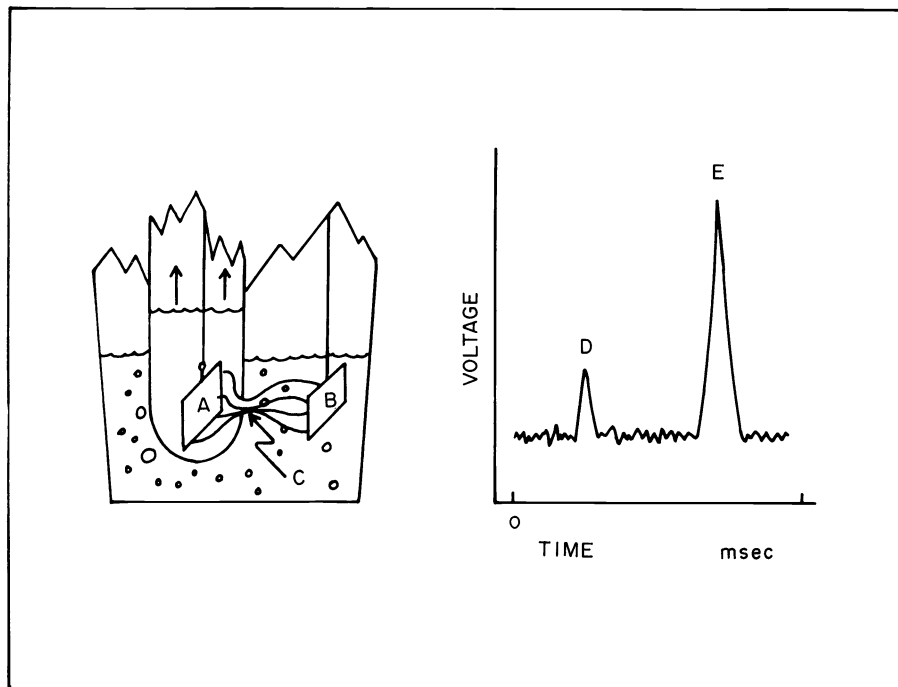


Fig. 4. Diagrammatic representation of the Coulter effect. Current flowing between electrodes A and B through orifice C is partially interrupted when particles are carried through C by fluid flow from outer to inner chamber. D represents the resulting voltage spike from a small particle, E that from a large one.

them, and calculate and print out seven parameters of hematological analysis; red cell count, white cell count, hemoglobin concentration of blood, average hemoglobin concentration of red cells, average size of red cells, mean corpuscular volume, and hematocrit. One simply cannot compare this productivity with the performance of 1957 because there was no way that a human could count the vast number of cells involved in any reasonable time.

IMPACT OF AUTOMATION IN THE LABORATORY VS. THE CLINIC

Automation has increased the productivity of the laboratory in the last two decades by about two orders of magnitude. One might conclude that the clinical laboratory is two orders of magnitude more useful to medical practice. Unfortunately this is not correct, a fact which requires some accounting. Automation has attacked two general areas in the laboratory in an attempt to decrease the load on humans. In one, the area of analytical processing, it has been dramatically successful. In the other, the area of manipulation of the finished data, it has not. As a result the whole hierarchy of tasks in the laboratory has changed. In the laboratory of 1957 the analytical process was so overwhelmingly the limiting step that the stream of data was small and its handling was no problem. It was then easy and somewhat appropriate for the average clinical chemist to confine his major attention largely to chemistry and pay little attention to the clinic. This is no longer so. Automation has removed the processing bottleneck, and as a result, the flow of data has increased tremendously. (Incidentally, this increase in flow is quite inevitable. A vocal minority has argued that more data is bad medicine, which is, of course, as fruitless as trying to deny the law of supply and demand.) But the facilities for handling and interpreting the data have undergone no fundamental change, in spite of concomitant major advances in computer technology. Therefore, the bottleneck has shifted from inside the laboratory to just beyond its output stage.

This is not to say that computers have had no impact in clinical chemistry. They certainly have, but their real success has been to further automate the analytical processing. The application of computers to the large-scale handling of finished laboratory data has been a graveyard for the hopes, dreams, and even reputations of many fine people for the last 10 to 15 years. We simply do not have dramatic accomplishments in the areas of automated sample collection and identification, retrieval of specimen data, reporting, interpretation, storage, and collective archival, retrospective or statistical use. Almost the sole impact of automation outside of the laboratory has been to vastly increase the number of data which must be handled by humans.

CHANGING CHALLENGES FOR THE CLINICAL LABORATORY

If the clinic is really to benefit from the automation of the laboratory, then data handling will have to be automated not only within the laboratory but also on the way into and out of it. Incoming data involve specimen identification and demographic patient data. Outgoing data handling involves collation and evaluation of data relating to each patient, as distinct from the intra-laboratory task of evaluating data to control the test.

Since automation has removed the old challenges for the laboratorian, by making data production so much easier, the new challenges no longer lie exactly within his domain, the laboratory, but lie across existing boundaries of interest and discipline. The boundaries of the laboratory are no longer as useful and significant as they were. Therefore, clinical scientists and physicians alike must now change their emphasis to meet challenges which heretofore lay between their domains of interest expertise, and authority. But shifting or sharing boundaries is inevitably a slow, painful procedure.

At first glance this problem seems less serious at the sample collection end of the process where the danger of improperly practicing medicine is not so great. In the long run, however, the problems of loss of privacy created by automatic data retrieval in demographic files may prove a very thorny problem. At any rate, some early success has been achieved in this area. Practical technics have been demonstrated and the next phase is standardization of equipment.

Today's major challenge lies at the interface between the clinical laboratory and the user of its data, the practicing physician. This is where the bottleneck exists, now that automation has removed the earlier one at the data pro-

duction stage. Again, digital computers have had some impact here, but relative to the size of the overall problem it has been negligible.

Figure 5 shows a portion of a report from a modern analytical automaton. Not only is the result presented but an attempt is made to provide some interpretation by relating the result to the range of results expected in normal individuals. This is provided in easily scanned visual form. Other report formats exist which give a day by day picture of patient status. These are generally called "Cumulative Reports." They contain a bewildering plethora of information, and make an impossible slide or text figure. But again, the so called "Normal Range" is used as a means of interpreting patient status.

<u>PATIENT</u>	<u>NO.</u>	<u>WARD</u>	<u>DATE</u>	<u>ETC</u>
Smith, J.T.	132546	3M	7/7/77	Other patient data
<u>TEST</u>	<u>RESULT</u>	<u>NORMAL RANGE</u>		
Urea	35	() X		
Calcium	2.5	(0)		
Thyroxine	3.0	X	()	

Fig. 5. Portion of a laboratory report. The computer automatically prints a diagram representing the relationship between the observed result and the "Normal Range."

THE "NORMAL RANGE" PROBLEM

The goal of this presentation is to suggest that improper preoccupation with the "95% Normal Range" has prevented the clinic from taking full advantage of the advances in laboratory automation described above. This preoccupation has indeed been intense, on the part of laboratorian and clinician alike. It has been fostered over the years by the high cost of obtaining data and by the boundary between the medical and laboratory disciplines. Of these two only the latter remains.

Numeric normal ranges are usually given as those numbers which include 95% of the well population, as illustrated by Fig. 6A for tri-iodothyronine. An unreasonable intrinsic value has been attributed to this concept of the 95% normal range, perhaps because data were so difficult to acquire and a statistical approximation was needed. But the mystique lingers on even though automation has made the data easy to get (see Note a). An inexcusable number of papers has been written about the shape of the distribution curve of normal people, a matter which is easily settled experimentally. Because 5% of persons are considered "not normal," by this definition, for each serum constituent there have even been papers pointing out that if multiple determinations are performed on one specimen the chances of one of them being abnormal is greater than 5%. Notice that two successive layers of semantic slippage are involved in that statement, namely that a datum can be abnormal and that abnormal means not good. Some serious and regrettable fuzziness has been allowed to grow in this area between chemistry and medicine, where a physician may regard a number as abnormal because it is beyond the limits of a gaussian distribution (seldom seen in practice) and may then accept or reject its validity based on the condition of a given patient. Rejection often has very

Note a. Some modern instruments actually discard data, data from which distributions could be plotted. These devices discard selected results on designated specimens, after performing the analysis. The data are obtained but their print-out is suppressed. This illustrates almost more than anything else the complete inversion in laboratory workload priorities during the last 20 years.

real implications of laboratory error in the mind of the average physician. This is a major factor in the strange ambivalence they have for the laboratory, and the negative side of this ambivalence is automatically accentuated with respect to multiple analysis automation. In single analyses the laboratory is inevitably going to put out 5% of its results on well patients as "abnormal," i.e. errors in some physician's minds, (disregarding the fuzzy semantics). In multiple analysis the "error" rate rapidly climbs towards 100% as the number of tests per specimen goes up. This fact has evoked heated criticism of multiple test automation over the last decade.

When one sets the limits of the 95% normal range one is really setting the fraction of false positives for the test at 5%, regardless of the fraction of false negatives. Perhaps because the designation "false negative" requires a prior medical decision that a disease exists, this area has been considered somewhat outside the laboratory, and in the domain of the clinician. However, reality is beginning to prevail. Physicians and laboratorians (often in the same person) are beginning to look at the other side of the coin, namely distributions of results for abnormal conditions (Ref. 3,4). Note that in this case the label "abnormal" 1) is applied to the correct entity, 2) has no statistical connotation, and 3) refers to something which can be defined on purely medical grounds.

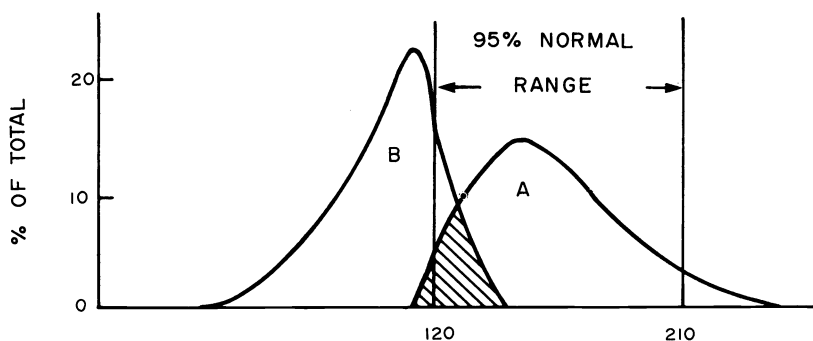


Fig. 6. Population distributions of tri-iodothyronine concentrations. A, 171 euthyroid adults. B, 108 hypothyroid patients before treatment. Data from Ref. 5.

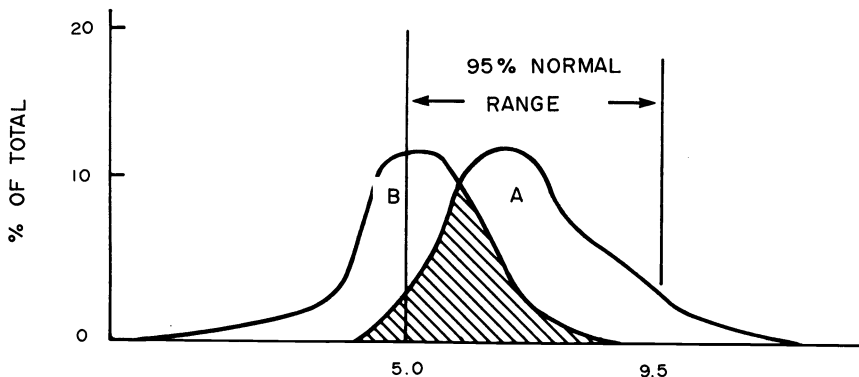


Fig. 7. Population distributions of thyroxine concentrations. A, 164 euthyroid adults. B, 99 hypothyroid patients before treatment. Data from Ref. 5.

Figure 6B shows the distribution of tri-iodothyronine (T3) in serum of adults who have been diagnosed as having hypothyroidism. This diagnosis was made on clinical and laboratory grounds and verified by response to treatment. Note how greatly this distribution overlaps that for normal persons. It is obvious that the proper decision-point for minimizing false positives and negatives depends on far more than the location of the lower limit of the 95% normal range. Among other things it depends on the medical risks. A false negative is, for example, far more serious to a hypothyroid person than is a false positive to a normal. This emphasizes further the inadequacy of the normal range.

Figure 7 shows the same types of distributions for thyroxine (T4). Here the overlap is obviously worse. This reflects on the relative value of these two tests for the diagnosis of hypothyroidism.

THE NEED FOR THE ESTABLISHMENT OF DATA BASES FOR DISEASE STATES

The type of information shown for two tests in hypothyroidism needs badly to be collected for all abnormal conditions where the laboratory presumes to be medically helpful. Physicians considering a laboratory report must have some version, however limited, of these decision-assisting data in their minds or the result in question must be useless. With automation there is no excuse for not collecting full data of this type. Until it is collected, automation cannot fulfill its potential role in medicine. As yet there is limited recognition of this need, but it is coming (Ref. 3,4). Without it serious and expensive mistakes can be made, such as widespread screening programs based on insufficient information (Ref. 4). With it automated assistance to the physician becomes possible, especially if he is willing to list his suspected diagnosis as part of the patient data. A report from a computer with access to this type of data base could, for example, give the physician the exact likelihood that the T3 and T4 values found in a given case are consistent with his suspicion of hypothyroidism.

A tremendous job of data collection and collation lies ahead. It will take a new level of cooperation and understanding between clinicians and laboratorians to accomplish it. However, proper exploitation of automation depends on it, and cannot be attained without it.

THE NEED FOR AUTOMATION OF THE MEDICAL RECORD

The final product of all of this new collection of data by automated means, and even its interpretation, is still limited in the case of each specific patient by what the physician can write by hand, or remember. The greatly increased flow of information has caused real problems at this point in the health-care delivery system. The physician has far more information to record and consider for each patient than did his counterpart of two decades ago, and he sees more patients. A wholly new approach to medical records is badly needed (Ref. 6), and one which will allow for automated handling. There is cause to be optimistic. Who would have believed two decades ago that every student today could have a pocket calculator so powerful as to make the sliderule obsolete? It seems likely that the hardware of the future will permit an automated medical record, which might even be kept by the most concerned person, the patient. One would hope that it would be designed to take full advantage of the whole body of experience of clinical laboratory data, while contributing its tiny share to that body. While this is currently a dream, it is clear that until that stage is reached exploitation of the present stage of automation within the laboratory will remain incomplete.

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