

**SECTION 7**

**ABSTRACTS OF GRADUATE STUDENTS  
RESEARCH PRESENTATIONS**

**Chairpersons**

Dr. Peter Bentley

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# The Effects of Sodium Bicarbonate and Added Dietary Fat on Racing Performance in Thoroughbred Racehorses

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Fifteen Thoroughbred racehorses in a training program ran a 1600-m race while on a control diet (CR) then were fed an isocaloric experimental diet for a 3 week period in which fat accounted for 12% of the digestible energy before running a second 1600-m race (FR). The mean race time for the FR was significantly faster ( $P < 0.05$ ) than the mean race time for the CR. Plasma glycerol, triglycerides, non-esterified fatty acids, beta hydroxybutyric acid, and glucose were significantly altered by the fat-added diet. Muscle glycogen storage was also significantly increased, however that does not appear to be the reason for the decreased running time. Rather, the improvement in race time was due to increased availability of blood glucose, better utilization of ketone bodies for

fuel, and a reduced state of ketosis in the fat-fed group postponing the onset of fatigue.

Sodium bicarbonate was administered at a dose of 0.4 g/kg body weight (BW) to 16 Thoroughbred racehorses 3 hours prior to a 1600-meter competitive race to study the effects of induced alkylolysis on exercise performance. Each horse competed in two 1600-m races in which pre-exercise treatment consisted of 0.4 g/kg BW  $\text{NaHCO}_3$ , there was a significant increase in venous blood pH and plasma lactate but no change in race times, venous  $\text{PCO}_2$ , or  $\text{HCO}_3^-$ . It is concluded that the administration of  $\text{NaHCO}_3$  to Thoroughbred racehorses prior to a 1600-meter race has no ergogenic benefit.

## An Isolated Perfused Tumored Skin Flap Model

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The use of intraarterial infusion and regional perfusion for localized cancer chemotherapy is theoretically advantageous to systemic delivery because increased local and decreased systemic drug concentrations allow for enhanced efficacy and reduced systemic toxicity. An isolated perfused tumored skin preparation would allow for the development of a model in which the effects of intraarterial infusion and regional perfusion could be evaluated under a variety of conditions (ie, altered perfusate temperature and pH, hyperthermia, hyperbaric oxygen concentration, drug induced vasodilation). The purpose of this abstract is to report preliminary results in the development of the isolated perfused tumored skin flap.

During a pilot study, we injected one of the following human tumor cell lines into 6-8 week-old Yorkshire-cross pigs that were immunosuppressed with cyclosporine: UM-UC-9, bladder carcinoma; JAR, choriocarcinoma; HXG, melanoma. All cell lines produced tumors, but the injection of JAR cells resulted in tumors that were larger and grew more rapidly. We then

determined the growth kinetics of tumors that developed after the injection of approximately  $3 \times 10^6$  JAR cells with and without the co-injection of  $1 \times 10^6$  normal human fibroblasts. Again, tumors developed at all injection sites. Some tumors were noted 2 days after injection, all were present by day 6. Maximal tumor size was reached by day 12, after which time tumor regression was noted. By day 24, the average tumor volume was 14% of maximal; 6 tumors had regressed completely. A beneficial effect of the co-injection of fibroblasts was noted in that tumors were larger and persisted longer. Tumor histopathology and morphometry will be discussed.

We conclude that the development of an isolated perfused tumored skin flap model is feasible. Future studies will evaluate the disposition of chemotherapeutic agents into the tumor compartment with and without hyperthermic conditions.

# The Effect of Hyperthermia on Normal Tissue Toxicity and Pharmacokinetics of Adriamycin in Dogs

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Several investigators have reported increased cytotoxicity of various anti-tumor drugs, including adriamycin, when combined with hyperthermia *in vitro*. This study examined the effects of adriamycin (ADR, 30 mg/m<sup>2</sup>), whole body hyperthermia (WBH, 42°C, 1 hr.), and the combination of the two (ADR/WBH) on gastrointestinal and hematopoietic toxicity and the effects of WBH on ADR pharmacokinetics.

Duodenal biopsies were taken from animals in each group via endoscopy and were incubated in the presence of <sup>3</sup>H-thymidine as an index of cell viability. Additional duodenal biopsies were assayed for the enzymes gamma-glutamyltranspeptidase, N-acetyl-B-D-glucosaminidase, and succinate dehydrogenase. Complete blood chemistry profiles and differential blood cell counts

were done prior to and following treatment. Gastrointestinal cytotoxicity was greatest 3 days after ADR or ADR/WBH.

WBH-induced alterations in adriamycin pharmacokinetics were examined by collecting plasma for drug analysis from the ADR and ADR/WBH groups at specific times up to 6 hours after drug administration. Adriamycin and its major metabolite adriamycinol were quantified using HPLC. Duodenal biopsies were collected immediately and 1 hour after drug administration for measurement of tissue concentrations of adriamycin. Significant changes in adriamycin pharmacokinetics occurred when drug was administered concurrently with WBH.

# The Effect of Environmental Factors on the Percutaneous Absorption and Cutaneous Metabolism of Parathion.

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The effect of environmental factors on the percutaneous absorption and metabolism of parathion in excised porcine skin was assessed in a flow-through diffusion cell system by varying the temperature (T), relative humidity (%RH), perfusate flow rate and composition (Bovine Serum Albumin (BSA) or porcine serum), and parathion dose (4µg/CM<sup>2</sup>, 40µg/CM<sup>2</sup>, 400µg/CM<sup>2</sup>). The percutaneous absorption and metabolism of parathion under various conditions were compared with controls (air temperature T=37°C, diffusion block T=37°C; %RH=60; flow rate=4ML/hour and standard bovine serum albumin media). High relative humidity significantly increased parathion penetration, as did two elevated temperature conditions, although not to such a great extent. The effect of perfusate flow rate was dependent upon dose of parathion applied. Porcine serum

significantly increased the extent of metabolism at all three doses as compared to a BSA based perfusate. Porcine serum decreased penetration in the 4µg/CM<sup>2</sup> dose. The influence of perfusate composition at 400µg dose was greater than that of increased flow. Elevated temperature doubled the amount of paraoxon formed at all doses and doubled the formation of p-nitrophenol at the 400µg dose. Except at the lowest dose, relative humidity had a significant effect on paraoxon formation. The effect of low flow rate on parathion metabolism was probably caused by slow parathion penetration and extensive metabolism. These results indicate that environmental parameters may have independent and different effects on the percutaneous absorption and metabolism of parathion. Experimental conditions must be strictly controlled and dose response studies need to be conducted when investigating these phenomenon. (Support by NIEHS grant ES 00044).

# Assessment of Biochemical Changes in Chicken Lymphocytes Induced by In Vivo Exposure to a Neuropathy-Inducing Organophosphate.

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Neuropathy target esterase (NTE) in lymphocytes of man and the White Leghorn chicken has been suggested as an early biochemical marker of organophosphate (OP) exposure. Of advantage in laboratory situations is the ability to evaluate the progression of biochemical changes in intact animals following exposure. Adult roosters were given one intramuscular injection of phenyl saligenin phosphate (PSP, 2.5 mg/kg). Blood was collected at 24 hours and at 6 days. Lymphocytes were isolated and assayed for free intracellular calcium concentration ( $[Ca^{2+}]_i$ ) using fura-2/am. Remaining cells were frozen at -

70° C and later assayed for NTE activity. At 7 days, brains were collected and assayed for NTE activity. At 24 hours and 6 days, mean  $[Ca^{2+}]_i$  was increased over control values by 16% and 40%, respectively, in PSP-treated birds. Lymphocyte NTE activities were inhibited by 61% and 63% at 24 hours and 6 days, respectively. Brain NTE activity at 7 days was inhibited by 42%. These results indicate that changes in lymphocyte NTE activity and  $[Ca^{2+}]_i$  occur as early events after exposure to an organophosphate inducing delayed neuropathy, with effect on NTE preceding effect on  $[Ca^{2+}]_i$ .

## Peptide Isoelectric Focussing as a Necessary Prerequisite to their Transdermal Iontophoretic Delivery

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Peptides and proteins serve as hormones, enzymes, antigens, antibodies, and structural elements in the body. In addition, they are involved in metabolic processes, immune defense mechanisms, cell growth and other biological activities. With the advent of genetic engineering and other biotechnological techniques, economically feasible commercial production has made certain peptides and proteins available for treating many medical conditions. Peptide drugs are not orally active due to proteolytic enzymes in the gastrointestinal tract and extensive first pass hepatic metabolism. Transdermal delivery of bioactive proteins and peptides has been suggested as a viable alternative route of administration.

Transdermal iontophoresis is a novel technique for achieving percutaneous absorption of drugs, whereby charged molecules are made to move into and through the skin by the application of an external current. The charged species are contained in an electrolyte solution and the electromotive force generated causes positively charged molecules to move away from the anode, or negatively charged molecules to move away from the cathode. It is therefore critical to know the sign and magnitude of the charge carried by the drug in question. Due to the

amphoteric nature of peptides and proteins, charge is largely dictated by the pH of the surrounding environment according to the Henderson-Hasselbach equation.

Isoelectric focussing (IEF) is a technique in which a pH gradient is created in a gel matrix, protein is loaded onto the gel and a direct electric current is applied. The protein migrates until it reaches the point where the pH equals its isoelectric point. At the isoelectric point the protein carries no net charge, and ceases migrating. Because slight movements from this point impart a charge on the molecule, it is forced to focus in a very narrow range. It has been suggested that proteins with isoelectric points differing by less than 0.1 pH unit can be separated depending on the condition of focussing. There are many choices when setting up an IEF system. Considerations of gel type, pH gradient formation, and staining techniques are important in achieving successful protein focussing and will be discussed. The challenges inherent in determining the isoelectric point of basic peptides, such as LHRH, will be "focussed on."

# Characterization of Beta-Endorphin Receptors on Equine Lymphocytes.

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Beta-endorphin is one of a unique group of peptide neurohormones termed "endogenous opioids" which have morphine-like actions in the body. The physiological effects of the endogenous opioids are mediated through interactions with highly specific membrane receptors. These receptors are thought to be the same receptors that mediate the effects of morphine and other opiate substances. Current research suggests that there exists a bidirectional communications system between the immune system and the central nervous system. Receptors for opioid peptides have been identified on human and murine leukocytes. Receptor-ligand interactions with opioid peptides appear to modulate various immunological parameters via both naloxone reversible (classical) or naloxone irreversible (nonclassical) interactions. In order to further characterize the role of beta-endorphin in equine immune function, we investigated the characteristics of opioid receptors on equine lymphocytes. Equine lymphocytes were isolated from heparinized venous blood by density gradient centrifugation and suspended in Medium 199 supplemented with 0.1 % BSA. (3-[<sup>125</sup>I]iodotyrosyl<sup>27</sup>) beta-endorphin binding to freshly

isolated equine lymphocytes was found to be time-, temperature- and pH-dependent, with optimum conditions occurring at a pH of 8.0, 25°C and an incubation time of 4 hours. Incubation of  $7.5 \times 10^6$  cells/ml with increasing concentrations of <sup>125</sup>I-beta-endorphin revealed that the binding site was saturable. A Scatchard-Rosenthal linear transformation of the saturation isotherm data revealed one class of binding sites with a  $K_d$  of approximately 17 pM. Displacement of binding was achieved with naloxone and fentanyl, which act primarily at the mu receptor, equine beta-endorphin which acts at both the mu and delta receptors, and methionine-enkephalin which acts primarily at the delta receptor. Highest potency at the receptor was observed with equine beta-endorphin and methionine-enkephalin. The kappa agonists, ethylketocyclazocine and U-50,488, were unable to displace beta-endorphin from the binding site. These results suggest that equine lymphocytes possess a highly specific receptor, possibly of the delta class, for endogenous opioids. The existence of such a receptor implies a role for beta-endorphin and other endogenous opioids in equine immune function.

## The Testicular Paracrine Factor, P-Mod-S, Modulates Sertoli Cell Function and Differentiation.

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Under androgen stimulation testicular peritubular cells produce a nonmitogenic paracrine factor, P-Mod-S, which has been shown to influence Sertoli cell transferrin and androgen binding protein (ABP) secretion to a greater degree than any other single regulatory agent. The combination of P-Mod-S with FSH, insulin, retinol and testosterone (FIRT) stimulated levels of the Sertoli cell proteins in an additive fashion as opposed to cells treated with FIRT alone. In addition, similar increases in the gene expression for transferrin and ABP were demonstrated by Northern blots and solution hybridizations for Sertoli cells treated with P-Mod-S or FIRT. These observations imply that P-Mod-S may modulate Sertoli cell function through a different mechanism of action than FIRT. Sertoli cells in culture were treated for various incubation times with P-Mod-S or FIRT to evaluate respective signal transduction pathways. P-Mod-S stimulated cGMP levels at short and long term treatments, while FIRT had no effect on cGMP

levels. In contrast, P-Mod-S had no effect on cAMP levels, while FIRT stimulated cAMP levels. Current investigations are examining the influence of P-Mod-S on guanylate cyclase and phosphodiesterase activity in the Sertoli cell. In summary, these studies demonstrate that Sertoli cell function and differentiation may be influenced by a local paracrine factor, P-Mod-S, which is apparently mediated through a unique signal transduction system in the Sertoli cell. Further investigations of P-Mod-S may provide important information on the significance of this paracrine factor in testis function and subsequent spermatogenesis.

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