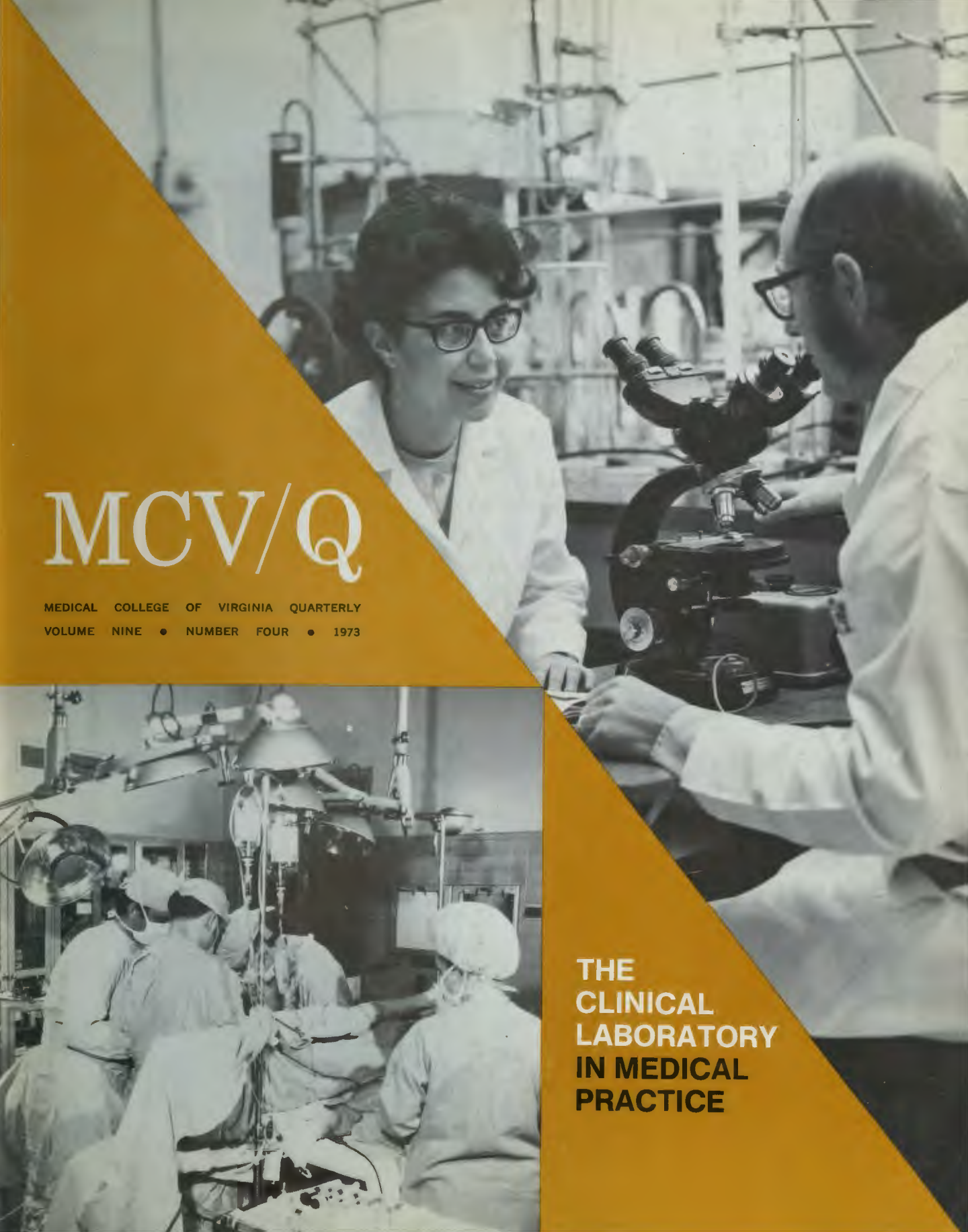

MCV/Q

MEDICAL COLLEGE OF VIRGINIA QUARTERLY
VOLUME NINE • NUMBER FOUR • 1973

THE CLINICAL LABORATORY IN MEDICAL PRACTICE

Spasm reactor?

Donnatal®!

	each tablet, capsule or 5 cc. teaspoonful of elixir (23% alcohol)	each Donnatal No. 2	each Extentab
hyoscyamine sulfate	0.1037 mg.	0.1037 mg.	0.3111 mg.
atropine sulfate	0.0194 mg.	0.0194 mg.	0.0582 mg.
hyoscine hydrobromide	0.0065 mg.	0.0065 mg.	0.0195 mg.
phenobarbital	(¼ gr.) 16.2 mg.	(½ gr.) 32.4 mg.	(¾ gr.) 48.6 mg.

(warning: may be habit forming)

Brief summary. Adverse Reactions: Blurring of vision, dry mouth, difficult urination, and flushing or dryness of the skin may occur on higher dosage levels, rarely on usual dosage. Contraindications: Glaucoma; renal or hepatic disease; obstructive uropathy (for example, bladder neck obstruction due to prostatic hypertrophy); or hypersensitivity to any of the ingredients.

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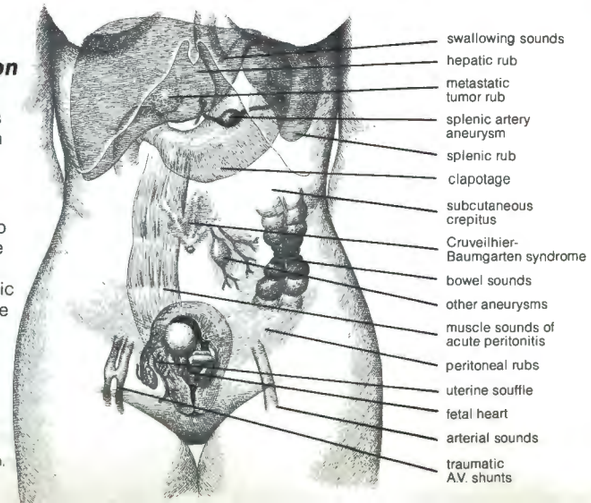
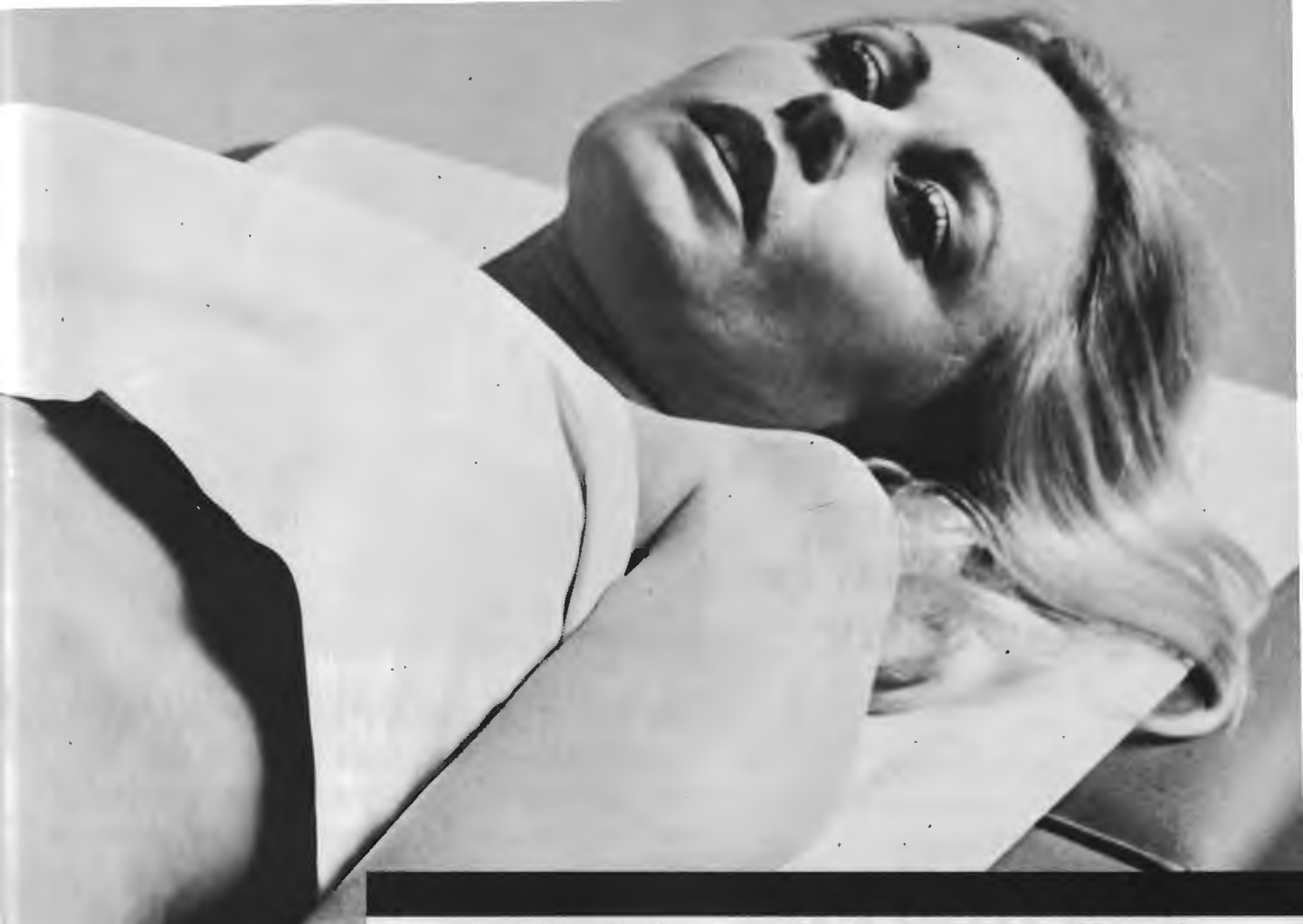
Pointers on auscultation

"It is fair to say that the stethoscope is as important as any instrument for examination of the gastrointestinal tract."*

Sources of diagnostic noises to be considered during a routine abdominal auscultation.
In addition to visceral-peristaltic activity, these sounds may have their origin in visceral-parietal friction as well as in vascular and muscular activities.



*Palmer, E.D.: Clinical Gastoenterology, ed. 2, New York, Hoeber Medical Division, Harper & Row, Publishers, 1973



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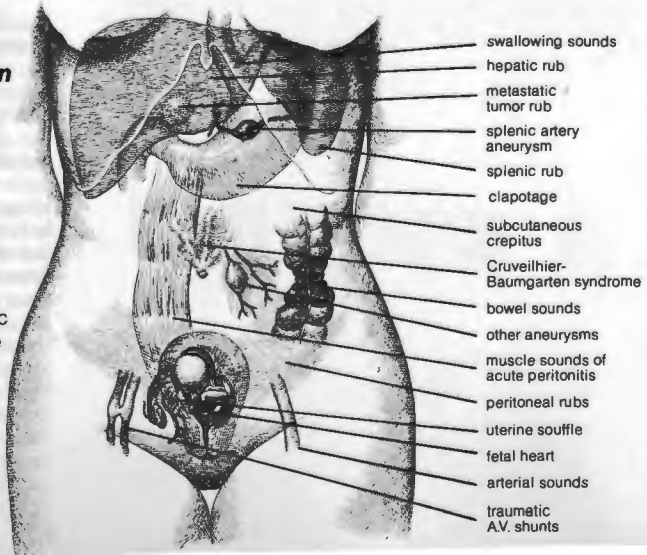

*Palmer, E.D.: Clinical Gastoenterology, ed. 2, New York, Hoeber Medical Division, Harper & Row, Publishers, 1973

When cardiac complaints occur in the absence of organic findings, underlying anxiety may be one factor

The influence of anxiety on heart function

Excessive anxiety is one of a combination of factors that may trigger a series of maladaptive functional reactions which can generate further anxiety. Often involved in this vicious circle are some cardiac arrhythmias, paroxysmal supraventricular tachycardia and premature systoles. When these symptoms resemble those associated with actual organic disease, the overanxious patient needs reassurance that they have no

Before prescribing, please consult complete product information, a summary of which follows:

Indications: Relief of anxiety and tension occurring alone or accompanying various disease states.

Contraindications: Patients with known hypersensitivity to the drug.

Warnings: Caution patients about possible combined effects with alcohol and other CNS depressants. As with all CNS-acting drugs, caution patients against hazardous occupations requiring complete mental alertness (*e.g.*, operating machinery, driving). Though physical and psychological dependence have rarely been reported on recommended doses, use caution in administering to addiction-prone individuals or those who might increase dosage; withdrawal symptoms (including convulsions), following discontinuation of the drug and similar to those seen with barbiturates, have been reported. Use of any drug in pregnancy, lactation, or in women of childbearing age requires that its potential benefits be weighed against its possible hazards.

Precautions: In the elderly and debilitated, and in children over six, limit to smallest effective dosage (initially 10 mg or less per day) to preclude ataxia or oversedation, increasing gradually as needed and tolerated. Not recommended in children under six. Though generally not recommended, if combination therapy with other psychotropics seems indicated, carefully consider individual pharmacologic effects, particularly in use of potentiating drugs such as MAO inhibitors and phenothiazines. Observe usual precautions

organic basis and that reduction of excessive anxiety and emotional overreaction would be medically beneficial.

The benefits of antianxiety therapy

Antianxiety medication, when used to complement counseling and reassurance, should be both effective and comparatively free from undesirable side effects. More than 13 years of extensive clinical experience has demonstrated that Librium (chlordiazepoxide HCl) fulfills these requirements with a high degree of consistency. Because of its wide margin of safety, Librium may generally be administered for extended periods, at the physician's discretion, without diminution of effect or need for increase in dosage. (See summary of prescribing information.) If cardiovascular drugs are necessary, Librium is used concomitantly whenever anxiety is a clinically significant factor. (See Precautions.) Librium should be discontinued when anxiety has been reduced to appropriate levels.

in presence of impaired renal or hepatic function. Paradoxical reactions (*e.g.*, excitement, stimulation and acute rage) have been reported in psychiatric patients and hyperactive aggressive children. Employ usual precautions in treatment of anxiety states with evidence of impending depression; suicidal tendencies may be present and protective measures necessary. Variable effects on blood coagulation have been reported very rarely in patients receiving the drug and oral anticoagulants; causal relationship has not been established clinically.

Adverse Reactions: Drowsiness, ataxia and confusion may occur, especially in the elderly and debilitated. These are reversible in most instances by proper dosage adjustment, but are also occasionally observed at the lower dosage ranges. In a few instances syncope has been reported. Also encountered are isolated instances of skin eruptions, edema, minor menstrual irregularities, nausea and constipation, extrapyramidal symptoms, increased and decreased libido—all infrequent and generally controlled with dosage reduction; changes in EEG patterns (low-voltage fast activity) may appear during and after treatment; blood dyscrasias (including agranulocytosis), jaundice and hepatic dysfunction have been reported occasionally, making periodic blood counts and liver function tests advisable during protracted therapy.

Supplied: Librium® Capsules containing 5 mg, 10 mg or 25 mg chlordiazepoxide HCl. Libritabs® Tablets containing 5 mg, 10 mg or 25 mg chlordiazepoxide.

The Clinical Laboratory in Medical Practice

MCV/Q

MEDICAL COLLEGE OF VIRGINIA QUARTERLY

A Scientific Publication of the School of Medicine
Health Sciences Division of Virginia Commonwealth University

1973 •Volume Nine • Number Four

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The Clinical Laboratory in Medical Practice
GEORGE P. VENNART, M.D., *Guest Editor*

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Introduction

The Clinical Laboratory in Medical Practice

This issue of the Medical College of Virginia Quarterly is devoted to the 44th Annual McGuire Lecture Series held in March 1973, under the auspices of the Division of Clinical Pathology, Department of Pathology, and the Department of Continuing Education. The program was designed specifically for the nonpathologist physician and addressed itself to two broad goals: first, to provide a summary of recent advances in practical laboratory medicine and second, to offer a blueprint for quality assurance to those physicians who, of necessity, must operate their own office clinical laboratory. We were particularly pleased that the McGuire Lecturer this year was Dr. George Z. Williams, the first Professor of Clinical Pathology and Director of Laboratories at the Medical College of Virginia, and presently Director of the Institute of Health Research in San Francisco, California.

GEORGE P. VENNART, M.D.

Chairman, Division of Clinical Pathology
Professor of Pathology
Medical College of Virginia

Advancing Technology of Clinical Laboratory Practice*

GEORGE Z. WILLIAMS, M.D., D.Sc.

Director, Institute of Health Research; Clinical Professor of Clinical Pathology, University of California, San Francisco

In spite of the temporary antitechnology attitude of a portion of our society, the future will be an era of automated technology. Buckminster Fuller (1) has reviewed the progress of industrial technology and points out that continuing scientific advances are inevitable and that technical applications will provide more equitable distribution of wealth, more time, more opportunity for education and intellectual pursuits. He predicts that technology will produce enough for all of mankind by efficient use of energy. He observes that the overall efficiency of the use of energy by man was 1% until the turn of the century; it has grown to 4% since then. Some modern devices and systems of energy conversion to productive work reach 10–15% and the potential of such machines as rockets is 75–80%. By 1900 the industrial revolutions of civilization had provided their benefits to approximately 1% of mankind, spreading to 5% of the world's population by 1920, 20% by 1940 and 44% by 1970. The growth curves depicting several technological indices in figure 1 provide convincing evidence of the remarkable accomplishments to be expected by extrapolation into the next several generations. The advances in communication up to the Telstars presently encircling the earth, in transportation by rocketing to the moon and in the scientific discoveries exemplified by the use of the chemical elements, illustrate the accelerated expansion of the benefits of science to man. A similar curve could be constructed for the advancement of medical science and its miraculous achievements when compared to health conditions in past centuries.

Science and technology have brought us to where we are and can be credited for all the "good" things we have. If scientific advances are not used for the benefit of mankind, the fault lies with the users, the people, not with the technology of science. Technology has brought a distribution of wealth to larger and larger numbers of the world's population in terms of food, communication, education and medicine. Today, fewer starve than ever before; the average life expectancy in developed countries has increased from 42 years to 80 years. Few in the world are ignorant of the happenings over the planet; radio and television are worldwide, and air transportation now reaches all points. Fuller reminds us that if we discarded all these advances at their present state, two-thirds of the world's population (two billion) would starve within six months, all communications would stop, people would be isolated into intolerable pockets of high density and cities would become inescapable centers of anarchy and violence. On the other hand, it is amusing to conjecture that if we discarded all the political ideologies, politicians and bureaucratic systems but retained our technological advances, the world would, in all probability, go on progressing, probably with less difficulty and turmoil. Nuclear energy was discovered by scientists, bombs were dropped by politicians. The thought occurs—is it rational to leave the decisions of the future to the politicians, or should they be left to those whose intellects, judgment and industry have bettered the physical and intellectual life of half the world beyond their fondest expectations of a short century ago? Recognition of what science has so far accomplished for man justifies the optimism that scientists will continue to mold a better life tomorrow for all the world's people.

* Presented by Dr. Williams at the 44th Annual McGuire Lecture Series, March 23, 1973, at the Medical College of Virginia, Richmond.

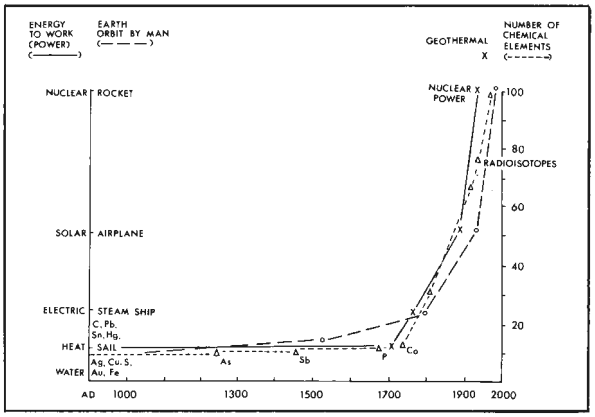

Fig. 1—Exponential growth curve of man's utilization of technology and scientific discovery.

Focusing on health and medical care, although development in medicine has necessarily lagged somewhat behind industry, there has been a parallel upswing of remarkable advances in the fields of human genetics, chemistry, physiology and mental capability. There is no question but that the sciences of physics, chemistry, physiology, morphology, psychology, logic and computer applications will continue to rapidly advance health knowledge. Automation must inevitably utilize fewer resources at a lower cost to provide more and better health and medical care to all.

The New Era of Scientific Medicine. Science is the systematic study of nature; bioscience applies scientific methods to the understanding of animate nature. Applications of scientific methods of discovery and analysis and the synthesis of knowledge concerning the nature of man have just begun. Growth ahead will be as rapid and phenomenal as advances in energy use, transportation and communication have been in the past. The computer potential in the study of man is in its initial exploratory phase, and practical applications in medicine have barely begun.

It is understandable that the laboratory, in its role of providing standardized and quantitative scientific measurements, will continue to grow as the primary source of reliable information concerning health and disease. To realize the importance of further advances, we can remind ourselves that, as with the technology of food distribution and communication in society, if we should destroy all modern applications thus far accomplished in medicine, chaos would reign. Think of the plight of today's dense population centers with no vaccines, no public health control of water, of insect disease vectors or food, no medical instruments, no drugs, no hormone or vitamin biologics, no antibiotics. The resulting disaster is unimaginable.

The laboratory of the future will probe not only deeper with newer tools of medical research, but it will apply the burgeoning new knowledge to maintain health and more effective therapy as well as to improve early diagnosis. To accomplish this at acceptable costs, however, automation, miniaturization, computerization and systemization are necessary.

Primary Laboratory Functions. The laboratory is the facility for the measurement of body functions and states of various constituents and for such activities as testing chemical reactions. Quantitative information is extracted from body fluids, products and tissues. Blood and urine contain enormous amounts of chemical and physiological information, which Dr. Irvine Page (2) compares with the use of coded computer tapes. This "coded information" must be discovered, identified and decoded. The data must be correlated and synthesized into understandable information, describing in medical terms the whole integrated organism of man during his varying reactions in health and disease. This task is only in the design stage. Our tools are still relatively crude. There are many steps in the development of this expanded role of the future laboratory. Immediate needs include:

1. New tests for functions and constituents yet undiscovered;
2. Frequently repeated sets of test measurements to reveal the kinetic nature of physiological and chemical activities and to delineate the limits of variation;
3. Improvement of precision and accuracy;
4. Limitation of blood and tissue samples to micro amounts for large batteries of analyses;
5. Miniaturization of equipment;
6. Automation for speed and elimination of error and laboratory bias (variability);
7. Computerized instant and long-term quality control;
8. Computerized data collection, correlation and reconstitution into usable physiological and biochemical information;
9. Identification of deviated test results which warn of trouble;
10. Standardization of methodology and inter-

pretation to permit comparability of data;

11. Regional health and medical data banks to prevent wasteful and costly duplications of examinations and testing and to provide lifetime health records.

The direction of development of the clinical laboratory in the future is already apparent. Mechanization and automation of the repetitive manipulations such as sample transport, pipetting, mixing, temperature control, end-point determination and reaction rate measurement are greatly improving precision and eliminating human errors. New transducers for detecting changes in constituents—their states and rates of change—are being devised and applied. Advances in gas-liquid chromatography, thermal microcalorimetry, laser beam technology, nuclear beam technology, nuclear magnetic resonance measurements, x-ray spectrography, radioimmune assay, mass spectrography and ion-electrode devices remain to be miniaturized, standardized, automated and calibrated. In one decade, the amount of blood sample required for a set of 10 or 12 analyses has been reduced from 100 to 1 ml—two orders of magnitude! Crude dual channel test instruments have advanced to 20 and 22 channels. Rates of measurements of reactions have shortened from minutes to seconds; precision has improved from 10% to less than 1% error for many tests.

Automation has amplified the productivity of the technical staff by factors of two, four and ten for different types of tests, and occasionally, by a factor of 100 for some, such as antibiotic sensitivity microtiter automation and the newer instruments for enzyme reaction rate analysis. At the laboratories of the National Institutes of Health, even the earlier phases of automation permitted doubling the test work load without increasing the technical staff.

Computational capability has advanced in two decades from the use of the mechanical desk calculator and slide rule to the programmed electronic calculator first, and more recently, the minicomputer. Five years ago, a laboratory processing a million tests per year needed a million dollar computer system. With the application of computer technology and economy, the same laboratory today, needs only ten tiny preprocessing computers, each built into a set of specific instruments, at a comparable computer capability at a cost of less than $300,000. Nowhere is economic efficiency progressing as rapidly as in computer automation technology. Five years ago, computers served laboratories as expensive data collection, storage, calculation and printing machines. Today, they are beginning to be used for their true value in high speed correlation analyses, process control and conversion of data into information.

Several examples may serve to indicate the trends for the future. In figure 2, the rate and character of aggregation of human platelets are recorded on a chart; this is partial mechanization. Pipetting and transport are still manual as is the conversion of the charted analog curve to numerical representation. This exciting new test of platelet activity, which detects hyperactive or deficient platelet functions, and thereby identifies high risk "clotters" and "bleeders," requires automation and integration into the computer system so that one technologist can more precisely perform ten tests in the time now required for one. The model "S" electronic blood cell counter, now a familiar instrument in many larger laboratories, provides four measurements and three derived results (total of seven results) in hematology with a precision at least ten times that of the traditional manual methods and at a speed and capacity which can approach a hundredfold improvement if need be.

Enzyme rate reactions used to be very slow and tedious to measure by stopwatch, water bath and manual colorimeter. Precision was difficult to obtain. The semiautomatic bichromatic reaction rate analyzer improves precision to less than 1% error for some enzyme tests and at rates of 60–120 multiple

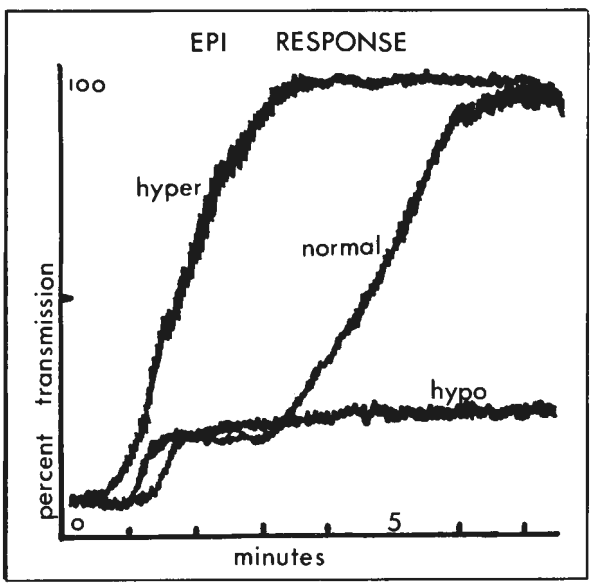

Fig. 2—Measurement of the rate and degrees of platelet aggregation by mechanized instrumentation.

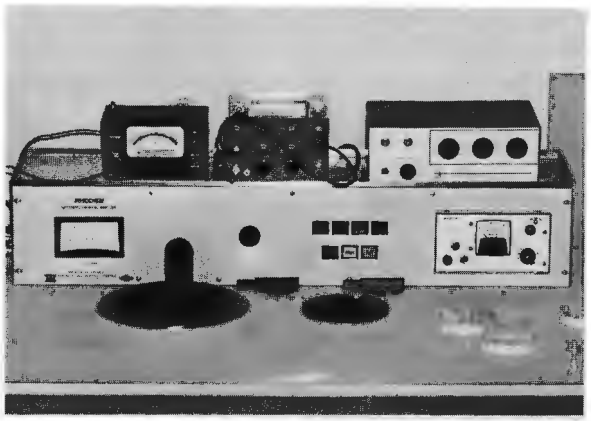

Fig. 3—Automated chemical analyzer console containing centrifuge and controls for speed, temperature and automatic washout of cuvettes.

point analyses per hour. With computer processing, human reading, calculation and transcription errors can be eliminated.

A new and innovative breakthrough in high speed, microsample handling and multisample analysis was developed at the Oak Ridge National Laboratory by Dr. Norman Anderson, Director of the Molecular Anatomy Program. Although precision for the tests adapted to this instrument, so far, is equal only to the best conventional methods, the sample size of 1–10 ml, comparably small amounts of reagent and high speed performance promise remarkable improvements in multitest microanalysis for the future. Figures 3, 4 and 5 illustrate the innovative principles and operation of this device which, with its built-in computer, exemplifies future automation in the laboratory. This device is a high-speed centrifugal analyzer using centrifugal forces to mix a series of samples and reagent in microchambers and to transfer them into quartz window cuvettes oriented radially in a teflon rotor. Computer-controlled synchronization permits serial multiple readings of density or color reaction changes within each cuvette. The rate of change in each sample is depicted graphically on an oscilloscope or CRT screen (fig. 6) and is computed as a digital result printed on an attached teletypewriter or via the computer system. Pipetting of sample and reaction is performed with a mechanized autopipetter of high precision.

Fig. 4—Systems Reaction-Analyzer—control panel, minicomputer and oscilloscope and television type screen displays of chemical reactions occurring in each cuvette of centrifugal head.

Fig. 5—Automated chemical analyzer. Transfer disc of centrifuge head showing the two well chambers placed in radial position for each of the 15 positions. The serum sample placed in the inner well is run into the reagent in the outer well and mixed by centrifugal force and finally forced into the peripheral quartz cuvette chambers of the rotor (not shown).

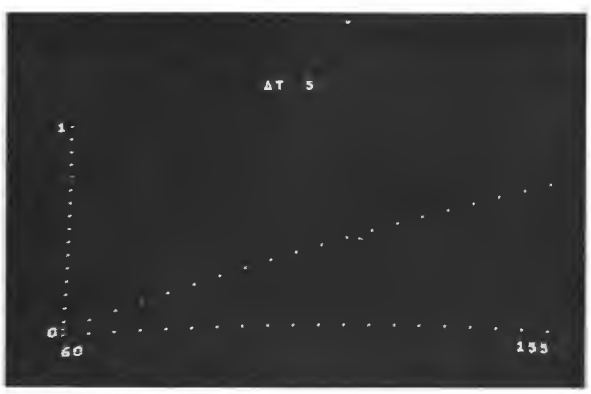

Fig. 6—Enzyme reaction rate curve depicted immediately on cathode ray tube screen.

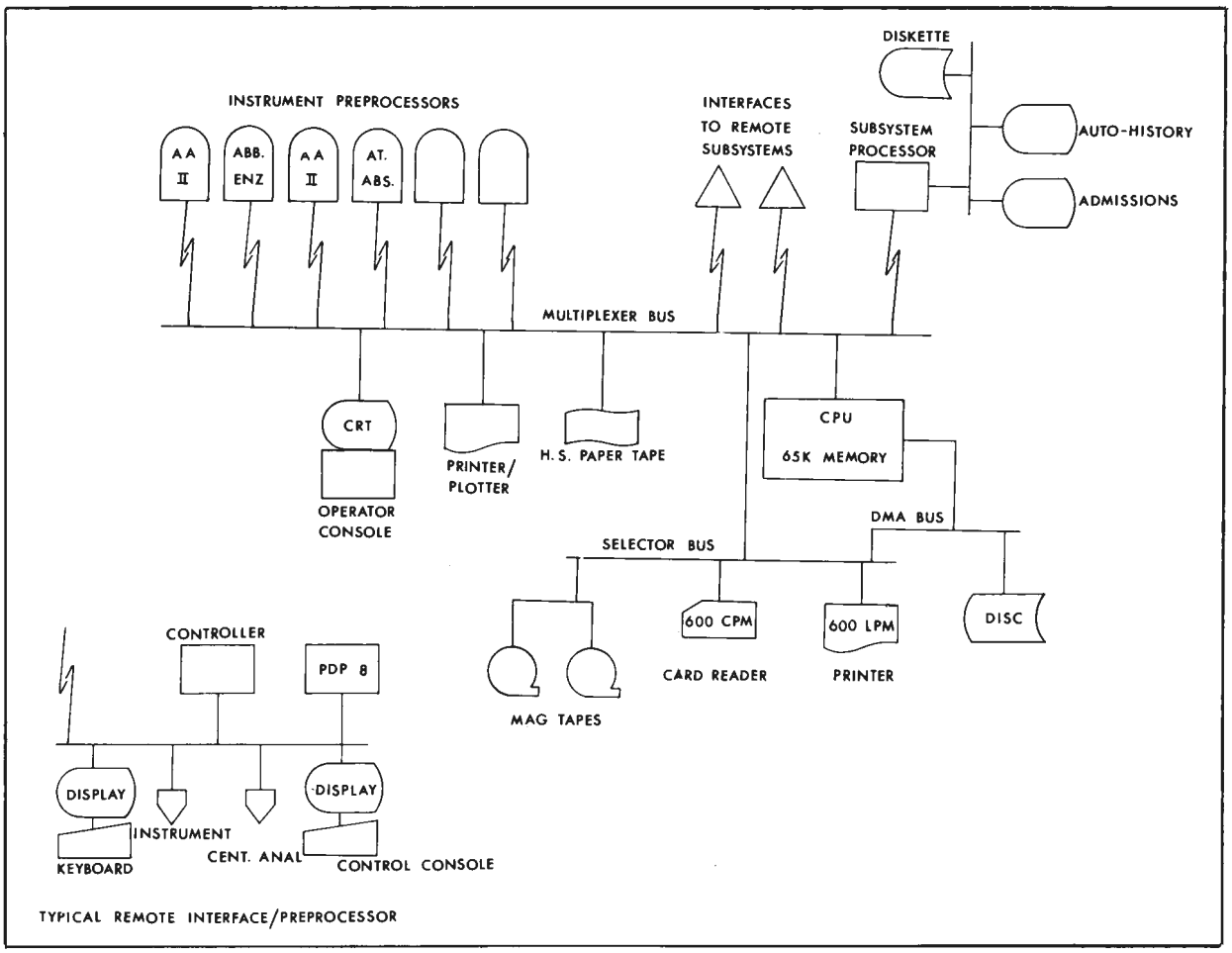

Fig. 7—Diagram of network of individual microcomputer preprocessors attached to each laboratory instrument and coupled to a control microcomputer for final processing, mass storage and report generation.

Finally, the heart of the laboratory of tomorrow—the computer. Due to the high costs of computer equipment and programming, initial attempts at application in the laboratory were designed to accomplish all possible operations with one large general purpose machine. As was discovered in industry, this was found to be expensive and inefficient in spite of the theoretical economic advantages of having one machine for the performance of many functions. With the exponential reduction in costs of processors and memories and the miniaturization of equipment, it is now feasible to employ many special-purpose mini-minicomputers, programmed to accomplish specific tasks for particular analytical instruments and to couple these in a network with one minicomputer for final processing, collation and report generation. This system frees the central coordinating minicomputer to accomplish the data correlation and interpretative functions while the other dedicated instrumental computers (buffered interfaces) are "baby sitting" the analyzers and preprocessing the raw data. Figure 7 depicts the diagram of such a system which is being installed in our new laboratory in San Francisco. Computers are here to stay because they can perform, at the speed of light, complex and repetitive computations, and can sort, coordinate and analyze large quantities of data too time consuming to be feasible if done manually. They can synthesize new information from multidimensional data, opening vast new areas of analytical potential.

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Utilization of Blood and Its Products*

ROBERT D. LANGDELL, M.D.

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The development of a system of plastic bags connected by integral tubing for the collection, processing and storage of blood has made it possible for selective transfusion of appropriate blood components. It is now possible to select the blood component that will correct a patient's physiologic deficiency. This has resulted in better patient care and in better utilization of blood since a single unit of blood can be used to supply erythrocytes for an anemic patient, platelets for a patient with thrombocytopenia and factor VIII concentrate for a patient with hemophilia.

In order to utilize blood and its components optimally, there must be a close working arrangement between the blood bank personnel and the clinical staff. At the time when blood is obtained from a donor, a decision should be made concerning how the blood will be used. Several of the components can be prepared only from fresh blood. The equipment, supplies and personnel time should be used to produce the maximum benefit to the recipients of the blood. Although each unit of blood could be separated into several useful components, it is a wasteful procedure if the components are not used. Each institution must determine its need for blood and blood components. Those responsible for the operation of the blood bank should not make unilateral decisions about the preparation of blood components and the clinical staff should not make unilateral decisions about the use of blood components.

Packed Red Blood Cells. When blood is collected in the proper closed bag system, it is possible to remove much of the plasma after sedimentation or centrifugation without opening the container. The resulting packed red blood cells remain viable for transfusion purposes for 21 days when stored under standard conditions. The obvious advantage of transfusing packed red blood cells is that the circulating erythrocyte mass of the recipient can be increased without increasing the circulating plasma volume. This minimizes the possibility of circulatory overload. It has been estimated that up to 80 percent of the transfusions should be in the form of packed red blood cells (2).

Along with the plasma, most of the sodium added in the anticoagulant solution is removed. This can be an important factor when transfusions are necessary in patients requiring limited sodium intake. In addition to the excessive amounts of electrolytes and citric acid, the plasma contains antibodies. One of the dangers in the universal donor concept is that the plasma of group O individuals contains antibodies against group A and group B cells. These antibodies are removed along with the plasma. Although type specific compatible erythrocytes are the treatment of choice, in an emergency situation when such blood is not available, group O packed red blood cells are an acceptable form of therapy.

Factor VIII Concentrates. The treatment of hemophilia with plasma rather than whole blood was an early form of component therapy. It was found that plasma contains the material lacking in hemophilia and except for the occasional patient with anemia, whole blood is seldom needed for the treatment of hemophilia. Although the antihemophilic factor in plasma is relatively labile, it can be maintained in plasma stored below $-18°C$. Until relatively recently, plasma removed from whole blood within a few hours of collection and then promptly frozen was the only available source of factor VIII for human use. Because of the relatively small amount of antihemophilic activity in plasma, large amounts of fluid had to be infused

* Presented by Dr. Langdell at the 44th Annual McGuire Lecture Series, March 23, 1973, at the Medical College of Virginia, Richmond.

to significantly increase the circulating level in the patient. In addition, the infused activity was retained in the circulation for only a short period of time (4). Thus, therapy was restricted to the amount of fluid that could be infused without causing overload.

In 1965, Pool and her associates (5) reported that when frozen plasma was thawed slowly, a poorly soluble, gelatinous precipitate was formed. This material, called cryoprecipitate, was found to contain a considerable amount of the antihemophilic activity of plasma. Thus, it became possible to concentrate and collect factor VIII of plasma in a closed bag system. The resulting material contains, on the average, about half of the antihemophilic activity of the original plasma and in a relatively small volume.

Although the treated plasma can be returned to the cellular component and used as whole blood, most have found it better to utilize the plasma for other purposes. The residual plasma can be used for all conditions where plasma is indicated other than for the treatment of hemophilia.

Platelet Concentrates. Fresh whole blood contains viable platelets which will function when transfused. When platelets are needed to control bleeding in a patient with thrombocytopenia, however, a large amount of blood must be infused to increase the platelet level in the circulation. Platelets can be separated from whole blood and transfused in a relatively small volume.

Platelets have a specific gravity of 1.040 while the specific gravity of erythrocytes is about 1.095 (3). Since platelets are lighter than erythrocytes, the red blood cells can be sedimented by low speed centrifugation while the platelets remain suspended in plasma. In this way platelets can be separated from whole blood in a closed bag system. Platelets, however, have a very short shelf life and to be effective must be infused promptly after collection. Although platelets lose viability rapidly, approximately half of the recovered platelets are still effective after 72 hours of storage. Most workers, though, feel that platelets should be used within the first 48 hours after collection. Although there is some evidence that room temperature storage has some advantages, it is felt that best results are obtained when platelets are stored at 4°C. Additional studies of storage are necessary to determine optimal conditions (1).

Platelets can be given along with the plasma as platelet rich plasma. It is usually necessary, however, to give the platelets recovered from six units of blood to achieve the desired effects and it is normally not desirable to give this amount of fluid. The usual practice is to concentrate the platelets by centrifugation and remove all but approximately 30 ml of plasma. Following centrifugation, it may be difficult to resuspend the platelets. Gentle agitation after storage for about one hour has been found to result in a satisfactory product. It is generally recommended that platelets be infused through a filter to remove any large aggregates.

Plasmapheresis. One of the limitations of obtaining blood is that approximately six-to-eight weeks are required for the regeneration of erythrocytes. For this reason, the interval between individual donations of blood should be at least eight weeks. The regeneration of the fluid volume and plasma proteins is more rapid. With a series of bags connected by integral tubing, it is possible to withdraw blood from a donor, separate the erythrocytes from the platelet rich plasma and return the erythrocytes to the donor. This process, called plasmapheresis, can be repeated at frequent intervals without danger to the donor.

This technique has several advantages when the needs for platelets and plasma are great. A relatively small number of donors can supply considerable amounts of platelets and plasma. There is increasing evidence that when platelets are given over a prolonged period, antibodies to platelets develop in the multitransfused patient. These antibodies appear to be directed against the histocompatibility antigens of the HL-A system. Those institutions which use platelet transfusions on a long-term basis have found that best platelet survival occurs when there is an HL-A compatibility between donor and recipient. It is generally felt that if a series of platelet transfusions are required, it is best to obtain platelets by plasmapheresis from a few histocompatible donors rather than utilizing platelets from a number of random blood donors (7).

Frozen Red Blood Cells. With currently available techniques, erythrocytes retain sufficient viability for transfusion purposes for only 21 days. For this reason blood supplies must be continuous and the blood bank must be able to predict its needs so that sufficient blood will be available and yet there will not be wastage due to excessive

amounts of erythrocytes lost by out-date. An obvious solution would be to develop a method of preservation of blood for longer periods.

A number of chemical additives have been used in an attempt to prolong the viability of erythrocytes. The most promising has been adenine (6). With the addition of adenine at the time of collection, there is evidence that erythrocytes remain viable up to 40 days under the usual storage conditions. At the present time, however, there is not sufficient evidence to be certain of the safety of this material for human use.

Other workers have demonstrated that erythrocytes suspended in cryoprotective solutions retain viability almost indefinitely when stored at low temperatures. Before the erythrocytes can be infused, the cryoprotective materials must be removed. The supplies and equipment necessary for preparation, storage and washing make this technique rather expensive. Although the techniques are practical, the cost for a unit of such blood is almost prohibitive for routine use. If methods can be developed to decrease the cost, there are many advantages to such a technique.

Leukocyte-Poor Blood. There are many known antigens associated with erythrocytes, but most of these are only occasionally of clinical significance. For practical purposes, it is only necessary to routinely test for ABO and Rh antigens. In a similar way there are a number of antigens associated with leukocytes, but these are usually of little clinical significance. In multitransfused patients, however, the leukocytes may cause reactions. To prevent these reactions it may be necessary to give leukocyte-poor blood. Recently it has been recognized that leukocyte antigens may have an adverse effect on organ transplantation. It has been suggested that patients with chronic renal disease who may be candidates for renal transplantation should be given only leukocyte-poor blood.

The specific gravity of granulocytes is 1.087–1.092 and the specific gravity of lymphocytes is 1.070 (3). Thus differential centrifugation to prepare leukocyte-poor erythrocytes is rather difficult. There are techniques by which most of the leukocytes can be separated from erythrocytes but these techniques are tedious and time consuming. In order to remove the leukocytes and their antigens it is necessary that fresh blood be used. In those institutions that have a frozen blood program, it has been found that this preparation is an excellent source of leukocyte-poor erythrocytes.

Summary. At the present time there are a number of blood components that are more suitable for transfusion purposes than is whole blood. In order to make maximum use of blood, it is necessary that there be close cooperation between the blood bank personnel and those responsible for patient care.

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Meaningful Toxicology*

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Before discussing this subject in a "meaningful" way, we must have some agreement as to what is meant by the term "toxicology." Toxicology includes such widely diverse areas as the chemistry of toxic substances, the signs and symptoms elicited by such substances, the detection and identification of toxic substances in a wide variety of specimens, the interpretation of analytical results and the treatment of toxic episodes. Although in recent years, toxicology has dealt with the problem of drug overdosage almost exclusively, it must be appreciated that any chemical substance, when absorbed in sufficient quantities to produce an adverse effect, is toxic. This would include common substances such as ordinary table salt and range through substances generally recognized as poisonous such as strychnine. In addition, the broad field of toxicology is concerned with the effects of acute overdosage of toxic agents as well as the effects of long-term, chronic exposure to these agents. The scope of toxicology, therefore, may extend from the study of a single element such as lead to a complex, chemically uncharacterized substance such as snake venom; from an overdose of heroin to the effects of long-term exposure to low levels of pesticides on anticonvulsant therapy. For the purpose of this discussion, we shall narrow the limits of the broad field of toxicology considerably, but it is important to be aware of the breadth and depth to which toxicology may at times extend.

Clinical toxicology laboratories generally have two main functions. The first and most apparent is the identification and quantitation of toxic substances in specimens from patients brought to the Emergency Room after a possible acute toxic episode. In general, the clinician needs the laboratory results promptly in order to answer questions such as: What toxic substance, if any, is present? If present, is the quantity consistent with the signs and symptoms displayed by the patient? Will the quantity of toxic substance present influence the choice of the treatment? Do the laboratory findings confirm the history, diagnosis or clinical impression?

The second general function of the clinical toxicology laboratory is to monitor certain chemical substances in patients. This may be done for the purpose of maintaining therapeutically effective drug levels such as in epileptic patients, or for the purpose of determining the effectiveness of a treatment procedure in reducing levels of toxic agents. A relatively recent area of interest may also include the determination of metal levels in cases of industrial exposure or when alteration in metal metabolism may be associated with a clinical condition.

Both of these general functions utilize common laboratory facilities and personnel, so that it is reasonable that the clinical toxicology laboratory be engaged in both activities. Since the goals to be achieved by these two functions are quite different, the approaches to the achievement of these goals must be considered separately. In general, in order to fulfill its role in dealing with the emergency situation, the toxicology laboratory must provide 24-hour service and utilize reliable, rapid methods resulting in a short turn-around time to be of maximum use to the clinician. The monitoring of chemical substances in patients can usually be achieved within a normal workday using dedicated procedures on a relatively continuous basis. Ideally, of course, with unlimited facilities, personnel and support, these goals can easily be achieved. Since most laboratories can never hope to enjoy this luxury, a more prag-

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matic approach must be taken which involves compromises between the ideal and the practical.

Since there are literally thousands of toxic substances which may be encountered in clinical toxicology, it is absolutely essential at the outset that these substances be examined realistically, making a selection of those which are commonly encountered in the community served by the clinical laboratory. Some substances are common to almost all treatment facilities while others may be unique to a particular locality. Rural and urban communities, for example, may differ as to the relative importance of problems relating to narcotics and pesticides. An important industry in a specific area may present specific toxicological problems which would not usually be encountered elsewhere.

It is commonly acknowledged that good communication between the clinician and the laboratory facility is essential for the production of meaningful laboratory results. Unfortunately, the gap between acknowledging this truism and its implementation is difficult to bridge. Lines of communication between the clinical toxicology laboratory and the clinician can be established initially by identifying the priority by which new toxicological procedures should be made available. It is essential that the laboratory understand the problems of the clinician and that the clinician understand the problems of the laboratory. For example, it may be important in a specific case to identify whether a patient has ingested an overdose of lysergic acid diethylamide (LSD). It is possible to assay LSD levels by radioimmunoassay, but does the need occur often enough to justify the expenditure of funds for equipment and personnel to make this assay procedure available on a "STAT" basis? Conversely, the laboratory may have an elegant method for measuring fluoride levels in serum, but is it of practical use to the clinician when 24 hours are required to obtain test results?

It is better for a clinical toxicology laboratory to conduct a relatively small number of tests by reliable methods in reasonable time periods than to attempt to handle all types of situations which might arise. Once having agreed upon a basic nucleus of tests, the laboratory can adapt and evaluate published procedures to give the clinician results which are meaningful both in reliability and in turn-around time.

It is essential that once lines of communication are opened, they be maintained in both directions. It is the responsibility of the laboratory to inform the clinician as to what can or cannot be done within the constraints imposed upon it in a given situation. It is the responsibility of the clinician to request reasonable and specific tests, supplying sufficient information to permit the laboratory some leeway in establishing priority with which specimens will be processed and to provide proper and adequate specimens for analysis.

The "unknown" toxic agent is always a difficult problem to handle. Again, good communication can help to partially resolve the problem. Most clinical toxicology laboratories have the ability to conduct a variety of screening tests. None of these is all-inclusive and most of them are chiefly of value when they are negative. Some screening tests are of the spot-test type, which can be conducted on urine or gastric contents after a minimum of handling. Others can be used to screen for metals, alcohol, other volatile substances or narcotic drugs. The simple request for "toxicological screening" or "test for poisons" presents a dilemma to the clinical toxicology laboratory. Should the screening tests be directed toward detecting drugs of abuse or heavy metals? Should pesticides be considered or is the patient a known alcoholic? Valuable time can be saved and costs minimized by a brief history or clinical summary of the patient's condition. If nothing else, this would indicate to the toxicology laboratory what substances are not likely to be present.

Ideally, a clinical toxicology consultant should be available to contribute personal expertise in the resolution of these difficult problems. Many toxicology laboratories have the experience and necessary resource material to aid the clinician in making a proper evaluation. Additionally, there are other resources available provided they are utilized. The poison control center, drug information center, and specialists in specific areas can make important contributions to patient management. Thoughtful planning and interdepartmental or interagency support can significantly assist, provided arrangements can be made in advance to utilize these resources properly. Obviously, an emergency at 2:00 a.m. is not the time to decide where to look for help or how to carry out a difficult laboratory test.

The development of a regional poison control center has been found to be the most effective way to resolve these problems in many communities. Properly coordinated, such a regional center could supply information, treatment and laboratory facili-

ties to a large area. This would ensure that costly equipment and skills could be most effectively used to serve a large geographic area. Costs for a specific test, for example, which may be difficult to justify due to infrequent occurrence in a given institution, might be practical if the frequency of occurrence were increased by serving a larger area.

Functioning lines of communication are also important in deriving meaningful toxicological data from laboratory procedures not related to the emergency room. Frequently, the toxicology laboratory is requested to develop or add to the armamentarium of tests available, a procedure identifying a specific drug in serum. The fact that the laboratory may have a certain instrument or procedure available does not necessarily enable the laboratory to apply that instrument or procedure to a specific test. Since the development of gas-liquid chromatography which now makes possible the detection of a vast number of drugs, it is frequently assumed that this is the answer to all drug analyses. Frequently, it is not possible in a practical manner, to modify a gas chromatograph, in order to shift it from a routine, smoothly operating procedure to a special application. Not only will the parameters of the new test be somewhat different but sample preparation is almost always the major problem. One of the difficulties confronting the toxicology laboratory is removing the toxic agent from the specimen submitted in such a manner and in such a state of purity that a particular instrumental procedure can be used for its identification and quantification. In addition, many substances which are rapidly metabolized may not even be detected in biological specimens. Thus, although the parent drug may be easily identified in a standard solution, the presence or absence of that drug in biological material may be a much more complex problem. Detection and estimation of metabolites may be the only method by which the original drug can be detected. These and similar problems should be discussed between the clinician and the toxicology laboratory in order that both may understand the usefulness or limitations of a given analytical procedure.

Finally, in order for the toxicology laboratory results to be meaningful to the clinician, some interpretation of the results is necessary. Tables of normal values are of little use since frequently there are no "normal" values for toxicological analyses. In the case of drugs, therapeutic levels may be available but these must be interpreted cautiously, since some patients may develop tolerance to a given drug after prolonged therapy and others may exhibit a hypersensitivity to very low levels of drugs. It is becoming increasingly common that drug combinations may be present and little information is now available as to drug interactions. Here, again, the clinical toxicologist and those sources of information previously mentioned can be utilized, so that the clinician may benefit more from the services of the toxicology laboratory.

In summary, "meaningful" toxicology can be realized only when the clinical toxicology laboratory provides more than a place where tests are run. Thoughtful and well-planned coordination of the laboratory's efforts with the efforts of the clinician can be provided by establishing and maintaining good communication between the parties. Outlining the proper procedure for establishing this communication is relatively simple. Making it work requires a constant, willful expenditure of effort in both directions.

Microbial Monitoring* **

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The "tubular" schematic of mammalian structure is a well-known concept that has been in use for years, but nowhere is this concept more useful than in understanding the man microbial interphase (fig. 1). The diagram emphasizes the fact that humans are subjected to microbial invasion from two areas where bacterial populations exist in large numbers. The first of these is the internal flora of the respiratory, intestinal and lower urinary tract which, along with the skin organisms, make up the endogenous microorganisms of the body. The second area, the periphery of the diagram, contributes the external or exogenous flora. The numbers and types of organisms found here are unpredictable since they depend upon environmental factors. Infections which are caused by the endogenous flora are considered opportunistic infections, while those derived from the exogenous flora are termed nosocomial infections.

The need for monitoring both areas for alteration in microbial flora, which may result in invasion or adverse effect on internal organs of the host, has been an accepted scientific fact of modern medicine for years. Questions remain, however, on how much monitoring needs to be done. What areas of the patient and the environment should be tested, and what are the practical uses of the results of such monitoring? Before attempting to answer these questions, let me give you an overview of how microbial monitoring has been used at the Medical College of Virginia hospitals and other institutions to identify and solve problems related to nosocomial and opportunistic infection.

Changes in Etiology of Bacteremia. One area that has been under constant surveillance in our institution is that of blood cultures or bacteremia. The numbers and types of microorganisms present in blood cultures have been recorded over the last 20 years. The data document that there have been profound changes in types of bacteria primarily associated with this infection (fig. 2). This graph shows the percentage of patients from whom gram-positive and gram-negative organisms were isolated during a 20-year period at the Medical College of Virginia hospitals. Since 1960, the percentage of patients with gram-negative bacteremia has continuously increased, while during the same period the percentage of patients subjected to gram-positive bacteremia declined (fig. 3). This graph demonstrates the number of patients who had bacteremias with some of the more common organisms associated with this type of infection. Perhaps the most striking results are those for 1970. In this year, the number of bacteremias by *Escherichia, Klebsiella, Enterobacter* and *Serratia* was in the neighborhood of 260 patients. In the same year, there were about 60 *Staphylococcus aureus* bacteremias, a ratio of over four gram-negative bacteremias for every one staphylococcus bacteremia. It is also distressing to note that there has been no decrease in the upward slope of the gram-negative organisms over the last 20-year period but rather an acceleration in this upward slope for the last five-year period.

Alterations of Endogenous Flora. The reason for this shift in the etiology of bacteremia is not known. A number of possibilities immediately present themselves, such as age of the population tested, types of surgical procedures performed and types of medication given. All may play a role in the alteration of results. In regard to medication, particular attention has been paid to such agents as corticosteroids, as they have been related to changes in the immunological response of the host which may allow alteration in microbial flora.

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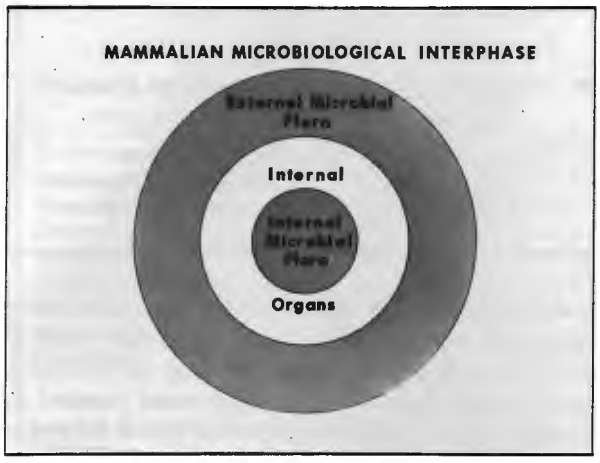

Fig. 1

More recently it has become evident that antibiotics may be responsible for allowing endogenous organisms to manifest pathogenicity. There is evidence that many of the gram-negative infections are endogenous in source, and the organisms of these infections in the immunologically depressed or debilitated patients are selected for by the use of antibiotics. An example of this is shown in figure 4.

The study was done in cooperation with Dr. B. W. Haynes of the Department of Surgery at the Medical College of Virginia. A patient, who had suffered a 23% total body burn, was given penicillin to protect him from the common exogenous pyogenic organisms such as *Staphylococcus aureus* and *beta hemolytic streptococcus*. Bacterial monitoring of the

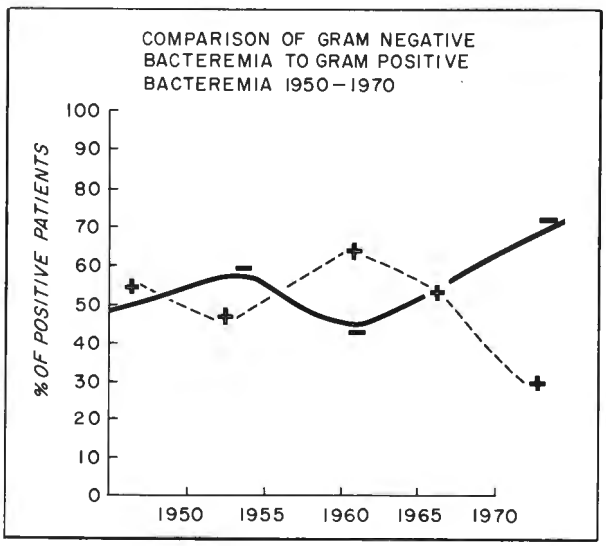

Fig. 2

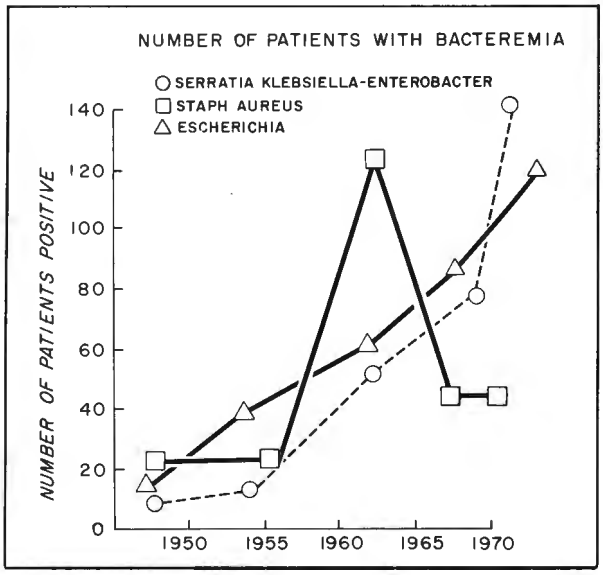

Fig. 3

burn lesion, using a standard technique, shows the success of this therapy, for early in the course of this patient's treatment both of these organisms were present but did not colonize the burn lesion (2). Penicillin, however, did not stop colonization of the lesion by gram-negative organisms consistent with the endogenous flora of the patient. Such organisms as *Proteus, Enterobacter* and *Pseudomonas* rapidly increased in number and only decreased after eschar removal with healing of the lesion. Only then was there a return of gram-positive organisms associated with normal flora (fig. 5). When broad-spectrum antibiotics are included in the antibiotic regimen, there may occur an emergence of a large number of organisms resistant to all the antibiotics used. In this

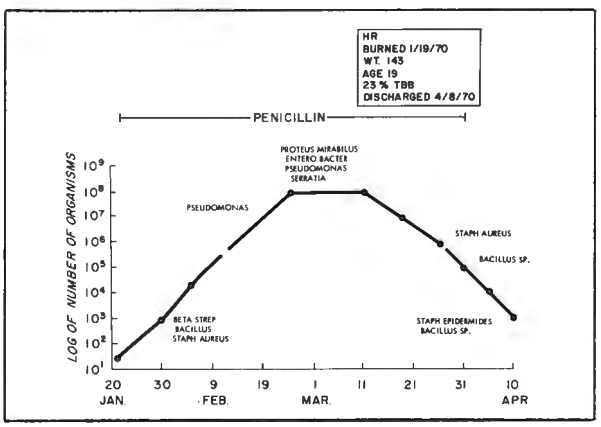

Fig. 4

patient, penicillin, streptomycin and gentamicin were given. The burn area was colonized by neither gram-positive nor gram-negative bacteria but rather by the yeast, *Candida albicans,* an organism known for its resistance to antibiotics. Again, a normal gram-positive flora was not established until eschar removal and healing of the lesion.

In our laboratory, we have been able to alter the normal flora of the hamster by giving large doses of oxicillin. In the upper respiratory tract of ten treated animals, there was a decline in the number of *alpha hemolytic Streptococcus* and *Neisseria,* with an increase in the number of *Escherichia, Proteus,* and *Enterobacter.* In three of the ten test animals, gram-negative bacteremias were demonstrated by heart blood cultures and in three of the ten test animals, death occurred from gram-negative pneumonia within 72 hours of treatment. The normal untreated controls during this period remained healthy and had no evidence of infection (6).

The medical literature also describes numerous examples of alteration in the etiological agents of infections which may be associated with treatment. Rogers (8) contrasts the etiologic agents of pneumonia found during a preantibiotic era (1938–1940) with that of a postantibiotic era (1955–1958). In the first period, there was an 8% incidence of gram-negative pneumonia, while in the latter period during the antibiotic era there was a 38% incidence of this type of infection. Lerner (5)

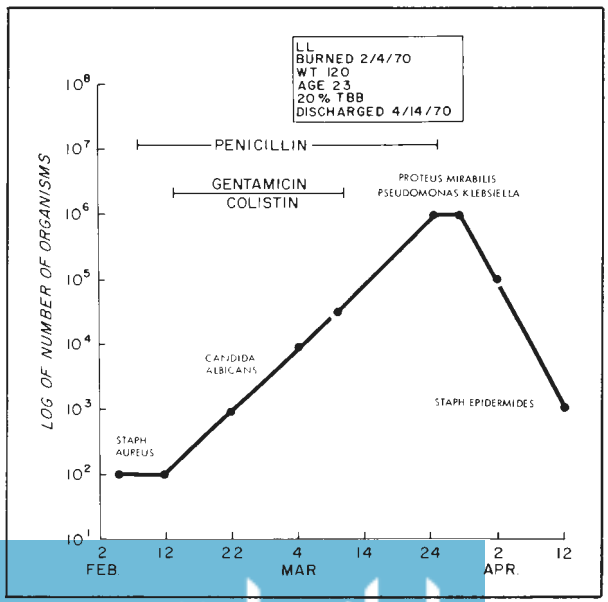

Fig. 5

TABLE 1

TYPES OF THERAPEUTIC REGIMEN ASSOCIATED WITH ENDOGENOUS MICROBIAL FLORA ALTERATIONS

Irradiation	Corticosteroids
Antimetabolites	Immunosuppressants
Antibiotics	Dermal preparations

also has reported an increase in gram-negative pneumonia in nonhospitalized patients. Price (7), in 1970, presented strong evidence that the extensive use of antibiotics in a neurology ward resulted in an epidemic of *Klebsiella* infection which only subsided with the withdrawal of antibiotics from ward use and a more judicious use of antibiotics after the withdrawal period. Can one predict with certainty whether a particular therapy will cause serious overgrowth? Of course, the answer is "No." One can, however, be alert to the therapeutic regimen that has a potential to cause such trouble (Table 1). This table shows those therapeutic regimens which have been associated with gram-negative opportunistic infection (1, 4). The list is by no means exhaustive, and many other examples can be found. Those listed in this table are known to have been associated with gram-negative infections at this institution or they have been cited repeatedly in literature. The common causes for alteration in flora according to these sources are: irradiation, antimetabolites, antibiotics, corticosteroids, immunosuppressants and dermal preparations.

The combined findings leave little doubt that the physician must appreciate the microbial ecology of the human and treat it as much as possible with an agent that will not drastically alter the normal microbial flora. In other words, the attending physician should be an ecologist concerned with the very personalized microflora of his patient. This is not a very radical statement since the medical profession

TABLE 2

TYPES OF PROCEDURES ASSOCIATED WITH EXOGENOUS MICROBIAL INFECTIONS

Intravenous infusion	Respirators
Dialysis	Incubators
Parental feeding	Suturing
Catheterization of blood vessel	Punch biopsies
Catheterization of urinary tract	Bone marrow aspiration

TABLE 3

FREQUENCY OF ISOLATION FROM 100 SKIN CULTURES OF VARIOUS SPECIES OF BACTERIA

Staphylococcus epidermidis	86 (85%)
Bacillus species	42 (42%)
Streptococci	29 (29%)
Enterococci	16 (15%)
Diphtheroids	13 (13%)
Herellea	4 (4%)
Escherichia	2 (2%)
Staphylococcus aureus	2 (2%)
No growth	4 (4%)

Herellea vaginicola isolated from skin of two patients on hospital day 1 for one patient and hospital days 26 and 29 for another.

Escherichia isolated from skin of two patients on hospital days 4 and 11, respectively.

(All gram-bacilli were isolated from upper extremities.)

has been concerned with the total environment of the patient for centuries.

Introduction of Exogenous Microbes. I have spent the time so far discussing only endogenous flora. What about exogenous flora or nosocomial infection? What role do these agents play in present day disease? One aspect of this problem is the number of new medical techniques and devices that require direct communication into the internal organs. Not only have a large number of new devices been added in the last 20 years, but also there has been a vast increase in the use of older procedures such as venipunctures. These procedures, unless careful aseptic technique is employed, serve as vehicles for the entry of exogenous organisms into the internal organs. In Table 2, you will find the techniques that have been repeatedly associated with nosocomial infections (1, 4). As in the last table, the list is not exhaustive and there are other procedures which have been associated with this condition. The role that these manipulations may play in contributing to nosocomial infections cannot be overemphasized. Let me give you two examples of how these procedures may allow the entry of organisms into the vascular system. The first example has to do with the effect of venipuncture on 100 hospitalized patients. This work was done in conjunction with Dr. Richard Duma of the Division of Infectious Disease. We have listed in Table 3, the types of organisms recovered from the antecubital area of the right arm, prior to cleaning the site for venipuncture. *Staph epidermidis* and *Bacillus species* were most commonly recovered, followed surprisingly by *Enteric streptococci, Herellea* and *Escherichia*. The purpose of the venipuncture was for obtaining blood cultures. This allowed, therefore, the correlation of the organisms recovered from blood cultures with those found on the skin surface. Table 4 shows the results of this correlation. Ten individuals had positive blood cultures; in eight of these, there was no

TABLE 4

RELATIONSHIP OF POSITIVE BLOOD CULTURES TO CULTURES OF OVERLYING SKIN IN 10 PATIENTS

Culture number	Blood culture (organisms)	Skin culture (organisms)	Site of cultures	Day of hospitalization
1	*Pneumococcus*	*S. epid.*, Diphth.	L. & R. A.	1
2	*Pneumococcus*	*S. epid.*, Bacillus sp., α strep. (Ent.)	L. & R. A.	1
3	*Streptococcus*	*S. epid.*, Bacillus sp. (α strep. cultured from skin of opposite arm)	L. A.	1
4	*Anerobic diphtheroids*	*S. epid.* & Bacillus sp.	L. A.	3
5	*Bacillus* species	Bacillus sp.	L. & R. A.	10
6	*Micrococcus* species	*S. aur.*	R. A.	3
7	*Herellea vaginicola*	*H. vaginicola*, *S.* epid., α strep. (Ent.), & Bacillus sp.	L. & R. A.	26
8	*Cryptococcus*	*S. epid.*	L. A.	1
9	*Escherichia* species & *Cryptococcus*	*S. epid.*, & Bacillus sp.	L. & R. H.	16
10	*Aspergillus*	*S. epid.*, & Bacillus sp.	R. A.	1

L—Left, R—Right, A—Arm, H—Hand

TABLE 5

COMPARISON OF COMMON ORGANISMS ISOLATED FROM BLOOD CULTURES 1969–1968

Genus	Number of Patients 1969	1968	% Change
Escherichia	84	70	+20
Aerobacter, *Klebsiella*, *Serratia*	102	109	−7
Proteus	30	27	+10
Pseudomonas	38	40	−5
Staph aureus	34	37	−8
Pneumococcus	58	47	+23
Number of cultures taken	11,380	11,117	+1

relationship between the organisms recovered from blood cultures and those isolated from the skin. But in two patients, numbers three and seven in Table 4, the same organisms found on the skin were also present in the blood cultures, indicating that the needle at venipuncture may have been contaminated at the site of entry and may have pushed organisms into the vessel. The data certainly indicate that this occurs infrequently, but the mere fact that it can occur at all should sensitize medical personnel to the need for good aseptic technique.

The second example that we will use to demonstrate how nosocomial infection can be spread will involve the use of i.v. infusion (Table 5). This table shows the percent increase of common bacteremia organisms for the years 1969–1968. You will note that when the two periods are compared, there has been a small increase in many of the organisms, but well within the percent increase that was discussed previously. Table 6 compares the first six-month period of 1969 and the first six-month period of 1970 for the same organisms shown in the previous table. You will note that there is a tremendous increase in the percentage of recovery, particularly with the gram-negative organisms. During this 1970 period, Dr. Richard Duma and Dr. Jack Warner (3) did an extensive in-use study on the i.v. systems throughout the hospital. They sampled

TABLE 6

COMPARISON OF MICROORGANISMS CULTURED FROM BLOOD ACCORDING TO NUMBER OF PATIENTS AND CULTURES JANUARY TO JUNE 1969–1970

Genus	Number of Patients 1969	1970	% Change
Staphylococcus aureus	14	33	+135
Herellea	5	11	+120
Aerobacter	10	22	+120
Candida	12	23	+91
Klebsiella	31	58	+87
Proteus	14	21	+50
Escherichia	42	55	+30
Alpha *Streptococcus*	27	32	+19
Serratia	10	10	0
Pneumococcus	33	28	−16
Number of cultures	5,584	6,688	+19

TABLE 7

BACTERIOLOGIC STUDIES ON 24 VOLUME CONTROL INTRAVENOUS INFUSION SETS IN 24 PATIENTS

Set Number	Type of Set	Culture Results: Inside of Cylinder	Inside of Bottle Cap
3A	25		*Corynebacterium*
6A	25		*Mima*
7	25	*Mima*	*Mima*
9	50		*Klebsiella*
10	50		*S. epidermidis*
12	50		*Pseudomonas* *E. coli*
14	50	*Serratia*	*Serratia*
16	75	*Enterobacter*	*Enterobacter* *Serratia* *E. coli*
17	25		*Flavobacterium* *E. coli*
20	50		*Corynebacterium*
23	50		*Flavobacterium*
24	25	*E. epidermidis*	
25	50	*Flavobacterium*	*Bacillus species*
26	50	Hemolytic *Streptococcus*	
31	50	*Candida species*	*Candida species*
37	50		*Mima*
38	25	*Corynebacterium*	
41	50		*Flavobacterium* *Candida species*
48	25		*Enterobacter*
55	25		*S. epidermidis*
58	25	*Corynebacterium*	
60	50		*Bacillus species*
61	50		*S. epidermidis*
62	50		*Flavobacterium*

Blank space represents sterile culture.

the internal reservoir of the i.v. sets while they were being used on the wards (Table 7). The results of this survey demonstrated that many of the same organisms which increased in 1970 were to be found as contaminant in the i.v. fluid itself. A correction of this situation brought a decline the next year in the *Klebsiella, Enterobacter,* and *Serratia* organisms isolated from bacteremia.

Studies such as these are found throughout the literature and are associated with many procedures. We all tend to neglect these results, however, thinking our ward and office procedures are above these dreaded possibilities; unfortunately, unless constant in-use monitoring of these procedures is done, we just do not know that they are.

Value of Microbial Monitoring. With this overview as background, let me restate my original questions and answer them as best I can. What patients need to be monitored bacteriologically? Any debilitated individual under therapy or subjected to the procedures described here should be carefully followed by clinical and laboratory methods for the possibility of an emerging opportunistic or nosocomial infection. What areas need to be monitored? All of the patient's environment conducive to microbial growth should be tested to insure that proper housekeeping procedures are being carried out but more importantly, equipment and infusions which go directly into the internal organs should be tested for sterility *while in use*. How often should monitoring be done? As often as necessary to convince the skeptical physician that good aseptic technique in microbial clean environment is available for his patient. The patient himself should be monitored also to insure that endogenous organisms are not adversely affecting him. What is the practical value of the results of such monitoring? One direct benefit to the patient is that such testing can alert the physician that a particular therapeutic regimen may be altering the microbial flora. There are also indirect benefits as problems are identified and solved by good in-use monitoring at the patient levels. There will be a decrease in the number of organisms with which the patient comes into contact. This should allow for a decline in the number of infections. Lastly, but perhaps most importantly, microbial monitoring of numerous patients allows the acquisition of valuable epidemiological data which contribute to our understanding of the dynamic ever-changing world of biology and medicine.

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Newer Techniques in Clinical Cytology: Aspiration Biopsy and Brushing Cytology*

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Since its introduction some twenty years ago the Papanicolaou technique for cervical-vaginal cytology has had a tremendous impact on the detection of precancerous lesions and *in situ* cancer in the female genital tract. It has forced the clinician and pathologist to study the "early lesions" of cancer, particularly in the female genital tract and has brought about a better understanding of their biology. Cytology has over the years been extended to other areas, particularly the respiratory tract. Two recent areas of emphasis are the thin-needle aspiration biopsy and direct brushing of mucosal surfaces through the various fiber optic scopes now available, particularly for the respiratory and gastrointestinal tract.

Thin-Needle Aspiration Biopsy. This is an old technique having been employed at Memorial Center for Cancer and Allied Diseases for over thirty years. It has never become popular in the United States, but in the last ten or fifteen years has become widely used in Europe, particularly in the Scandinavian countries. At the Karolinska Institute, approximately 12,000 such aspirations are done per year, mainly for mass lesions of the breast, prostate, lung, thyroid and lymph nodes. The technique renders a rapid diagnosis, particularly on benign and malignant tumors, and is usually done on an outpatient basis. In the setting described, a saving of time and expense to the patient is obvious.

The technique of thin-needle aspiration is done with a narrow gauge 22 or 23 needle of various lengths, percutaneously, without anesthesia. Because of the nature of the needle it is nontraumatic and less painful than a venipuncture. Important is the use of a syringe such as the Franzen type, which may be operated with one hand (11). The other hand is used to fix the tumor mass and thereby control the needle point. The type of syringe holder that will employ disposable 20 ml plastic syringes is illustrated in figure 1.[1]

The needle attached to the syringe is introduced into the mass to be aspirated without the application of vacuum. The vacuum is applied and the needle is moved back and forth in the mass several times. The operator should watch the barrel of the syringe and avoid getting material into the barrel. It is not necessary to obtain a core of tissue, but if tissue fragments are obtained that is an added bonus. The idea is to obtain cells and to keep the material within the needle.

If a cyst is aspirated this may be evacuated by filling the syringe. If the fluid is clear it is unrewarding to process it either as a smear from the cell button of the centrifuge specimen or by one of the filter techniques, either nucleopore or millipore. If the fluid is cloudy or hemorrhagic it is advisable to process it as described, either by filter techniques or smears from the cell button. Cell blocks are not useful but any fragments of tissue may be processed as a biopsy.

Once the needle has been moved back and forth in the mass several times the pressure in the syringe is allowed to equalize *before* the needle is removed from the mass. This is extremely important.

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[1] The Cameco Syringe Pistol® purchased from Precision Dynamics Corporation, 3031 Thornton Ave., Burbank, California 91504.

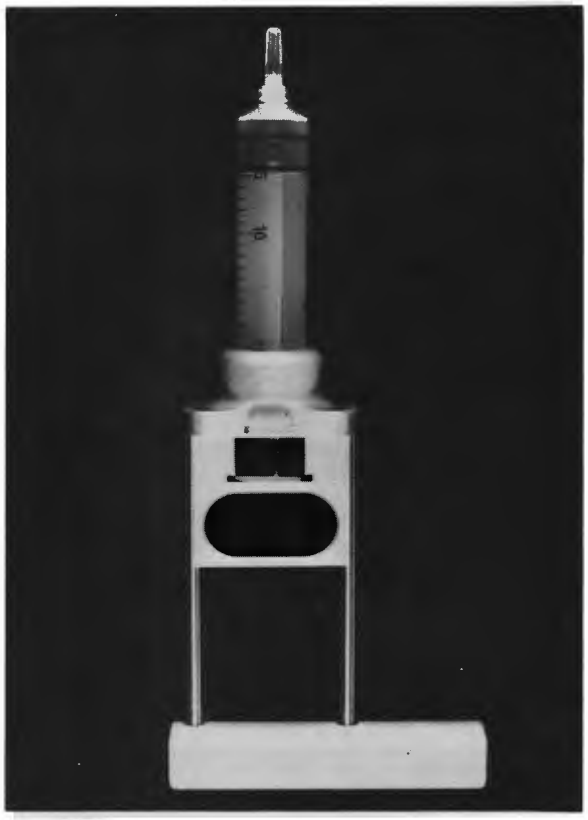

Fig. 1—Syringe pistol for thin-needle aspiration biopsy.

It prevents a chance to deposit the cells along the needle tract. The syringe is then withdrawn and the needle is held over plain glass slides while the syringe is removed. Air is then introduced into the syringe, the needle reattached and a small drop or two of material is expressed onto the slides. There is usually enough material to make two or three slides.

Smears are prepared much in the manner of making a blood smear, but the entire slide need not be covered and the material may be confined in a smaller area. Smears to be stained by the Papanicolaou technique should be immediately fixed *while still wet* in methyl alcohol. Smears for May-Grunwald Giemsa stain or Wrights-Giemsa or hematoxylin and eosin are allowed to air dry. In addition, on air dried smears we have used a metachrome B stain with success. This is the same stain that we employ on frozen section material. Smears prepared in this way may be made permanent by simply washing the metachrome B stained slide with water, allowing this to evaporate and then coverslipping with Permount®.

European investigators have favored the hematology stains while pathologists in the United States have favored the hematoxylin and eosin or Papanicolaou stain. I have found the metachrome B stain combined with the Papanicolaou stain to be extremely useful.

In the aspiration biopsy technique using the thin-needle, the clinical evaluation is extremely important. Since the material is small in amount and of a cellular type rather than a tissue fragment, clinical evaluation of the mass and its orientation becomes of utmost importance. The greatest success with this technique has been when the pathologist who is reading the aspiration has also taken the material. He has palpated the tumor mass and has a first hand knowledge of the clinical situation. If clinicians wish to adopt this technique and send their material to be read by a pathologist, then they *must* supply extremely detailed clinical information to avoid errors, especially of the false positive type. The technique has been used particularly to aspirate lymph nodes with metastatic tumors, for breast tumors, thyroid lesions, salivary gland tumors, lung tumors through the transthoracic route, prostate and kidney tumors and soft part sarcomas. There has been limited use in bone tumors. Prostate aspirations are done transrectally with a metal guide which fits over the examining finger and is so positioned that the tip of the needle will pass into the mass palpated by the tip of the examining finger.

Results. Results from several selected sites in a large series are summarized in Table 1. One of the most useful areas has been cervical lymph nodes, particularly for metastatic tumors. It can be seen that in a large series of cases there were no false positive reports and only a small percentage of false negative reports. Primary sites of these metastatic tumors were equally divided between those above the clavicle and those below (11). In 35% of these cases the aspiration was the first indication of cancer (11). Zajicek, *et al.* (27), obtained similar results in 1,200 consecutive aspirations of cervical nodes.

Two difficult areas are congenital cysts of the neck and carotid body tumors. Engzell and Zajicek (8) reported the aspiration of 100 consecutive congenital cysts of the neck and compared these with 100 aspirations of cases of metastatic squamous cell carcinoma. They made a definite diagnosis from the aspiration in 83% of the cysts and in 95% of the carcinomas. The diagnostic accuracy varied with the

TABLE 1

THIN-NEEDLE ASPIRATION BIOPSY RESULTS IN SELECTED SITES

	No. of Cases	Correct Diagnoses	False Positive	False Negative
Head and Neck				
Cervical Lymph Nodes (11)	257	93.7%	0	6.3%
Salivary Gland Tumors (4, 5, 6, 7, 26)	368			
Benign Mixed Tumor	(215)	93.0%	4.0%	3.0%
Malignant Mixed Tumor	(7)	100.0%	0	0
Adenoid Cystic Carcinoma	(45)	66.0%	0	33.0%
Acinic Cell Carcinoma	(34)	65.0%	0	35.0%
Warthin's Tumor	(45)	80.0%	0	20.0%
Oncocytoma	(4)	100.0%	0	0
Mucoepidermoid Carcinoma	(18)	62.0%	0	38.0%
Thyroid (3)	258			
Carcinoma	(177)	94.0%	0	6.0%
Cystic Nodules	(61)	98.0%	0	2.0%
Struma	(20)	100.0%	0	0
Breast (15)	1429			
Benign Nontumor	(305)	88.0%	0.2%	12.0%
Benign Tumor	(251)	89.0%	0*	11.0%
Carcinoma	(873)	89.2%	0	10.8%
Prostate (12)				
Carcinoma	101	93.6%	2.4%	4.0%
Lung (2)	125			
Carcinoma	(101)	89.0%	0	11.0%
Benign conditions	(24)	40.0%	5.0%@	55.0%

* 5.3% of cases suspected as carcinoma were all histologically proven fibroadenomas
@ one case

location of the cyst, being only 55% in medial cysts and 92% in lateral cysts. They found benign looking squamous cells in 6% of the smears from metastatic carcinoma.

Engzell, *et al.* (10), have reported their experience with 13 cases of aspiration of carotid body tumors. In one case, there were no cells and in two, metastasis was suspected from an unknown primary. Three cases were diagnosed as neurofibroma or neurofibrosarcoma and in seven cases a correct diagnosis of carotid body tumor was made. The authors indicate some caution in aspirating this tumor when it is suspected, as one of their cases became hemoplegic following the procedure.

Aspiration of salivary gland tumors has generally been quite successful by this technique as indicated in Table 1 (4, 5, 6, 7). Adenoid cystic carcinoma, acinic cell carcinoma and mucoepidermoid carcinoma carry fairly high false negative rates (26). Follow-up on benign mixed tumors indicates no increase in recurrence rate due to the aspiration technique. In seven cases following resection, the needle tract was serially sectioned and no tumor cells were found (5). Important in the aspiration smears in the diagnosis of adenoid cystic carcinoma are mucinous globules. These were present in 22 of 45 patients (6). While the false negative rate for adenoid cystic carcinoma over the 24-year period of the original study is high, it has improved over the last 11 years, being only one out of 23 aspirations or 4% (6). This is true also for 34 cases of acinic cell carcinoma in which aspirations of the last 11 years have resulted in a false negative rate of two in 16 or 12% (7).

Other salivary gland carcinomas are too infrequent to document the reliability of the technique. The most difficult has been mucoepidermoid tumors of low grade malignancy. In the report by Zajicek and Eneroth (26), 18 cases of mucoepidermoid carcinoma yielded only cystic fluid, cell detritus and lymphocytes. Poorly differentiated mucoepidermoid carcinoma as well as the unclassified adenocarcinomas were easily identified by the aspiration technique.

Clinicians have been reluctant to aspirate thyroid lesions, but this has been done with considerable success at the Karolinska Institute. Results indicate

no false positives in carcinoma, cystic nodules or struma, and a small percentage of false negatives in carcinoma and cystic nodules (3).

The major area of interest in the United States has been needle aspiration of the breast. Franzen and Zajicek (15) have attained a high degree of accuracy with this technique, not only with carcinoma, but with the benign disease. A conservative attitude is required in dealing with breast lesions since radiation or surgical treatment is frequently done on the basis of aspiration diagnosis. False negative aspirations are generally on the basis of tumors less than 1 cm, very fibrotic, poorly cellular carcinomas or failure to aspirate the residual mass after evacuating a cyst. With increased experience in the recent series, the false negative rate has been cut to 5%.

It can also be seen from Table 1 that the aspiration technique is highly accurate in prostate carcinoma. Since the approach is transrectal, there have been a few cases, less than 2%, of gram negative septicemia attributable to the aspiration (12).

The lung has been the most recent area where the thin-needle technique has been used. Lesions of the lung yield grudgingly to diagnostic techniques of exfoliative cytology or conventional bronchoscopy and biopsy. Prior to the thin-needle technique, a large bore-type needle was used with a frequent tendency to hemorrhage, pneumothorax or air embolism. Dahlgren (2) has used the thin-needle technique under fluoroscopic control with only topical skin anesthesia. In 101 malignant tumor cases, there have been no false positives and in the 24 benign conditions, only one false positive. In the total series of 3,000 thin-needle aspirations, there have been no complications (2). Sputum cytology in the same material has yielded only a 40% diagnostic rate. Nasiell (22) correctly diagnosed from 83 lung cancer cases, 72% by the thin-needle technique. About half of the false negative cases had inadequate material. The results were better than conventional sputum cytology. The technique is thought by that author to be particularly good for peripheral and small tumors or for clinically inoperable cases. The author reported no complications. Sanders, *et al.* (25), also reported a large series of 164 patients with an accuracy of 84% in malignant neoplasms. The authors did not find the technique accurate in nonmalignant localized parenchymal disease and not particularly good in chest wall or diffuse parenchymal disease. They did find it helpful in the mediastinal mass lesions, particularly pericardial, bronchogenic or thymic cysts. They listed the contraindications as: 1) hemorrhagic diathesis, 2) a patient on anticoagulants, 3) severe pulmonary hypertension, 4) pulmonary hydatid cyst, 5) uncontrolled cough, 6) advanced emphysema, 7) patient with suspected arteriovenous malformation.

These authors used the large bore needle (2.1-3.0 ml) and had a 30% complication rate of small pneumothoraces. This needle is twice as large as that recommended in the thin-needle technique from the European workers (25). Most of these pneumothoraces were asymptomatic. Five percent in this series, however, required tube drainage. In the follow-up of this series, there were no verified cases of tumors spread by the needle technique employed.

Janower and Land (20) also used the thicker needle of the Vin-Silverman type. As expected, they had a 10-15% minimal complication rate of hemoptysis and pneumothorax. These larger needles cannot be recommended any longer, therefore, in this technique.

The most frequent criticism leveled at the thin-needle technique is the implantation of tumor cells along the needle tract. Berg and Robbins (1), in a 15-year follow-up of 370 cases of breast cancer that were aspirated and 370 matched controls, found that the long term prognosis slightly favored the aspirated cases. This would seem to indicate no untoward effects from the thin-needle aspiration technique. Engzell, *et al.* (9), have also studied aspiration experimentally in the popliteal lymph node in rabbits seeded with the Vx2 carcinoma cells. The efferent lymphatics and efferent vein were cannulated and material was collected during the aspiration with massage of the lymph node. In one case out of 16, tumor cells were found in the blood and lymph after massage and thin-needle aspiration.

The authors (9) also reported the clinical follow-up of 124 cases of benign mixed tumors after ten years and found three local recurrences at 4, 5 and 9 years. No tumor was found in the needle tract on serial sectioning. In addition, they studied 242 patients with prostate carcinoma who had been followed for five years after thin-needle aspiration. One patient of this group developed transrectal growth three years after needle aspiration. It would seem from these studies that this technique is safe from the criticism of implantation of tumor cells in the needle tract.

Brushing Cytology. The second area of cyto-

logic investigation has been brushing cytology using the various fiber optic scopes, particularly in the respiratory and gastrointestinal tract. In the respiratory tract, this is the outgrowth of the selective catheterization technique of Nordenstrom and Carlens (23) and the disposable bronchial brush designed by Fennessey (13, 14).

We prefer the bronchial brushing under direct vision using the fiber optic bronchoscope. This allows visualization of any lesion in the major and most of the secondary bronchi as well as the respiratory excursions. Any lesion seen may be sampled directly and the brush may be pushed into the parenchyma of most of the bronchial segments for peripheral sampling. Our experience to date, involves 39 cases with cytologic abnormalities, including 16 carcinomas mostly of the squamous type and 18 cases with various atypias of reserve cell hyperplasia, squamous metaplasia or bronchial hyperplasia. We have had four unusual cases as follows: cytomegalic inclusion disease in a transplantation case, pneumocystis carinii in a patient with myeloma, adenovirus infection and the identification of ferruginous bodies along with atypical alveolar pneumonocytes.

We have had the best success in smear preparation by having a technologist available in the room where the brushing is being done to make the smears. Two or more smears are made using totally frosted Dakins slides[2] which have been premoistened with ethyl alcohol. These are immediately fixed in 95% ethyl alcohol. Just because the slides are damp, cellular material is not lost and drying artifacts which occur very rapidly with bronchial epithelial cells are avoided. This is extremely important. Failure to follow this simple technique will result in poor quality smears and will discourage the physicians from using this very valuable technique. Following the making of the smears, we shake the brush in a small amount, 10 ml, or physiologic saline or Polysal®[3]; from this material filters are prepared, either nucleopore or millipore. The material is all stained by the Papanicolaou technique. If fragments of tissue are obtained, as they occasionally are, they may be processed as a biopsy.

The results from several series are reported in Table 2 (16, 17, 18). A false positive rate has been obtained in these series of less than 1% or one case. The best results are reported by Hattori *et al.* (18), who attained an accuracy of 91.6% in 12 cases of lung cancer. In this series there were no false positive cases. Even in the community hospital where there might not be special interest in this technique, Funkhouser and Meininger (17) reported 70% accuracy. Combining this with bronchial and sputum exfoliative cytology, an overall accuracy of 83.8% was obtained in the diagnosis of lung cancer.

Contraindications to the technique as outlined by Janower and Land (20) are patients who are in poor general condition or who cannot tolerate any bronchial irritation. The advantages to the technique, in our opinion, are that the quality of the material is greatly improved from the cytologic point of view. Secondly, a more specific diagnosis can be rendered, particularly in inflammatory and infectious conditions. Thirdly, secondary segmental bronchi and peripheral lesions can be reached and bronchi can be selectively sampled, particularly in "early" lung cancer cases.

The technique is also very valuable in lesions of the esophagus and stomach. The Japanese have pioneered the technique through their use of fiber optics. In their hands, with the early diagnosis of gastric cancer by this method, they have reported a survival in 365 patients of 92.5% for five years (19). While this technique has not generally been used in the United States, the results reported by Prolla *et al.* (24), summarized in Table 2, are quite impressive. The false positive rate is very low and is better than conventional lavage cytology. The false negative rate is much lower than the conventional lavage cytology. The advantages are the simultaneous brushing with endoscopy. The cytology is selected from the lesion seen. The cytologic procedure takes only a short time in contrast to the collection, preservation and centrifugation for conventional lavage cytology. The preservation and cellularity of the material is much better and there are no complications (21). Our own experience with this technique in a limited series to date would confirm all of these findings. Particularly to be stressed is that the quality of the material cytologically is much improved.

Summary. Reviewed in this paper are two cytologic techniques, thin-needle aspiration and brushing cytology, which have added immeasurably to the diagnosis of particularly malignant disease in a variety of areas. The advantages of the techniques are that they bring the cytology closer to the source of

[2] Trident Microscope Slides-Rectangular Dakin, Aloe Scientific, St. Louis, Missouri 63103.

[3] Polysal® Balanced Electrolyte Injectable. Cutter Laboratories Inc., Berkeley, California 94710.

TABLE 2

BRUSHING CYTOLOGY
RESULTS IN SELECTED SITES

	No. of Cases	Correct Diagnoses	False Positive	False Negative
Bronchi and Lung* (16, 17, 18)	114	75.0%	<1.0%@	25.0%†
Stomach and Esophagus (24)	269			
Carcinoma and Lymphoma	(54)	90.7%	0	9.3%
Benign Conditions	(215)	98.6%	1.4%	0

* Combination of three series
† No false negatives among malignant tumors
@ One case

the lesion, particularly in the brushing cytology; the quality of the material is much improved over conventional cytologic techniques and the complications to the patient and the expense of the procedures are minimal.

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Laboratory Aspects of Venereal Disease*

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Introducing the laboratory aspects of venereal diseases, I would like to define the criteria according to which certain infectious diseases are considered to be "sexually transmitted." The expression "sexually transmitted disorder" is less weighted with moral overtones than the term "venereal" and may therefore be more acceptable to both medical practitioners and the general public.

Sexually transmitted diseases are best considered as a group of lower genitourinary inflammations related to sexuality, rather than in the narrower sense of two or three disorders associated mainly with sexual promiscuity. Not all lower genitourinary tract infections are characterized by a discharge, but those which are may be due to a variety of causes. When these infections are caused by a microorganism which exists primarily in the genital epithelium, that infection is rarely transmitted except by sexual intercourse. Intercourse must, therefore, have taken place with an individual already infected who had contracted the disease in the same way. On the other hand, when the responsible microorganism might possibly exist in other sites, although usually sexually transmitted, coitus with an infected individual is not essential. Both conditions, however, are strictly "venereal."

Although many different microorganisms or parasites may be sexually transmitted, only those commonly transferred in this manner can be said to be venereal. A list of microbial agents associated with venereal disease is shown in Table 1. Only the diseases caused by those agents which appear in italics are reportable in this country, according to the U. S. Public Health Service. There is, however, a difference from country to country as to which venereal diseases are notifiable. A few of these diseases are endemic in almost every part of the world. Some of them, notably syphilis, have become sporadic in certain countries.

The disruption of the natural clinical and immunological responses of the infected individuals by the liberal use of antibiotics places a greater responsibility on the interpretation of laboratory results and on the judicious performance of serological tests in the absence of clinical signs. As far as we know, no treponemal resistance has yet arisen to the antibiotics usually recommended. Nevertheless, higher doses of antibiotics are considered more essential today than two decades ago.

Ulcerative and vesiculopapular lesions of the genitalia are commonly secondary to infective causes within the lower genitourinary tract, or are due to such lesions as herpes simplex, thrush or scabies. Chancroid and the conditions discussed along with it are becoming less common; their importance lies in the necessity to exclude syphilis. The cause of urethritis, vaginitis or cervicitis cannot always be determined. Consideration must be given to such factors as trauma, irritation, allergy and primary or secondary infection. It is often possible to make an etiological diagnosis, or at least an assessment of the probable cause, after both partners have been seen and examined. The incidence of these conditions shows no sign of diminishing. The inflammation resulting from sexually transmitted infection varies greatly in severity and extent. The potentially most damaging disease is syphilis, which unless treated, is frequently a life-long disease and may also infect the fetus *in utero*. Other infections presenting as sores which include chancroid, granuloma inguinale and lymphogranuloma venereum result as a rule in few complications. Any infectious discharge from the genital tract may proceed to a variety of complica-

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TABLE 1

MICROBIAL AGENTS OF VENEREAL DISEASE

Protozoon:	*Trichomona vaginalis*
Yeast-like:	*Candida albicans*
Mycoplasma:	*M. hominis*, "*T*" *strain*
Bacteria:	*Treponema pallidum*
	Neisseria gonorrhoeae
	Haemophilus ducreyi
	Donovania granulomatis
Bedsonia:	*Lymphogranuloma venereum*
	Trachoma-inclusion conjuctivitis
Virus:	*Herpes hominis*, type 2

tions, local or general, mild or severe. About 5% of males with gonorrhea seen in the venereal disease clinics are asymptomatic. Eighty percent of females with gonorrhea, however, are asymptomatic and remain in the population as an infectious reservoir. Gonorrhea in the male is predominantly symptomatic, motivating most patients to seek medical attention (5). The principal disorders which occur are inflammations of the pelvic organs and tissues and their sequelae, or rheumatism and arthritis.

If asked about the type of microorganisms associated with venereal disease, probably many would refer immediately to syphilis and gonorrhea and state that chancroid and lymphogranuloma venereum are not commonly found in medical practice. As a matter of fact, even recent textbooks on the subject of microorganisms associated with venereal disease refer to agents such as *Chlamydiae* (trachoma-inclusion conjunctivitis (TRIC) agents and *lymphogranuloma venereum,* mycoplasma, *Candida albicans* and *Trichomonas vaginalis,* but fail to add herpesvirus to the list. I wish to emphasize, however, that TRIC agents and *lymphogranuloma venereum* are not viruses and are infrequent etiological agents of venereal disease. On the other hand, during the last several years, herpes simplex (also called *herpes hominis*), type 2, has been shown here and elsewhere to be sexually transmitted and of high incidence in the general population. More importantly, it has been incriminated in uterine cervical carcinoma in connection with coitus, showing an increased frequency where there is an earlier onset of sexual intercourse, a large number of sex partners and when the individual remarries in later life.

Because of the limited amount of time available for a comprehensive discussion on the laboratory aspects of all venereal diseases, I will comment only briefly on those infections of minor clinical importance or on those which occur only rarely in this country. The sequence in which these will be presented, however, does not necessarily correlate with their frequency of occurrence. *Trichomonas vaginalis* is a common cosmopolitan parasite of both males and females. Infection rates vary greatly but may be high in some areas especially where female hygiene is poor. Coitus is the common mode of transmission, but contaminated towels, douche equipment, examination instruments and other objects may be responsible for some new infections. Infants may be infected at birth. Most infections in both sexes are asymptomatic or cause minor symptoms. Control of this parasite requires detection and treatment of the infected male sexual partner at the same time that the infected female is treated. Laboratory diagnosis consists of microscopic examination of vaginal or urethral secretions or discharge for characteristic motile trichomonads. Dried smears may be stained with hematoxylin or one of the Romanowsky stains for later study. Culture of vaginal or urethral discharge, of prostatic secretion or of a semen specimen may be indicated when direct examination is negative.

Candida albicans is probably one of the most common causes of vulval and vaginal symptoms, flourishing particularly in pregnancy, following antibiotic therapy and when glycosuria is present. A low-grade urethritis and balanoposthitis in men not infrequently accompanies a genital candida infection in their consorts. Fresh wet preparations of the vaginal fluid mixed with 10% potassium hydroxide or Gram-stained vaginal smears may lead to the detection of yeasts or blastospores or, less commonly, filaments or hyphae can be seen.

Mycoplasma hominis and T strains are yet to be conclusively demonstrated as the primary etiological agents of genitourinary infections. Some mycoplasmas are inhabitants of the normal genitourinary tract, especially in females. Moderately high serum antibody titers to *Mycoplasma hominis* and T strains (tiny colonies, requiring 10% urea for growth), however, can accompany their presence and they have been cultured occasionally from focal genital abscesses. Special enriched media are required for growth. These organisms are inhibited by tetracycline but not by penicillin in clinical dosages. This is consistent with the clinical response to therapy of a significant proportion of patients with a nonbacterial inflammation.

Chancroid, granuloma inguinale and *lymphogranuloma venereum* are sexually transmitted in a large proportion of cases. They are usually described together since they are usually associated with genital "sores." They are relatively rare and are most prevalent in warmer climates or seaboard cities.

Haemophilus ducreyi, the cause of chancroid, can be detected by smears or cultures from a suppurating ulcer which usually show a mixed bacterial flora, including Gram-negative rods in chains. Serologic tests are rarely done. The Ducrey skin test (*Haemophilus ducreyi* suspension) usually is positive within three-to-five weeks after infection. A positive skin test, however, cannot differentiate between old and recent infection.

Donovania granulomatis, the cause of granuloma inguinale, can be cultivated in complex bacteriologic media but this is rarely attempted in practice. Histologic demonstration of intracellular "Donovan bodies" in biopsied material most frequently supports the clinical diagnosis. Serologic tests are not useful. *Lymphogranuloma venereum* can be recovered by inoculating pus from suppurating lymph nodes into embryonated eggs but the procedure is not practical. Serologic tests, usually complement-fixation, can demonstrate a fourfold antibody titer rise between paired sera collected two weeks apart. The Frei skin test is frequently used to support the clinical diagnosis and it usually becomes positive two-to-three weeks after infection and remains positive for life, but recently the test has been negative in a significant proportion of proved infections. This skin test reflects reactivity to a group-specific antigen which is shared by all members of the group. Past infection with psittacosis or trachoma, therefore, may give rise to a positive Frei test as readily as past infection with *lymphogranuloma venereum.*

Inclusion conjunctivitis usually manifests itself as an acute conjunctivitis of the newborn, or as a venereal disease and eye infection of adults. Immunofluorescence of Giemsa-stained smears of scrapings from the eye, the cervix, or the male urethra may show typical crescent-shaped inclusion bodies in epithelial cells. Culture in irradiated cells may permit isolation of chlamydiae.

From 1947 to 1955, one of the most dramatic and precipitous declines in the history of our country in the reported morbidity of a chronic, social, communicable disease was recorded. This disease was syphilis. Since 1955, a pronounced upward trend has been observed for syphilis, occupying together with gonorrhea a very prominent place among the ten chief causes of morbidity as illustrated in figure 1. There is also growing divergence between the curves representing the incidence of syphilis and of gonorrhea, with a greater rise in the latter. The majority of undiscovered syphilis in the past, today and for the foreseeable future is latent syphilis which presents no signs, symptoms nor findings in the patient other than those demonstrated by appropriate serologic testing. The value of a serological test depends on its sensitivity (ability to react in the presence of syphilis), specificity (inability to react in the absence of syphilis), and reproducibility. The types of serologic tests for syphilis are listed in Table 2. The fluorescent treponemal antibody-absorbed (FTA-ABS) is the most sensitive test available. It becomes reactive early in primary syphilis, and it remains positive almost always indefinitely even after apparently adequate therapy. Occasional false positive results have been reported, but rarely in more than one percent of cases. Venereal Disease Research Laboratory (VDRL), Kolmer complement-fixation (KCF), and Kolmer Reiter protein (KRP) or Reiter protein complement-fixation (RPCF) are less sensitive and less specific. In numerous conditions, both acute and chronic, the reagin or nontreponemal tests react nonspecifically in 30–40% of late or inadequately treated cases of syphilis; these tests are nonreactive. Our own clinicolaboratory studies at the Medical College of Virginia have confirmed these findings. Figure 2 illustrates graphically the humoral immune responses, as detected by the FTA-ABS and the VDRL tests, respectively. Observe that, unless one is dealing with a biological false positive result, a reactive VDRL is always

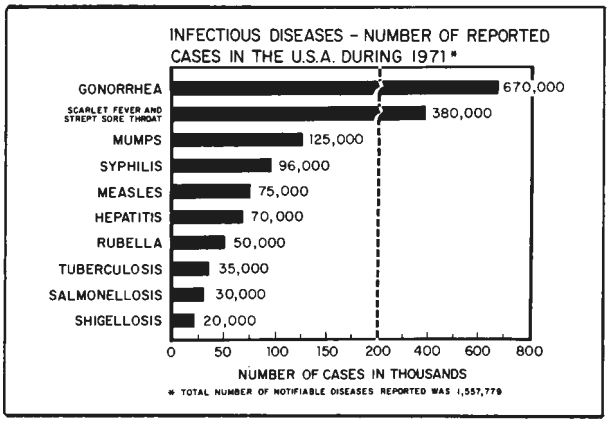

Fig. 1

TABLE 2

TYPES OF SEROLOGIC TESTS FOR SYPHILIS

I. NONTREPONEMAL (nonspecific)		II. TREPONEMAL (specific)	
Flocculation	Complement-Fixation	T. Pallidum	Reiter Strain
VDRL Rapid Reagin	Kolmer	FTA FTA-ABS TPI	KRP or RPCF

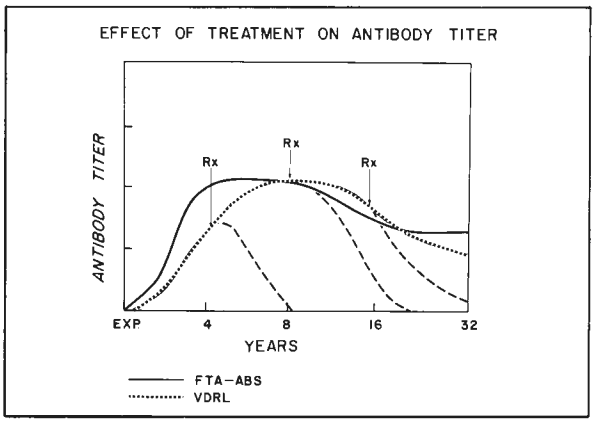

Fig. 3

accompanied by a reactive FTA-ABS. A nonreactive VDRL together with a reactive FTA-ABS, however, may occur in early primary or late syphilis. No reagins can be found in about 40% of untreated patients with tertiary syphilis whose FTA-ABS antibodies are present for life.

Among the complications of late syphilis, neurosyphilis has been particularly difficult to diagnose, due to the lack of sensitivity of certain laboratory tests such as the VDRL and treponema pallidum immobilization (TPI), the obscurity of the clinical symptoms, the rising incidence of its atypical forms and the well-known occurrence of nontreponemal seronegative neurosyphilis. The FTA and FTA-ABS tests were first adapted for use with cerebrospinal fluid (CSF) at the Medical College of Virginia, demonstrating considerably higher specificity of the FTA for the CSF than for blood with a degree of sensitivity comparable to that of the blood FTA-ABS. For the sake of brevity, I would like to refer the reader to three major publications resulting from clinicolaboratory studies done in our institution (1, 2, 4). Recommendations for the use of these tests can be found there and in some more recent work presented at the American Society of Microbiology Annual Meeting held in Philadelphia in April 1972 (3). The effect of antibiotic therapy on antibody titer can be seen in figure 3. No posttherapeutic change can be observed for the FTA-ABS, but the VDRL reactivity gradually disappears after treatment during a period of time proportional to the length of active syphilis infection; that is, the longer the active infection, the longer the time required for serological reversion from reactive to nonreactive.

The number of reported cases of gonorrhea from 1950 to 1972 is shown in figure 4, which

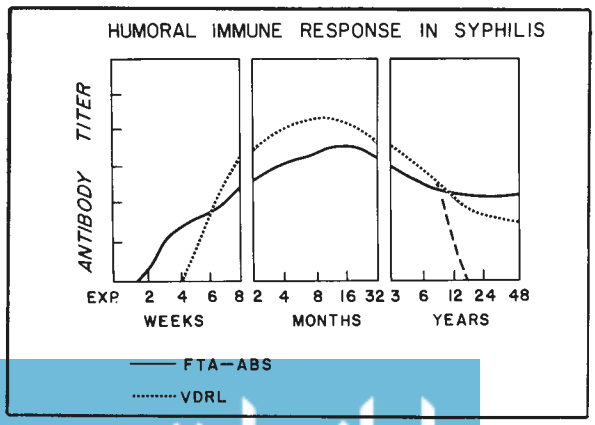

Fig. 2

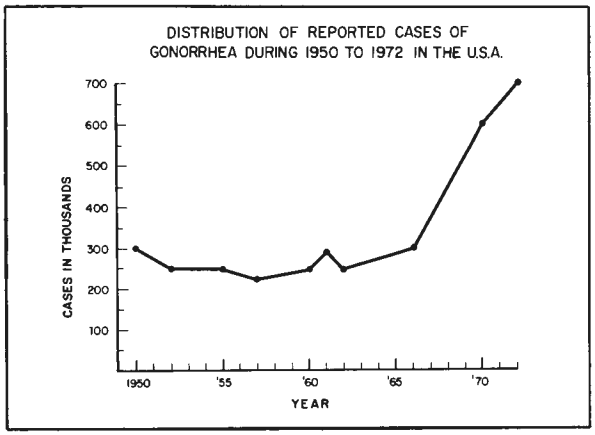

Fig. 4

indicates an upward trend in the incidence of gonorrhea in the United States, as in other parts of the world; thus, gonorrhea has become the leading reportable disease in this country. For example, an incidence of 137 cases of gonorrhea per 100,000 in 1957 had risen to more than 307 per 100,000 in 1971. Also, it is well known that the actual number of cases of gonorrhea is almost four times the total number reported to health authorities by all sources. The age-specific case rates by sex in the United States during 1971 are illustrated in figure 5. One of the major problems in the control of this disease is the existence of a large number of unidentified asymptomatic females estimated to range between 640,000 and 1,000,000 cases. The comparative sensitivity of the Thayer-Martin medium versus smear techniques using cervical specimens is 88% and 54%, respectively. A list of cultural diagnostic techniques is presented in Table 3. Presumptive and definitive tests are included. Although serological procedures are still under evaluation, complement-fixation tests are sometimes performed on individuals with negative cultures who are suspected of harboring chronic gonococcal infection. These tests, however, lack specificity and reliability. Antibodies detected by immunofluorescence are promising in gonococcal arthritis.

Intensified research is required on the biochemical and immunological aspects of venereal diseases. This research might soon lead us not only to a simplified serologic screening test for gonorrhea (the absence of which in a large part accounts for the rise in its incidence as compared to syphilis) but might also lead to an immunizing procedure against treponemal diseases such as syphilis. For years attempts at controlling gonorrhea were abandoned in favor of "treating it out" of existence with increasingly larger doses of penicillin. A decade ago the less sensitive strains requiring 0.1 unit of penicillin per ml for inhibition *in vitro* were extremely rare in clinical practice, but strains requiring 0.5 unit per ml are now common. Today many strains of gonorrhea are so resistant to penicillin that a dose so large as to approach the outer limits of safety is needed to effect a cure.

TABLE 3

Presumptively Positive Cultural Diagnosis
Typical Colonial Morphology
Oxidase-Positive Colonies
Gram-Negative Diplococci
Definitely Positive Cultural Diagnosis
Sugar Fermentation Reaction
Fluorescent Antibody Staining

The recent recognition of the relatively frequent sexual transmission of herpes simplex and its association with cervical carcinoma following the improvement in virological techniques suggests the important role of viral agents in venereology. The tests, specimens required and time needed for the laboratory diagnosis of herpes simplex infection are listed in Table 4. Most of these procedures are performed routinely in our laboratory, including those for the differentiation of types 1 (oral) and 2 (genital).

Other viruses may with time be shown to be sexually transmitted under certain circumstances, such as the hepatitis virus. Progress cannot be made against any of the venereal diseases without interdisciplinary cooperation between the venereologist and other medical specialists such as obstetricians, gynecologists and general practitioners. At the same time, cooperation of these individuals with public health workers, epidemiologists and researchers as well as with the teacher, health educator and social worker must be forthcoming not only at the patient and institutional level, but also on an interstate, national and international basis. The paramount importance of transfrontier cooperation is illustrated by a recent report of a California prostitute with secondary syphilis who kept a diary. It was found that among some 310 males who were involved as contacts, 168 (all long distance truck drivers) were traced. This threat of spreading disease extended over 34 states in this country and into Canada and Mexico. This epic of epidemiology was written by a

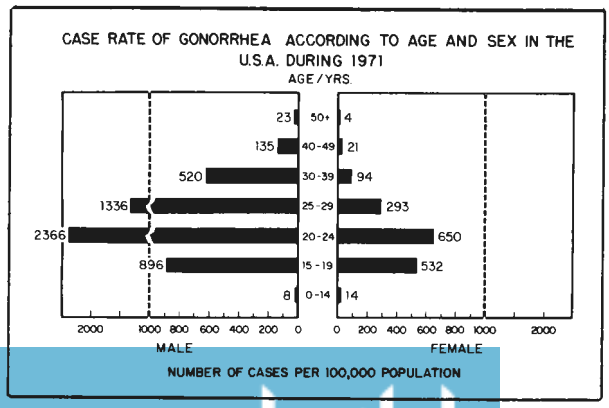

Fig. 5

TABLE 4

LABORATORY METHODS FOR HERPES SIMPLEX VIRUS (HSV)

Method	Specimens	Time Required
Light microscopy for multinucleated giant cells and inclusion bodies	Scrapings from base of lesions on glass slides	½–1 hr.
Immunofluorescence for antigen	Vesicle fluid, scrapings, biopsy of brain or other tissue	2 hrs.
Electronmicroscopy for viral particles	Vesicle fluid, scrapings, biopsy of brain, liver and other organs	1–2 hrs.
Tissue culture* for virus isolation	Vesicle fluid, scrapings, CSF, biopsy or post-mortem tissues	24–96 hrs.
Serology for anti-bodies	Acute and convales-cent sera (at least a week apart) CSF	24 hrs.
Histology	Biopsy or postmortem tissues in fixative	24 hrs.

* Special procedures (plaquing, type of CPE, etc.) available for differentiation of HSV-1 from HSV-2.

staff reporter and appeared in a periodical infrequently seen by "highly dedicated physicians"—namely the Wall Street Journal (1970).

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The Modernization of the Autopsy: Application of Ultrastructural and Biochemical Methods to Human Disease* **

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The information gained through the autopsy has played an important role in the evolution of knowledge of human disease. At the time of Semmelweiss, bedside symptoms and signs plus postmortem examinations were the only investigative tools available to study and understand the causes and progression of disease, to assess the efficacy of therapy and to monitor the quality of medical care. Feedback provided by postmortem examination was highly instrumental in the development of physical diagnosis. The autopsy was virtually the sole means of classifying disease. In the process, the autopsy spawned and furthered the development of almost every contemporary technique for the diagnosis of disease of which radiology and electrocardiography are notable examples. Once developed and refined, such clinical sciences have assumed a direction and ideology aimed at the living patient and paradoxically, the contribution of the autopsy to patient care has steadily diminished. Advances in the clinical sciences have depended more on achievements made in biologic science and less on necropsy in large part because modern biological techniques have not been employed in postmortem examinations. As a result, the clinical sciences have been cut loose from the conceptual base previously provided by information obtained at autopsy.

The autopsy has provided, and still provides, the stimulus for many attempts to reproduce disease in experimental animal models. This approach has become increasingly difficult, however, in the case of human disease, principally shock. The study of some pathological states in animal models requires testing in several species and final confirmation in man before this knowledge can be applied to living patients. In our studies the application of cell biology techniques at autopsy has permitted the generation of new hypotheses which are more amenable to further exploration in experimental models and can be more precisely related to human disease.

The chief limitation for the interpretation of observations made in the routine autopsy is caused by the delay in obtaining tissue following somatic death. The resultant autolytic changes invalidate most ultrastructural, biochemical and functional studies. Investigations utilizing contemporary refined techniques from the experimental laboratories of university pathology departments are, however, the very ones needed to restore the postmortem examination to a primary role in patient care and in quality con-

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trol of health care. The use of biopsy obviates time delay. While much valuable information can be and is obtained from biopsy, there are distinct limitations: a) The small size of biopsies restricts the number of analytical techniques that can be applied; b) Often, medical indications for biopsy do not outweigh the risks involved for seriously ill patients; c) Finally, the fact that complete organ sampling cannot be performed make this contribution of limited value in the study of systemic disease.

A potential limitation in applying an immediate autopsy technique is the selection of patients for any contemplated multidisciplinary approach. The voluminous data generated by intensive care units have made valuable contributions to medical knowledge and patient care, yet little of these data has been related to phenomena at the tissue and cellular level. It seems logical then to direct an intensive approach to this population of patients. Especially needed, we feel, are morphologic, biochemical and functional observations on tissues removed immediately after death in order to correlate them with clinical data.

The initial objective of the immediate autopsy is directed to the evaluation of cellular and subcellular changes in major organ systems in cases of shock. Shock frequently accompanies trauma and constitutes a significant determinant of the posttraumatic morbidity and mortality, a major cause of death. Antecedent diseases resulting in shock are legion and shock is a component of the terminal phase of numerous illnesses of many causes. Morphologic techniques used in the routine autopsy do not permit separation of the cellular effects of shock from the effects of certain underlying diseases which cause shock. For example, in acute myocardial infarction with cardiogenic shock it is often impossible to distinguish the extent of primary myocardial injury from the damage secondary to shock. Although we know that there are common pathways of cellular reaction to a diversity of injurious agents, variations exist. Our studies have demonstrated that a distinction can be obtained between the effects of antecedent disease and the effects of shock when precise clinical data are correlated with ultrastructural, biochemical and functional assays at immediate autopsy. This permits better understanding of the cellular shock, which is of importance in the utilization of autopsy technique to the study of other diseases. Without knowledge of the changes resulting from shock, adequate evaluation of autopsy data in other diseases may be difficult or impossible.

The Immediate Autopsy Program at the University of Maryland is conducted by the Pathology Department in conjunction with the Maryland Institute for Emergency Medicine. The primary purpose of the program is to document and clarify the pathological changes leading to organ failure in cases of shock and trauma. This paper demonstrates that tissues obtained at immediate autopsy yield valid observations on the pathogenesis of cellular injury in shock. Methods have been discussed in detail elsewhere (5); the autopsy procedure, however, will be briefly outlined below.

Immediate autopsy is made possible by two legal instruments existing in the State of Maryland: a) the Medical Examiners' Law which charges the Medical Examiner with the responsibility of determining the cause of death and investigating medicolegal and biological factors in deaths due to violence, traffic accidents and unexplained natural death; b) the Anatomical Gift Act, similar to laws enacted in many states and aimed at obtaining organs for transplantation, whereby the patient or his next of kin can give permission for autopsy prior to death.

All the individuals studied include cases of "brain death" as determined by the Harvard criteria (1). Once somatic death is determined by the absence of heart beat, pulse and respiration, as well as an isoelectric EKG, the rapid sampling phase of the immediate autopsy is begun. The prosector, together with the ten-member team, does a rapid sampling of several organs for study by light and electron microscopy, immunofluorescence, enzyme histochemistry and studies of organelles after homogenization and differential centrifugation. Following the rapid sample phase, a routine autopsy dissection is performed.

Simultaneously, the intracranial contents are perfused *in situ* via polyethylene tubing inserted through the right common carotid and after ligation of the three other major cervicocranial arteries. The fixative is composed primarily of aldehydes.

Concepts of Cell Injury. Since Rudolph Virchow's famous papers of about one hundred years ago, we think of disease in terms of cellular changes, and define disease as the summation of the effects of injury as well as the responses of cells to injury. Injury is defined as an event that alters cellular homeostasis. If the injury is lethal, such as complete ischemia or anoxia, there will be a reversible phase prior to the time of cell death. If the blood supply or oxygen are restored during this reversible stage the cell can recover, return to the normal state of homeostasis and

continue functioning. At some point, however, damage becomes irreversible and the cell is said to die; even if the blood supply is restored recovery does not occur. Instead, the cell undergoes necrosis and gradually approaches equilibrium with the environment. Thus there is a reversible and an irreversible phase following a lethal injury. Shown in figure 1 are some of the key organelle changes which characterize these

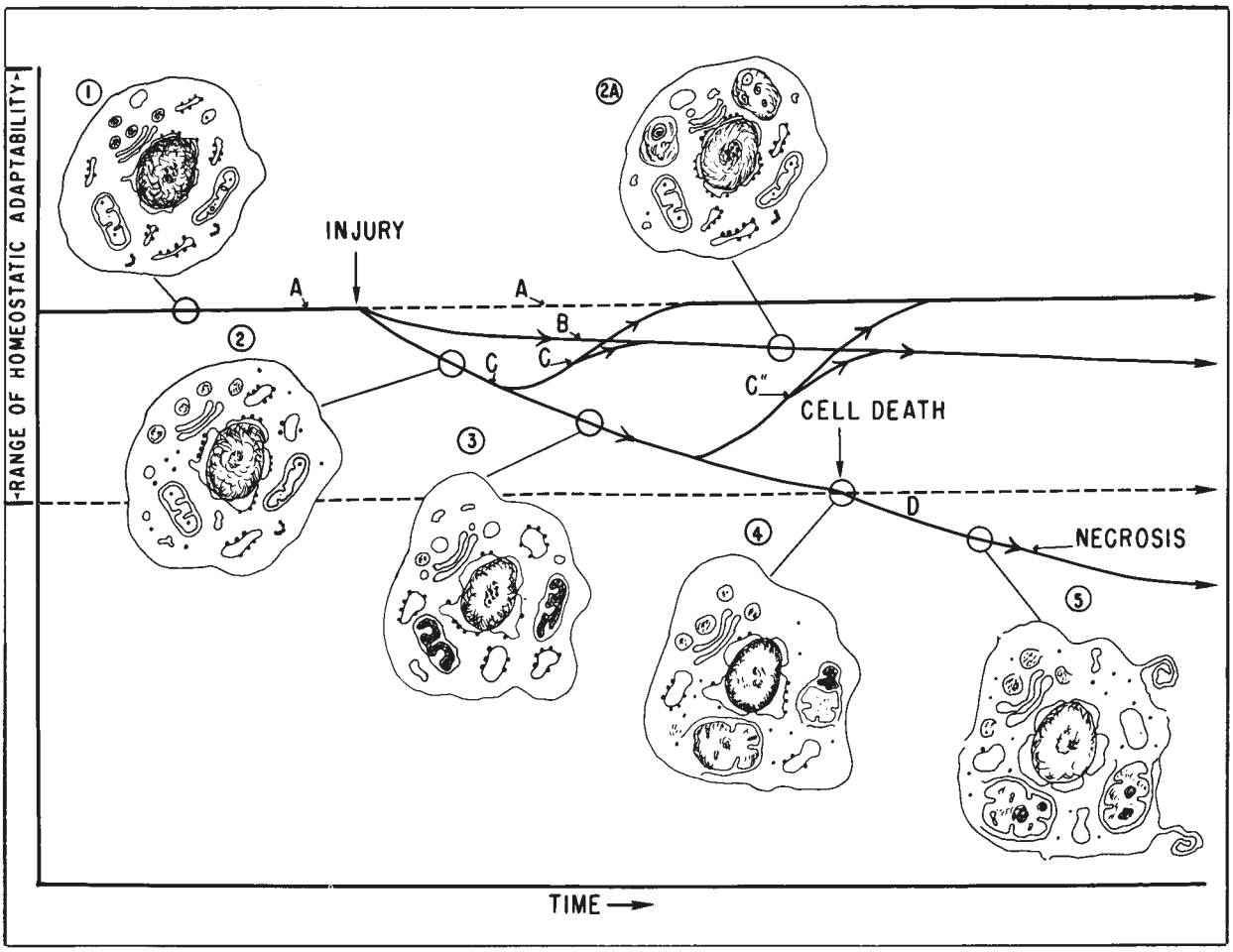

Fig. 1—Stages of cell injury. This diagram represents a conceptualization of the results of cell injury. Time is plotted along the abscissa and the range of homeostatic ability along the ordinate. An injury is applied at the arrow. This injury may be sublethal or acutely lethal. Lethal injury will be considered first. Curve C represents a homeostatic ability. Recovery can occur, however, if the injurious stimulus is removed prior to the point of cell death. Such recovery may proceed along the curve C′ or C″. Stages 2 and 3 represent progressive changes during this period. Stage 2 is characterized by dilatation of the endoplasmic reticulum and slight clumping of the nuclear chromatin. Some ribosomes may also be detached from the endoplasmic reticulum and the entire cell may be slightly swollen. Condensing of the mitochondria, additional swelling of the cell and the appearance of blebs along the cell membrane are characteristic of stage 3. After the point of cell death, recovery can not occur even if the injurious stimulus is removed and the cell is said to enter the phase of necrosis. Stages similar to those in stage 3 occur in stage 4. In stage 4, however, some mitochondria are markedly swollen, others have portions that are condensed and other portions that are swollen. Stage 5 depicts a typical morphologic picture of cell necrosis during which the cell undergoes degenerate degradation by autolysis and denaturation. In this and later stages, myelin figures appear along the cell membrane, intracellular membrane systems are fragmented, interruptions occur in continuity of the plasma membrane and the mitochondria show high amplitude swelling of the inner compartment with prominent flocculent densities. Lysosomes are probably beginning to leak at this point although they may still appear intact. In some injuries, the cell is able to adapt even to the presence of continued injury by attaining some altered steady state. This is depicted as curve B. A common sublethal adaptation in which numerous secondary lysosomes are filled with digestive debris is represented in stage 2A. Note that incomplete recovery during the reversible phases after lethal injury might also result in a new steady state depicted by the right-hand limb of curves C′ and C″. From Trump, B. F., *et al.* (6). Used by permission.

phases as observed in studies of experimental animals. For example, one of the earliest changes that occurs following injury is dilatation of the endoplasmic reticulum (ER), which is associated with early ion and water shifts within the cell. These constitute a manifestation of reversible injury. Thereafter, the inner compartment of the mitochondria undergoes condensation probably due to potassium loss from that compartment; this, too, is reversible and if blood supply is restored, the cell can survive. Cells that are past the "point of no return" or have entered the phase of necrosis typically show massive mitochondrial swelling with formation of dense matrical inclusions in the inner compartment, presumably due to denaturation of matrix protein. The permeability of the cellular plasma membrane leads to increased levels of intracellular enzymes in the plasma. These enzymes include glutamic oxalacetic transaminase and lactate dehydrogenase. Nuclei swell and undergo karyolysis. In late phases of necrosis lysosomal enzymes such as acid phosphatase can be demonstrated in the serum.

Immediate autopsy vs. routine autopsy. The state of preservation of tissue, in this case liver, removed at immediate autopsy within minutes of death (fig. 2), is contrasted with a sample of liver removed from the same patient after the customary somatic death-fixation interval (12 hours) had elapsed (fig. 3). A "differential necrosis" of centrilobular zones is noted in the later specimen which is not present in the immediate autopsy sample. Given only the routine sample, this could lead to the erroneous conclusion that a terminal episode of shock in this patient together with two earlier episodes had resulted in extensive central and midzonal hepatic necrosis.

Instead, chronic injury over a one-month period had resulted in a differential accumulation of lysosomes in centrilobular hepatocytes. Lysosomes were precisely localized with an enzyme marker, acid phosphatase, in the immediate specimen, but diffuse reaction characterized the routine sample. Study of the immediate sample by electron microscopy revealed enlarged lysosomes in this human liver (fig. 4). The remarkable degree of good ultrastructural preservation obtained is evident and demonstrates the feasibility of applying this technique to the study of human disease. In a series of such patients sustaining repeated episodes of shock, rather striking increases in the amount of chemically assayed acid phosphatase have been found in liver homogenates as compared with patients not sustaining or in cases with a single acute injury or shock (7). The numerous large lysosomes in liver cells contain various types of debris much of which presumably resulted from what is called autophagocytosis, a situation whereby normal organelles, such as mitochondria, are segregated within the lysosomal system and are digested. This phenomena of autophagocytosis occurs through budding of portions of cytoplasm into

Fig. 2—(left) Light micrograph of centrilobular cell of liver from a 48-year-old man who had suffered several episodes of shock over a 32-day period. Note the excellent state of preservation of hepatic parenchymal cells. Note, however, that many of these cells contain easily visible, eosinophilic cytoplasmic inclusions. Paraffin embedding, H & E, × 345. Fig. 3—(right) Portion of liver from the same case from the routine autopsy taken 12 hours later. Note that in this picture typical coagulation necrosis of some of the centrilobular hepatic cells can be seen. Paraffin embedding, H & E, × 345.

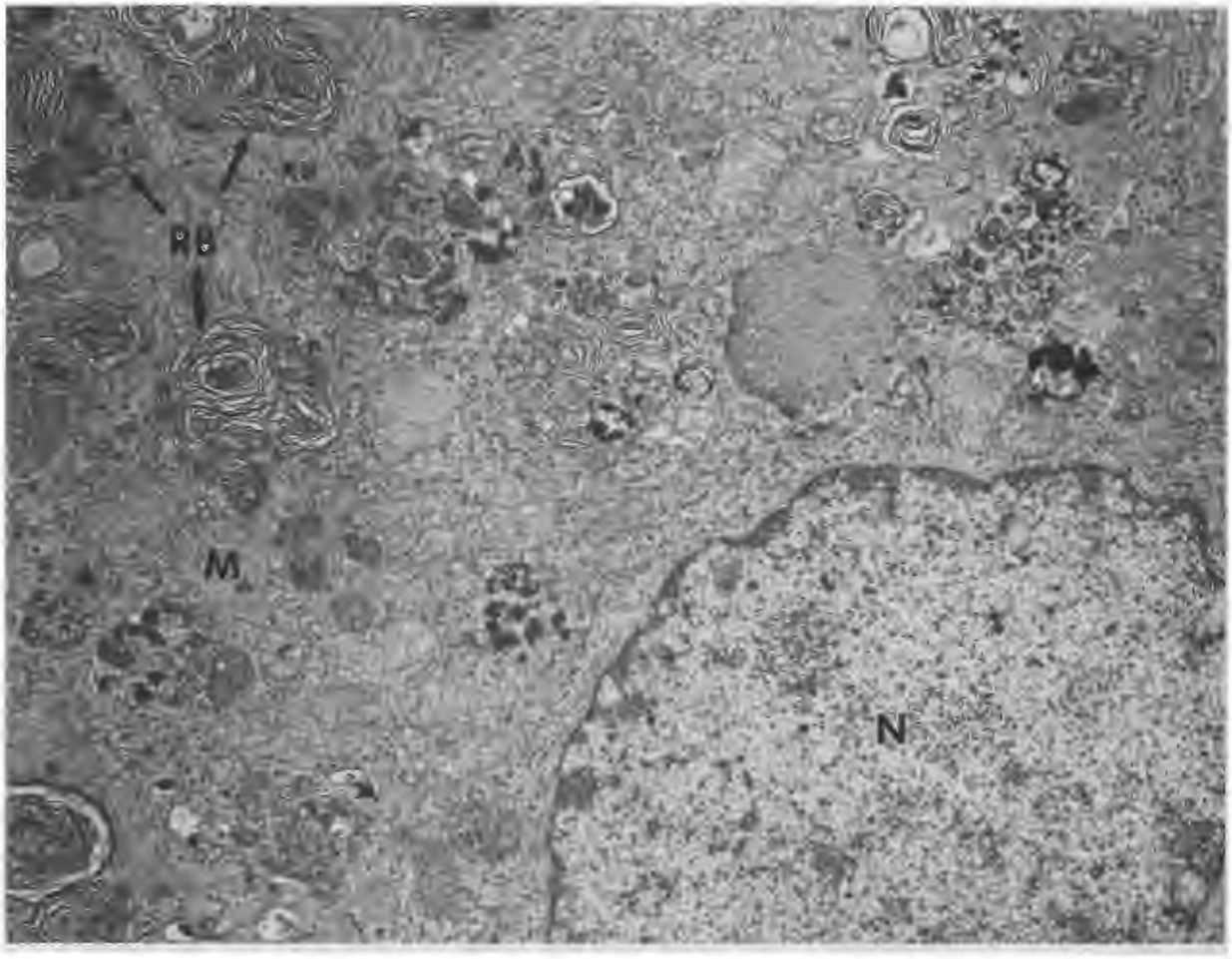

Fig. 4—Electron micrograph taken from a specimen fixed at immediate autopsy from the same case shown in figure 2 illustrating numerous large single membrane-bound inclusions in the hepatic parenchymal cells. These inclusions contain a variety of debris principally lamellar and are interpreted as residual bodies (RB) resulting from incomplete digestion of material entering the lysosomal system by autophagy and/or heterophagy. Nucleus (N), mitochondria (M), × 12,000.

intracellular cavities such as the endoplasmic reticulum followed by pinching off of the bud releasing the segment of cytoplasm containing sequestered organelles and fusion with primary and/or secondary lysosomes. This rather complex process involving cell membrane movements is ATP-dependent and can be reproduced experimentally by administering glucagon.

In the case of the rat model it appears that the glucagon-induced autophagy is mediated through cyclic AMP. It is entirely conceivable, therefore, that in this particular patient, as well as in others with shock, the increased autophagy is the result of changes in hormonal levels; for example, increased levels of glucagon in the plasma. With digestion, lipids are released giving rise to phospholipid bilayers sometimes called "myelin figures." The lysosomes in this patient superficially resemble those seen in many of the "lipid storage" diseases, such as the gangliosidoses in which various types of cellular debris accumulate on the basis of a lysosomal enzymatic defect. In this case presumably there is no enzymatic defect, but rather an overloading of the system due to induced autophagocytosis. This results in cells which are not killed but are perturbed by the initial injury. Autophagocytosis can be defined as a manifestation of sublethal injury. Only an occasional hepatocyte was "necrotic" in the immediate specimen, a fact which cannot be validly assessed from the routine sample. The observation that necrosis was more advanced in the routine (delayed) sample is interpreted as the effect of increased digestion by lysosomal enzymes following postmortem ischemic necrosis. In a sense, therefore, these

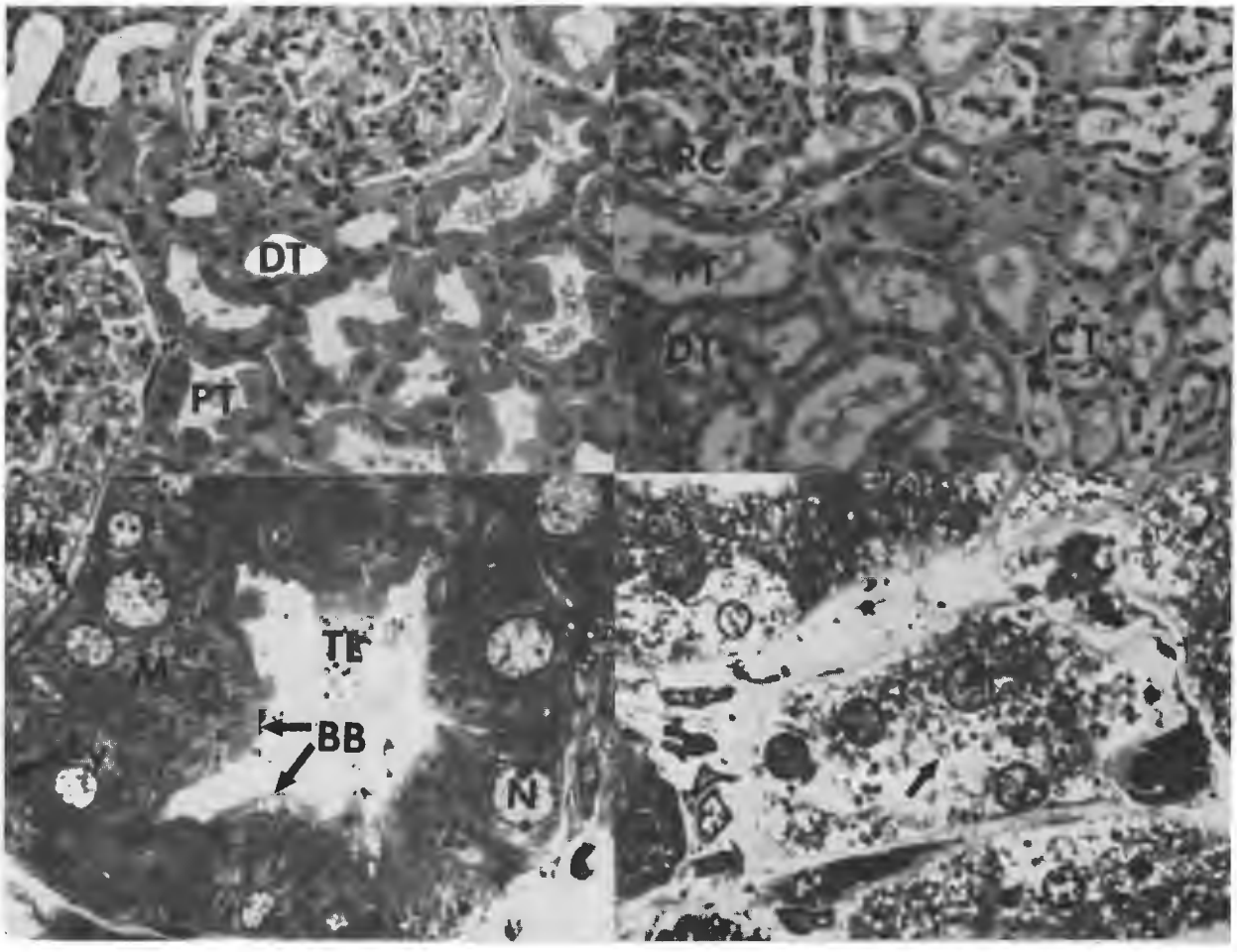

Fig. 5—(left, top) Light micrograph of renal cortex from a 28-year-old man who did not have shock, but died following severe head injuries. Note good preservation of renal corpuscle from the proximal tubules (PT) and distal tubules (DT). Paraffin embedding, H & E, × 345. Fig. 6—(left, bottom) Semithin section from the same case shown in figure 5 illustrating the increased cytologic detail visible with this technique. Note that now the brush border (BB) can be fairly delineated and numerous granules, most of which are mitochondria (M), can be seen in the cytoplasm. Note also the lysosomes (Ly). The basement membrane (BM) can be clearly seen along the outside of the tubule. Tubular lumen (TL), nucleus (N). Epon embedding, toluidine blue, × 865. Fig. 7—(right, top) Light micrograph of the renal cortex from a 16-year-old girl who died six hours after an anoxic episode. Note the normal appearance of the renal corpuscle (RC), proximal tubules (PT) and distal tubules (DT). One collecting tubule (CT) can be seen. With this technique the tubules appear within normal limits. Paraffin embedding, H & E, × 345. Fig. 8—(right, bottom) Semithin section from the same case. Note again the increased detail. Now, however, it is evident that the tubule is not within normal limits but that the mitochondria are swollen and have small extrinsic densities (free arrow) apparently equivalent to the flocculent densities seen in electron micrograph. Epon embedding, toluidine blue, × 865.

liver cells with more lysosomes become somewhat like the normal pancreas which is known to undergo much more rapid autolysis following death.

High Resolution Light Microscopy. Much additional information can be obtained when tissue for light microscopy is embedded in epoxy resins as a supplement to routine paraffin embedding. The structure seen in paraffin, as opposed to plastic embedded immediate samples of kidney from a patient who did not die in shock (figs. 5, 6), are contrasted with a similar sample from a patient having a single acute terminal shock episode (figs. 7, 8). Generalized cell swelling (both cytoplasmic and nuclear) can be noted in either paraffin or plastic embedded kidney from the shock patient but can be more precisely assessed in the plastic embedded specimen. Paraffin embedding results in comparatively poor preservation of cellular structures. Numerous rounded bodies are

noted within the cytoplasm of the plastic embedded proximal tubule cells in both specimens. These are mitochondria but are not readily visualized in the paraffin sections. Furthermore, the mitochondria in the shock kidney cells (fig. 8) are notably larger and even exhibited punctate densities in epoxy semithin sections. Using electron microscopy (fig. 9), mitochondria are markedly swollen and exhibit flocculent matrix densities; there are features characterizing acute lethal cell injuries as derived from varied model experiments *in vivo* or *in vitro* (5). The close correlation between light and electron microscopy is then obvious and the light microscope can thus be employed to evaluate tissue samples larger than those that can be meaningfully studied by electron microscopy.

Correlation of Organelle Structure and Function. The immediate autopsy approach further permits a valid assessment of mitochondrial function which is of profound importance in the pathogenesis of cell injury in shock because mitochondria often seem to be the deciding factor for cell survival. Whether or not the mitochondria can recover and make ATP following treatment of the cause of injury seems to be the crucial question.

Mitochondria can readily be prepared from the immediate autopsy specimens. Tissue is removed and shortly thereafter homogenized and centrifuged to obtain a mitochondrial suspension. Mitochondrial function is then assayed using an oxygen electrode. The chamber is designed so that substrates and ADP can be added to the mitochondria suspension

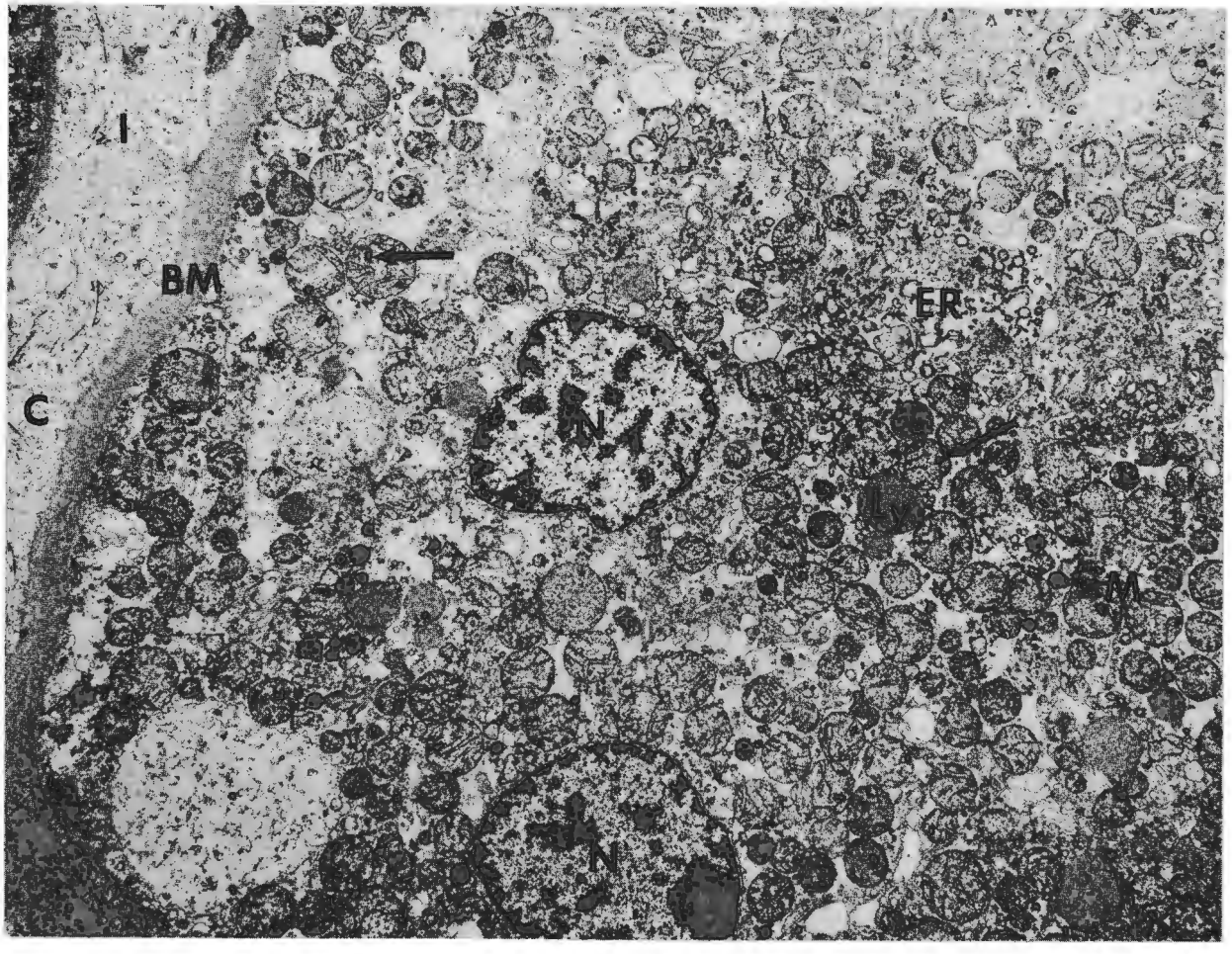

Fig. 9—Electron micrograph of proximal tubules from the same case showing stage 5 changes as depicted in diagramatic form in figure 1. Note the markedly swollen mitochondria (M) with flocculent densities (free arrow). Fragmentation of cisternae of endoplasmic reticulum (ER); nuclei (N) which show marked clumping of chromatin and several apparently intact lysosomes (Ly). Basement membrane (BM), interstitium (I), collagen fibers (C), × 10,000.

to stimulate respiration. With mitochondrial respiration, a downward slope as a function of time depicts the oxygen consumed. Given the amount of mitochondrial protein added, oxygen consumption per milligram protein can be computed. Normal mitochondria exhibit tightly coupled respiration, meaning that they do not respire in the presence of a substrate like succinate unless ADP is present. In other words, the rate of respiration is limited either by the amount of substrates or ADP which is going to be phosphorylated; addition of mitochondria to the chamber results in a gradual slope due to endogenous substrates. When a substrate, for example, succinate, is added there is only slight respiration, due to the absence of ADP which is phosphorylated. With addition of ADP, there is a great increase in the rate of respiration which continues until completion of the phosphorylation of the ADP at which point the slope of oxygen consumption levels off again. By comparing the phosphorylation slope with the resting slope, a ratio is obtained which is a measure of the efficiency of the mitochondria. If the mitochondria are completely damaged, there will be no change in rate on adding the ADP. If they are normal mitochondria or normally functioning, the rate will increase three- to ten-fold. Plotting phosphorylation rate over resting rate is termed the respiratory control index which is utilized as a measure of mitochondrial functional integrity. A P/O ratio or an ADP/O ratio is computed from the amount of ADP added, divided by the atoms of oxygen consumed. When the amount of ATP that formed in the oxygen electrode per milligram protein in both the liver and kidney from a series of patients is plotted on a two dimensional scale, one can discriminate two groups of patients, one group with head injury which did not have shock and one group of patients sustaining shock (fig. 10). In shock, apparently there is a marked reduction in the ability of mitochondria to make the ATP; this fact correlates with the morphology of mitochondria studied in plastic embedded preparations and by light and electron microscopy (figs. 6, 7). P/O ratios which should be around 2.5-3 for glutamate and about 1.5-2 for succinate, are significantly reduced in shock (4).

Studies of the Central Nervous System. The final two cases are examples of the improved results obtained in the interpretation of microscopic abnormalities of the brain whenever intravascular perfusion fixation and electron microscopic methods are used according to the systems mentioned above. The first patient, neurologically normal for 72 years, died after a prolonged period of marked hypotension with a ruptured abdominal aneurysm. The second patient, who had a lengthy history of inappropriate belligerent behavior, was stabbed and developed suppurative peritonitis and pulmonary edema. In both instances perfusion fixation of the brain was started about 25 minutes after somatic death.

Fixation of the central nervous system which allows adequate cytological evaluation is difficult to accomplish in part due to the relative inaccessibility of the tissue. In other animals, it has been shown that handling of the normal brain frequently results in abnormalities referred to as "dark" neurons (2). Thus, avoidance of tissue handling before fixation has distinct advantages in the fixation of neuronal and glial elements in the human brain. As for the effects of prolonged death-fixation interval, we have studied the ultrastructural effects of total ischemia in feline cerebral cortex and have determined that pro-

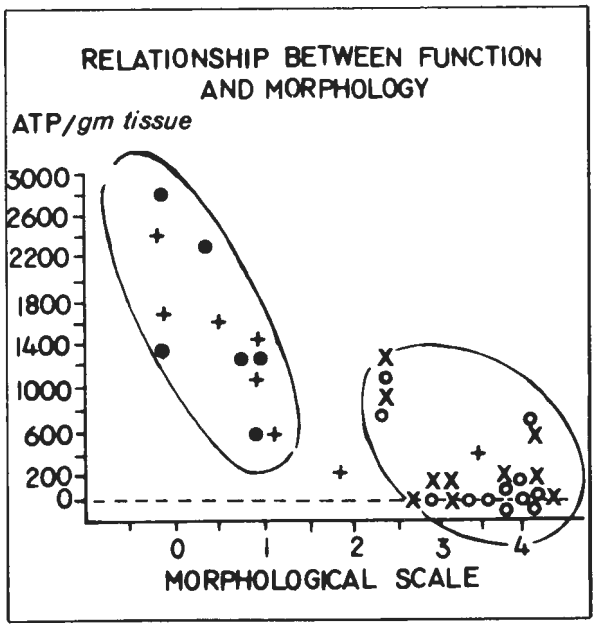

Fig. 10—Two dimensional scatter plot of morphological changes in mitochondria compared with ability of the mitochondria to synthesize ATP *in vivo*. On the morphological scale, 0 represents mitochondria showing normal morphology and 4, mitochondria with the most advanced changes. One, 2 and 3 are intermediate. Note that two clusters are formed; the patients dying without shock have better morphology and higher rates of ATP production in contrast to mitochondria from individuals with shock. + = succinate with pure head injuries; ● = glutamate with patients with head injuries without shock; X = succinate with shock and ○ = glutamate with shock.

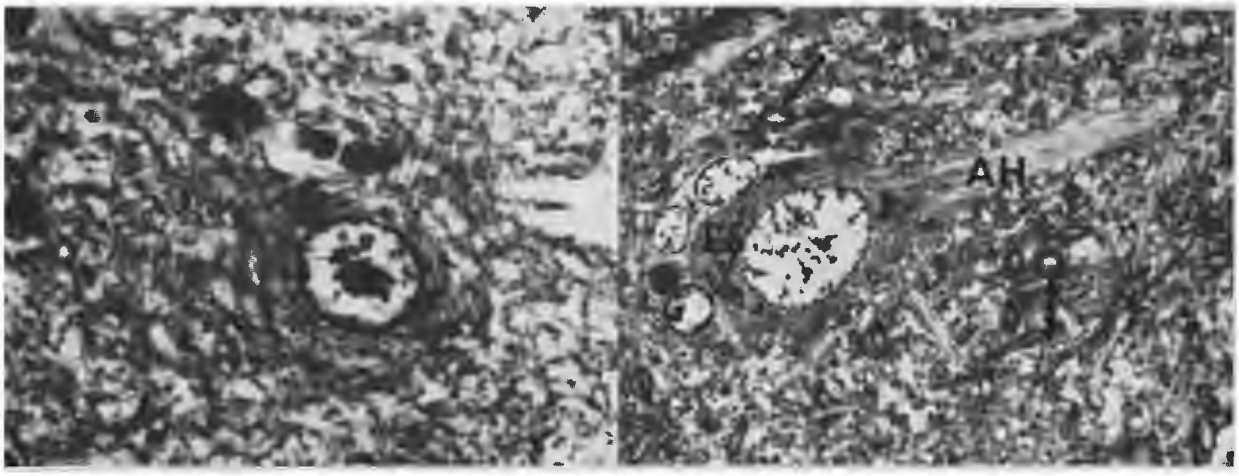

Fig. 11—(left) Light micrograph of tissue from the cerebral cortex of a 72-year-old man who died with irreversible brain damage following an 18-hour ischemic episode subsequent to a ruptured abdominal aneurysm. Note that in this pyramidal cell we can appreciate the nucleus and cytoplasm. Only rather indistinctly can portions of the chromidial substance be seen. Paraffin embedding, H & E, × 865. Fig. 12—(right) Semithin section of cerebral cortex from the same case as in figure 11 showing greatly improved cytologic detail. Now we can recognize an axon hillock (AH), cytoplasmic lysosomes (Ly), and in the adjacent neuropil, myelinated nerve fibers (free arrows). Epon embedding, toluidine blue, × 865.

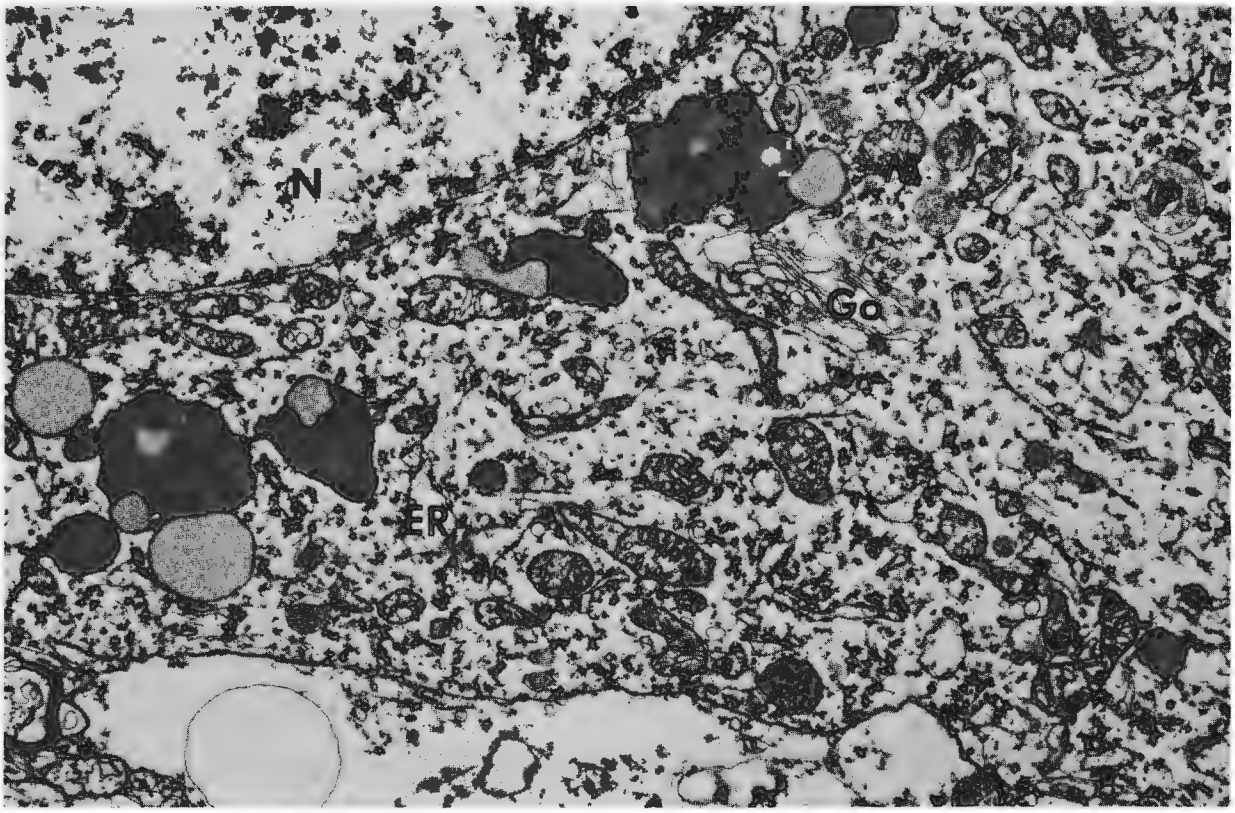

Fig. 13—Electron micrograph from the same case as in figures 11 and 12 showing a portion of a cortical neuron which exhibits stage 5 changes. The mitochondria (M) show flocculent densities, the endoplasmic reticulum (ER) is dilated as are the saccules of the Golgi apparatus (Go). Several residual bodies (RB) which are presumably related to the patient's age are also seen; nucleus (N), × 15,000.

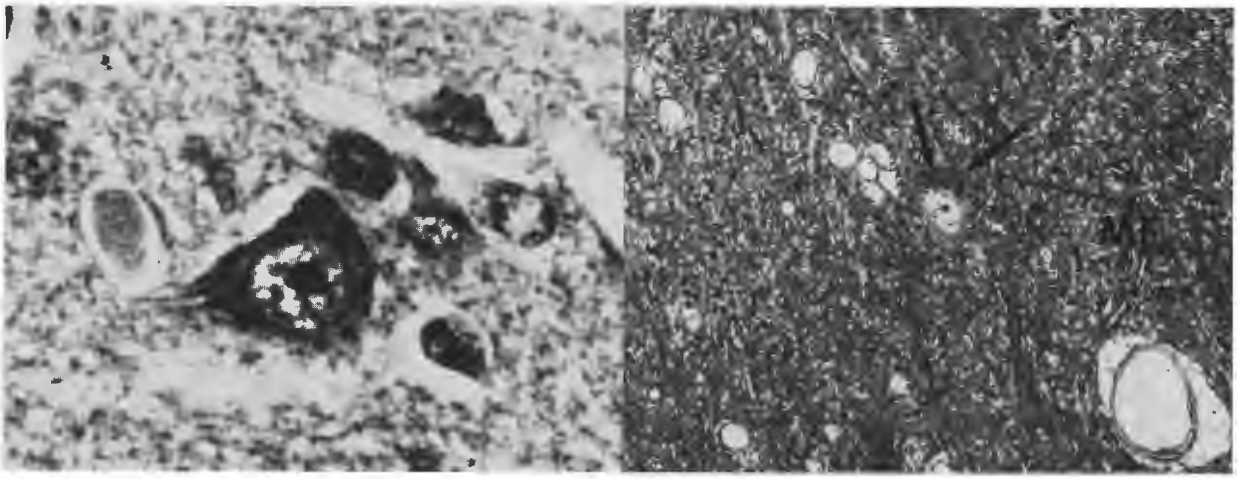

Fig. 14—(left) Light micrograph of pyramidal cell from the cerebral cortex of a 50-year-old man who died with peritonitis and pulmonary failure. At the time of autopsy, severe atrophy of the brain was grossly apparent. In contrast to the patient shown in figures 11–13, this patient had only a brief terminal episode of hypotension. In this preparation, the neuron has an appearance which is within normal limits. Paraffin embedding, H & E, × 865. **Fig. 15**—(right) Light micrograph of a semithin section from the same patient. Note the large amount of lysosmal granules (free arrow) in the neuron, which correspond to brownish pigment seen in hematoxylin-eosin preparations. Note also numerous myelinated fibers (MF). Epon embedding, toluidine blue, × 345.

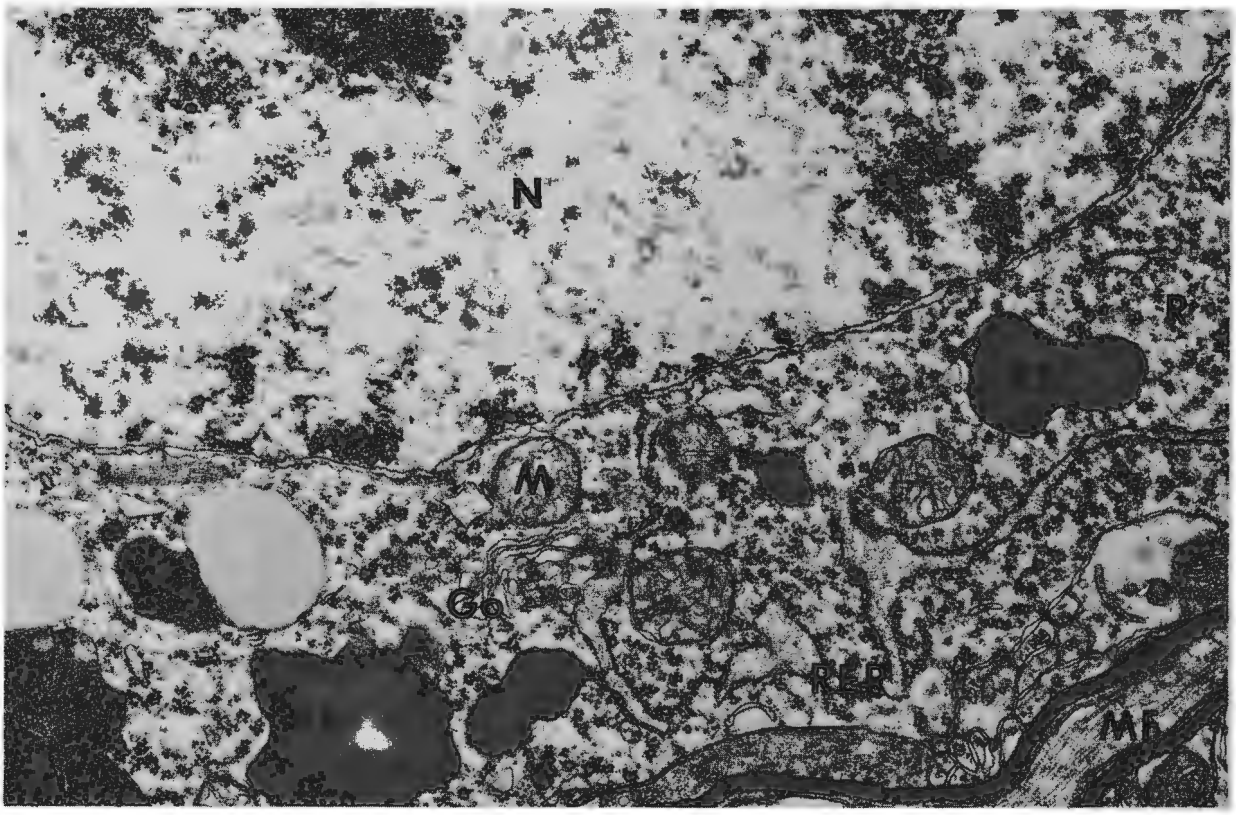

Fig. 16—Electron micrograph of same case as in figures 14 and 15 showing numerous residual bodies (RB), profiles of rough endoplasmic reticulum (RER), Golgi apparatus (Go), mitochondria (M) and numerous free ribosomes (R). All of these appear within normal limits. The numerous residual bodies which are much too frequent for this patient's age presumably reflect a diffuse metabolic disorder of nerve cells. At the bottom of the picture a normal-appearing myelinated fiber (MF) can be seen; nucleus (N), × 20,000.

found structural changes occur after 60 minutes, but that these may be minimal before 30 minutes (3).

Finally, the improved resolution and, therefore, information gained through electron microscopy can also be appreciated by comparing the images obtained after paraffin embedding and processing, Epon embedding and ultrastructural evaluation. Note that in this instance electron microscopy not only reveals alterations invisible in the paraffin-embedded material, but also permits separation between two different degrees of circulatory injuries to the brain—one lasting up to 18 hours before death (figs. 11, 12, 13) and one probably lasting less than one hour before death (figs. 14, 15, 16).

The application, therefore, of concepts and investigative techniques generated in research laboratories for studying cellular response to injury, provide powerful methods which will markedly improve our understanding of human disease. It is possible to conclude, on the basis of our studies to date, that human shock has important and often disasterous effects on cell function level. The cellular pathology of shock has been a mystery for a long time. The principle reason for the continuing mystery surrounding the pathology of shock is that the changes appear to be mainly at the organelle level. In the future we may think of shock more as a mitochondrial, lysosomal or cell membrane disease than as a disease with primary organ targets.

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HAA (HB Ag) Evaluation—State of the Art*

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Viral hepatitis has been a major problem for public health workers and for blood bankers. There is much to be learned despite recent major advances in the natural history of the disease, in some of its epidemiological characteristics, and particularly in its laboratory diagnosis. These discoveries will significantly reduce the rate of posttransfusion hepatitis. A complete solution of the overall hepatitis problem will be attained only when the causative role of the agent, presumably a virus, has been conclusively demonstrated. This will in turn produce more sensitive and specific diagnostic procedures and lead to the development of effective, preventive and therapeutic measures. Specific laboratory diagnosis of hepatitis became possible with the discovery of Australia antigen—a term used in 1964 (4) by its discoverer Baruch Blumberg. The observation that Australia antigen or Au-1 was intimately related to hepatitis B virus stimulated large numbers of investigators who developed overlapping systems of nomenclature. Some of these terms, including those recommended by the Committee on Viral Hepatitis of the Natural Research Council, are given in Table 1. Henceforth, the symbol HB Ag will be used as the synonym to Au-1.

The discovery of HB Ag has provided a specific marker of infection with serum hepatitis, the most common cause of posttransfusion hepatitis. This in turn has permitted a partial solution to the blood bankers' dilemma, namely, the detection of HB Ag carriers before blood is collected for infusion.

The frequency of HB Ag in different blood donor populations in the U. S. has been estimated to be 0.1-0.5% among volunteer donors, and up to 2% among paid donors of commercial blood banks (6, 9, 14, 15, 16). In other words, blood collected from the latter group may have as high as 20 times the risk of transmitting hepatitis as that obtained from volunteer donors. A list of techniques for the detection of HB Ag is presented in Table 2, including their relative sensitivity in reference to agar gel diffusion (AGD) and the time required for results to be obtained.

AGD was the first method available (3). This method lacks sensitivity. Another disadvantage is that it requires a minimum of one to seven days of incubation before any results are seen. Moreover, it requires that both antigen and antibody be sufficiently potent and also of approximately equivalent concentration; otherwise, the results may be falsely negative. AGD has the significant advantage, however, in that it can discriminate between the components in complex mixtures. It offers a direct demonstration of identity, partial identity or nonidentity between different antigens or antibody reactants. In addition, sensitivity may be increased by certain modifications. Precipitation lines of identity, partial identity, and nonidentity on an agar gel plate are depicted in figure 1.

Counterelectrophoresis (CEP) is the most commonly used version of the immunoelectrophoresis

* Presented by Dr. Hossaini at the 44th Annual McGuire Lecture Series, March 22, 1973, at the Medical College of Virginia, Richmond.

TABLE 1

VIRAL HEPATITIS—NOMENCLATURE

Old Terms	New Terms	Committee on Viral Hepatitis Div. Med. Sc., NAS—National Research Council
Virus B	SH antigen	Hepatitis B virus, HBV
Serum hepatitis (SH)	Au/SH antigen	Hepatitis B antigen, HB Ag
Posttransfusion hepatitis (PTH)	Hepatitis antigen (HA)	Hepatitis B antibody, HB Ag or anti-HB Ag
Long incubation disease	HAA	
Hemologous serum jaundice	MS-2	
Syringe jaundice		
Virus A		
Short incubation disease	MS-1	Hepatitis A virus, HAV
Infectious hepatitis (IH)		
Acute catarrhal jaundice		
Epidemic jaundice		
Epidemic hepatitis		

procedures. The principle of this method is somewhat more difficult to understand than AGD although it is as easy to perform. It has the advantage of requiring only 30 minutes to two hours to obtain results (8, 10). Like AGD, the CEP test utilizes agar gel coated slides and the reactions appear as precipitation lines.

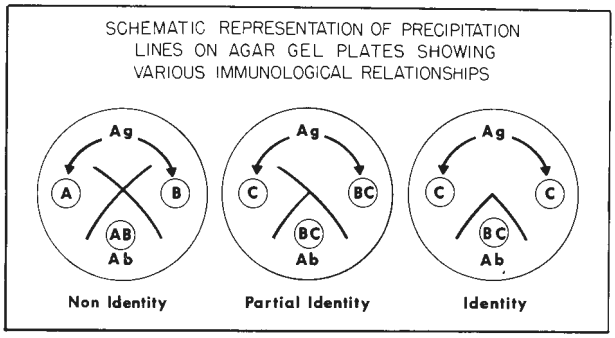

Fig. 1

The complement-fixation (CF) test is considerably more complicated than AGD and CEP technically and in principle. It is not, therefore, as practical for mass screening. Generally it is more sensitive than CEP (11, 12, 18, 20). The sensitivity of certain CEP procedures, however, has been improved to approach that of CF (11, 15, 19). Another disadvantage of CF tests is the scarcity of suitable antisera, since many antisera are of low titer and are anticomplementary (AC).

The hemagglutination-inhibition (HAI) test and the radioimmunoassay (RIA) procedures have been reported to be much more sensitive than the CEP and CF tests for the detection of both antigen and antibody. The comparative sensitivity of CEP and HAI is given in Table 3 as reported by Vyas *et al.* (23). Vyas and Shulman (22) estimated that HAI was 100 times more sensitive than AGD for the detection of HB Ag. In addition to its relatively high sensitivity, HAI has the advantage of simplicity. Not all batches of antigen are satisfactory for coating the reagent red cells, however, and some lots of anti-

TABLE 2

RELATIVE SENSITIVITY OF TESTS FOR AUSTRALIA ANTIGEN IN REFERENCE TO GEL DIFFUSION

Test	Relative Sensitivity	Time	Reference
Agar gel diffusion	1.0	24 hrs.	Blumberg and Alter (4)
Electron microscopy	1.5	2-6 hrs.	Almeida and Waterson (1)
Electroimmunodiffusion	10.0	½-3 hrs.	
a. Immunoelectrodiffusion			Duquesnoy and Becker (8)
b. Immunoelectroosmophoresis			Prince (17)
c. Counterelectrophoresis			Gocke and Howe (10)
Immunoelectronmicroscopy	11.0	2-6 hrs.	Almeida and Waterson (1)
Complement-fixation	100.0	16 hrs.	Shulman and Barker (21)
Hemagglutination inhibition	1000.0	2-4 hrs.	Vyas and Shulman (22)
Radioimmunoassay	1000.0	5 days	Lander, *et al.* (13)
Radioimmunoassay (solid phase)	600.0	2-4 hrs.	Cawley (5)

TABLE 3

COMPARISON OF CEP AND HAI FOR HB AG TESTING ON SERA OF 700 PATIENTS WITH CLINICAL HEPATITIS.*

	CEP+	CEP−
HAI+	201	50
HAI−	9	440

* The same goat anti-HB Ag was used for both CEP and HAI.

serum are not suitable for agglutination and inhibition tests.

A number of RIA techniques have been described. All utilize a radioiodinated marker, usually HB antigen or its antibody labeled with I^{125}. The Ausria-125® of Abbott seemed to be a promising technique because of its high sensitivity with potential screening of blood donors (14, 19). Recent studies, however, have revealed that there may be an inherent serious pitfall in this particular procedure (1). A small percentage of the reactions seems to be falsely positive thus necessitating further tests if mislabeling of individuals as carriers of HB Ag is to be avoided.

Soon after reagents for the detection of HB Ag became commercially available, the Medical College of Virginia Blood Bank began screening donors by the AGD procedure. The Virology Laboratory at the Medical College of Virginia instituted the CF test using antiserum provided by NIH to test patients suspected of having viral hepatitis. When CEP kits became available early in 1971, because of the low sensitivity of AGD, it was decided to conduct a study to evaluate the relative sensitivity of the new method; thus, each of 300 sera were tested by AGD, CF and CEP. The sera were obtained from patients suspected clinically of having viral hepatitis.

Some of the results of this initial study are given in Table 4 showing the relationship between CF titers and the reactions of the 300 sera in the AGD and CEP tests. Of the 300 sera tested, 22.3% were positive by CF, whereas 12% and 10.6% were positive by CEP and AGD, respectively. The highest serum dilution giving a positive reaction by AGD was 1:16, while CEP failed to detect dilutions higher than 1:64. This study, like a number of others, indicated that AGD is the least sensitive method and that CF is the most sensitive of the three techniques.

The sensitivity of AGD in this study was somewhat higher than that reported by other workers. This enhancement was probably related to antiserum titer and the placement of patient sera adjacent to wells containing HB Ag-positive serum (reinforcement pattern). Although this study did not provide evidence for a significant difference in the sensitivity of the CEP as compared to AGD, it was decided to abandon the latter procedure in favor of the former because the performance of CEP could be shortened to 60 minutes.

A second study was then started around the middle of 1972 because, at the time, there were no reports in the literature on the relative sensitivity of the various commercial CEP methods that have become available for routine HB Ag screening of donors and patients and because it was felt that a significantly more sensitive, yet relatively simple, technique was needed. Abbott's Ausria-125® was chosen for this purpose because it was reported to meet these criteria (14). It was hoped that the results of this comparative study would serve as a guide in selecting one of the six CEP methods and/or the RIA for donor screening. It was decided that a change from the CEP to the RIA technique should be made if the latter could be shown to be practical and devoid of false positive reactions. Since a reverse-passive hemagglutination (RPHA) technique was available at that time, it was also included in these comparative studies.

The Blood Bank routinely uses the CEP method of Spectra Biologicals to screen donors. Five other CEP methods used in this study were kindly supplied to us by Abbott Laboratories, Ortho Diagnostics, Pfizer, Squibb and Hyland. All CEP tests were performed according to the manufacturers' instructions. In addition to CEP, Abbott kindly supplied us with the reagents for RPHA as well as the reagents and the gamma counter used with their RIA procedure. Again the prescribed protocol of the manufacturer was followed with one modification which consisted of a reduction in the incubation time of test sera with the antibody. The incubation time was reduced from 16 hours to 90 minutes.

Initially 90 sera were tested by the six CEP methods. Of these, 72 were derived from blood secured commercially, 11 were from donors suspected of having Gilbert's disorder and the remaining seven were from individuals known to be HB Ag positive on the basis of previous testing. As seen from Table 5, none of the sera from commercial blood donors or from donors with Gilbert's disorder

TABLE 4

COMPLEMENT-FIXATION (CF) TITERS OF HEPATITIS B ANTIGEN (HB AG) AND REACTIONS OF 300 SERA IN IMMUNODIFFUSION (ID) AND COUNTERELECTROPHORESIS (CEP) TESTS

Total No. of Sera Tested	CF Titer of HB Ag (22.3% positive)	No. of Sera Positive by CEP (12% of total)	No. of Sera Positive by ID (10.6% of total)
233	<1:2	0	0
19	1:2–1:8	0	0
12	1:16–1:32	2	0
16	1:64–1:128	14	12
14	1:256–1:2048	14	14
6	1:4096–1:16384	6	6

gave a positive test. The seven sera previously known to be positive by Spectra CEP technique were positive again on retesting but the other five CEP procedures detected only six of these. Shortly after these tests were performed, kits for RIA and RPHA testing were made available to us. At the same time, and by lucky coincidence, a panel of 20 sera arrived from the American Association of Blood Banks (AABB) for proficiency and quality control testing. CEP and RIA results on these 20 sera are shown in Table 6. AABB subsequently reported that 11 were positive. All 11 were detected by RIA and all but one were detected by Spectra CEP which at the time seemed to be the most sensitive of the CEP methods. Next in sensitivity appeared to be the Ortho and Pfizer CEP procedures, each of which detected nine of the 11 positives. The other methods showed relatively poor sensitivity, failing to detect almost 50% of the positive sera.

Testing of 204 serum samples, representing a mixed population of patients suspected of having viral hepatitis, and "normal" hospital employees, was then done by CF, RPHA, RIA and by the six CEP methods. Results are presented in Table 7. Among the six CEP methods, Ortho's proved to be the most sensitive while Abbott's was the least, failing to detect HB Ag in six sera. There was also a significant difference between Ortho's sensitivity and that of the other four CEP procedures each of which failed to detect the antigen in five sera. These findings and the fact that Ortho's method was nearly as sensitive as the CF test in the present series indicated that when the test conditions were adequate, relatively high sensitivity could be attained with the CEP procedure. Lewis and Coran (14) attributed the higher sensitivity of the Ortho technique to the considerably larger volumes of test sera and antisera used in this method as compared to the others. Nevertheless, these results confirmed our previous findings, namely that generally, CEP methods are less sensitive than CF. These results also revealed that it is possible to get CEP positive–RIA negative results. These findings are in disagreement with those of Lewis and Coran (14).

The present study did not provide evidence that Abbott's RPHA method, a version of hemagglutination, is more sensitive than CF or Ortho's CEP. It was noted that RPHA results were more difficult to interpret, since the differences between weakly positive and negative reactions were not clear and could not provide for a clear-cut diagnostic reading.

Like other studies (5, 13, 24), RIA appeared

TABLE 5

RESULTS OF TESTING 90 SERA BY SIX CEP PROCEDURES (A-F)

Category of Donors	Test Procedure & No. Positive A	B	C	D	E	F	Total No. Samples Tested
Commercial	0	0	0	0	0	0	72
MCV Known Positive	7	6	6	6	6	6	7
Gilbert's	0	0	0	0	0	0	11
CEP Methods:	Spectra	Hyland	Ortho	Pfizer	Abbott	Squibb	

TABLE 6

COMPARATIVE SENSITIVITY OF THE RIA WITH CEP PROCEDURES ON PROFICIENCY SAMPLES FOR DETECTION OF HB AG

Total No. Samples*	CEP System A	B	C	D	E	F	RIA Test	Results
	8	6	7	6	4	5	11	Positive
20	2	1	2	3	2	1	—	W** Positive
	10	7	9	9	6	6	11	Total Positive
CEP Methods	Spectra	Hyland	Ortho	Pfizer	Abbott	Squibb		

* Submitted by the AABB for quality control
** Weakly

to be the most sensitive technique, despite the fact that the CEP and CF procedures could detect a total of five positive sera which were negative by RIA; RIA, however, was the only procedure giving a positive reaction in all 11 sera (Table 7). The higher sensitivity of this technique seemed to be further supported by testing 50 additional sera only by RIA Spectra and Ortho. As seen in Table 8, two sera failed to react by both Ortho's and Spectra's CEP but gave a positive RIA result. In this series, however, none of the sera positive by Spectra's and/or Ortho's CEP methods was negative by RIA.

In order to determine the specificity of the RIA positive reactions, two approaches were taken. First, three sera that were positive by RIA only were concentrated by lyphogel to see if these concentrates would revert from negative to positive CEP on retesting. Only one of the three concentrated sera converted to positive by both CEP procedures; the other two sera remained negative. These results indicated that some of the positive reactions were specific, thus implying that the nonreactivity of unconcentrated sera by CEP was due to the lower sensitivity of this procedure as compared to that of RIA. The second approach consisted of testing double serial dilutions of five sera of known complement-fixing titer by the two CEP methods and by RIA. AB serum known to be negative by Spectra's and Ortho's CEP methods and by RIA was used as a diluent (7). These sera had been frozen at −20°C for variable time periods up to several months. The results (Table 9) indicate that Ortho's CEP and Abbott's RIA are two-to-four times and 16-to-32 times as sensitive as Spectra's CEP, respectively. These findings are in disagreement with those reported by others who showed that RIA is 100-to-1000-fold more concentrative than CEP (5, 14, 24). The lower comparative sensitivity in this study may be due to the shorter incubation period.

TABLE 7

COMPARATIVE SENSITIVITY OF THE RIA WITH CEP, CF AND RPHA PROCEDURES FOR DETECTION OF HB AG (204 samples*)

CEP Methods: A = Spectra B = Hyland C = Ortho D = Pfizer E = Abbott F = Squibb

CEP System A	B	C	D	E	F	CF Test	RPHA Test	RIA Test	Results
19	17	22	15	15	18	28	28	38	Positive
3	5	5	7	6	4	—	—	—	W** Positive
22	22	27	22	21	22	28	28	38	Total Positive
1	2	0	4	0	0	—	—	—	Nonspecific
1	1	1	0	0	0	0	1	1	Positive by one method only

Proportion positive by one or more methods: 41/204

* From a mixed population: "normal" hospital employees and patients suspected of having hepatitis.
** Weakly

No correlation was found among CF, CEP and RIA titers. For example, serum #5 with a CF titer of 1:8,192 had an RIA titer only one dilution higher than that of serum #1 which had a CF titer of 1:128. Environmental factors, such as temperature and duration of storage as well as differences in handling of sera, might have been responsible for some of these changes. A second study was then conducted in which five freshly drawn samples (three known positive and two known negative) were tested in a similar fashion. The CF titer of the three positive sera was determined (Table 10).

Unfortunately, the RIA results were erratic and difficult to explain. For example, positive serum #2

TABLE 8

COMPARISON OF RESULTS OBTAINED BY RIA AND ORTHO AND SPECTRA CEP METHODS (50 samples)

No. of Tests	% of Tests	RIA	Ortho CEP	Spectra CEP
41	82	0	0	0
4	8	+	+	+
3	6	+	+	0
2	4	+	0	0
% Total	100	18	14	8

reacted first, at dilutions 1:64 and 1:128. Negative results followed for seven serial dilutions to appear positive again at a dilution of 1:32,768. Analogous results were obtained with the presumably negative sera #4 and #5. Serum #4 was negative when tested, undiluted and at 1:2 and 1:8 dilutions, but it was positive at 1:4 and 1:16. Serum #5 reacted at a 1:4 dilution but was negative undiluted and when tested at 1:2, 1:8 and 1:16.

It has been reported that when normal human sera are used as diluent they may enhance reactivity of positive sera (6). This, however, does not explain the erratic behavior of the RIA test in this series. A technical error cannot be excluded entirely as the possible cause and would suggest that this technique may be too exacting to be used as a screening procedure. This could conceivably be responsible for at least some of the false positive reactions described in the last series.

Since not all RIA positive–CEP negative sera became positive after concentration and in view of the erratic results obtained in the last series, it is impossible to ascertain the specificity of all RIA positive reactions. This cautious statement is consistent with a recent report which described the occurrence of false positive reactions using Abbott's RIA technique. It was demonstrated that reactivity of an antibody present in human serum directed against a guinea pig protein was responsible (2).

TABLE 9

TITER OF FIVE HB AG POSITIVE SERA* BY CEP (SPECTRA & ORTHO) AND BY RIA

Serum Dilution	Method 1 CEP A	Method 1 CEP C	Method 1 RIA	Method 2 CEP A	Method 2 CEP C	Method 2 RIA	Method 3 CEP A	Method 3 CEP C	Method 3 RIA	Method 4 CEP A	Method 4 CEP C	Method 4 RIA	Method 5 CEP A	Method 5 CEP C	Method 5 RIA
Undiluted	+	+	ND**	+	+	ND	+	+	ND	+	+	ND	+	+	ND
1:2	+	+	ND	+	+	ND	+	+	ND	+	+	ND	+	+	ND
1:4	+	+	ND	+	+	ND	W+	+	+	+	+	ND	+	+	ND
1:8	+	+	ND	+	+	ND	−	+	+	+	+	ND	+	+	ND
1:16	+	+	+	W+***	+	+	−	−	+	+	+	ND	+	+	ND
1:32	−	+	+	−	+	+	−	−	+	+	+	ND	+	+	ND
1:64	−	+	+	−	−	+	−	−	+	+	+	ND	W+	+	+
Undiluted	−	−	+	−	−	+	−	−	+	W+	+	+	−	+	+
1:256	−	−	+	−	−	+	−	−	−	−	+	+	−	−	+
1:512	−	−	+	−	−	−	−	−	−	−	−	+	−	−	+
1:1024	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+
1:2048										−	−	+	−	−	+
1:4096										−	−	−	−	−	−
1:8192										−	−	−	−	−	−
1:16384										−	−	−	−	−	−
1:32,768										−	−	−	−	−	−
CF Titer	1:128			1:512			1:512			1:8192			1:8192		

* The five sera in this experiment have been frozen for various time periods at −20°C. The above CF titers were those obtained on the fresh samples prior to freezing.

** ND = not done.

*** W+ = weakly positive.

TABLE 10

TITERS OF FIVE FRESH HB AG POSITIVE SERA BY CEP (SPECTRA & ORTHO) AND BY RIA

Serum Dilution	Serum 1 CEP A	Serum 1 CEP C	Serum 1 RIA	Serum 2 CEP A	Serum 2 CEP C	Serum 2 RIA	Serum 3 CEP A	Serum 3 CEP C	Serum 3 RIA	Serum 4 CEP A	Serum 4 CEP C	Serum 4 RIA	Serum 5 CEP A	Serum 5 CEP C	Serum 5 RIA
Undiluted		+	ND*	+	+	ND	+	+	ND	−	−	−	−	−	−
1:2	+	+	ND	+	+	ND	+	+	ND	−	−	−	−	−	−
1:4	+	+	ND	+	+	ND	+	+	ND	−	−	+	−	−	+
1:8	W+**	+	ND	+	+	ND	+	+	ND	−	−	−	−	−	−
1:16	−	+	+	+	+	ND	W+	+	+	−	−	+	−	−	−
1:32	−	−	+	+	+	ND	−	+	+						
1:64	−	−	+	W+	+	+	−	+	+						
1:128	−	−	+	W+	+	+	−	−	+						
1:256	−	−	+	−	−	−	−	−	+						
1:512	−	−	−	−	−	−	−	−	+						
1:1024	−	−	−	−	−	−	−	−	+						
1:2048				−	−	−	−	−	+						
1:4096				−	−	−	−	−	−						
1:8192				−	−	−	−	−	−						
1:16384				−	−	−	−	−	−						
1:32,768				−	−	+	−	−	−						
CF Titer	1:128			1:512			1:512			2			2		

* ND = not done.
** W+ = weakly positive.

Until further tests can be performed to demonstrate the presence of HB Ag in the RIA positive–CEP negative sera by other methods designed to eliminate the human antiguinea pig protein interference, the positivity of these sera must remain suspect.

In conclusion, this study supports the claim that the RIA is the most sensitive of all assay methods for detection of HB Ag in use today. Because of the inability to verify the presence of HB Ag in those sera positive by RIA alone, however, this claim must be taken only at face value. Further studies are needed to confirm the specificity of these positive reactions.

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Quality Control in the Office Laboratory*

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"The poorest of all laboratory work is that done in the private physician's office." That allegation was made by the Director of the New York City Department of Health Bureau of Laboratories in a statement to a Senate Committee of the Judiciary Committee on Antitrust and Monopoly on February 7, 1967 (1). Statements such as this typify the public relations problem facing directors of office laboratories today. The problem is twofold: First, how can the quality of the office laboratory be assured and documented? Second, how can this information be disseminated to legislators and the general public? The first is an easily solved technical problem toward which this paper is directed. The second and probably the most difficult is a media problem beyond the scope of a technical journal.

Laboratory quality assurance and documentation are best considered as a subset of the more general field of quality control, an industrial discipline with its roots in the industrial revolution. Industrial quality control is the technique, for instance, whereby the automotive industry may precisely design obsolescence into an automobile without impinging on the warranty period in order to maximize profits. Quality control in the drug industry assures not only that the amount of drug in a pill falls within acceptable tolerances but that the pill itself is not malformed. A malformed pill results in very bad public relations for the drug company, even if its function is not impaired. The pigeon, an animal with a very fine eye for detail, has been used successfully for assembly line quality assurance in removing faulty pills prior to packaging (2). Laboratory medicine also produces a product which can be subjected to quality control, the laboratory result.

Error Frequency Distributions. Any discussion of laboratory quality control should begin with a brief account of practical statistics, since statistics is the science whereby a decision can be made as to whether a laboratory result is acceptable or unacceptable. An introduction to these statistics should begin with the error frequency distribution.

If a unit of outdated blood bank blood is mixed well and dispensed into 100 test tubes, this set of tubes is called a "pool." The plasma glucose content of any one of these tubes is the same as that of any other, since all tubes came from the same bag of blood. If one attempts to assay each of those 100 tubes of blood for glucose, however, the assay results will not be identical for all tubes. Some will be above an average value and some below, as shown in the histogram in figure 1. A few results will deviate markedly from the average value but most will cluster around the average. The smooth curve (sometimes called Gaussian or bell-shaped) drawn through the steps of the histogram is called a frequency distribution plot, and it can be used to make a decision as to when a laboratory result deviates too far from the average value of the pool. If one calculates a so-called standard deviation (SD), one can define location of the cutoff point between acceptable and unacceptable results. The use of the standard deviation has two advantages over a single intuitive guess about where the line between good and bad data lies. First, use of the standard deviation (actually $\pm$ 2SD) as a decision point guarantees that 95% of the results will be in the acceptable range on the average. Second, standard deviation is used by most practitioners of laboratory medicine and is understood by them, thereby providing a common ground for discussion. It might be mentioned that the frequency distribution plot shown in figure 1 is a real life situation. That is, it is technically impossible to get

* Presented by Dr. Riddick at the 44th Annual McGuire Lecture Series, March 22, 1973, at the Medical College of Virginia, Richmond.

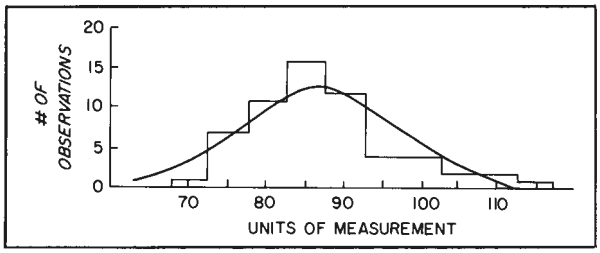

Fig. 1—Frequency histogram of Gaussian distribution with overlying frequency distribution plot. Note that the majority of values cluster around the peak of the curve.

identical results for the plasma glucose in each of those 100 tubes. One can use more costly and difficult methods and the range on the horizontal axis will narrow, but the results cannot be made identical. This means that there is no "true" value for the glucose in the plasma. Analytical balances used for weighing the glucose also have error frequency distributions.

Frequency Distributions of Normal Patient Laboratory Determinations. Figure 2 shows the results obtained for blood urea nitrogen determined on each of 495 "healthy" patients plotted in the same fashion as the pool results in figure 1. As might be expected, most of the results cluster around an average value with a few markedly deviant values to the left and right of center. Notice that the frequency distribution plot is skewed with more deviant values to the right (occult disease?) than to the left. This is because there is no such thing as a negative blood urea nitrogen, so the curve is steeper on the left than on the right.

Now, suppose one took a large number of patients with renal disease, drew their blood, performed a blood urea nitrogen on them, and superimposed their frequency distribution plot on an idealized plot of the frequency distribution of urea nitrogen of "normal" patients (fig. 3). How might one define the upper limits of "normal" using this data? One might draw a vertical line at the lowest point between the normal peak and the sick peak and call this the upper limit of normal. It is obvious, however, that the two curves overlap and in the areas of overlap, some normal patients will be called sick when they are not, and some sick patients will be called normal when they are not. Anyone experienced with the SMA 12–60 has seen elevated uric acids in perfectly normal people who never develop gout. To further complicate things, a large laboratory error component will tend to broaden this range of ambiguity to an even greater extent.

Effect of Methodology on Normal Ranges. Figure 4 shows frequency distribution plots of two different assay methods for blood glucose—the glucose oxidase and the ferricyanide methods. The glucose oxidase method is a so-called "true glucose" method and produces comparatively low results, partly because it is subject to inhibition by some patients' blood. The classical ferricyanide glucose method, on the other hand, produces comparatively high results because it measures not only true glucose but also other reducing substances such as fructose and glyceraldehyde. From the point of view of the patient and his physician, the important thing is the normal range. The glucose oxidase blood glucose normal range is 10–20 mg percent lower than that of the ferricyanide method. If one used both methods

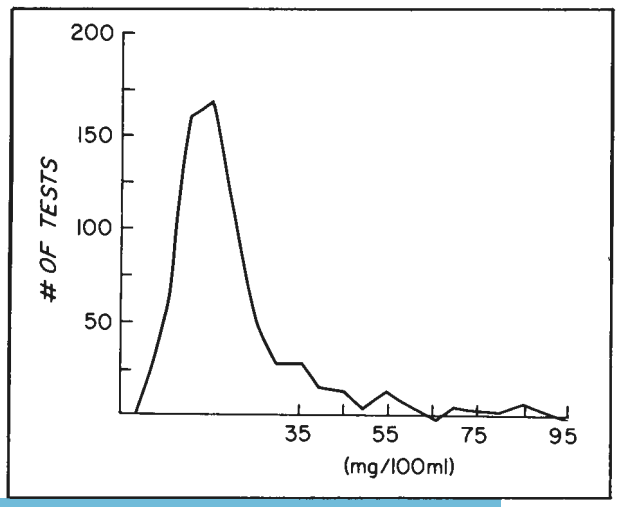

Fig. 2—Urea nitrogen frequency distribution plot. Notice that there are far more deviant results on the high side of the curve than on the low side; that is, the curve is skewed.

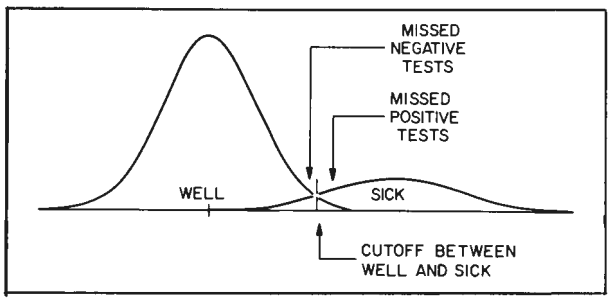

Fig. 3—Superimposed frequency distribution plots of normal and abnormal urea nitrogen determinations. Note the area of ambiguity or overlap between the sick and the well person.

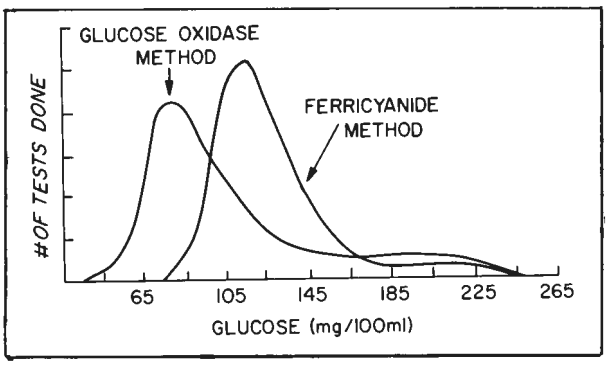

Fig. 4—Frequency distribution plots for two different blood glucose methods. Note that the normal range is different for each.

simultaneously, one would widen the normal range as shown in the composite curve in figure 4 and a substantial increase in false negative blood glucose results would occur. This could easily happen if two different kits using different methodology were used interchangeably. The result would be a component of laboratory error which would widen considerably the range of ambiguity of the blood glucose data.

Effect of Methodology on "Biologic Variation." Figure 5 is a time-course plot of the blood sodium level of the author drawn over a period of a month. The upper curve (JHR) represents the human blood; the two lower curves (Control 1 and Control 2) are two commercial pools assayed simultaneously with the human blood. Had there been no laboratory error, the lower plots (Control 1 and Control 2) would be perfectly straight lines parallel to the horizontal axis. Note that the two curves are sawtooth instead, describing day-to-day laboratory error. Because of within-day random error, or perhaps because of pipetting error they are not exactly parallel to one another. The most striking feature of figure 5 is that the human serum drawn on the same day shows the same pattern of variation. The implication is that a substantial component of so-called "biologic variation" for serum sodium actually is laboratory error. Carefully controlled studies such as this, in fact, indicate that the flame photometer is incapable of measuring the subtle changes of biologic variation and that, in fact, all variations measured in normal human blood sodium are actually laboratory error not biologic variation. Many other blood components, potassium, for example, show similar effects of day-to-day laboratory error. If one could reduce the day-to-day error component in measuring blood sodium, one might be able to pick up more subtle changes, the normal range would undoubtedly shrink and fewer false negative and false positive results would occur. The cost of increased accuracy and precision, however, is prohibitive at present.

Setting of Control Limits and the Effect on Patient Care. Figure 6 is a graphic example of the effect of control limits set too wide in the laboratory. Bilirubin is traditionally a poor test from an accuracy standpoint because of the difficulty of maintaining adequate standards which will not deteriorate. The error shown in figure 6, however, is caused by an improperly calibrated reference serum used to calibrate the SMA 12–60 bilirubin. On day 20, the laboratory used the last of a particular manufacturer's reference serum and began to use a new lot from a different manufacturer. The effect was an abrupt increase in every normal patient's bilirubin of 0.4 mg percent and an increase in the bilirubin of the patients in the moderately elevated range of 0.7 mg percent. Obviously the normal range was widened by this change. Tighter control limits would have detected this change sooner. Furthermore, an alert visual scan by a physician of the patient results for the day would have proven to be an extremely useful form of quality control. It was concluded that one could not rely upon the manufacturer's brochure provided with the lots of reference serum.

Some Simple Techniques for Quality Control. One of the oldest forms of laboratory quality control is the "repeat." If one doubts the validity of the first result, send another one and compare the two.

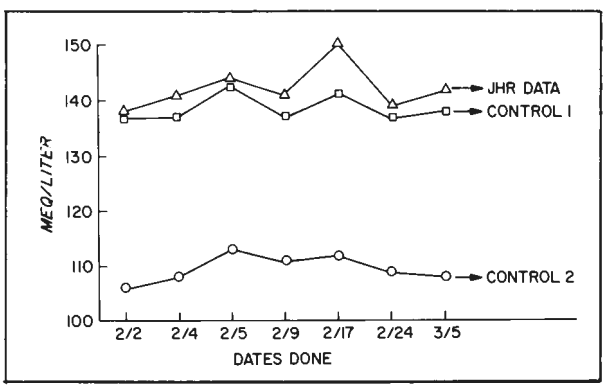

Fig. 5—Time course plot of the blood sodium level of the author drawn over a period of 30 days. The upper curve represents the human blood (JHR). The lower curves (Control 1 and Control 2) represent pooled control sera. The parallel variation indicates that the greater part of the "diurnel variation" of the human sodium actually represents analytical variation.

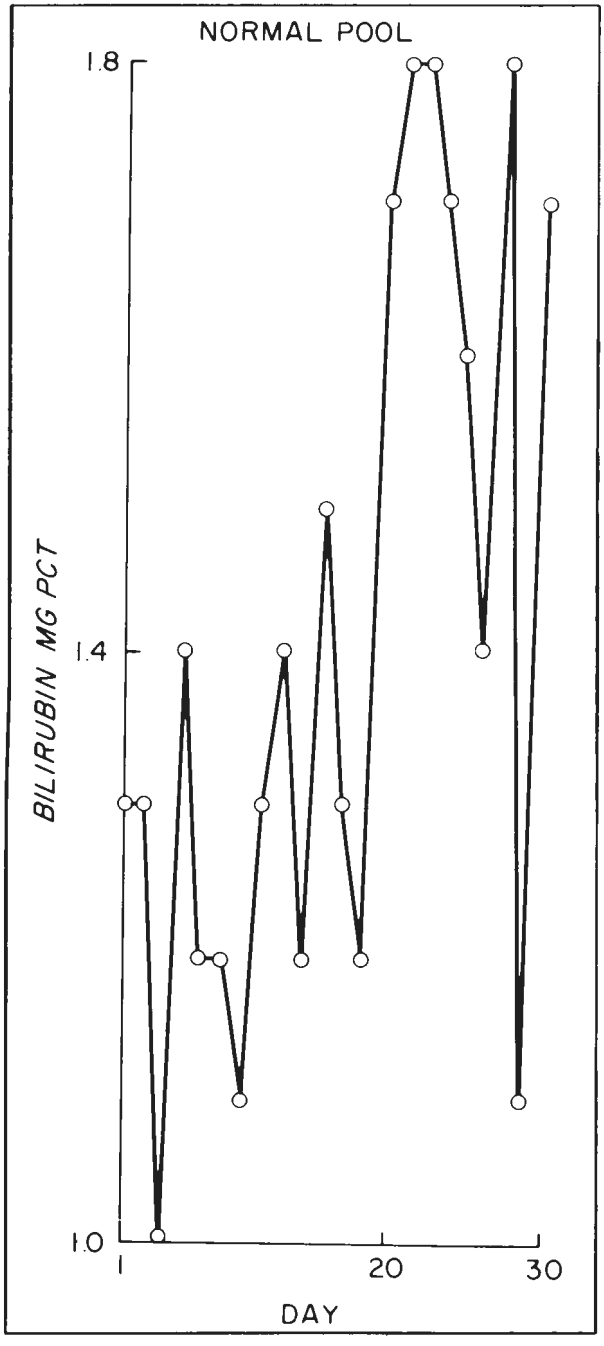

Fig. 6—The effect of a change of control pool manufacturer on day 20. Notice the striking change in levels of bilirubin for the control pool.

This is of no use, however, if a very poor laboratory is very good at reproducing its bad results on repeats.

The split sample is a slightly more sophisticated form of the repeat. In this case, the original sample is mixed well and divided into two parts. A phony name is attached to one specimen and the second member is submitted to the laboratory sometime later. This technique is designed to foil the laboratorian who claims that "biologic variation" caused the difference in the two results. A rule of thumb for the split sample is that if the results of the two split samples vary by more than 10% the results are suspect. This figure varies from test to test. The split sample is also useful in those tests for which reference standards are not yet available such as urinalysis, bacteriology and even some coagulation studies.

Another more complex method of quality control is the mixed specimen. This works best with blood chemistries. If you mix known amounts of each of two patient specimens, the results of all components (excluding enzymes) will be proportional to the original concentrations and volumes mixed. The calculation for the predicted results is fairly straightforward.

Most people rapidly tire of the exercise of preparing their own quality control samples and purchase these from some national program such as the Proficiency Evaluation Program (PEP) for the Physician's Office Laboratory.

Proficiency Evaluation Program for the Physician's Office Laboratory. Beginning in April, 1973, the College of American Pathologists and the American Society of Internal Medicine are jointly sponsoring a quality control program designed specifically for the physician's office laboratory. The data obtained are treated in an entirely confidential manner and meet the demands of public health agencies, legislators, professional associations and patients for a third party evaluation of the office laboratory. Quarterly kits are mailed out to subscribers containing seven vials of test unknowns and a blood smear. These vials contain material for evaluating hemoglobin, red cell counts, hematocrit, glucose, bilirubin, cholesterol, urea, uric acid, urine specific gravity, urine protein, urine reducing substance, urine bilirubin, urine hemoglobin, urine pH, prothrombin time, white cell count, urine bacteriology and peripheral smear. One hundred tests per year are performed on a quarterly basis and mailed into the testing agency for evaluation. Four weeks after receipt, results from all subscribing laboratories are pooled, processed by computer, and a computer print out is mailed to all subscribers giving name of constituent, method used, subscriber's result pass or

fail code, average of all laboratories for comparison, a good performance range and an acceptable performance range. In addition, a quarterly bulletin is sent to all subscribers, describing findings and including a personalized certificate for wall-mounting.

The advantages of such a system are apparent. First, the director gets a confidential warning that his laboratory is having difficulty, thus giving him the opportunity to correct the difficulty. Comparison of results with peers is always informative as to the wide interlaboratory differences. The effort expended is minimal and does not disrupt the normal function of the laboratory and the cost is far less than manual preparation of samples and data collection.

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Commercial and Reference Laboratories*

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It occurred to me in thinking over the area to be covered that the title, although in general use today, refers to generic terminology. I thought it might be wise to define the two terms in relationship to the term "laboratory." Webster defines "commercial" for our purposes as "having financial profit as the primary aim" and "reference" as "any person or thing referred to for information, recommendations, etc." In my own mind in the strictest sense of the word, a reference laboratory is one where materials tested by stringent techniques are forwarded to participant laboratories throughout the country. These tests are analyzed and the results returned to the reference laboratory for comparison with their results. These results are then made available to the participants to compare with their own and others in the program; in short, a quality control program. The two best examples I have are the College of American Pathologists' Proficiency Testing Service[1] and Dr. F. William Sunderman.[2] A step further down the line is a combination of commercial and reference laboratories. Here, exotic tests or tests not ordinarily performed in a laboratory are referred to a laboratory for testing. This, of course, is done for a profit. The third step is the situation where all types of tests referred to a laboratory are performed for a profit. A good example of the commercial-reference laboratory is Bio-Science Laboratories in California. The third step involves a myriad of laboratories—state, interstate, and national—with which by now all physicians are familiar.

A physician who is practicing away from an area where diagnostic procedures are immediately available, and even in areas where good laboratory medicine is practiced, has to decide what laboratory or laboratories he will use. What steps should be taken to make certain of good quality service from the many laboratories that are at his disposal? The first thing to remember is that the performance of any medical laboratory test is not a simple procedure. It is a technique of medical practice which should be as important to the physician as the delivery of a baby is to the obstetrician. The determination of a simple hemoglobin can present many difficulties which can be compounded by time and delay of transportation. A physician must examine his conscience and determine firstly that he must demand quality to ensure good patient care. Once this has been settled in the individual's mind, he should analyze the speed of performance, the quality of performance, the amount of quality control, how the laboratory is operated and equally important, the cost to the patient.

The speed of performance in some instances is important but in other areas need not be a factor. In office practice where a patient will not return to the office for ten to fourteen days, practically all laboratories will have returned the test results. This is even true of some of the more exotic tests such as aldosterone, estrogen levels, etc. In Virginia at the present time, a number of laboratories are performing daily pickup service, with the return results reported on routine procedures the following day. This might pose some minor delays in rural areas but speed must not overshadow quality of work performed.

Quality and quality control are tied so closely together that they can be discussed together. A laboratory performing good quality laboratory medicine should provide you with well-trained technical

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* Presented by Dr. Carroll at the 44th Annual McGuire Lecture Series, March 22, 1973, at the Medical College of Virginia, Richmond.

[1] The College of American Pathologists Proficiency Testing Service, 230 North Michigan Avenue, Chicago, Illinois 60601.

[2] Dr. F. William Sunderman, Proficiency Test Service, 1833 Delancey Place, Philadelphia, Pennsylvania 19103.

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personnel who are under well-trained supervisors directing and reviewing the test procedures being performed. Some laboratories operate on a twenty-four-hour basis with night-shift personnel who may not be adequately trained or if they are, are working a second shift and so will not be working at peak efficiency. Quality control charts of the laboratory operator should be made available to you at regular intervals to assure you that tests are performing within acceptable standard deviation variations (usually not over 2 SD from mean).

I wish to point out at this time why results from a commercial laboratory may differ from your hospital laboratory. The point to emphasize is that different methodologies between one automated piece of equipment and another, and between automated equipment and standard wet-bench-chemistry methods may have different normal values. This has caused considerable furor in laboratory circles but is a fact of life that must be accepted. In addition, one should remember that enzyme tests, CPK, SCOT, LDH, are very difficult to reproduce on automated equipment. Of these three, the CPK is the most difficult to reproduce in terms of uniform levels within a given laboratory and between different laboratories. This is especially true of the ultraviolet light (UV) determinations of CPK with conversion to international units.

The delivery of good quality and good quality care are dependent on how the laboratory is operated. The question to be raised here is: "What are the professional qualifications of the people you select to do your laboratory diagnosis?" Is the head of the laboratory one who has been trained in laboratory medicine, a pathologist or otherwise, and most important is this person a physician? It is quite important that you as a physician know the director, his background and education, his staff and the facilities from which the laboratory operates. This is coming under much better control today at both the federal and state levels. I feel that knowing your consultant in laboratory medicine is just as important as knowing other physicians with whom you consult. This, of course, is not always possible but the closer to home the commercial laboratory you use, the closer the contact and knowledge you can have of its operation and staffing. There are many companies sponsoring commercial laboratories today. All of the larger companies, such as Dow Chemical, Damon, Smith, Kline and French and Upjohn have a pathologist as director. All of these you can be assured are turning out quality assurance work. There are others in this vicinity, pathologist directed, with excellent staffing, not associated with a major company that are also turning out quality work with good quality control.

One of the problems with the number of commercial laboratories in this country has led to some of the other smaller units bringing to light the "symptom of what is going on in many practices." Competition has made some of the laboratories lower the quality of work to stay in business, and in some instances has caused price reductions at the cost of good quality assurance. This seems to occur because some physicians care very little about the quality of the tests performed on their patients, but prefer more tests for the cheapest price. In the final analysis, the cost to the patient remains a major factor. Here also have arisen many of the problems to which we as physicians must direct ourselves. I think it ridiculous for a physician to change laboratories in order to have a test done for twenty-five cents less. This has actually occurred recently here in Richmond. Whether there was a difference of quality one can only speculate. The bigger problem which we as physicians must face is that in using any laboratory, the price to the patient must be fair for the work rendered. It is ironic to realize that most physicians are marking-up tests done by outside laboratories by three-to-four to even ten times the amount charged by the laboratory. This has caused an understandably adverse reaction by the American Medical Association and the Blue Plans. This has also stirred reaction in our own state and has caused action by the Medical Society of Virginia. It is apparent from many studies that some differential is needed between hospital and doctor's office charges such as is now in effect in California. My point in raising this issue is that as physicians, the high volume, low cost of tests performed by commercial laboratories should still carry only a reasonable increase to the patient and not the marked increase which is much more prevalent than many physicians admit.

I have not covered the area of advertising in the material presented here, because there is mixed emotion by even those within the commercial laboratory field as to how this can best be accomplished. I would be wary of the laboratory which advertises by price cutting, for prices are now close to an all time low level. I would insist on quality of work with evidence of quality control. I might point out here that one of the problems in mailing long dis-

tance is that of deterioration and hemolysis of specimens. I know of one instance where material was sent to a western laboratory for platelet counts and where an observer noted all specimens which were being counted by phase were hemolyzed. I am sure the referring physicians were unaware of the method used and that using any method platelets will have disintegrated within six-to-eight hours. Such specimens reaching the west coast from nearly anywhere would be useless for evaluation of platelets. This should reemphasize the need for knowing your referring laboratory and its staff.

Remember that the cost to the patient is the important factor and the price passed on to him should be a reasonable one and in line with the cost for operating your practice. As I said earlier, every doctor today, in the face of mounting pressures, must examine his conscience and determine what quality he will insist on for good patient care.

In summary, there are now in existence many good commercial and reference laboratories. I would use those laboratories with which you are most familiar, where you know the staff, those which can demonstrate reasonable speed in testing and can assure good quality testing and control at what you feel is a reasonable cost to the patient.

Evaluation of Prepackaged "Kits"*

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The proliferation of commercially-available "kits" for the clinical laboratory has resulted in such a technological surfeit that one or more kits are available for virtually every type of commonly performed test. Such kits offer prepackaged convenience and, under some circumstances, economies in the laboratory. A few offer technical procedures which are superior to standard methods.

In spite of their apparent simplicity, commercial availability of a reagent kit does not provide any assurance that it will perform satisfactorily or that the resulting data will be accurate. In contrast to therapeutic agents, federal regulation of the manufacture of diagnostic kits has only recently been instituted, and it remains for the user to determine whether a particular kit does in fact meet the specifications stated in promotional material. The selection and the continuing evaluation of diagnostic kits present problems to every laboratory, whether the laboratory is in the physician's office or in a large hospital.

Perhaps the simplest type of kit is a prepared reagent for a certain determination. This category would include specific antisera for blood grouping or other purposes, as well as standard chemical reagents. Usually, however, the word "kit" is used to describe a prepackaged set of multiple reagents for carrying out a certain test in the laboratory. Many such multiple-reagent kits are based upon standard, accepted methods; those from reliable manufacturers offer the advantages of standard methodology and elimination of reagent preparation. Some kits are based upon manufacturer-developed methods which are usually patented or kept as proprietary secrets. Some of these manufacturer-developed methods are acceptable; some, no doubt, have been developed primarily to permit a wider profit margin. Lastly, several well-known firms are marketing kits which are suitable only for their own analytical instruments. This presents a double dilemma for the purchaser, since it is necessary not only to evaluate the kit but the instrument as well.

Selection of Kits. Most physicians are deluged with flyers and advertisements which suggest that the purchase of a few kits is an efficient and entirely satisfactory method of installing an instant clinical laboratory. These blandishments frequently lead to an illogical and expensive system for providing laboratory data. The first question to be asked is not which kits to buy but rather which determinations should be done in the laboratory. If a test is definitely needed, purchase of a kit is one of the alternative methods for making it available. Particularly in the physician's office, the convenience, the elimination of reagent preparation and savings in personnel time are advantageous. Use of a kit usually results in a higher per-test cost than having the test done in a large, automated laboratory, but the advantages of using kits sometimes outweigh these higher costs. Kits from different manufacturers will offer different analytical methods, differing numbers of tests per kit, different instrument requirements and, of course, different prices.

An important principle in selecting any type of laboratory kit is to require the manufacturer to provide relevant experimental data which substantiate any claims regarding performance of the kit. Descriptions such as accurate, precise, simple, inexpensive and reliable are all relative. Unless the manufacturer can produce data, preferably substantiated by an independent investigator, regarding these parameters of performance, further consideration of using the kit should not be entertained. Most reputable manufacturers will supply reprints of articles describing such evaluations.

Evaluation of Kits. If a laboratory is to produce

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reliable data, a critical evaluation of each method must be carried out prior to its introduction as a routine procedure. It is immaterial whether or not the procedure utilizes a prepackaged kit. Such an evaluation should make full use of any data collected by other laboratories, but an experimental evaluation by the laboratory which is to use the kit is essential.

Although kits come in various forms, there are two major categories of laboratory tests for which they will be used—qualitative tests and quantitative tests. Qualitative tests are those for which the results can be expressed as a yes or no, positive or negative or present or absent report. Pregnancy tests and tests for urinary glucose are examples of qualitative tests. Quantitative tests are those which are used for measurement of a specific constituent and results are expressed in numerical terms. The evaluation of a kit will differ depending on whether it is used for a qualitative or quantitative procedure.

Comparison with Reference Method. Evaluation, no matter what is being evaluated, is a comparative process. If a kit merely supplies reagents necessary to do a standard laboratory test, data on the standard test are readily available in published form. More often, the kit will be a modification of a standard method or, occasionally, a new approach to measuring the same constituent. In either situation, the manufacturer should provide experimental data which compare the kit procedure to an accepted, established method.

For quantitative tests, the parameters which should be examined are accuracy, precision and range of linearity over which measurements can be made. Accuracy is an elusive parameter to evaluate, and a comparison of results using the kit with those obtained by a standard method is acceptable. Precision can be evaluated by replicate determinations and linearity by measuring a series of samples with varying concentrations of the constituent being measured. An excellent example of such a comparison is the study by Barnett, Cash and Junghans (2) in which cholesterol measurements using 12 different kits were compared to those using the Abell-Kendall method. They concluded that only two of the 12 kits being marketed at the time of the study were acceptable for clinical use. Other published evaluations should be equally disconcerting to any laboratory which uses a kit without first subjecting it to rigorous performance trials. Kim, Waddell and Logan (6) measured sodium and potassium with chemical kits manufactured by the Stanbio Laboratory, San Antonio, Texas and by Medi-Chem, Santa Monica, California. Results were compared with those obtained by standard flame photometric techniques, and the authors concluded that both kits gave "diagnostically unsatisfactory results." A study by Dietz, Rubenstein and Lubrano (5) which involved serum cholinesterase measurements using the Acholest® kit manufactured by E. Fougera and Co., Hicksville, New York, provides a shocking indictment of the lax standards set by some commercial firms. The Acholest® method "failed to detect 12 of 20 cases at high risk of prolonged apnea after succinylcholine." Similar comparative studies of kits for less complicated procedures such as glucose and urea indicate that some are entirely suitable for these measurements (7, 9, 11). It is noteworthy that Logan, Waddell and Krynski (9) found that the kits which were the most expensive and which revealed the least information concerning their constitution gave the poorest performances.

The evaluation of comparative data for qualitative tests frequently is more difficult than for quantitative tests. The two parameters corresponding to accuracy and precision are validity and reproducibility. Reproducibility can be studied through replicate tests on the same group of samples; validity, however, like accuracy, may have to be evaluated by comparison with a reference method. The objective for all qualitative tests is a positive result when the constituent or disease is present and a negative result when it is not. For example, serological tests for syphilis are usually compared to the fluorescent treponemal antibody-absorbed (FTA-ABS) test. Since the serological test is used primarily as a screening procedure, acceptable performance would result in no false negatives and as few false positives as possible. Frequently, the evaluation must include consideration of the clinical context in which the test will be used. Tests for pregnancy are usually evaluated by testing large numbers of pregnant and nonpregnant women. A test which gave positive results in 97% of women in the second trimester of pregnancy clearly would not be as useful as one which gave similar accuracy during the first three weeks. The undesirability of false positive pregnancy tests is readily apparent.

In addition to comparisons with reference methods, information provided by the manufacturer should include predisposing test conditions or patient abnormalities which will affect the test and give inaccurate or undependable results. Such interfering

conditions are particularly troublesome with newly developed tests such as radioimmunoassays. Tests for digoxin, for example, may measure not only digoxin but also its metabolites (10). More worrisome, however, are reports that therapeutically administered compounds such as cortisol and spironolactone may react with the digoxin-binding antibody to give erroneous results.

Laboratory Trials. Although few laboratories will carry out an evaluation of a kit as elaborate as those which are described in scientific journals, it is falacious to assume that results similar to those in published articles can be obtained automatically by any kit purchaser. The procedures for experimental evaluation may vary from kit to kit, but critical testing of every type of kit by the laboratory in which it is to be used is essential.

Meticulous examination of the instructions which accompany the kit will frequently eliminate unnecessary work. The instructions should present a logical and detailed outline of each step in the procedure, with a clear indication of where errors might occur, what types of instruments are suitable, in which steps timing is critical and how results are to be calculated from instrument readings. The instructions should be followed compulsively under all circumstances. Suitable standards should be included with all kits for quantitative measurements and the standards should have concentrations which span the range of clinically useful measurements. Controls should be run with each batch of any procedure, whether it is a quantitative or a qualitative one and, if possible, control solutions should be obtained from a manufacturer other than the supplier of the kit. Qualitative tests generally should have both a positive and a negative control; these usually accompany the kit, however, since they may be the only suitable controls available.

Replicate determinations on different days of one, or preferably several, control solutions provides an indication of the precision which might be expected. If reproducibility is unsatisfactory, the problem may reside either in the kit or in the technique. If errors can be traced to faulty technique which is corrected, the experiments should be repeated; if not, the kit should not be accepted for routine use in the laboratory. A second useful step in evaluating a kit is separation of patient samples into two aliquots, one to be run by the kit method and the other to be submitted to a reference laboratory. A minimum of a dozen, and preferably several times that number, split samples should be analyzed before acceptance of the kit for routine use. Many manufacturers will supply free samples of kits for preliminary evaluation; this practice, however, should have no influence on the laboratory in regard to which kits are tested and which are finally selected for routine use.

Evaluation of an instrument which is designed for use with kits for a variety of procedures may be quite time-consuming, since each procedure for which a kit is available must be evaluated separately. Logan and Sunderland (8) evaluated the Unitest System® marketed by Bio-Dynamics, Inc., Indianapolis, Indiana, and Diagnotest® marketed by Dow Chemical Company, Diagnostic Products Division, Indianapolis, Indiana. For each instrument, some of the kits performed unsatisfactorily, and the authors concluded that personnel without technical training could not obtain reliable data with either system.

Continuing Evaluation. Initial evaluation and acceptance of a kit for routine use in the laboratory does not assure continuing satisfactory performance. Most important, the use of kits does not obviate the need for strict quality control measures. Suitable quality control solutions should be run with each batch of procedures, strict limits for variation of the control solution should be set and all data should be rejected if control readings are out of the predetermined range. Lot numbers of each reagent or kit should be entered into the laboratory log book and when a new lot number is used, samples should be run in duplicate with the old and the new reagents to permit comparison between lots.

FDA Regulations Regarding Kits. It perhaps should be stressed that most of the laboratory kits on the market today were developed during a period when there were no federal regulations setting minimum performance standards. As early as 1966 the American Association of Clinical Chemists (1) published policies regarding reagent sets and kits which, had they been followed by all manufacturers, might have greatly reduced the number of subsequent articles devoted to documenting the inadequacies of many kits. Manufacturers also could voluntarily submit kits to the College of American Pathologists for evaluation; however, compliance with the recommendations of the college in the case of inadequate kits was also voluntary. Published evaluations of all types of kits clearly indicate that some are unsatisfactory, some are satisfactory and some are outstanding in meeting performance standards.

In January 1972, the Food and Drug Administration announced that existing legislation gave them authority to exercise regulatory control over diagnostic kits to ensure that they deliver a consistently high level of quality and performance (3). A statement of procedures for developing policy and interpretive regulations was published in August 1972 (4). Briefly, these regulations require that diagnostic kits be accurate and reliable, that manufacturers test and evaluate kits prior to marketing them and verify results against a generally accepted procedure, that premarket testing is done to find if predisposing patient abnormalities will affect the test and that the labeling of all kits contains adequate directions for use. The labeling directions must include complete information on accuracy, reproducibility and sensitivity performance. The FDA intends to establish standards of performance for each type of laboratory kit and require manufacturers to meet these standards. Establishing pertinent standards will be time consuming (the FDA intends to start with glucose and hemoglobin), but merely requiring manufacturers to provide evidence that kits will perform as claimed will be helpful for anyone who must decide which kit to purchase. The FDA is already enforcing these regulations and has required two manufacturers of pregnancy test kits to recall their products.

Cost Evaluation. The cost of performing laboratory tests is causing increasing concern because of the dramatic increase in the use of laboratory data in patient care and because many of the newer tests are more complicated and thus more expensive. Evaluation of a laboratory procedure should include the cost of doing it. In a physician's office or a small laboratory, such cost accounting can be quite simple, since it is easy to calculate the cost per test done by a kit method and to measure personnel time involved. Since most kits have expiration dates, the cost per test of the kit should take into account the necessity for discarding outdated reagents. Generally speaking the cost of a quantitative measurement carried out by a kit method will be considerably higher than the same test carried out on automated laboratory instruments. On the other hand, even large laboratories use prepared reagents and kits for performing some of the simpler tests and these tests can be performed in the office laboratory at the same or perhaps lower cost than in a large laboratory. Higher costs for performing tests in physicians' offices may be offset by convenience to the patient or the necessity for having data immediately available.

Summary. Prepackaged laboratory kits for performing diagnostic procedures are frequently the most suitable alternative in the selection of laboratory methods, especially in physicians' offices and small laboratories. Because of the previous lack of governmental regulations covering the manufacture of kits, many kits now on the market do not perform adequately and may produce misleading results. Each laboratory must evaluate each type of kit before it is put into routine use. This evaluation should include a review of published experimental data, comparison of results using the kit to results using a reference method and an experimental evaluation of the kit in the laboratory in which it is to be used.

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Unique Characteristics and Implications of Individual Health Profiles*

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During the era of prescientific medicine, people were divided into two categories: those who were sick and those who were not sick. Physicians found it necessary to establish criteria to differentiate the ill and separate them according to symptoms and signs characteristic of classifiable diseases. Because of preoccupation with disease, the concept of the "normal" versus the pathological, as two opposite and definable conditions, was inevitable in the absence of scientific knowledge of human chemistry and physiology. This concept has carried over into the present era of scientific medicine. Growing health awareness and concern and the demand for preventive medicine, however, has sharply focused attention upon the question of normalcy. This vestigial desire for a neat, comfortable definition of "the normal" was initially satisfied by the artifact of utilizing the statistical mean and two standard deviations as descriptive of the "normal" population. As our knowledge of human physiology, chemistry and hematology has enlarged, however, critical analysis of precise measurements has demonstrated that there is no universal "normal" state, nor a "normal" range of values. *Normalcy* is a philosophical and social condition of not being recognized *abnormal*. Scientific definition of "normal" is not only impossible but meaningless. The large heart which is normal for a big man is decidedly abnormal for a small man. The normal erythrocyte count for a native of the high Andes is abnormal for a native of the Virginia tidelands. Furthermore, the body is seldom free of some reaction to bacterial invasion, foreign protein, or localized tissue destruction. Dr. Edmond A. Murphy (Perspect. Biol. Med., 15:566, 1972) asks what reason there is to believe that the notion of normalcy has any useful meaning. He questions the purpose of trying to convert a continuum of graded risk and change into a fictitious dichotomy of the normal and the abnormal.

There is a need, however, to recognize and describe the deterioration of optimum health in the individual person as a basis for rational preventive medicine and predictive diagnosis. The prerequisite is documentation of a reference base of values descriptive of optimum health of each individual.

The International Federation of Clinical Chemistry has established a committee on relative values to recommend definitions and procedures for determining reference values. This committee is agreed that the artificial concept of a normal average and normal range should be avoided and considered impossible to define scientifically. Preference is given the term "reference values," defined as those values of quantities which describe a definite "reference state" of health or disease of an individual or of a group of like people. The reference state may be that of optimum health defined by a given set of limits related to a person's genetic potential, environment and life habits, or of relatively good health, compromised only by stabilized defects which do not shorten survival. Other reference states related to disease are useful in diagnosis and monitoring therapy. Our present interest is focused on the reference state of optimum health.

Only in the past decade have laboratory meth-

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ods and automated instrumentation become precise enough to permit discovery of individual differences in homeostatic control of blood chemistry and physiology and of the consequent documentation of a reference profile of reliable values for each person. Work started at the National Institutes of Health during 1960, which is being continued in our Institute of Health Research,* has demonstrated that chronological profiles of blood chemical, hematological, and certain physiological measurements are unique for each individual and are surprisingly stable over prolonged periods. Such a profile, when established during a time of vigorous health and well-being, becomes a clearly defined and reliable reference base for all future measurements, and more importantly, for the discovery of early abnormal trends in that particular person. We have found that the individual range of variation for each constituent, when corrected for laboratory bias, is quite small, stable and far less than the range of values found to be usual in a large population of "well" people.

Documentation of Individual Reference Profiles. Because test methods are not sufficiently precise, it is necessary to obtain a series of repeated sets of measurements during a period of optimum health for any given person, in order to establish his individual reference values of blood chemistry, hematology and physiology. The series of measurement data permits the determination of the mean and the range of variation of each measurement to best approach the true values. Since it has been clearly demonstrated in our laboratories, and confirmed by others, that the observed range of repeated measurements of blood chemical constituents consists of at least two components, the bias of laboratory manipulations and a physiological variation, all sets of observed measurements should be corrected for the analytical bias. Physiological shifts are characteristic of the person being tested. Laboratory manipulations, instruments and conditions cause analytical bias. For most blood chemical tests, this bias is at least as large as, and for some tests substantially larger than, the physiological variation manifested over periods of weeks or months. This is illustrated in figure 1, which is the plot of cholesterol values obtained weekly for 12 weeks on a group of 29 healthy individuals. The horizontal axis represents the range of two standard deviations, above and below the mean, for the entire group of 200 healthy persons in the study. Each bar represents the mean and standard deviation of the observed set of values for that individual. The middle, darker portion represents the physiological changes which occurred over the period of observation and the open ends are the proportion of the total variation due to analytical bias. This plot also illustrates the uniqueness of the mean and physiological variation for each individual.

We have interpreted the range of variation in each person to portray a long-term homeostatic control of the particular blood constituent. This shows the individual's healthy tolerance limits and built-in control of variation, based on his genetic potential and the usual circumstances for his life-style in terms of diet, physical activity and reaction to his environment.

The variation of any blood constituent in an individual apparently is controlled by physiological feedback communication mechanisms limiting the degree of variation. The range of values established during a period of optimum health may be presumed to represent the efficiency of this control and is assumed to be homeostatic in nature. Homeostatic control apparently is effective in the individual over long periods of time (years) for many blood con-

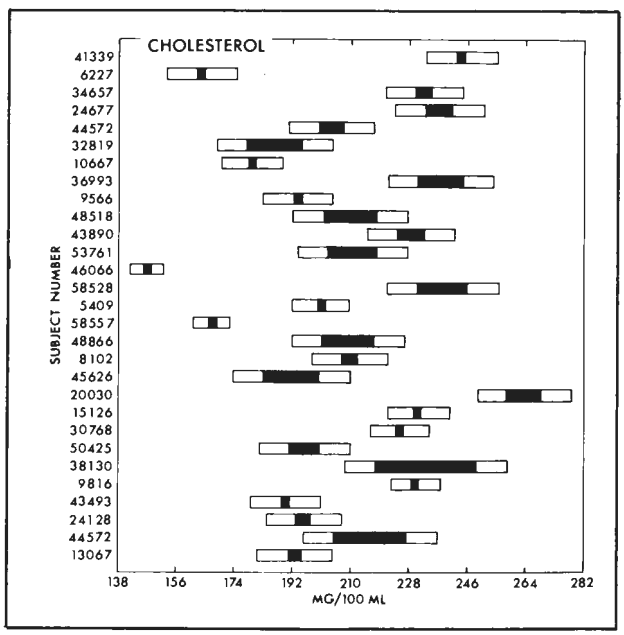

Fig. 1—Blood cholesterol values of 29 healthy people. Each bar represents the mean and 2.5 standard deviation ranges of the 12 weekly measurements for each person. The open portion at both ends of each bar represents the measured laboratory bias of the test.

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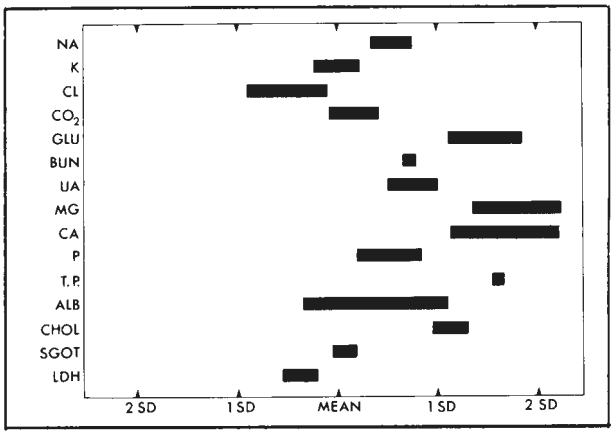

Fig. 2

stituents. It is reasonable to believe that the degree of this homeostatic control of the range of variation must have important implications in relation to state of health and optimum function. Thus, substantial changes in the range of variation of a blood chemical must have medical meaning; for instance, for a consistently narrow range of glucose or uric acid or cholesterol or an enzyme to broaden to a wide range of daily or weekly variation may augur trouble.

When repeated measurements obtained are similar for other blood chemical constituents over a defined period of optimum health, and the data are plotted in a like manner, the resulting chart depicts the unique blood chemical profile for that individual. Figures 2 and 3 are the profiles of two people. No two identical profiles have been found in several hundred healthy people.

Such a set of quantitative values, when established with rigidly controlled, precise analytical methods during a period of optimum health, constitutes the individual's reference base line to which all subsequent values can be compared. Deviation of any particular constituent or set outside the individual's reference range may be presumed to be undesirable for this particular person—even though the deviation may not extend beyond the conventionally accepted boundaries of "normal" for the general population. Should several determinations, at appropriate intervals, confirm the deviation to remain outside this individual's reference range, it should be interpreted as the beginning of a definite pathologic trend, although otherwise undetectable, that requires further investigation.

The "natural history" of the deterioration of an optimum state of health associated with the development and onset of disease may be depicted diagrammatically as in figure 4. During some period of a person's life, prior to onset of symptoms, there are at least two phases—optimum health and a zone of uncertainty during which health gradually and "silently" deteriorates to a condition of "predisease." This phase may be detectable by predisease changes in chemical and physiological quantitative values by comparison with the person's health reference profile of values and variations.

Figure 5 represents two possible deviation events. Superimposed upon the healthy reference profile of an individual are the results of annual sets

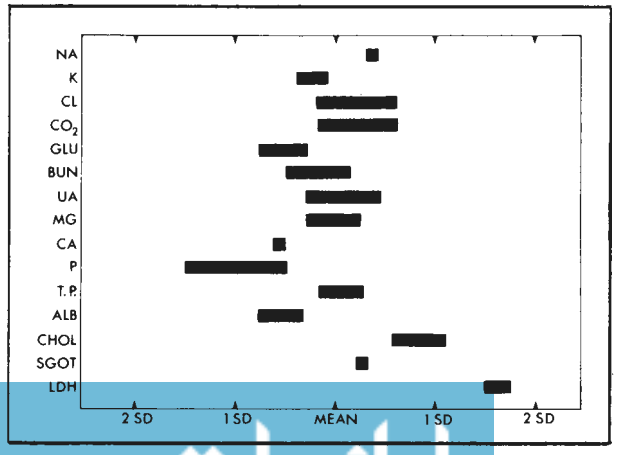

Fig. 3

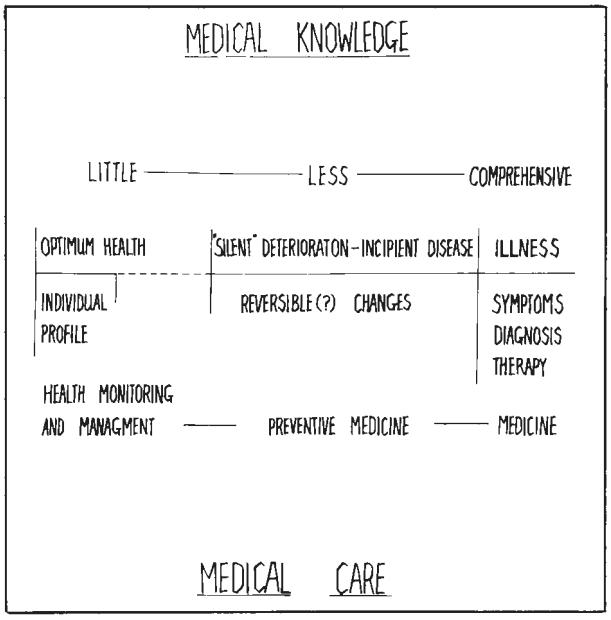

Fig. 4

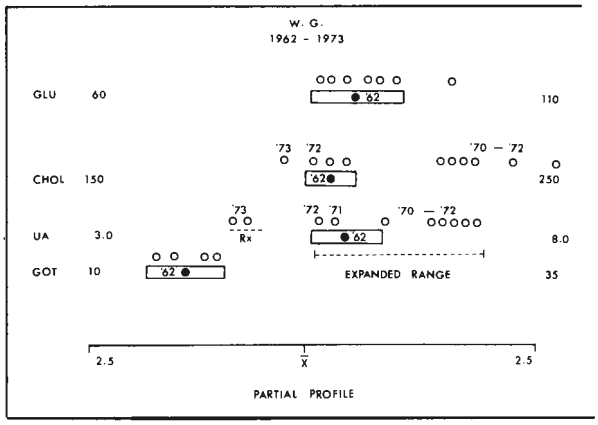

Fig. 5

of tests for several years. Note that in the instance of uric acid there is a linear and persistent deviation of these results in the direction of higher values. For cholesterol, deviation is geometric in progression. Even though these results are all within the range usually considered normal for an adult, in this instance, they must be interpreted as definitely abnormal for this particular person. Here we have a new tool for monitoring a person's health and for detecting early trends in the deterioration of his health, which may warn of impending pathology. Furthermore, this technique allows rationalized management procedures to attempt reversal of early abnormal trends or correction of the conditions which brought about this change. Figure 6 is the chronological graphic record of such a case over a ten-year period. There was a gradual increase in blood uric acid and cholesterol for several years, followed by reversal of both to the individual's own normal range

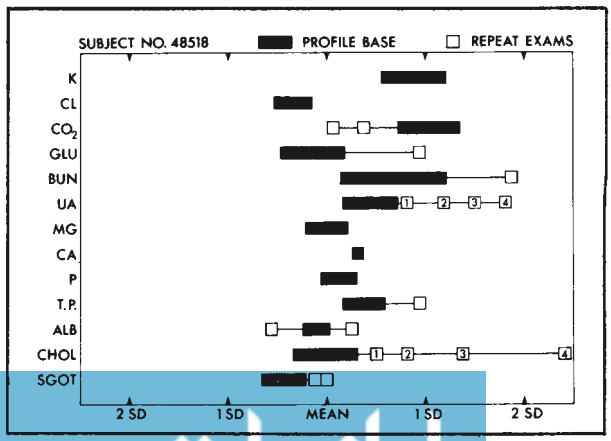

Fig. 6

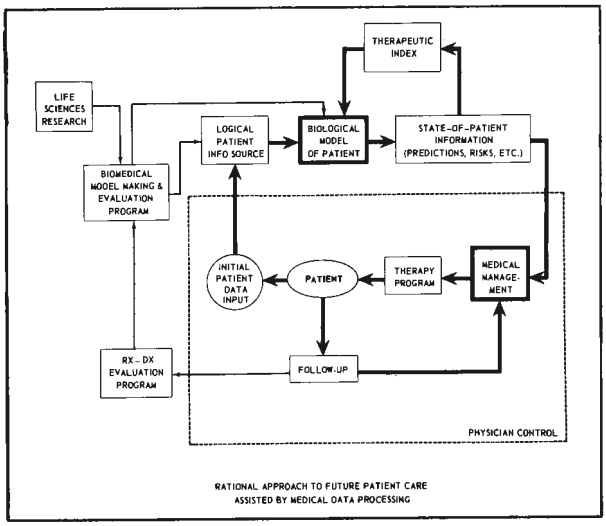

Fig. 7

by institution of management procedures, including monitored administration of allopurinol and a diet and running exercise regimen. Other cases in our experience encourage the belief that such "silent" trends can be discovered during a reversible phase and may result in either delay or prevention of progressive deterioration to overt disease.

The Future. We now have a scientific procedure for establishing a reference base of optimal values for people and the maintenance of prospective, lifetime profiles in regional data centers. It is possible to design computer simulation models for each of these profiles, including the body chemistry, physiology, and later, mental and emotional test patterns to be correlated with the person's life history. The individual models can be updated periodically to provide follow-up health management guides for each person. Such a data base will be invaluable for the physician in guiding his diagnostic and therapeutic decisions when a person requires medical care. Figure 7 is a tentative diagram of such a model. When irreversible changes do occur and definitive diagnosis is feasible, the development of disease will be detected far earlier than is now possible and presumably the patient will react more amenably to rational therapy. The physician team will have at hand not only a subjective, recent history but also a well-documented family and clinical history and a very useful physiological and chemical history. This is only one of the many advances we may expect of rapidly accelerating technological progress in the new era of preventive medicine.

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The Value of Chemical Screening Profiles on Blood*

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Chemical screening profiles on blood specimens are designed to yield information that may lead to new and additional clinical diagnoses, to revision of clinically established diagnoses, to confirmed impressions of the physician and to following the course of diseases during a patient's hospitalization. Screening profiles on blood are designed to indicate diseases of the liver, kidney, heart, striated muscle and other organs; they may be helpful in the diagnosis of anemia, diabetes mellitus, gout, congestive heart failure, osteomalacia and hyperlipidemia, hyperparathyroidism and other diseases. When these screening profiles were first introduced, there was widespread skepticism among physicians as to their value and their yield and some skepticism remains as to the usefulness of a broad spectrum of laboratory tests.

The relative number of new clinical diagnoses generated from blood chemistry screening profiles has been reported in five studies of three different patient populations: hospital patients, hospital clinic patients and patients presenting to doctors' offices (Table 1). The percentage of new diagnoses ranged from 3–16.9% with the percentage tending to increase with the number and selection of tests. New clinical diagnoses were obtained in 4% and 8.3% of hospital patients, 3% of hospital clinic patients, and 5.5% and 16.9% of patients presenting to doctors' offices. The blood chemistries that were most frequently abnormal were glucose, bilirubin, alkaline phosphatase, serum glutamic oxalacetic transaminase (SGOT), lactic dehydrogenase (LDH), uric acid, cholesterol, creatinine, urea nitrogen and hemoglobin; the diagnoses that were suggested and subsequently confirmed by the abnormal blood chemistries are given in Table 2. Diagnoses other than those listed in this table were generated but at a lower frequency.

Occasionally, elevated calcium led to a diagnosis of primary hyperparathyroidism and an elevated protein led to a diagnosis of multiple myeloma. If these blood screening profiles had uniformly included other blood tests, such as iron and iron binding capacity for anemia, triglycerides for hyperlipoproteinemia and protein bound iodine (PBI) or thyroxine (T-4) for thyroid disease, then the percentage of patients yielding new diagnoses would have increased. It must be emphasized that the initial chemical screening tests in themselves did not usually lead directly to the assigned diagnosis but more definitive tests were required.

Table 2 indicates the laboratory screening values that generated new clinical diagnoses and in most instances, required a change in treatment. Thus, the screening tests contributed to the recognition of otherwise unsuspected disease. The abnormal test results may be of value in arriving at earlier diagnoses, earlier treatment and reduction of time for subsequent investigation since the results are available sooner than under previous conditions where laboratory tests were ordered individually. If clinical features alone were used to guide the physician in ordering laboratory tests, abnormal test values would be obtained in less than 1.5% of the patients (4). Selection of the most appropriate tests for chemical screening is still not complete since different populations of patients may require different groupings of chemical screening tests.

* Presented by Dr. Bakerman at the 44th Annual McGuire Lecture Series, March 22, 1973, at the Medical College of Virginia, Richmond.

TABLE 1

NEW DIAGNOSES OBTAINED BY CHEMICAL SCREENING PROFILES ON BLOOD IN DIFFERENT PATIENT POPULATIONS

Patient Population	Percent of Patients Yielding New Diagnoses
Hospital Patients	4.0 (Belliveau *et al.*, 1970)
	8.3 (Carmalt *et al.*, 1970)
Hospital Clinic Patients	3.0 (Young and Drake, 1966)
Patients Presenting to Doctors' Offices	5.5 (Percy-Robb *et al.*, 1971)
	16.9 (Carmalt *et al.*, 1970)

TABLE 2

ABNORMAL BLOOD CHEMISTRIES BY CHEMICAL SCREENING PROFILES AND FREQUENTLY FOUND NEW DIAGNOSES

Abnormal Blood Chemistries	Diagnoses
Glucose	Diabetes Mellitus
	Inadequate Control
Bilirubin	Liver Disease
	Congestive Heart Failure
Alkaline Phosphatase	Liver Disease
	Osteomalacia
Serum Glutamic Oxalacetic Transaminase (SGOT)	Liver Disease
Lactic Dehydrogenase (LDH)	Gout
Uric Acid	
Cholesterol	Hyperlipoproteinemia
	Liver Disease
	Hyper- or Hypothyroidism
Creatinine	Renal Disease
Urea Nitrogen	
Hemoglobin	Anemia
	Polycythemia

Data are not included on abnormal laboratory values that tended to confirm the impressions of the physician based on his history and physical examination of the patient. These profiles also contribute to patient care by providing indicated laboratory tests rapidly and efficiently (3). These profiles also help to detect organ systems that may be functioning normally and thus, may not require a doctor's attention. If these data were available, then the value of chemical screening profiles would be even more apparent.

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Multitest Screening in Hematology*

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The concept of multitest screening for hematological disorders is not necessarily a new one. Implementation of such ideas has recently become possible, for the automated electronic instruments performing sequential multiple analyses within very short periods of time are a very significant advancement in the technology of the hematology laboratory. It must be emphasized, however, that the value of such instrumentation lies not only in the rapidity, but also in the accuracy and precision as well as the number and nature of the tests performed. While the data on samples have been reported on individual patients three and a half years in our hematology laboratory, it is only within the past few months that examples of its utilization for screening studies have begun to appear in the literature (1, 3, 5).

Several automated instruments are available that perform the routine counting and sizing of cells in the blood. The Coulter model "S" is the one most widely used (2). This is the instrument we use and it is the one used in the studies to be cited here. It is an instrument that reports on seven parameters, measuring the WBC, RBC, and mean corpuscular volume (MCV), and utilizing the latter two computing the hematocrit and then the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The time required from the time of sample aspiration until the results of the seven determinations are printed is 40 seconds. I must emphasize that not only speed is achieved, but precision and accuracy are far greater than many previously used methods. Indeed, variation on replicate samples is less than 1% and accuracy appears to be of the same order.

Considering the availability of such accurate data and the comparative ease with which they may be obtained, it can be seen that these instruments can be valuable tools in screening for hematological abnormalities. Indeed here at the Medical College of Virginia, two interdepartmental cooperative studies have been conducted within the past year. One of these was presented in abstract form at the December 1972 meeting of the American Society of Hematology, and has recently been submitted for definitive publication (5). The work in large part was the project of Mr. Alvin Schmaier, currently a third-year medical student, with assistance from Drs. Maurer, Johnston and Scott. The other study has been conducted as part of a larger study in approximately 530 Black children in the Head Start Program. These studies exemplify particularly well the principle of multitest screening in hematology, and it is to these and to a comparison of these with previous studies (1, 3) that I wish to address myself.

I will not detail the technical operation of the model "S" but note here that we have had the instrument in operation for more than three and a half years. This has given us extensive experience in all of the positive as well as the negative qualities of the instrument. Because some of the parameters are computed, our initial efforts were to compare these with other methods then in operation and standardize the data. This was accomplished by comparative data obtained on 1,500 samples of EDTA anticoagulated blood. No significant difference was found in the hemoglobin determination, but the linearity was better in the model "S" determinations than with flow-through determinations giving greater accuracy to the former, particularly in the high > 16 gm and low < 7 gm ranges. For reasons which have never been satisfactorily explained either by us or by the manufacturer, the WBCs average approximately 8% lower by the model "S" than when determined by our alternative or electronic back-up. This discrepancy is noted also in data from other institutions. The lower percentage has been consistent

* Presented by Dr. Johnston at the 44th Annual McGuire Lecture Series, March 23, 1973, at the Medical College of Virginia, Richmond.

and therefore is not a factor. Finally, the comparison of the spun hematocrit with the computed hematocrit (MCV × RBC) showed the latter to be, on the average, 1% higher—a difference that is minimal and one that certainly can be disregarded. I should note here that this is somewhat different from the study of England and Fraser (1) who set the electronics so that the hematocrit is 1.5% below the spun hematocrit. This quite possibly might allow for differences of our MCV norm 92 ± 6 (range 86–98) from theirs (80–90). Indeed Pearson, *et al.* (3), describe a range of 72–101 with a mean of 88.67 and a standard deviation of 5.3.

At the outset then, it becomes important to note what the norms are for the population being studied and these should be norms for the institution. The data just given are presumed to be an adult population; however, it is not clear from either of the published reports cited above whether age differences were taken into consideration in their studies. The reason for concern with the age variability of the MCV data will become apparent since this parameter is one of the major criteria used to screen for the thalassemia syndromes.

The other measured parameter of extreme importance in the screening efforts is the RBC. We have come to regard this as extremely accurately measured by the model "S." In contrast to the earlier manual methods with an accuracy no greater than 20%, we now feel the accuracy and reproducibility of this measurement is on the order of 1%, or in the range of 50,000 RBC/μl.

With these points in mind, I would like to discuss the application of multitest screening in hematology and share some of the results we have accumulated in specific studies. The first such study undertaken here was a cooperative effort involving the divisions of Clinical Pathology, Pediatrics and Medicine. Mr. Alvin Schmaier, working as a summer extern in the Department of Pediatrics, was responsible for the collection of samples and collation of the data. This portion of my report is in large part his effort. It is a most commendable piece of work which recently has been submitted for publication (5). The data given here are reproduced with his permission.

This study took advantage of the fact that defective alpha chain synthesis may occur in the neonate and result in the presence of the tetrameric hemoglobin, hemoglobin Barts (gamma 4). This form of hemoglobin rapidly disappears with onset of beta chain synthesis and by six months of age no longer can be identified. Cord blood studies have been performed but these naturally are limited in scope. Alpha thalassemia heterozygotes have abnormal red cell indices, and if these could be identified early, it might be possible to select infants in the neonatal period, or certainly within the first few weeks of life, whose blood then could be subjected to hemoglobin electrophoresis.

Taking advantage of the fact that blood could be obtained by capillary puncture with the use of unopettes from Becton Dickinson especially for model "S" counters (order no. 5840, Becton Dickinson, Rutherford, N. J.), Mr. Schmaier began collecting capillary samples in triplicate from the newborn nursery, of course after receiving parental consent. Table 1 shows the tabulation of the results. In particular, I want to call attention to the MCV and MCH. If one were to choose values for identification as suggested by other studies, one would select only those with values below 80 fl! Obviously, then the norm for this population group is quite different from adults, a fact that is well known (6). We were unaware, however, of the limits using the model "S" and these needed definition for the study.

A total of 200 newborn Black infants was studied. Electrophoresis of the hemoglobin in these newborns showed 181 to be normal; that is, only hemoglobins A and F. Of the remainder, 13 showed an additional abnormal hemoglobin either S or C, while six showed the presence of hemoglobin Barts. Of the six, one also showed C and one S hemoglobin. As defined in Table 1, the lower limit of normal was 97 fl. In the samples studied, nine infants had MCV less than 94 fl and MCH less than 29.5 fl. Since these figures are less than normal the data would be "suspect" and thus hemoglobin electrophoresis was performed. In the group of 200 studied, all had hemoglobin electrophoresis performed. Table 2 shows the hemoglobin electrophoresis and pertinent model "S" data on those with abnormal MCV and MCH. It is to be noted that hypochromia can no longer be assessed by the MCHC (4); that is clearly shown in this table. The most striking findings are that of nine patients, six had hemoglobin Barts and thus the alpha thalassemia trait. These were clearly identified by the low MCV and low MCH. Two of the patients had a second abnormality in that Hgb C was identified in one and S in the other. In the total sample, 13 infants were identified who had either

TABLE 1

RED CELL INDICES IN NORMAL BLACK FULL-TERM NEWBORN INFANTS

	Mean	Standard Deviation	ks	Lower Tolerance Limit
Red Blood Cell Count ($\times 10^6/\mu l$)	5.2	0.6	1.0	4.2
Hemoglobin (g/dl)	18.0	2.0	3.3	14.7
Hematocrit (%)	55.3	6.1	10.0	45.3
Mean Corpuscular Volume (fl)	106.4	5.7	9.4	97.0
Mean Corpuscular Hemoglobin (pg)	34.5	2.2	3.6	30.9
Mean Corpuscular Hemoglobin Concentration (g/dl)	32.5	1.0	1.7	30.8

s = Standard Deviation
Constant k = 1.65
Lower tolerance limit = mean-ks
Sample size = 91
For example, for the mean corpuscular volume of 106.4μ^3, the lower limit of normal is 97μ^3 (lower tolerance limit).

Hgb S or C but no Barts and an MCV and MCH within the normal limits. Thus in this study, all infants with alpha thalassemia were readily identified so that no "false negative" values were recorded. There apparently were three false positives, with data just below the norms set. Currently, there is no explanation for these.

It can be seen then that Mr. Schmaier made a significant contribution in the study and that it is possible to screen infants using the multitest principle. As a matter of fact, the incidence of 3% hemoglobin Barts is consistent with the reported incidence in American Blacks.

Let me now turn to another study involving multitest screening with the evaluation of RBC abnormality indices. Whereas the previous study was concerned with alpha thalassemia trait, this study was designed to study thalassemia traits perhaps encompassing both alpha and beta thalassemias. Again, this was a cooperative study involving the same divisions as those in the study just cited.

The group screened was quite different, however. This was composed of 540 Black children most of whom were enrolled in the Head Start Program in Richmond. Our study was only part

TABLE 2

HEMATOLOGIC FINDINGS IN NINE INFANTS WITH MEAN CORPUSCULAR VOLUMES <94 FL AND MEAN CORPUSCULAR HEMOGLOBINS <29.5 PG

Infant	Hgb Pattern	MCV (fl)	MCH (pg)	MCHC (gm/dl)	Hgb Barts (%)	Other Abnormal Hgb (%)
1	F, A, S, Barts	93	28.5	31.0	4.6	4.8
2	F, A, C, Barts	93	28.2	30.7	4.4	10.6
3	F, A, Barts	87	27.7	32.0	5.1	—
4	F, A, Barts	94	29.1	31.4	6.6	—
5	F, A, Barts	93	29.5	32.1	4.8	—
6	A, F, Barts*	90	28.0	31.6	3.0	—
7	F, A	91	29.0	31.2	—	—
8	F, A	93	29.2	31.4	—	—
9	F, A	91	29.3	32.2	—	—

MCV = mean corpuscular volume
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
RBC = red blood cell count
Hgb = hemoglobin
Hct = hematocrit
* Electrophoretic pattern at one month of age

of a larger medical evaluation conducted under the aegis of the Department of Pediatrics at the Medical College of Virginia. As part of the study, capillary blood samples were drawn by a registered medical technologist at the various schools. Samples were taken for Coulter model "S" determinations and hemoglobin electrophoresis. As in the earlier study, unopettes for the model "S" (order no. 5840, Becton Dickinson, Rutherford, N. J.) were used. These were drawn in duplicate, the model "S" parameters determined on both samples. Capillary samples also were used for the hemoglobin electrophoresis. Indices available for comparison were dated prior to the advent of electronic counters performing multiple simultaneous tests. Our experience with initial standardization of our model "S" showed that our norm for the automated procedures of 92 ± 6 was higher than that generally used of 88 ± 8. It seemed likely, therefore, that these older data could represent values lower than might be obtained by the currently used automated methods. Additionally, other than the standards cited by Shumway (6), I am aware of no others. As a result, it seemed preferable to determine our own mean model "S" parameters for this age group.

As noted, the recently published works did not specify the ages of the patients studied. It was assumed, therefore, that these were adults. In both studies, the lower limit of normal was 80. With Shumway's data (6) suggesting a lower mean MCV for pediatric age groups when compared with adults, we felt that perhaps it would be wise to include lower values. Thus for those studies, an MCV of 76 or greater was used for establishment of the mean and those 75 or lower were excluded. It is gratifying to note that 26% of the children studied had MCVs 76-79 and would have been excluded if the criteria of other screening studies had been used.

For the moment, I would like to refer to the results not as normal but rather as "mean model 'S' parameters." The health of the children was not evaluated and the sampling was random. Data from two children were excluded because of hemoglobin values less than 10 but with MCV over 80.

As a result, determination on 451 Black children, ages four to eight, with MCV greater than 76 were available for analysis. The results of these determinations are shown in Table 3. As noted, these are mean model "S" parameters for the group studied. These show the means with one standard deviation as well as the range. Also shown is the lower tolerance limit using a 95% tolerance interval (k = 1.65). With these figures we could be 95% certain that 90% of the values would be above the lower tolerance limit shown in the right hand column.

TABLE 3

MEAN MODEL "S" PARAMETERS
(451 Black Children MCV > 75)

Determination	Mean ± SD	Range	Lower Tol. Limit*
RBC	4.51 ± 0.31	3.81–5.19	4.0
Hgb	12.3 ± 0.86	10.4–14.8	10.9
Hct	36.7 ± 2.4	31.1–45.1	32.7
MCV	81.8 ± 3.3	76.0–91.0	76.4
MCH	27.3 ± 1.3	24.3–31.3	25.2
MCHC	33.7 ± 0.8	31.9–35.7	32.4

* Lower Tolerance Limit = $\bar{x} - ks$
$\bar{x}$ = mean
k = 1.65 (95% tolerance level)
s = standard deviation

It now becomes of great interest to compare these results with those alluded to earlier as having been determined by nonautomated methods and reported by Shumway (6). This comparison is shown in Table 4. The similarities are remarkable, especially when one considers that our population sample was not a selected one and was from children with Hgb greater than 10.4 gms and MCV 76 or greater. I think we can conclude that it might be reasonable to accept these as normal values for the age group.

Our original intent for the determination of the indices was to see if it would be possible to screen for thalassemia trait using microcytic hypochromic indices as the major criteria. The spectre of iron deficiency, however, loomed large in front of us since both thalassemia trait and iron de-

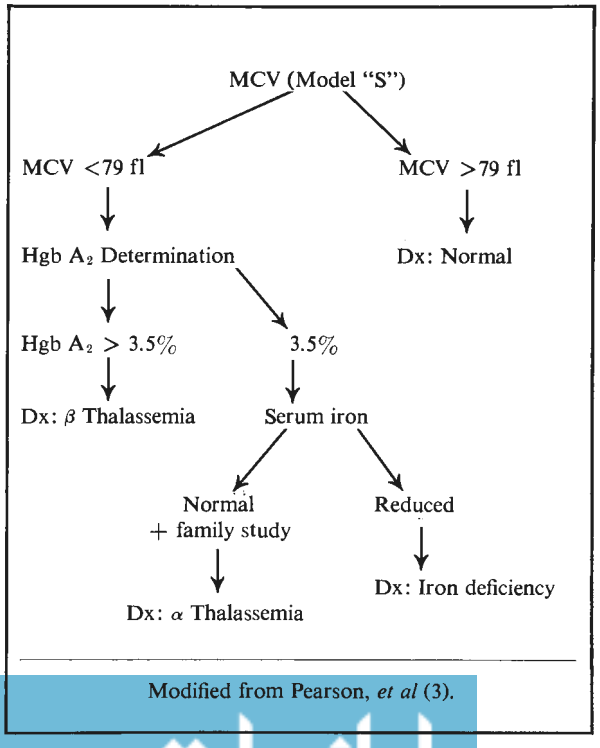
TABLE 4

	Current Data	Shumway (6)
RBC	4.51 ± 0.31	4.65 ± .5
Hgb	12.3 ± 0.86	12.7 ± 1
Hct	36.7 ± 2.4	37.0 ± 3
MCV	81.8 ± 3.3	80.0 ± 4
MCH	27.3 ± 1.3	27.0 ± 2
MCHC	33.7 ± 0.8	34.0 ± 1

ficiency are characterized by microcytic hypochromic indices. At the time that these data were being collated for presentation, the results of the hemoglobin electrophoresis were not available. Thus, there were 84 children with MCV 75 or less who needed to be differentiated if possible. Pearson, *et al.* (3), in their studies, proposed a scheme for thalassemia trait screening. This is shown in a slightly modified fashion in figure 1. Note that the first step in screening is to classify the subjects into two groups according to the MCV which is precisely what we did. The next step involves quantification of A_2 hemoglobin which, as noted, is not at the moment available.

This led to a search for an alternative method for attempting to differentiate the MCV 75 and below group. Recently, such a method has been published by England and Fraser (1). They reported that by the use of the statistical discriminant function (D.F.) they were able to differentiate between the disorders with a 99% success rate in 72 cases. As a result of their study, a slightly modified formulation is available and is shown as well as the data for the study in Table 5. Thus, it seemed that it might be possible to predict, in advance of the receipt of the electrophoretic data, the groups into which our 84 subjects might fall and effectively separate the thalassemia trait from an iron deficiency. Accordingly, the appropriate data on those children with MCV 75 or less were substituted into the formula. The results are shown along with the normal in Table 5. It can be seen that 11 of the group had negative, while 73 had positive, D.F.s. To show the distribution, a chart like that of England and Fraser (1) was prepared and is shown in figure 2. The closed circles represent those subjects with a positive D.F.′ and presumable iron deficiency. Those with the open circles represent the negative D.F.′ and presumable thalassemia trait. Since the critical data currently are missing, I think it is only possible to state that we are predicting that these are the groups into which the subjects fall. The data in Table 5 show certain of the mean values to be sufficiently different to suggest that these really are two distinct groups despite a few (four) that were too close to 0 to call (fig. 2). As soon as the additional data are available, we will know how well we are able to predict.

If I may be allowed to speculate, I expect a high success rate. As evidence for this rather rash statement, again direct your attention to figure 2. Note that in the D.F.′ negative area [D.F.′ = MCV −RBC − (5 × Hgb) − 3.4] there are four x's. While this manuscript was in preparation, I noted four patients' model "S" data that were quite like those seen in the 11 children with negative D.F.s. I felt these were suspect on the basis



Modified from Pearson, *et al* (3).

Fig.1—Suggested schema for thalassemia trait screening.

TABLE 5

COMPARISON OF GROUPS BY MCV AND D.F.′*

# of Children	451	11	73
		MCV < 76	
	MCV > 75	D.F.′−	D.F.′+
RBC	4.51 ± 0.31	*5.54 ± 0.19	4.86 ± 0.28
Hgb	12.3 ± 0.86	12.5 ± 0.67	11.6 ± 0.70
Hct	36.7 ± 2.4	37.7 ± 1.79	35.2 ± 2.14
MCV	81.8 ± 3.3	*68.4 ± 3.8	*72.9 ± 2.57
MCH	27.3 ± 1.3	*22.6 ± 1.75	*24.0 ± 1.15
MCHC	33.7 ± 0.8	33.3 ± 1.03	33.1 ± 0.93

* D.F.′ = MCV − RBC − (5 × Hgb) − 3.4

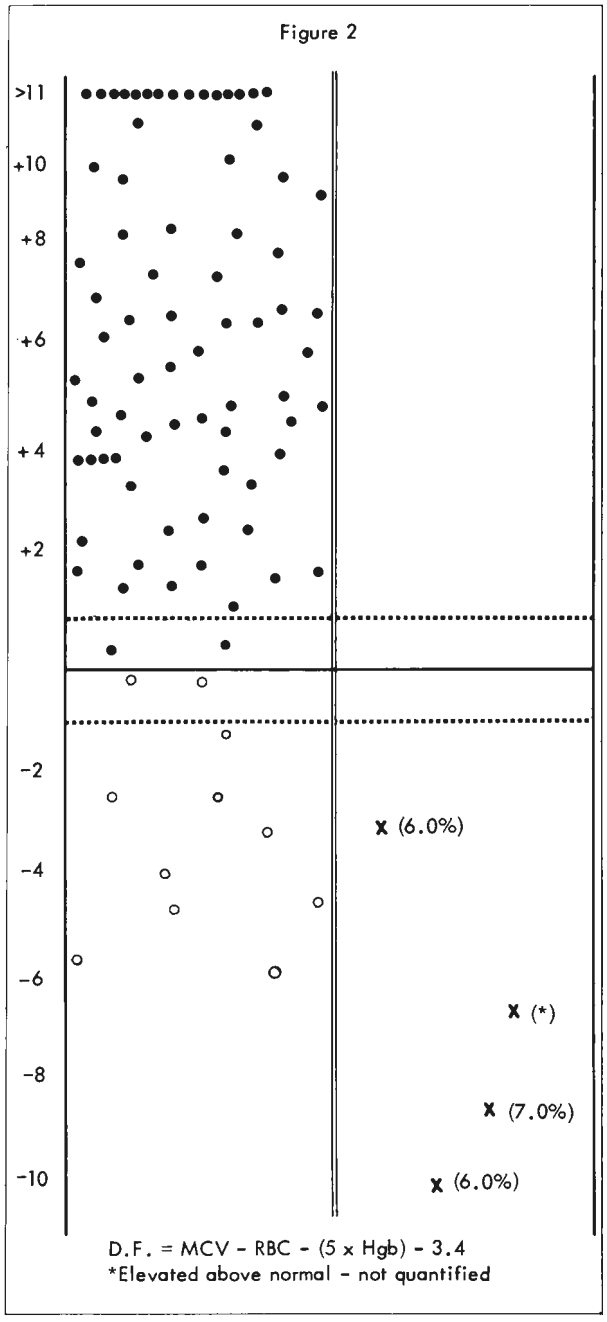


Fig. 2

of the experience I was accumulating. Hemoglobin electrophoresis was performed in these, therefore, and the A_2 hemoglobin quantified. Summary data on these patients are given in Table 6. Such ability to predict certainly suggests that the automated multitest apparatus has given us remarkable ability

TABLE 6

Patient	RBC	MCV	MCH	D.F.	% A_2Hgb
D.H.	5.65	62	20	−3.05	6
W.F.	6.52	64	20	−9.92	6
A.J.	6.17	65	21	−8.5	7
P.F.	6.16	64	20	−6.5	(*)

* Elevated above normal—not quantified

to select those most likely to have beta thalassemia trait. I await with eagerness complete collation of data, predictions and electrophoresis in the 84 children noted.

In summary, it can be said that a sample of multitest screening in hematology has been presented, made possible by the use of automated counting apparatus. The ease, precision and accuracy of the determinations favor much wider application. To this end studies are now cropping up in the literature, almost with every new journal that hits one's desk. Our efforts presented here show that studies of "at risk" populations are not only feasible but practical. Such examples as those given, certainly advance our knowledge and facilitate our know-how in difficult diagnostic areas.

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Pitfalls in Unqualified Acceptance of Laboratory Data*

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Professor, Department of Pathology,
Louisiana State University Medical Center, New Orleans

One can never be absolutely certain that any single laboratory report is correct. As a general rule, therefore, do not undertake potentially serious action on behalf of any patient solely because of a single laboratory report. This is especially important if the result is unexpected or not in harmony with the rest of the clinical information available.

What are the reasons for this seemingly deplorable situation and how can it be remedied? The reasons are legion and I do not foresee the day when they can be remedied to the point where no errors ever occur. On the other hand, the situation has improved considerably during the past decades and there are reasons to hope for further improvement. Such improvement will require greater effort from physicians ordering the tests as well as those working under their professional supervision, greater effort on the part of reagent, laboratory-ware and instrument manufacturers and greater effort on the part of laboratory personnel.

Let us begin with specimen collection. I fear that even in these days, when each hospital patient has a wrist band with his name and identifying number on it, and when hospital rules usually forbid two patients with the same last name being placed in the same room—or even on the same floor in some cases —mistakes are occasionally made in patient identification. The errors arise in various ways. The blood collector may not check the wristband and a new patient may have been placed in the room, or the blood collector may have entered the wrong room. The collector may have asked, "Are you Mrs. Jean Jones?" and have been answered, "Yes" by Mrs. Sally Smith who didn't understand the question and who always answers questions positively. I have heard that this is not an uncommon reaction among patients, who wish to please those who are taking care of them. Or the patient may actually be Mrs. Jean Jones, but the collector may have picked up Mrs. Sally Smith's pre-labeled slips and collection containers. Pre-labeling, while time saving, can generate problems, since it makes this sort of mistake easier. I have seen a nurse hand a sputum jar to a patient about to undergo gastric lavage for suspected tuberculosis and be told by the patient that the name on the slip was not his own. Once such an incorrectly identified specimen reaches the laboratory the error is hardly likely to be caught.

Almost no other clinical feat appears more difficult than obtaining an accurate 24-hour urine collection. Bottles continually arrive in the laboratory bearing on the laboratory slip the information that the patient's collection started at 8 a.m. on the morning of the first day and terminated at 8 a.m. on the morning of the second day. Nonsense! I'll bet not one in one hundred patients voids on the hour exactly. A nurse or nurse's aide has probably prelabeled the containers and slips and left them with the patient with rapid-fire oral instructions or a neat little card bearing written directions which the patient can't read, doesn't read or doesn't understand. It is essential that someone who really understands the procedure explain it to the patient in simple terms. It is not important that the collection start and stop on the hour or that it be exactly 24 hours—a fact which in my experience seems to escape most medical students and probably most nurses. It is important that the time be known exactly, that the first specimen at the beginning of the collection

* Presented by Dr. Beeler at the 44th Annual McGuire Lecture Series, March 23, 1973, at the Medical College of Virginia, Richmond.

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period be discarded and that all of the urine be collected during the following collection period, uncontaminated by feces. Patients often forget when they use a bed pan that urine passed along with the bowel movement also counts. As a matter of fact, errors in 24-hour urine collections are so universal that many laboratories will not report results per 24 hours, but merely the volume of the urine and the results per 100 ml or per milliliter, in order not to return ridiculous results.

The type of container and what it contains is also important. I know of an expensive study of calcium and phosphorus metabolism conducted some years ago at a famous clinic which was invalidated because of improper cleaning and rinsing of the urine containers. Heparinized blood used for collection for blood ammonia determinations may be unsatisfactory as some heparins contain significant amounts of ammonia. I know of a case in which a fibrinogen band on electrophoresis of what was thought to be serum was interpreted as a monoclonal gammopathy or *M* peak when plasma was inadvertently substituted for serum. Nonsterile containers are often used to collect and transport specimens for bacteriologic study. Containers not chemically cleaned are often used to collect specimens for trace metal analysis. Manufacturers have suddenly, without prior notification, introduced changes such as siliconizing a widely used brand of blood-collection tube and consequently wreaked havoc with unsuspecting hematology laboratories using the tubes for whole blood clotting times! The wrong anticoagulant can make the interpretation of a peripheral blood smear difficult or impossible. Improper preparation of the patient is also a common cause of unreliable laboratory results. Glucose tolerance tests on patients who have been on starvation or reducing diets in the days preceding the specimen collection are not reliable for purposes of diagnosing diabetes mellitus. Serum lipids may be misleadingly normal if the patient has been losing weight or has been on a starvation or fat restricted, low calorie diet. It is not possible to interpret the 24-hour urine calcium, if the calcium content of the diet prior to the collection is not known.

Inappropriate specimen handling and inadequate preservation also causes error. Glucose rapidly metabolizes if plasma or serum is allowed to sit in contact with red cells. Although variable, the average rate of reduction of blood glucose at 37°C is 15 mg/100 ml/hr. Bilirubin is oxidized rapidly when exposed to direct sunlight or even to fluorescent lighting. Ammonia generation starts immediately following withdrawal of the blood sample. The pH rises if blood is exposed to air. There is a paradoxical rise in serum alkaline phosphatase (as much as 10%) when serum is refrigerated overnight.

Hemolysis of the sample can interfere with many laboratory procedures by different mechanisms including absorbance by hemoglobin at the wavelength used, inhibition of enzyme activity by hemoglobin (lipase) or contribution of intracellular substances present in higher concentration in red cells than in serum (potassium, LDH). Turbidity of the serum may also interfere, particularly in photometric procedures.

The problem of drug interference is so formidable that I hesitate even to mention it. Drugs may interfere by altering the patient's biochemical and physiological processes or by interfering with the analytic procedures. This may result in raising or lowering test results significantly or only slightly, or may render the specimen totally unfit for testing. The entire October 1972 issue of *Clinical Chemistry,* the journal of the American Association of Clinical Chemists is devoted to a computer printout of laboratory tests and drugs affecting them. It represents 9,000 filed entries developed in the Clinical Pathology Department of the Clinical Center of the National Institute of Health and over 250 pages are devoted to this problem in that one issue. It is beyond the capability of the human mind to remember even a fraction of such a list. Even if it were not, the lack of quantitative data concerning the degree of interference and its consistency and the innumerable possible combinations and their varying effects would cause this to be an almost unsolvable problem. Even so, major effects of the commonest medications on the frequently used laboratory tests should be kept in mind. Examples of interference by physiologic mechanisms are the effect of "the pill" on thyroid function tests, and of morphine or codeine on serum amylase. Examples of interference with chemical analyses directly include the effect of administration of iodine containing substances on the serum protein bound iodine (PBI) and the effect of bromide on the ferric iron cholesterol methods.

If the patient has been properly prepared, the specimen properly collected and preserved and the patient has received no interfering medications, many potential pitfalls still await the procedure within the laboratory. Once again, there is the pos-

sibility of misidentification of specimens. I know of no completely foolproof specimen identification system, although in recent years improved systems have been evolved.

There is the ever present problem of unacceptable error originating in the actual laboratory procedure. First, let us acknowledge the hard fact that no human act—or even the act of any machine (although machines may come closer)—is perfectly reproducible. There is an irreducible minimal variation inherent in the actions of the technologists, in the limitations of glassware, reagents and instruments with which we must all live. It is the business of the clinician to acquaint himself with this variability as estimated by his own laboratory for each of its laboratory procedures, so that he may decide whether two tests on the same patient can reasonably be judged to be different. A simple, somewhat oversimplified rule of thumb is not to consider two test results which are within three standard deviations of each other (the standard deviation in this case estimated from daily quality control samples) to be significantly different—or to indicate a possible laboratory error—unless they *should* be different and do not appear to be. There are two general sorts of analytic error—those that effect all the unknowns in the batch in the same direction (bias) and those that strike randomly. The systematic error or bias can result from deteriorating standards, a bad reagent, improper instrument setting or operation. All laboratories have or should have an adequate daily control program whose primary purpose is to detect this sort of error, so that it can be corrected before erroneous results are reported. Random errors, on the other hand, are generally not detected by the usual quality control program. They can result from pipetting errors, an intermittent instrument failure, a random calculation error or from the lack of specificity of the tests coupled with an abnormal concentration of some other substance in the sample. They can be minimized by good procedures, good instruments, good instrument maintenance and well-trained, careful technologists. All calculations should be performed independently by two different laboratory workers and results should, whenever possible, be compared with previous results on the same patient or with other tests performed for the same patient on the same day with an eye to their compatibility. Unfortunately, since errors can be in either direction and of any magnitude, there is really no greater reason to subject abnormally high or low results to closer scrutiny than normal results (unless results are incompatible with life or ridiculously abnormal). There does exist the possibility of greater liability of significant inappropriate therapeutic intervention on the basis of abnormal laboratory results, but lifesaving intervention not initiated because of an erroneously reported normal laboratory value can be similarly threatening. The physician ordering the test can be of assistance by informing the laboratory if, judged by other information available to him, it appears likely that a laboratory error has occurred. The laboratory director should encourage this type of feedback and should see that each instance is investigated thoroughly. The laboratory should indicate a willingness to repeat the test on a freshly collected sample without additional charge—certainly if the first result was erroneous, and probably even if the first one was not in error, provided, of course, that the clinicians do not abuse this opportunity.

Even if the analytic procedure is reasonably specific and the result is accurate, there are still pitfalls awaiting the unwary interpreter. These pitfalls result from intra- and interpatient variability and the many unsolved problems related to normal values and interpretation of laboratory test results.

In summary, numerous pitfalls await anyone brash enough to accept laboratory data in an unqualified fashion. Errors result from improper patient preparation, improper specimen collection and preservation or identification, drug interference and technologist, glassware, reagent or instrument failure. Errors can be minimized by education, interest and cooperation among clinicians, laboratory directors, nurses, technologists and all others involved. Such errors cannot ever be completely erradicated. It bears repeating, therefore—*never undertake potentially serious action on behalf of a patient solely on the basis of a single laboratory test result.*

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Genetic–Metabolic News*

This is the first in a series of newsletters designed to inform the Virginia medical community of recent advances in the area of GENETICS and METABOLISM and to provide information regarding a variety of services that are available at the Medical College of Virginia. The number, complexity and expense of tests that are now required in order to make precise diagnoses in many of the newly described genetic and metabolic disorders make it impossible for most clinical pathology laboratories to provide these services.

Accurate and meaningful genetic counseling *must* be based on *precise* diagnoses. Genetic counseling is imperative if we are to have some measure of success in reducing the familial transmission of these disorders. "An ounce of prevention is worth a pound of cure."

Diagnosis and continued therapy of many of the inborn errors of metabolism (i.e. phenylketonuria, galactosemia, glycogen storage disease, maple sugar urine disease, methylmalonic aciduria, etc.) require highly sophisticated biochemical tests and enzymatic analyses. Newer techniques for the detection of carrier states (i.e. Tay-Sachs disease, sickle-cell disease) have stimulated the development of several programs for mass screening for carriers. These screening programs have already been successful in identifying married couples where both husband and wife are carriers of a gene for an inborn error of metabolism and thus have a 25% likelihood of producing an affected child.

One of the more exciting developments in the past several years has been the ability to make chromosomal and metabolic diagnoses on cells in amniotic fluid obtained transabdominally (i.e. by amniocentesis) at 14–16 weeks of pregnancy. This procedure enables one to monitor certain "high risk" mothers for an affected fetus prior to the 20th week of gestation—a time sufficiently early to allow the therapeutic termination of pregnancy when indicated. Amniocentesis involves a very minimal risk to mother and fetus. Indications for this type of study performed in the Department of Obstetrics and Gynecology are noted in the following pages.

Beginning on Tuesday, January 8, 1974 the National Foundation—March of Dimes Genetic Counseling Clinic will accept for investigation and counseling any families desiring these services. No charge will be made for the counseling services or for the services of the metabolic tissue culture laboratory. Where a precise diagnosis is not already well established, the family may be directed to various subspecialty areas, either as a private or general staff patient, where more definitive studies can be carried out on a fee-for-service basis. Charges for chromosomal studies are outlined in the following pages.

Medical personnel who desire further general information regarding the Counseling Clinic may write or call Dr. Peter Mamunes at (804) 770-3033 or 770-5076 or Dr. R. B. Young at (804) 770-4206 or 770-5076 in the Department of Pediatrics. A listing of the various genetic and metabolic services which are currently available at the Medical College of Virginia is provided in the following pages for your future reference. Where specific services are noted, direct contact should be made with the persons offering the services.

MEDICAL COLLEGE OF VIRGINIA GENETIC AND METABOLIC SERVICES

1. **National Foundation—March of Dimes Genetic Counseling Clinic (Department of Pediatrics and Program in Human Genetics).** The counseling services of this clinic are available at no charge to patients referred for genetic counseling. This clinic meets on the first and third Tuesday afternoons of each month. For inpatients and families where a precise diagnosis is not clearly established, appropriate referrals are made. Contact Dr. Peter Mamunes or Dr. R. B. Young.

2. **National Foundation—March of Dimes Metabolic Tissue Culture Laboratory (Department of Pediatrics).** This laboratory provides the definitive identification of certain metabolic defects which can be measured by enzyme analyses of white blood cells, red blood cells or fibroblasts from skin or amniotic fluid. Since these studies are complex and time consuming, they are performed only after preliminary screening studies have indicated the likelihood of a genetic defect.

Due to the very large number of genetic defects now recognized to occur, this laboratory performs tests only on a few specific disorders of carbohydrate,

* Supported by National Foundation—March of Dimes.

lipid and amino acid metabolism which have been recognized to occur with a reasonable incidence in this state. These include the glycogen storage diseases, defects in intermediary carbohydrate metabolism which lead to hypoglycemia or developmental delay, various problems which cause organic acidosis, the aminoacidopathies and a small number of abnormalities which are associated with abnormal lipid storage (especially Tay-Sachs disease). Where other diseases are suspected, specimens are sent to other laboratories in the U.S. that have specific interests in the particular disorder in question. Contact Dr. Robert Eanes or Dr. Peter Mamunes.

3. **MCV Metabolic Service Laboratory (Department of Pediatrics.** Contact Dr. Peter Mamunes).

A. *Urine for Metabolic Screening.* To screen for various inborn errors as a cause for psychomotor retardation, failure to thrive, recurrent acidosis, seizure disorder, etc., a battery of qualitative tests and high voltage electrophoretic separation of amino acids is performed on a random urine specimen (minimum 15 cc volume). After acidification to pH 1 (with a few drops of concentrated hydrochloric acid) the urine can be mailed to this laboratory at room temperature. Testing is performed once per week. More specific quantitative procedures are undertaken where the screening tests determine the need. The fee for preliminary metabolic screening tests on urine is $20.00.

B. *Measurement of Specific Metabolite or Enzyme.* As a part of its research activities (in mitochondrial metabolism, pathophysiology of Reye's syndrome, organic acidemias and hypoglycemia) this laboratory has established a substantial number of quantitative procedures for metabolites and enzymes. When there is a need for a test not performed locally, referral to the appropriate source laboratory is made.

C. *Treatment of Inborn Errors of Metabolism.* Supervision of dietary and other treatment modalities for the inborn errors of metabolism is monitored by careful analysis of accumulating substrate or intermediary metabolites. This program is supported to a large extent by the Bureau of Child Health of the Virginia State Health Department.

4. **MCV Chromosome Service Laboratory (Department of Pediatrics and Program in Human Genetics).** Chromosomal analysis is performed on a fee-for-service basis on patients with: 1) multiple congenital anomalies; 2) sex anomalies not fully identified by sex chromatin (buccal smear) studies; 3) suspected chronic myeloid leukemia for identification of the Philadelphia-1 (Ph^1) chromosome. Blood specimens can be collected in micro capillary tubes (heparinized) and mailed in special culture tubes which are available on request. Culture and analysis require approximately two weeks. Charge for complete culture and photographic karyotyping on peripheral blood leukocytes or bone marrow will vary from $75.00 to $150.00 according to complexity. Special arrangements are necessary for the scheduling of this test. Charge for routine buccal smear for sex chromatin (Barr body) is $10.00 or by fluorescent technique for identification of the double Y bodies in the XYY syndrome is $20.00. Contact Dr. Andrew Chen or Dr. R. B. Young.

5. **MCV Amniocentesis Laboratory for Intrauterine Study of Chromosomal Anomalies (Department of Obstetrics and Gynecology).** Patients may be referred by their obstetrician or family physician to be considered for amniocentesis and chromosomal analysis. Parents in certain high risk situations should be referred for counseling *before conception when possible* or as early in pregnancy as possible. Arrangements could then be made for the amniocentesis to be performed around the 14th–16th week of pregnancy primarily in the following *high risk* situations:

A. one parent a known carrier of a chromosomal translocation (i.e. 14/21 translocation in mongolism) which carries a *one-in-three* to *one-in-five* chance of having another affected child;

B. any family with a prior mongoloid child (recurrence risk is approximately 1:200);

C. any mother 40 years of age or older (occurrence risk is approximately 1:100);

D. any family where parents are known to be carriers of a sex-linked recessive condition such as hemophilia (occurrence risk is 50% in *male* children);

E. any family where both parents are known carriers of a biochemical disorder that is capable of being detected in amniotic fluid (now some 20 or more disorders). Most of these disorders have autosomal recessive transmission with a *25% recurrence risk.*

Amniocentesis will be performed on an outpatient basis in the Department of Obstetrics and Gynecology. In most cases amniocentesis will be preceded by placental localization by diagnostic ultrasound. Referred specimens of amniotic fluid may be accepted if prior arrangements are made. Total charges will range from $200 to $250. The time required for tissue culture and photographic karyotyping of the chromosome is approximately three weeks. It is anticipated that almost all parents will elect early termination of pregnancy if the fetus is found to be affected. Immediate consultations between referring physician and the Department of Obstetrics and Gynecology will be imperative in these situations. Contact Dr. Fay Redwine, Dr. Edward Davis or Dr. Leo Dunn, Chairman.

6. **Consultation and Evaluation Clinic Chromosomal Laboratory—Bureau of Child Health and Department of Pediatrics.** Children being evaluated for psychomotor retardation in any of the C&E clinics throughout the state are eligible for chromosome analysis without charge when these studies are clinically indicated. Any mongoloid (Down's syndrome) patient may also be referred by his physician for chromosome analysis without charge. Further family studies will be carried out when indicated.

7. **Special Study Project of Multiple Spontaneous Abortions—Sponsored by Developmental Disabilities Study Grant and Program in Human Genetics.** Chromosomal studies will be performed free of charge for the next six months (December 1, 1973 to June 31, 1974) on parents who have had two or more spontaneous abortions to search for possible translocations which may then indicate high risk of recurrence. Amniocentesis might then be considered if a translocation is identified in either parent. Contact Dr. Andrew Chen or Dr. R. B. Young.

8. **Tay-Sachs Screening Program—Supported by the Department of Pediatrics, the Department of Pathology, the Virginia State Health Department and voluntary contributions from individuals and organizations.** A Tay-Sachs carrier detection program has been established. Over 1,800 adult Jews in the state have thus far been screened after an intensive educational campaign in the greater Richmond area. Each of the 60 identified carriers has been appropriately counseled. Presently, we are advising and supporting other Virginia communities in their efforts to educate, test and counsel for this lethal, inherited metabolic disorder of the central nervous system which affects primarily Jews. Because the test (done on one milliliter of serum) measures enzyme (hexosaminidase A) activity, special handling of the specimen is required. All married Jews in the childbearing age should *definitely* be tested. Charge is a voluntary contribution. Contact Dr. Peter Mamunes.

9. **Sickle Cell Testing and Counseling.** The Virginia Sickle-Cell Anemia Awareness Program (VASCAP) tests for carriers of sickle trait in the *childbearing age* and provides genetic counseling. Blood samples may be sent by the patient's physician (mailing tubes can be provided) or the patient may go to the testing clinic at 1008 East Clay Street in Richmond. Call (804) 770-7797 for information.

For Specific Information in the Above Areas Contact:

Name	Department	Telephone (804)
Dr. Andrew Chen	**Program in Human Genetics**	**770-4646 or 770-4645**
Dr. Edward Davis	**Department of Obstet.-Gynecol.**	**770-4111**
Dr. Leo Dunn	**Department of Obstet.-Gynecol.**	**770-4111**
Dr. Peter Mamunes	**Department of Pediatrics**	**770-3033 or 770-5076**
Dr. Fay Redwine	**Department of Obstet.-Gynecol.**	**770-4111**
Dr. R. B. Young	**Department of Pediatrics**	**770-4206 or 770-5076**
Dr. Robert Eanes	**Department of Pediatrics**	**770-7610 or 770-5076**

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the long-range analgesic

in chronic pain: continued relief without risk of tolerance

Though Talwin® Tablets can be compared to codeine in analgesic efficacy, Talwin is not subject to narcotic controls. For patients who require potent analgesia for prolonged periods, Talwin can provide consistent, long-range relief, with fewer of the consequences you've come to expect with narcotic analgesics.

- **Comparable to codeine in analgesic efficacy:** one 50 mg. Talwin Tablet appears equivalent in analgesic effect to 60 mg. (1 gr.) of codeine. Onset of significant analgesia usually occurs within 15 to 30 minutes. Analgesia is usually maintained for 3 hours or longer.
- **Tolerance not a problem:** tolerance to the analgesic effect of Talwin Tablets has not been reported, and no significant changes in clinical laboratory parameters attributable to the drug have been reported.
- **Dependence rarely a problem:** during three years of wide clinical use, only a few cases of dependence have been reported. *In prescribing Talwin for chronic use, the physician should take precautions to avoid increases in dose by the patient and to prevent the use of the drug in anticipation of pain rather than for the relief of pain.* (See last page for a complete discussion of Warnings under Brief Summary.)
- **Not subject to narcotic controls:** convenient to prescribe—day or night—even by phone.
- **Generally well tolerated by most patients:** infrequently cause decrease in blood pressure or tachycardia; rarely cause respiratory depression or urinary retention; seldom cause diarrhea or constipation. If dizziness, lightheadedness, nausea or vomiting are encountered, these effects may decrease or disappear after the first few doses. (See last page of this advertisement for a complete discussion of Adverse Reactions and a Brief Summary of other Prescribing Information.)

50 mg. Tablets

Talwin®

brand of **pentazocine** (as hydrochloride)

in moderate to severe pain

the long-range analgesic

in chronic pain: continued relief without risk of tolerance

Though Talwin® Tablets can be compared to codeine in analgesic efficacy, Talwin is not subject to narcotic controls. For patients who require potent analgesia for prolonged periods, Talwin can provide consistent, long-range relief, with fewer of the consequences you've come to expect with narcotic analgesics.

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50 mg. Tablets

Talwin®

brand of **pentazocine** (as hydrochloride)

in moderate to severe pain

the long-range analgesic

in chronic pain: continued relief without risk of tolerance

Talwin® Tablets brand of pentazocine (as hydrochloride)
Analgesic for Oral Use—Brief Summary

Indications: For the relief of moderate to severe pain.

Contraindication: Talwin should not be administered to patients who are hypersensitive to it.

Warnings: *Drug Dependence. There have been instances of psychological and physical dependence on parenteral Talwin in patients with a history of drug abuse and, rarely, in patients without such a history. Abrupt discontinuance following the extended use of parenteral Talwin has resulted in withdrawal symptoms. There have been a few reports of dependence and of withdrawal symptoms with orally administered Talwin. Patients with a history of drug dependence should be under close supervision while receiving Talwin orally.*

In prescribing Talwin for chronic use, the physician should take precautions to avoid increases in dose by the patient and to prevent the use of the drug in anticipation of pain rather than for the relief of pain.

Head Injury and Increased Intracranial Pressure. The respiratory depressant effects of Talwin and its potential for elevating cerebrospinal fluid pressure may be markedly exaggerated in the presence of head injury, other intracranial lesions, or a preexisting increase in intracranial pressure. Furthermore, Talwin can produce effects which may obscure the clinical course of patients with head injuries. In such patients, Talwin must be used with extreme caution and only if its use is deemed essential.

Usage in Pregnancy. Safe use of Talwin during pregnancy (other than labor) has not been established. Animal reproduction studies have not demonstrated teratogenic or embryotoxic effects. However, Talwin should be administered to pregnant patients (other than labor) only when, in the judgment of the physician, the potential benefits outweigh the possible hazards. Patients receiving Talwin during labor have experienced no adverse effects other than those that occur with commonly used analgesics. Talwin should be used with caution in women delivering premature infants.

Acute CNS Manifestations. Patients receiving therapeutic doses of Talwin have experienced, in rare instances, hallucinations (usually visual), disorientation, and confusion which have cleared spontaneously within a period of hours. The mechanism of this reaction is not known. Such patients should be very closely observed and vital signs checked. If the drug is reinstituted it should be done with caution since the acute CNS manifestations may recur.

Usage in Children. Because clinical experience in children under 12 years of age is limited, administration of Talwin in this age group is not recommended.

Ambulatory Patients. Since sedation, dizziness, and occasional euphoria have been noted, ambulatory patients should be warned not to operate machinery, drive cars, or unnecessarily expose themselves to hazards.

Precautions: *Certain Respiratory Conditions.* Although respiratory depression has rarely been reported after oral administration of Talwin, the drug should be administered with caution to patients with respiratory depression from any cause, severe bronchial asthma and other obstructive respiratory conditions, or cyanosis.

Impaired Renal or Hepatic Function. Decreased metabolism of the drug by the liver in extensive liver disease may predispose to accentuation of side effects. Although laboratory tests have not indicated that Talwin causes or increases renal or hepatic impairment, the drug should be administered with caution to patients with such impairment.

Myocardial Infarction. As with all drugs, Talwin should be used with caution in patients with myocardial infarction who have nausea or vomiting.

Biliary Surgery. Until further experience is gained with the effects of Talwin on the sphincter of Oddi, the drug should be used with caution in patients about to undergo surgery of the biliary tract.

Patients Receiving Narcotics. Talwin is a mild narcotic antagonist. Some patients previously given narcotics, including methadone for the daily treatment of narcotic dependence, have experienced mild withdrawal symptoms after receiving Talwin.

CNS Effect. Caution should be used when Talwin is administered to patients prone to seizures; seizures have occurred in a few such patients in association with the use of Talwin although no cause and effect relationship has been established.

Adverse Reactions: Reactions reported after oral administration of Talwin include *gastrointestinal:* nausea, vomiting; infrequently constipation; and rarely abdominal distress, anorexia, diarrhea. *CNS effects:* dizziness, lightheadedness, sedation, euphoria, headache; infrequently weakness, disturbed dreams, insomnia, syncope, visual blurring and focusing difficulty, hallucinations (see *Acute CNS Manifestations* under **WARNINGS**); and rarely tremor, irritability, excitement, tinnitus. *Autonomic:* sweating; infrequently flushing; and rarely chills. *Allergic:* infrequently rash; and rarely urticaria, edema of the face. *Cardiovascular:* infrequently decrease in blood pressure, tachycardia. *Other:* rarely respiratory depression, urinary retention.

Dosage and Administration: *Adults.* The usual initial adult dose is 1 tablet (50 mg.) every three or four hours. This may be increased to 2 tablets (100 mg.) when needed. Total daily dosage should not exceed 600 mg.

When antiinflammatory or antipyretic effects are desired in addition to analgesia, aspirin can be administered concomitantly with Talwin.

Children Under 12 Years of Age. Since clinical experience in children under 12 years of age is limited, administration of Talwin in this age group is not recommended.

Duration of Therapy. Patients with chronic pain who have received Talwin orally for prolonged periods have not experienced withdrawal symptoms even when administration was abruptly discontinued (see WARNINGS). No tolerance to the analgesic effect has been observed. Laboratory tests of blood and urine and of liver and kidney function have revealed no significant abnormalities after prolonged administration of Talwin.

Overdosage: *Manifestations.* Clinical experience with Talwin overdosage has been insufficient to define the signs of this condition.

Treatment. Oxygen, intravenous fluids, vasopressors, and other supportive measures should be employed as indicated. Assisted or controlled ventilation should also be considered. Although nalorphine and levallorphan are *not* effective antidotes for respiratory depression due to overdosage or unusual sensitivity to Talwin, parenteral naloxone (Narcan®, available through Endo Laboratories) is a specific and effective antagonist.

Talwin is not subject to narcotic controls.

How Supplied: Tablets, peach color, scored. Each tablet contains Talwin (brand of pentazocine) as hydrochloride equivalent to 50 mg. base. Bottles of 100.

50 mg. Tablets

Talwin®

brand of **pentazocine** (as hydrochloride)

in moderate to severe pain

Winthrop Winthrop Laboratories, New York, N.Y. 10016 (1607M)

Before prescribing, please consult complete product information, a summary of which follows:

Indications: Tension and anxiety states, somatic complaints which are concomitants of emotional factors; psychoneurotic states manifested by tension, anxiety, apprehension, fatigue, depressive symptoms or agitation; symptomatic relief of acute agitation, tremor, delirium tremens and hallucinosis due to acute alcohol withdrawal; adjunctively in skeletal muscle spasm due to reflex spasm to local pathology, spasticity caused by upper motor neuron disorders, athetosis, stiff-man syndrome, convulsive disorders (not for sole therapy).

Contraindicated: Known hypersensitivity to the drug. Children under 6 months of age. Acute narrow angle glaucoma; may be used in patients with open angle glaucoma who are receiving appropriate therapy.

Warnings: Not of value in psychotic patients. Caution against hazardous occupations requiring complete mental alertness. When used adjunctively in convulsive disorders, possibility of increase in frequency and/or severity of grand mal seizures may require increased dosage of standard anticonvulsant medication; abrupt withdrawal may be associated with temporary increase in frequency and/or severity of seizures. Advise against simultaneous ingestion of alcohol and other CNS depressants. Withdrawal symptoms (similar to those with barbiturates and alcohol) have occurred following abrupt discontinuance (convulsions, tremor, abdominal and muscle cramps, vomiting and sweating). Keep addiction-prone individuals under careful surveillance because of their predisposition to habituation and dependence. In pregnancy, lactation or women of childbearing age, weigh potential benefit against possible hazard.

Precautions: If combined with other psychotropics or anticonvulsants, consider carefully pharmacology of agents employed; drugs such as phenothiazines, narcotics, barbiturates, MAO inhibitors and other antidepressants may potentiate its action. Usual precautions indicated in patients severely depressed, or with latent depression, or with suicidal tendencies. Observe usual precautions in impaired renal or hepatic function. Limit dosage to smallest effective amount in elderly and debilitated to preclude ataxia or oversedation.

Side Effects: Drowsiness, confusion, diplopia, hypotension, changes in libido, nausea, fatigue, depression, dysarthria, jaundice, skin rash, ataxia, constipation, headache, incontinence, changes in salivation, slurred speech, tremor, vertigo, urinary retention, blurred vision. Paradoxical reactions such as acute hyperexcited states, anxiety, hallucinations, increased muscle spasticity, insomnia, rage, sleep disturbances, stimulation have been reported; should these occur, discontinue drug. Isolated reports of neutropenia, jaundice; periodic blood counts and liver function tests advisable during long-term therapy.

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