VENIPUNCTURE IN THE MEDICAL PHYSIOLOGY LABORATORY

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Medical physiology laboratories, traditionally devoted to animal experimentation, face unprecedented difficulties linked to cost, staffing, instrumentation, and the use of animals. At the same time, laboratory experiences with living creatures play a unique role in medical education. In this article we describe the use of venipuncture and subsequent blood analysis, with medical students serving as both subjects and experimenters, in a sequence of first-year physiology laboratories. These experiments are safe, robust, inexpensive, and time efficient, and they teach the principles of cardiovascular, respiratory, renal, nutritional, and gastrointestinal physiology. In addition, they enhance medical education in several other important dimensions. First, they teach safe venous blood collection and handling, a training appropriate for students at this level. Second, by serving each week as subjects as well as experimenters, students experience aspects of both sides of the doctor-patient relationship. Third, the laboratories can be used to teach fundamentals of research design and data analysis. Finally, because blood analysis is central to medicine, and because the student's own blood data are discussed, students are enthusiastic and cooperative, and the clinical relevance of the data is clear.

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Laboratory experiences in physiology have long been the province of animal experimentation. Although such experiments can explore the principles of physiological regulation, animal studies are beset by problems ranging from cost to staffing, to instrumentation, and to questions concerning animal use itself. In Bloomington, where physiology has been taught to first-year medical students since 1903, retirements of faculty and staff experts in dog experimentation, along with aging instrumentation, brought the laboratories to a crossroads in 1990. Continuation of animal experimentation was unquestionably too costly, in terms of both personnel and equipment. At the same time, the laboratory experience offers several unique opportunities for students. The solution to this problem began with a single laboratory centered around human blood analysis, with venipuncture performed by students on students, after training and under supervision. In meetings with these same students during their third year of medical education, in which issues regarding basic medical education were explored, these students strongly suggested that more laboratories should be developed that involve analysis of venous blood. Consequently, blood studies have been introduced into a sequence of seven laboratories; these exercises achieve, in our view, more educational objectives than the animal experiments they replaced. The rationale for development of these

laboratories, several considerations common to the entire group of experiments, and then specific details of each exercise, are described here.

RATIONALE

Five criteria guide development of new laboratories involving venipuncture. These principles are as follows.

Safety. Because collecting and handling blood is an inevitable part of medical education, learning to apply safe practices regarding blood-borne hazards is an appropriate goal for the first year of medical education. Our laboratories were developed with the cooperation of the Bloomington campus, Indiana University School of Medicine, Committee for Biosafety, Human Tissues and Fluids Subcommittee. During the first laboratory involving venous blood collection, a member of the biosafety committee is invited to speak to the students, a nurse is present to teach proper venipuncture technique, and a film (*Blood Collection: The Routine Venipuncture*) from the American Society of Clinical Pathologists, devoted to universal precautions and proper blood collection and handling methods, is shown. The nurse, after discussing and demonstrating appropriate methods for blood collection, also provides two artificial arms for practice. This training before an initial puncture matches that provided in the professional training of phlebotomists. In addition, one of the faculty (J. B. Watkins III) present throughout all of the experiments is a trained phlebotomist, and each member of the staff is experienced in performance and teaching of venipuncture. Every week, samples are drawn with students supine to minimize the possibility of fainting. All students, regardless of prior experience in phlebotomy, are trained as if entirely naive.

Before the series of laboratories itself, it is made clear to students that certain medical conditions require nonparticipation in one or several laboratories. Specifically, bleeding disorders and diabetes mellitus are mentioned. Students are urged to discuss with an instructor, before a given exercise, any potential medical concerns.

A related concern is the loss of confidentiality regarding results from blood and other tests as measured in the laboratory. Student data is posted for all to see and is discussed in detail. At the outset of the course, students are encouraged to speak with a teacher regarding any test or tests for which they might prefer confidentiality.

Safety is, of course, paramount in all other experimental procedures as well. The exercises are not research and, consequently, not under the jurisdiction of a Committee for Protection of Human Subjects. Nonetheless, the concept of ''minimal risk,'' that is, activities or tests that present no more risk than those normally encountered in daily life, is applied to determine whether specific procedures are appropriate for these laboratories.

Illustration of physiological principles. The sequence of seven laboratories begins with focus on cardiovascular, hematologic, and hemostatic regulation in physiology. Subsequently, blood analysis is used to augment experiments devoted to renal, metabolic, and respiratory physiology, gastrointestinal absorption, and blood glucose regulation.

Student participation. Blood analysis is replete with easily measured variables, even when inexperienced personnel collect, centrifuge, and pipette blood and read, calculate, and record data. By choosing variables whose determination is relatively uncomplicated, the involvement of students can be increased.

Cost. The cost for subjects is zero; the cost for supplies is modest; the cost for analytic kits for blood-borne substances ranges widely, but only rarely must important assays be bypassed because of monetary considerations. The choice of a variable for determination depends on available instrumentation and must be adapted to existing facilities and equipment. Typically, a general-purpose centrifuge, a spectrophotometer, and approximately $5.00/student/week for supplies are sufficient.

Time constraints. Experiments are designed to keep students comfortably busy for 2–3 h. Blood can be obtained and analyzed, and the data discussed, within this time, with students active throughout. On some occasions, longer assays requiring multiple steps or substantial delays are performed by technical personnel and then presented to students and discussed in an

ensuing lecture or laboratory. Cross-references between lecture and lab clearly enhance both.

GENERAL CONSIDERATIONS

The Medical Sciences Program. The Bloomington campus of the Indiana University School of Medicine possesses several advantages for venous blood studies in Medical Physiology. At this site, 28 first- and 28 second-year students are trained. Medical physiology laboratory is taught on two afternoons each week for 14 weeks in the spring semester of the first year. The class is divided so that each afternoon session includes 14–17 students (medical and graduate). Because the Program also teaches undergraduate physiology laboratories, a full-time lab demonstrator is available for setup, cleanup, and technical assistance. Participating faculty are present throughout each lab session, a necessity in view of the inexperience of the students. Two faculty members, the demonstrator, and an associate instructor combine to yield an advisor-student ratio of ~1:4; in addition, a nurse is present during the first week involving blood.

Sequence of laboratories. The semester begins with at least one bloodless lab, allowing students to become familiar with the room and other procedures before needles are introduced. Currently, blood is utilized in seven successive weeks of laboratory.

Rotation of venipuncture partners. Each week, students are assigned new ''partners'' for venous sampling. Because in any population veins differ widely in accessibility, a student paired with a partner with small or deep veins will have difficulty collecting blood. For most students, fears about drawing blood (fear of using a needle on another person and fear that one will fail to collect a sample) exceed fears of giving blood. A satisfactory standard is that every week every student will successfully draw blood, whether from the partner, another student, or a faculty member, and every student is expected to allow at least one attempt at a vein.

Student anxiety. The intense student anxiety creates opportunities to discuss the fears of patients concerning medical tests and procedures and the role of the physician in allaying those fears.

Data reliability. Each week, questions of the biological and clinical significance of the data, and of its reliability, can be explored. The various potential technical errors in measurement—in blood sampling, pipetting, reagents, standards, and so forth—are all discussed, because students have immediate experience with each phase. Comparisons of techniques used in this learning environment with actual clinical laboratory procedures are made repeatedly.

During seven successive laboratories, the efficiency of blood collection steadily improves. For example, obtaining samples from 16 students, which requires perhaps 90 min the first week, may require only 20 min in the sixth week. Increased efficiency allows the focus to shift, week by week, from the venous sampling procedure itself to the biological and clinical science that blood analysis illuminates.

EXPERIMENTAL DETAILS

Week 1: Teaching venipuncture; analysis of hemoglobin and hematocrit. The focus of this laboratory is the teaching of venipuncture itself, with only simple blood analyses included. Once samples are in Vacutainers, hematocrit and hemoglobin are readily measured. A diagnostic kit is used to measure total hemoglobin (procedure no. 525, Sigma Diagnostics); the demonstrator prepares solutions, pipettes, and spectrophotometers (complete with blank, standard solutions, and standard curves). Repetitive steps involving micropipetting are assigned to the associate instructor or demonstrator. After distribution of aliquots of this first sample for hemoglobin and hematocrit readings, the remaining blood is taken to the local hospital for a complete blood count. These last results are returned to students for discussion in the second week involving blood.

In this and each subsequent lab, individual data are written on a large blackboard. The data blackboard serves several functions. Students quickly recognize when their data are missing and move faster to complete work. The variety of ''normal'' values, evident in every measurement, teaches that ''normal'' is a range. Discussion of means, standard deviations, and other statistical concepts proceeds naturally. Because the board this first week is organized to segregate male and female students, differences in

hematocrit and hemoglobin between genders become apparent.

Blood analyses utilized: hemoglobin and hematocrit.

Blood analyses tried in the past and rejected (for reason in parentheses): serum iron and total iron-binding capacity (difficult to perform; slow to complete; complex in explanation).

Week 2: Hemostasis. Three to five hours before the laboratory (after prior screening for persons with possible bleeding disorders and persons potentially sensitive to salicylates), all students receive in double-masked fashion either placebo or 650 mg sodium salicylate. After venous blood samples have been drawn, each student performs a bleeding-time test on the partner's forearm. Students are told that a small scar can result from this test and, as always, are given the opportunity not to participate. This simple, clinically relevant test gives excellent results: statistically significant differences are typically found between the aspirin and placebo groups despite the use of unpaired comparisons of only eight students in each group. In addition, breaking the double-masked code affords students an excellent introduction to research design.

Although prothrombin time and activated partial thromboplastin time are important hemostatic parameters, neither variable is easily or inexpensively measured in a student laboratory (and neither would be expected to change under aspirin administration). Instead, students determine blood groups and Rh factor, and individual results from the complete blood counts are returned and then discussed. As a result of the affiliation of our program with Bloomington Hospital, these blood analyses are obtained at a substantial discount. The complete blood counts are information intensive; with students relaxed after blood collection and analysis are complete, discussion can focus on differential white blood cell counts, the various measures of red blood cell number and volume (comparison of hospital- and student-measured hemoglobin and hematocrit is informative), and the factors involved in hemostasis.

Blood analyses utilized: bleeding time, blood groups and Rh factor, and discussion of complete blood count (results from blood samples obtained during the previous week).

Blood analyses tried in the past and rejected (for reason in parentheses): prothrombin time and activated partial thromboplastin time (complex steps; unavailable equipment).

Week 3: Renal physiology. The laboratory begins with students collecting an initial urine sample in a relatively dehydrated state (no liquid intake allowed for 4 h before laboratory) and then drinking a liter of either water or an electrolyte solution. After initial urine collection and fluid ingestion is complete, urine flow rate, osmolality (Wescor model 5100B vapor pressure osmometer; Wescor, Ogden, UT), and specific gravity are measured, and determination of urine creatinine concentration (procedure no. 555, Sigma Diagnostics) is begun. A second urine sample from the hydrated state is collected 1 h later. In the hour between urine samples, blood collection takes place. Blood urea nitrogen (procedure no. 535, Sigma Diagnostics) is measured by students after preparation by the demonstrator of reagent solutions, spectrophotometers, boiling water bath, and micropipettes. Urine creatinine is measured directly, and plasma creatinine assumed, in the calculation of glomerular filtration rate. This allows one blood analysis, and one urine-based analytic procedure, to occupy this afternoon. Augmenting discussion of renal function with blood measurements solidifies for students the integration of the renal and cardiovascular systems.

Blood analysis utilized: blood urea nitrogen.

Blood analysis tried in the past and rejected (for reason in parentheses): blood creatinine (time constraints).

Week 4: Metabolism. Heart rate, blood pressure, minute ventilation, oxygen consumption, and carbon dioxide production are measured after 2 min of exercise at each of three intensities (zero load pedaling, 60 W, and 150 W) on cycle ergometers in this laboratory. Calculation of cardiac output from predictive equations based on oxygen consumption allows further calculation of stroke volume, total peripheral resistance, and ventilation-perfusion ratio of the lung during graded exercise. With the students divided into

four groups of four, blood collection takes place as students complete exercise. Samples are later analyzed for total and high-density lipoprotein (HDL) cholesterol (procedure nos. 352 and 352–4, Sigma Diagnostics) by the demonstrator. These cholesterol results are returned to the students and discussed during the laboratory devoted to glucose tolerance (*week 7*), because that session is less labor and data intensive.

Blood analyses utilized: total serum cholesterol and HDL cholesterol.

Blood analysis tried in the past and rejected (for reason in parentheses) blood lactate (time constraints).

Week 5: Nutrition. This laboratory requires the selection of a local ''fast-food'' restaurant that can provide both a written menu and detailed nutritional information about each menu item. The menu is distributed to students, who choose various items for lunch, and these are purchased, brought to the laboratory, and consumed as the session begins. Students then analyze and record total calories, calories from fat, protein, and carbohydrate, and cholesterol, vitamin, and mineral content of their meals. Students also record their height, weight, triceps skinfold, and calculated midarm muscle area as an estimate of lean body mass. A venous blood sample is drawn as these procedures are completed and later is taken to the local hospital for a blood chemistry profile (included are 21 items, including blood glucose, cholesterol, triglycerides, and electrolyte levels).

Within the laboratory period itself, the wide range among the students in meal content (typical range: 200–2,000 kcal, 0–300 mg cholesterol, 50–2,500 mg sodium) animates discussion of dietary factors in disease. Meal analysis in concert with estimates of lean body mass and fat mass and comparison of normalized body weight with standard tables illuminates discussion of anorexia, malnutrition, and obesity. The blood chemistry profile is returned to students for discussion in *week 7*, during the glucose tolerance test. Among the issues discussed at that time is the relationship between acute dietary fat intake and serum triglyceride levels.

Week 6: Respiration. After 5 wk experience with antecubital vein puncture, students are prepared to attempt ''butterfly'' needle puncture of a dorsal hand vein. Wrapping the hand and wrist in a heating pad allows local venous blood to approximate arterial blood in pH and P_{CO_2} (1). With the laboratory primarily devoted to pulmonary function testing, with artificial simulation of obstructive and restrictive lung disease, blood gas analysis directly links the lungs to homeostasis in arterial blood. Students rotate between three stations. The first utilizes Stead-Wells spirometers (Collins, Braintree, MA) for measurement of vital capacity and the forced expiratory volume in 1 s. A second station uses a spirometer (Ohio Instruments) in conjunction with an *x-y* recorder adapted for measurement of a flow-volume loop, with attendant calculation of the maximal flow rate on expiration. At each of these stations, obstructive and restrictive disease are simulated, respectively, by placement of rubber stoppers with small central holes placed in the expiratory tube and by application of a tightly fitted thoracic fabric wrap. The third station is focused on collection of the blood sample. Each pair of students at this station is directly supervised by a faculty member. Blood samples are analyzed for pH and P_{CO_2} using an automated blood gas analyzer (Radiometer).

Blood analysis utilized: pH and P_{CO_2}.

Essential equipment: blood gas analyzer.

Week 7: Glucose tolerance test. This laboratory is focused on gastrointestinal absorption of ingestate and the subsequent blood glucose response. Inured to blood collection, students willingly provide as many fingertip samples as necessary; in this experiment, blood is collected every 15 min for 2 h. Blood glucose determination using kits purchased from local pharmacies gives students insight into the routines of the diabetic patient. The formal glucose tolerance test, although rarely used clinically today and occasionally provoking mild nausea, does generate wide-ranging blood glucose responses that facilitate discussion about borderline diabetes mellitus and the role of specific dietary choices and drugs in the treatment of the disease. Our program is equipped with a breath hydrogen analyzer (Quintron Instruments, Milwaukee, WI), allowing us to measure both H_2 and glucose responses to lactose ingestion. A subgroup of two or

four students are paired to demonstrate both lactose digestion and maldigestion. With the remaining students requested to choose either high- or low-fiber breakfasts on the day of the laboratory (bringing their dietary records to the session), the initial breath hydrogen level is used to discuss colonic physiology. The minutes between blood glucose determinations are used for distributing and discussing prior results concerning total and HDL cholesterol (from *week 4*) and the blood chemistry profile (*week 5*).

Blood analysis utilized: fingertip blood glucose.

SUMMARY

Laboratories involving human blood can be made safe, inexpensive, and time efficient. The sessions illuminate fundamental aspects of the physiological regulation of interrelated organ systems. In our opinion, these laboratories achieve more educational objectives for medical and graduate students than the animal experiments that they replaced.

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