SYLLABUS - FALL SEMESTER 2009

ANIMAL PHYSIOLOGY: 86356 341 01 (Lect) 82265 341 31 (Lab)
Lecture: Tuesday and Thursday 11:10 to 12:30 p.m. in room # 224 E. E. Just Hall
Instructor: Professor Abner B. Lall <alall@howard.edu> and Dr. Kenneth Sossa
              <ksossa@hotmail.com>
Laboratory: 12:40 to 3:30 p.m. in Room # 225
Teaching Assistant: Ms Shannon Smith <shannon.274@gmail.com>
Office Hours: Dr. Lall - Room # 219, Tues & Thurs 10:30 - 11:00 p.m.

This course covers the principles of animal physiology and deals with the physiological mechanisms, which make it possible for animals to function in a variety of environments. It also describes how different species of animals survive diverse habitats and the stresses, which result from changes occurring in the environment. The course deals with general and cellular physiology, cardiovascular, respiratory, osmoregulation, hormonal, reproductive, and muscular and energy systems. The interdisciplinary nature of the subject requires that the student have had college level knowledge of general chemistry and organic chemistry (or currently enrolled in a course in organic chemistry). It is taken for granted that a student has had general biology as well as other 200 level courses in biology. Those students who have had one year of physics will have easier time. This course and its sister course (Bio 444 01-Neurophysiology/Neuroscience) offered during the Spring term essentially are geared for preparing students for taking MCAT and for entry examinations into allied medical professions.

Required Text: Principles of Animal Physiology by Christopher D. Moyes

Auxiliary Texts:

Animal Physiology, 2nd Edition by Richard W. Hill, Gordon A. Wyse and
Margaret Anderson, Sinauer Associates, Sutherland, MA 2008

Animal Physiology: Genes to organisms, by Lauralee Sherwood, Hillar Klandorf
and Paul H. Yancey: Thomson Books/Cole, Belmont, CA 2005

Eckert Animal Physiology: mechanisms and adaptations, 5th Edition by David
2002

Human Physiology: An Integrated Approach by Dee Unglaub Silverthorn,
<table>
<thead>
<tr>
<th>Lecture #</th>
<th>Date</th>
<th>Subject</th>
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<tr>
<td>Week # 1</td>
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<td>Week # 2</td>
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<td>3.</td>
<td>September 2 (Tues)</td>
<td>Cell membrane etc. Chapter 2: 64-84</td>
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<td>Week # 3</td>
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<td>Week # 4</td>
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<td>7.</td>
<td>September 16 (Tues)</td>
<td>First Hour Exam</td>
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<td>8.</td>
<td>September 18 (Thurs)</td>
<td>Biochemistry Chapter 2: 35-63</td>
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<td>Week # 5</td>
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<td>9.</td>
<td>September 23 (Tues)</td>
<td>Cell Signaling and Endocrine Regulation</td>
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<td>Week # 6</td>
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<td>12.</td>
<td>October 2 (Thurs)</td>
<td>Reproduction Continued</td>
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<td>Week # 7</td>
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<td>13.</td>
<td>October 7 (Tues)</td>
<td>Circulatory system Chapter 8:348-409.</td>
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<td>14.</td>
<td>October 9 (Thurs)</td>
<td>Circulatory system continued</td>
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<td>Week # 8</td>
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<td>15.</td>
<td>October 14 (Tues)</td>
<td>Circulation system continued</td>
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<td>16.</td>
<td>October 16 (Thurs)</td>
<td>Second Hour Examination</td>
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<td>Week # 9</td>
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<td>17.</td>
<td>October 21 (Tues)</td>
<td>Cellular movement and Muscle Chapter 5:196-245.</td>
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<td>18.</td>
<td>October 23 (Thurs)</td>
<td>Continued Chapter 5</td>
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<td>Week # 10</td>
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Week #11
21. November 4 Respiratory Systems Continued
22. November 6 Respiratory Systems Continued

Week #12
23. November 11 (Tues) Holiday
24. November 13 (Thurs) Third Hour Exam

Week #13

Week #14
27. November 25 (Tues) Ion and water balance continued

Week #15
28. December 2 (Tues) Energy Metabolism Chapter 12: 580-622*
29. December 4 (Thurs) Thermal Physiology Chapter 13:624-661*
* Selected topics

FINAL FOURTH HOUR EXAMINATION on Friday, the 17th of December at 11 a.m. TO 12 NOON AND FINAL COMPREHENSIVE (Essay type) from 12 noon to 1:00 p.m. All students are to take FOUR one hour examinations and the ONE HOUR final comprehensive without any exception. It is mandatory to take an examination on the date it is given. Any absence from the examination without a medical reason (a physician's letter is necessary for verification) will be counted as zero. Any late make-up examination automatically would mean a reduction of 10 points. Whenever an examination is missed a grade of zero will be averaged. All home-work assignments are mandatory and to be completed on time.

Four one hour examinations are 100 points each
The final comprehensive is 100 points
The laboratory examination and the homework assignments equal 100 points.
Total score will be 600 points.

A is between a total of 448 points and higher.
B is between a total of 398 and 447 points.
C is between 348 and 397 points.
D is between 297 to 347 points.

The exact dates for the laboratory section will be announced later.
SYLLABUS (FALL 2009)

TIME: Tuesday, Thursday 2:10 PM to 5:00 PM (2:10 – 3:30; 3:40 – 5:00)

ROOM: 337, Lecture; 337, Laboratory

INSTRUCTOR: Dr. T. A. Bremner
Office hours: Rm 244: Mon. 3:30 PM to 5:30 PM; Thurs. 10:30 AM 12:30 PM; or by appointment.

All laboratory protocols will be provided.

RECOMMENDED READING:
3. AT THE BENCH: A Laboratory Navigator. Kathy Barker, Cold Spring Harbor Laboratory Press, 2005. Contains valuable information on laboratory techniques, procedures, safety, data handling and presentation, etc. [Should be on reserve in Founders]

EVALUATION

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<tr>
<th>Requirement</th>
<th>% of grade</th>
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<td>3 full-period examinations (to be announced)</td>
<td>50</td>
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<tr>
<td>Quizzes (unannounced)</td>
<td>10</td>
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<td>Laboratory (participation, assignments, presentations)</td>
<td>25</td>
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<td>Final examination:</td>
<td>15</td>
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<td>Total</td>
<td>100</td>
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100%-90% = A; 89%-80% = B; 79%-70% = C; 69%-60% = D; <60% = F

The following are required for participation: A laboratory coat, scientific calculator, laboratory notebook, and an assigned locker (no coats or bags will be allowed in the laboratory). Blue Book examination books (11" x 8.5") will be required for all examinations. Laboratory exercises require extensive preparation; there will be no "make-up" laboratory exercises. Blackboard access is absolutely necessary for participation in this course. Most of the instructional material will be posted on Blackboard. At registration, please be sure to provide your current e-mail address to Blackboard. Class announcements will be made via e-mail through Blackboard.

The use of cell phones, iP0Ds, and other communication or entertainment devices is prohibited in this class.
TECHNIQUES IN BIOCHEMISTRY is designed to provide basic laboratory skills that have wide applicability in the biomedical sciences. It is open to senior undergraduates who have completed the core curriculum in biology or who have had equivalent experiences (see instructor) and to graduate students at all levels. Although a survey course in biochemistry is highly recommended, it is not required. However, a student must have completed organic chemistry before registering for this course. This is not a course in instrumentation. Students are required to understand the theoretical basis of each procedure or technique covered, and to generate and analyze quantitative data.

Educational Objectives
Upon completion of this course students will be able to:
1. Calculate the pH of a solution of known hydronium ion concentration
2. Prepare commonly used laboratory solutions and buffers
3. Measure, spectrophotometrically, the protein concentration of a biological sample
4. Determine the initial velocity of an enzyme-catalyzed reaction
5. Determine the $K_M$ of an enzyme for its substrate
6. Measure the specific activity of an enzyme in a biological sample
7. Use a gel chromatography column to desalt a protein sample
8. Isolate and quantify total RNA from cells
9. Isolate mRNA by oligo(dT) affinity chemistry using magnetic bead technology
10. Calculate RCF
11. Prepare lysis and homogenization solutions of known pH and reagent concentration
12. Separate proteins and nucleic acids by SDS-PAGE and agarose gel electrophoresis
13. Perform a co-immunoprecipitation experiment to show protein:protein interaction
14. Design a Reverse Transcription-Polymerase Chain Reaction experiment to measure levels of specific mRNAs in cells using gene-specific primers
15. Prepare nuclear and cytoplasmic extracts of cells

Statement on ADA Procedures
Howard University is committed to providing an educational environment that is accessible to all students. In accordance with this policy, students in need of accommodations due to a disability should contact the Office of the Dean for Special Student Services for verification and determination of reasonable accommodations as soon as possible after admission to the University, or at the beginning of each semester. The Dean of the Office of Special Student Services, Dr. Barbara Williams, can be reached at (202) 238-2420.

A single make-up examination (worth 20 supplemental points) may be given, if necessary. Rules for the make up exam are as follows: The student must earn 70% or more on the make-up in order to earn a supplement: a failing score will not be added to another failing score to give a passing score. For example, if a student earns 65 (D) on the hourly exam and 69/100 (D) on the make-up, no supplement is earned, and the score remains 65. The score on the make-up is the percentage of 20 points (the value of the make-up) that will be added to the original score. For example, if the original score is 57, and the make-up score is 90%, a supplement of 18 points (90% of 20), will be added to the original score to give a
total of 75 (a C). If the original score was 67, and the make-up score is 60% (a D), no supplement is earned. The original score plus the supplement may bring a student's score to 78 (a C) but no higher.

INSTRUCTIONAL UNITS

1. **SOLUTIONS.** pH and buffers (Tris, HEPES, phosphate); concentration (M, N, % w/v, % v/v, osM); conversions. (Each student is required to master the relevant calculations and prepare specified buffers. pH of solutions of strong acids and bases; of weak acids and bases. pKs of dissociable groups in amino acids. Titration curves of weak acids. Use of the Henderson-Hasselbalch equation in preparing buffers of specified pH, for predicting the pH of mixtures of conjugate acids and conjugate bases, and for calculating pH after addition of acid or base to a buffer. Biological buffers: characteristics; buffering capacity and buffering; Good's buffers.)

Students will generate and interpret UV-Visible absorbance spectra of oxidized and reduced NADPH, and cytochrome c. Using molar extinction coefficient (molar absorptivity) to calculate the concentration of a chromophore in solution.

2. **METHODS OF PROTEIN DETERMINATION.** UV absorbance; Lowry; Peterson modification of Lowry; BCA assay; Bradford method. Detergent compatible protein assays.

3. **CENTRIFUGATION.** Relative centrifugal force (RCF); linear and continuous density gradients (sucrose, Percoll); cell fractionation; CsCl isopycnic density gradient centrifugation.

4. **PROTEIN PURIFICATION/CHROMATOGRAPHIC METHODS.** Protein structure: bonds involved in maintaining 3° and 4° levels of structure. Denaturation and renaturation. Cell and tissue disruption; lysis buffers and homogenizing media; ionic and nonionic detergents; effects of oxidation, dilution, temperature, etc. Protective reagents: reducing agents, protease inhibitors, chelating agents. Protein precipitation: isoelectric precipitation, salting in, salting out. Desalting: dialysis, molecular exclusion chromatography (desalting columns). Concentration by membrane filtration, dehydration, lyophilization. Protein purification and estimation of molecular weight: Molecular exclusion chromatography, affinity chromatography; ion-exchange chromatography.

5. **ENZYME ASSAYS.** Progress curves: Initial or instantaneous velocity (\(v_0\)), Maximum velocity (\(V_0\) or \(V_{MAX}\)), activity, specific activity. Estimation of apparent \(K_M\) from Lineweaver-Burk and Eadie-Hofstee plots. Chromogenic substrates and enzyme conjugates: Horseradish peroxidase, alkaline phosphatase; avidin/streptavidin-Biotin; β-galactosidase and X-GAL. Cellular Activation of Signaling ELISA (CASE™). Caspase assays for apoptosis. Competition assay ELISA for low-abundance proteins.
7. **ELECTROPHORETIC METHODS.** PAGE; SDS-PAGE; gel electrophoresis and activity staining for isozymes and other protein isoforms. Western blot analysis of labeled proteins. [Northern blot analysis of mRNA using a biotinylated ds DNA probe]. Gel mobility shift assays (discussion/demonstration).

8. **CELL CULTURE METHODS.** Anchorage-dependent and suspension cultures; cell counting and growth curves. Viability assays: Trypan Blue, MTT assay, Quantitative Neutral Red-Uptake. Cytospin preparations of suspension cells.

9. **AFFINITY-BASED METHODS:** Immunoprecipitation, immunodepletion, and GST pull-down assays. Detection of protein-protein interaction *in vitro* and *in vivo*. Identification of proteins in immunocomplexes by SDS-PAGE and immunoblotting. Co-immunoprecipitation (Co-IP) and Chromatin immunoprecipitation (ChIP). GST-based kits for pull-down assays of Rho, Rac, and Cdc42. ELISA for specific proteins, ELISA-based assays for quantification of transcription factors (HIF-1α, NF-κB).

10. **NUCLEIC ACIDS.** Isolation of high MW DNA from cultured cells. Spectrophotometric quantitation of DNA. Isolation of total RNA from cultured cells using the Versagen™ RNA Cell Kit. Purification of poly(A)$^+$ mRNA on oligo (dT)$_{25}$ affinity magnetic beads using Dynabeads® mRNA DIRECT kit. Spectrophotometric quantitation of RNA and measurement of $A_{260}/A_{280}$.

11. **PCR.** Reverse transcription-polymerase chain reaction (RT-PCR) for estimating the relative abundance of specific mRNAs. mRNA is reverse transcribed and the cDNA product is amplified by PCR using gene-specific forward and reverse primers. The amplified product is separated on agarose gel, stained with EtBr and photographed by UV illumination. Amplimers are identified by size using a DNA MW ladder. Transcriptional profiling: DNA microarrays and gene arrays.
References

RNA ISOLATION FROM TISSUE CULTURE CELLS

SINGLE STEP ISOLATION OF RNA WITH TRIZOL™ REAGENT

DIRECT VISUALIZATION OF NUCLEIC ACIDS ON NYLON MEMBRANES

PCR NONRADIOACTIVE LABELING SYSTEM FOR SYNTHESIS OF BIOTINYLATED DNA PROBES.

IMMUNOPRECIPITATION WITH ANTI-PHOSPHOTYROSINE ANTIBODIES

PCR and PCR-ELISA:

PROTEIN-PROTEIN INTERACTION:
PROTEIN FOLDING

DISULFIDE BOND FORMATION AND PROTEIN FOLDING
It has been generally accepted that cellular disulfide bond formation occurs only in the lumen of the endoplasmic reticulum (ER), because only the ER possesses the requirements for disulfide bond formation: an oxidizing environment and the enzyme protein disulfide isomerase (PDI). In recent years, however, compelling evidence has been presented for disulfide bond formation in mitochondria. Mitochondrial disulfide bond formation occurs in the mitochondrial intermembrane space and serves to facilitate protein translocation into the mitochondrion. Such translocation is necessary because many mitochondrial proteins are encoded in nuclear genes and synthesized (translated) on cytosolic ribosomes. Although the two systems are not evolutionarily related, the same result is accomplished.

Reference(s)

INTRINSICALLY DISORDERED PROTEINS AND PROTEIN FOLDING
Many proteins lack tertiary structure yet possess specific biological functions. Such Intrinsically disordered proteins, aka inherently disordered proteins (IDPs) or natively unfolded proteins, undergo disorder-to-structure transitions upon binding to physiological targets. Failure to fold into 3D structures is inherent in the amino acid sequences of IDPs. Intrinsic disorder may have implications for protein evolvability.

References: