Abstracts of Theses for Graduate Degrees

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Respiratory Gas Tensions and Flow of Pulmonary Lymph in Anesthetized Dogs

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Thirty-seven samplings of right duct lymph, thoracic duct lymph, arterial blood, mixed venous blood and expired air, made separately and simultaneously in 59 out of 300 anesthetized mongrel dogs, showed a mean value of 61 mm Hg for the right duct lymph PO_2 and 37 mm Hg in the thoracic duct lymph. The pulmonary lymph PO_2 was probably higher than that in the right duct and close to alveolar air, since the right duct carries lymph from non-gas exchanging areas as well as from alveoli. Lung lymph could not be collected in the remaining 241 dogs because of technical difficulties and communication between the right and thoracic ducts (in 16% of the animals). The possibility of diffusion of oxygen through the walls of the right duct and smaller lymphatics made interpretation of the PO_2 values for lymph of the right duct difficult. A counter-current diffusion exchange mechanism of oxygen between pulmonary arterial blood and pulmonary lymphatic vessels appeared possible. The right duct lymph PCO_2 was similar to arterial PCO_2. The right duct lymph pH was higher than the pH of either the thoracic duct lymph or the arterial blood.

Pulmonary lymph flow was estimated to constitute about 40% of lymph flow from the right duct. Simultaneous measurements in 28 dogs showed mean values of 4.5 and 24.6 ml/hr, respectively, for control lymph flow from the right and thoracic ducts. The flow from the right duct was related to tidal volume but not to the body weight of the animal. It was found that left atrial pressures below those generally believed to cause pulmonary edema, e.g., 10 or 15 mm Hg, increased flow from the right duct. This finding could only be explained by the presence of interstitial tissue pressure around the pulmonary capillaries. Hypoxia induced by low O_2 breathing and i.v. administration of dinitrophenol increased the pulmonary lymph flow, probably because of increased capillary permeability. Alloxan increased lung lymph flow markedly. Hyperoxia (100% O_2 breathing) did not change the rate of pulmonary lymph flow.

A Linkage Map of Seven Loci in the X–Chromosome of *Drosophila tropicalis*

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The order of seven mutant genes, one dominant and six recessive, in the X-chromosome of *Drosophila tropicalis* has been determined and a linkage map constructed. The recombination frequencies for some of the seven genes varied in different crosses, which may have been a result of variability associated with the different strains used in this study.

When compared to the X-chromosome linkage map of some other species that also possess a metacentric X-chromosome, the region of 36 units mapped in *D. tropicalis* appears much shorter and thus probably represents only a segment of the whole X-chromosome. This is almost certainly true because of the small number of sex-linked mutants known in this species. As more sex-linked mutants are found, the map distance will likely increase because of the probability of finding genes near both ends of the chromosome.

Comparison of the X-chromosomes of *D. tropicalis* and *D. willistoni* was made, using the presumed homologous mutants. Three schemes have been presented to illustrate inversion sequences that may have taken place between these two species. Both overlapping and included inversions would result in a similar gene order.

A Study of Some Factors Affecting Starch Swelling and their Relationships to Tablet Disintegration

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The project was planned to study some factors influencing starch swelling at 37° C. and their relationships to tablet disintegration. The swelling capability of starch was determined by microscopic examination. One hundred or 200-grain diameters were measured for each environmental condition. The mean grain diameters were compared in various full

factorial experimental designs to determine whether changes in environmental conditions produced significant changes in grain diameters. This statistical significance was determined by calculation of analyses of variance and application of F-ratio tests.

To determine the relationships between starch swelling and tablet disintegration, tablets of various materials were prepared with cornstarch as the disintegrant. These tablets were studied for the correlations between compressional force, disintegration time, starch grain damage, void space and elastic recovery.

Cornstarch and amioca starch, when submersed 5 to 30 minutes in simulated gastric fluid USP (SGF), had greater increases in grain sizes than when submersed in distilled water.

The effects of the individual components of SGF were examined. Changes in pH had little effect on swelling. Salts affected swelling, with polyvalent cationic salts ($MgCl_2$ and $AlCl_3$) producing greater diameter increases than monovalent cationic salts (NaCl and Na_2SO_4). Ionic concentration did not produce an effect on swelling. There was no statistically significant swelling demonstrated by pepsin or surfactants in the submersion medium.

No significant difference in swelling was demonstrated between the various time intervals. However, when unsubmersed starches were included in the analyses, significant differences were shown between the unsubmersed starches and the starches slurried for five minutes and longer.

The swelling of starch grains was in the order of 5% to 10% increase in mean grain diameter. This was calculated to represent a volume increase of about 1.1% to 5.5% in a tablet containing 10% cornstarch. Since most tablets contain more than 5.5% void space, this did not seem to be a large enough change to cause the tablet to rupture.

The literature review indicated that damaged starch grains will swell in cold water. This was demonstrated by experiments with cornstarch damaged by ball milling. Submersion of the starch samples that had been ball milled from 10 to 48 hours produced increases in grain diameters of 40% to 80%.

Since damaged starch grains were shown to have an increased swelling capability in SGF at 37° C., the effect of the tableting procedure on starch grain damage was investigated. Damage to the grains, resulting from compression of pure cornstarch, was shown to be insignificant.

The effect of compressional force and hardness of the tablet ingredients was examined. In each formulation the starch grain damage increased as the compressional force was increased. The ingredient apparently had no effect on the direct proportionality of this relationship but did have an effect on the degree to which it occurred. There was no correlation between degree of starch grain damage and hardness of the ingredient. The crystalline form of the ingredient may exert a greater influence on starch damage than hardness. There was no correlation between starch damage and stress produced by elastic recovery of the tablet after removal of pressure. Contrary to what might be expected, there was an inverse relation between the amount of stress and the degree of starch grain damage for all of the formulations except aspirin.

There was no evident relationship demonstrated between disintegration time and starch grain damage. Other than compressional force, the inherent effect of the tablet ingredient was the only factor that appeared to affect disintegration.

The long accepted swelling mechanism of starch as a tablet disintegrant was not demonstrated in this study. The results of the investigation revealed no measureable correlations between starch grain damage and disintegration or between starch swelling and disintegration.

Circulatory Effect of Hypercapnia and its Role in the Production of the Vasodilator Response to Ischemia

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Despite extensive studies, the magnitude of the vasodilator effect of CO_2 on blood vessels in skin and muscle and its role in the local regulation of blood flow are still controversial.

In the present work the vasodilator effect of CO_2 in the human forearm was evaluated quantitatively, its mechanism of action was investigated, and its role in the production of the vasodilator response to ischemia was determined.

One hundred and twenty-seven experiments were carried out in 66 normal human volunteers. Forearm blood flow was measured by venous occlusion plythesmography. The vasoconstrictor effect of increased activity of sympathetic nerves and of circulating catecholamines was inhibited by the induction of alpha-adrenergic blockade through intra-arterial administration of phenoxybenzamine. In most experiments the dilator effect of circulating catecholamines was also eliminated by producing beta-adrenergic blockade through intra-arterial administration of propanalol or pronethalol.

Hypercapnia, produced by breathing 2.8% to 9% CO_2 in air, caused increase in forearm blood flow and decrease in forearm vascular resistance. In the steady state the decrease in vascular resistance during hypercapnia was related to the change in venous blood

PCO_2 or pH by a semilogarithmic relationship. In several experiments the decrease in vascular resistance during CO_2 breathing was produced by a short-lasting, small increase in vascular resistance.

In another series of experiments, the vasodilator effect of local hypercapnia was evaluated in the intact forearm in the following manner: isotonic saline equilibrated with 100% CO_2 was infused intra-arterially and its effect on forearm blood flow determined. During the infusion of saline with high CO_2 tension, forearm blood flow displayed a biphasic response consisting of a small initial decrease in flow followed by a more pronounced increase in blood flow. These experiments indicated that CO_2 has a local vasodilator effect on vessels of the human forearm.

Another series of experiments was undertaken to determine whether the stimulus for the CO_2-produced vasodilatation was increased PCO_2 or decreased pH. For this purpose, subjects breathed 7% CO_2 for two 10 minute periods. During one of these periods, decrease in pH in response to CO_2 breathing was abolished by the intravenous administration of sodium bicarbonate. The changes in forearm blood flow in forearm vascular resistance during the two periods were not significantly different, indicating that the stimulus for the CO_2-produced vasodilatation is increased PCO_2 rather than decreased pH.

The roles of local hypercapnia and local hypoxia in the production of the vasodilator response to ischemia were evaluated in the human forearm in the following manner: ischemia was produced by digital compression of the brachial artery, and the collateral flow was allowed to perfuse the forearm, while venous blood was withdrawn during the period of ischemia to determine the magnitude of the increase in PCO_2 and decrease in PO_2. The vasodilator response to ischemia was then compared to that produced by: a) increase in venous blood PCO_2 produced by CO_2 breathing, when the circulation was free; or b) decrease in venous blood PO_2, similar to that produced by ischemia, induced by breathing gas mixtures containing low concentration of oxygen, when the circulation was free; or c) increase in venous blood PCO_2 and decrease in venous blood PO_2, comparable to those changes produced by ischemia, induced by breathing gas mixtures having high concentration of CO_2 and low concentration of oxygen when the circulation was free. It was found that local hypercapnia accounted for about 50% to 60% of the vasodilator response to ischemia, while the contribution of local hypoxia was much less important, amounting to about 20% of the response to ischemia. It was found, furthermore, that the response to ischemia was not changed during 100% oxygen breathing, in spite of the fact that a substantial increase in venous blood PO_2 occurred, confirming the view that the contribution of local hypoxia to the response to ischemia is not pronounced.

The Initial Destruction of Intracellular *Salmonella typhimurium*

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An experimental procedure was designed for the in vitro cultivation of macrophages infected with *Salmonella typhimurium*. Peritoneal macrophages from guinea pigs were permitted to phagocytize *S. typhimurium* and were cultured in suspension. At intervals, samples were taken for determination of total cell population and for quantitative recovery of cell-associated bacteria. The ratio of bacteria to cells was thus computed at each interval, and a curve was constructed representing the fate of intracellular parasites over a period of time after phagocytosis.

Two strains of *S. typhimurium* with different degrees of virulence against mice were compared by the above procedure. Data show that there is an initial destruction of intracellular bacteria of both strains. However, there is a difference in the extent of this intracellular destruction. With the avirulent strain there is a two-log decrease in the intracellular population, the minimum being reached in four hours after phagocytosis; whereas with the virulent strain there is only a 1.2 log decline in the intracellular population, its minimum being reached in three hours. After this period of decline, the surviving organisms in both strains begin to multiply.

Cell Culture of Oral Mucous Membrane Lesions

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The difficulty in recognizing the malignant potential of oral lesions is well appreciated by the clinician who faces this problem routinely. In an effort to provide information which may aid in determining the potential of these lesions, a tissue culture project was undertaken.

The specific aims of this project were: a) the development of a reliable in vitro system for the growth of normal cheek-pouch mucosa of the hamster, and b) the comparison of the growth of this normal mucosa with the growth of 9, 10-dimethyl-1, 2-benzanthracene (DMBA)-induced lesions of the hamster pouch.

The hamster mucosal lesions were produced by repeatedly painting the animal's cheek pouch with 0.5% DMBA, the opposite pouch in the same animal being used as a control. Biopsies of these areas were taken

from the time the painting with the carcinogen began until obvious tumors appeared. A part of this tissue was preserved for histologic sectioning. The remaining tissue was explanted into Sykes-Moore chambers. These chambers were then incubated at 36±0.5° C. in a standard water jacket incubator. Eagle's Minimum Essential Medium with 10% Fetal Bovine Serum was used as a growth medium. Developing cell sheets in the Sykes-Moore chambers were observed with phase microscopy up to a 30-day maximum.

The tissue preserved for histologic sectioning was fixed in formalin, and routine H & E sections were prepared. Based on these sections, the tissue from the painted cheek pouches was divided into three categories: hyperplastic, early carcinomic, and late carcinomic. On this basis the tissues in culture could be compared.

Results of the pilot study indicated that primary cell cultures of hamster cheek-pouch epithelial cells could be repeatedly grown using a plasma clot explant culture technique. Twenty-four of 25 control cultures produced epithelial cell sheets in the first three post-explant days. Fibroblasts appeared later in these cultures, normally about the fifth post-explant day. These cells did not compromise the growth of the epithelial cells.

Cell size in this pilot study seemed to be a good indicator of growth activity. As long as small or medium cells could be found in the cell sheet, growth activity would continue until the next observation of that culture.

Epithelial cell sheets developed from the control tissue in a typical pattern. This process was followed from the initial appearance of cells around the explant until the continuity of the cell sheets was broken and the cells themselves had degenerated.

Three other experiments were carried out to confirm the results of the pilot study and to better compare the cultures of pathologic tissue with the cultures of normal tissue. Two of these experiments used a blind experimental design, so that the cultures from pathologic tissue were not identified until culture growth had terminated. The results of all three experiments were similar.

The cultures of normal tissue behaved much like cultures of normal tissue in the pilot study. Early epithelial cell sheets developed with a similar pattern whether they were from normal or pathologic mucosa. Cell size continued to be a good indicator of growth activity. The control cultures uniformly exhibited epithelial cell sheets in the first three post-explant days. However, none of the normal tissue samples produced cultures whose cell sheets persisted through the 30–day observation period.

In contrast many cultures of pathologic tissue showed poor growth or no growth at all. A large amount of granular debris was often seen accumulated around the explant, particularly in cultures from the later stages of carcinoma development. However, in each experiment cultures of pathologic tissue exhibited intense growth activity that persisted for the maximum observation period. These cell sheets were filled with small pleomorphic cells which were often piled one cell on another. Often the nucleus filled the cell. This type of cellular growth was seen in cultures from all three classes of pathologic tissue. No real comparison could be made among the classes of pathologic tissue since the number of early carcinoma and late carcinoma biopsy samples was small.

In contrast to previous reports in the literature, several types of lesions were produced with DMBA painting. The classic lesion described is an exophytic lesion that progresses to a well-differentiated carcinoma. Both endophytic and exophytic lesions were seen in this project. Some of these were highly cellular; others appeared invasive, with a minimum total number of involved cells.

In summary, the growth of pathologic mucosa in culture differed from normal mucosa. The clinical significance of these findings remains to be determined.