

# Abstracts of Theses for Graduate Degrees

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## Radioimmunoassay of HGH and ACTH in Surgically Stressed Patients

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The development of a specific, highly sensitive radioimmunoassay (RIA) for adrenocorticotrophic hormone (ACTH) is described. With this assay it is possible to measure normal plasma concentrations of ACTH without first extracting the peptide from the plasma. The range of plasma ACTH of normal subjects with this assay is 0 to 35 pg/ml when determined between 9 a.m. and 12 noon. High concentrations of plasma ACTH are associated with Cushing's disease, after bilateral adrenalectomy and the ectopic ACTH syndrome. Low concentrations are associated with treatment with dexamethasone and hypercortisolemia caused by an adrenal tumor.

A study of the human growth hormone (HGH) and ACTH response to surgery has been done. The plasma levels of both ACTH and HGH increase in response to surgical stress under general anesthesia. The increase in HGH concentration could not be attributed to hypoglycemia since blood glucose levels which were also monitored remained elevated. No significant increase in either hormone is seen following surgical stress under spinal anesthesia. This suggests that spinal anesthesia is capable of blocking the response of the pituitary to surgical stress. The pre-operative medications used and the induction of anesthesia exhibit no significant effect on the plasma concentration of either hormone. The remarkable similarities observed in the increase in secretion of HGH and ACTH to surgical stress under general anesthesia suggest a similarity of pathways involved in this response. Also it appears that the stress of major surgery is capable of diminishing the effect of the glucose dependent negative feed-back mechanism involved with HGH secretion.

## Tongue Rolling and Tongue Folding in an American Caucasian Population

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A sample of 1040 Caucasian students at the Health Sciences Division and the Academic Division of Virginia Commonwealth University were classified by visual observation according to the extent, if any, to which the tip of the tongue could be turned up without aid of teeth or lips and by whether or not the tongue could be rolled. Individuals varied in the extent to which they could turn up the tip of the tongue from not at all to folding the organ flat upon itself. The abilities to roll and to fold the tongue were independent, but only if folding was classified as folding the tip of the tongue flat upon the organ. Proportionally more males (75.81%) than females (67.49%) in the sample could roll the margins of the tongue, but there was no difference between the frequencies of males (4.61%) and females (3.47%) who could fold the tongue flat. The frequencies of rolling and of folding in the sample were in close agreement with the frequencies in other Caucasian populations and differed to various degrees from the frequencies in other races. Comparisons of the frequencies of rolling and of folding in some African tribes, an American Negro population, and in Caucasian populations, including the present study, suggested that the different frequencies in closely related human populations are more likely to be results of genetic drift in isolates than of natural selection.

## Study of the Toxic, Pyrogenic, and Cytopathogenic Properties of Lipopolysaccharide of *Salmonella Abortus Equi*

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A study was made of the toxic, pyrogenic, and cytopathogenic properties of the lipopolysaccharide of *Salmonella abortus equi* endotoxin. The *S. abortus equi* was a commercially obtained Westphal phenolic extracted lipopolysaccharide (LPS). The LPS was treated according to the method of Neter, Westphal, Lüderitz, Gorzynski, and Eichenberger (1956). The preparations, NaOH/6 min., NaOH/60 min., PBS/6 min. (phosphate buffered saline), and PBS/60 min. were tested in three systems. Toxicity of the preparations was determined in mice, pyrogenicity in rabbits, and cytopathogenicity in Wi-38 cell culture. By the imposed treatment, the ability to exert a cytotoxic effect is retained while toxicity for mice is greatly reduced and pyrogenicity for rabbits reduced to a lesser degree. The rabbit pyrogen test was shown to be 100,000 times more sensitive than the mouse lethality system and the cell culture system 10 times as sensitive as the rabbit system. The cell culture test is a highly sensitive system and seems promising as a tool for studying biological activity.

## Influence of the Cutaneous Application of Ice on Isolation and Control of Single Motor Units in Humans

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In this study an electromyographic technique has been used to evaluate the effect of a brief cutaneous application of ice on motor neuron excitability in human subjects. Approximately 80% of the forty-one subjects participating in the study were successfully trained to isolate a single motor unit in the right Biceps Brachii muscle. Once the training period was completed, the subjects learned to control the single unit as evidence by the electromyographic record of his response to a start-stop command, to a random number sequence, and in synchrony with the sound of the metronome. The subject's success in learning to isolate and control a single motor unit to these

experimental tasks was observed to be dependent on his concentrated volitional effort.

When the subject learned to perform these tasks accurately, ice in the form of a cube was applied by light stroking to the skin area overlying the Biceps muscle until an even erythema was evident. Immediately after the ice application the subjects repeated the learned experimental tasks, and the influence of afferent stimulation on isolated motor unit activation and control was evaluated. The cutaneous application of ice affected the subject's performance of these tasks as evidenced by facilitation of background motor unit activity and spontaneous firing of the originally isolated motor unit. This facilitation was observed in approximately 60% of the subjects during a start-stop sequence and during the activation of the isolated motor unit in synchrony with the sound of the metronome, and in 50% of the subjects during a random number sequence.

It is suggested that the method of ice application used in this study caused facilitation of motor unit activity due to the excitation of all types of cutaneous cold thermoreceptors, mechanoreceptors, and proprioceptive receptors. Further studies are necessary in order to identify the influence of each of these receptor types on the excitability states of motor neurons within the central nervous system.

## Sodium Dodecyl Sulfate Inactivation of Bovine Liver Glutamate Dehydrogenase

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The physical and catalytic effects of the detergent, sodium dodecyl sulfate, on the enzyme, bovine liver glutamate dehydrogenase, were examined by physical and kinetic methods. Double reciprocal plots of velocity versus substrate concentration indicated that low concentrations of sodium dodecyl sulfate gave uncompetitive inhibition. It was also determined from Hill plots of activity versus inhibitor concentrations that there was probably cooperativity in detergent binding. Further studies using the competitive inhibitor, isophthalate, showed that binding of substrate to the active site of glutamate dehydrogenase was not changed by the presence of detergent. Molecular sieve studies showed that there was no change in the size of the enzyme at low detergent concentrations that produced uncompetitive inhibition. However, a higher concentration of detergent, which caused complete inactivation, was found to dissociate and alter fluo-

rescence of the enzyme molecule. These data indicate that sodium dodecyl sulfate affects glutamate dehydrogenase by two different mechanisms. Sodium dodecyl sulfate, at low concentrations, is an uncompetitive inhibitor. At higher detergent concentrations, denaturation and protein dissociation occur.

### A Study to Determine why a Group of Knowledgeable Women Did or Did Not Seek a Regular Cervical Smear Examination

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The purpose of this study was to determine why a group of knowledgeable indigent and medically indigent women did or did not seek regular cervical smear examination. Special emphasis was placed on how knowledge regarding the cervical smear examination was obtained in an attempt to determine which sources and methods of health teaching were effective or ineffective in motivating women to seek the smear examination regularly.

An interview schedule was utilized with twenty-five subjects. All had recently received or were receiving treatment for cervical cancer. Fifteen subjects had neither sought nor been subjected to the cervical smear examination regularly. The remaining ten had either sought or been subjected to the cervical smear examination regularly.

The results of the study revealed the following: (1) Personal contact seemed to be the best motivator for seeking cervical smear examinations but was most effective when the contact was an individual whose opinion was highly valued; (2) In general, indigent women who learned about the smear examination from mass media, friends or relatives were not favorably influenced to seek examination; (3) Indigent women have been subjected to cervical smear examination without any explanation of the procedure by the health professionals in attendance; (4) Those subjects who sought examination regularly seemed to be motivated by a fear of cancer and the desire for peace of mind.

### A Study to Determine the Effects of Deliberate Nursing Intervention upon Postoperative Pain in a Selected Group of Male Patients

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This exploratory study investigated the effects of deliberate nursing intervention upon postoperative pain in a selected group of male patients. Six adult males who underwent thoracotomy were selected as subjects for the study. Six additional patients, randomly selected from hospital records from the previous year, comprised the control group.

The investigator acted as each patient's nurse giving care during the 3-11 PM tour of duty on the operative day and for the first two postoperative days. When the patient complained of pain, the investigator initiated verbal exchange to determine the nature of the pain and for the patient to select the methods of relief to be employed. Patients chose either prescribed analgesics or deliberate nursing intervention.

At the time of the initial complaint, baseline recordings of the patient's blood pressure, pulse and respiratory rate were recorded. Thirty and sixty minutes after the selected intervention these parameters were again measured and verbal exchange took place between the patient and investigator. Intervention was considered effective when patient's verbal response indicated relief and or when the physiological parameters were decreased from the pre-intervention level.

In eleven out of twelve pain occurrences the blood pressure and pulse were both reduced from the pre-intervention level regardless of the method of intervention. Due to the nature of surgery the patients were subjected to, it is impossible to speculate concerning alterations in the respiratory rate as an indicator of pain relief.

In this small sample, medication was selected as a method of relief six times and nursing intervention was selected six times. The medication was ineffective in relieving pain four out of six times as evidenced by the patient's verbal responses. Deliberate nursing intervention was effective in relieving pain six times out of six as evidence by the physiological parameters and verbal responses of the patients. Deliberate nursing intervention was more effective than medication in relieving pain.

Analysis of data revealed that patients in the experimental group required less medication than those patients in the control group. The control group re-

ceived almost five times the medication as the experimental group.

Obvious limitations included a small highly selected population; the absence of a second investigator to eliminate observer bias and the interrupted time interval of study. Replication of this study is recommended in order to verify the contribution of deliberate nursing intervention to postoperative pain relief.

## A Study to Ascertain the Relationship of Certain Factors as they Contribute to Lack of Control or Improper Management of Diabetic Patients Admitted to In-Patient Hospital Service

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It was the purpose of this study to ascertain the relationship of selected factors as they contributed to lack of control and/or inappropriate body weight of adult diabetic patients admitted to in-patient hospital service. These factors were knowledge of the diabetic regimen, practice of the regimen, and knowledge of the relationship between practice of the diabetic regimen and symptoms and/or complications of diabetes.

Two hypotheses were formulated. It was predicted that patients having incorrect or limited knowledge of the regimen would not practice their regimen correctly and would have limited knowledge of the relationship between practice and symptoms and/or complications of diabetes. It was also predicted that more patients having knowledge of the regimen and knowledge of the relationship between practicing the regimen and symptoms and/or complications of diabetes would practice more of the regimen correctly than those patients with knowledge of the regimen but without knowledge of the relationship.

Data were collected by three means. Descriptive data were obtained from the current hospital record. An interview schedule which incorporated observation was constructed to include questions to elicit the patient's knowledge, practice of the regimen, and knowledge of the relationship between practice and symptoms and/or complications of diabetes. Socio-economic data were also obtained.

During a ten week period, staff or private hospitalized patients who were on insulin, between the ages of 21 and 65, diagnosed as being diabetic for at least six months, and out of control by predetermined criteria were studied. A total of 20 patients fulfilling these

criteria were interviewed and observed for the selected practices of the diabetic regimen.

Socio-economic and descriptive data for the twenty patients were tabulated. The scores for each patient in each category of the interview were calculated and the total scores derived. Correlation coefficients based on the patient's score in each category were determined to establish the possible existence of relationships between categories.

Due to the small study population only trends appeared in the data. The majority of the study population was Negro, female, and over 50. The median income and mean grade level of the study population was lower than the average for diabetics based on accepted national statistics. Total number of acceptable responses for the entire study population in all three categories of the interview schedule was low on a scale of 0-9. The mean total score indicated that the majority of the study population had less than 50 per cent acceptable responses and no patient had over 75 per cent or 20 acceptable responses out of a possible 27. The scores concerned with knowledge of relationship items were lowest with 14 patients having scores of 4 or less out of a possible 9.

Negro subjects had a higher number of unacceptable responses than did white subjects. Those who had been diabetic longer had the higher scores when the total scores were compared. Patients making the lower scores in each category of information had slightly lower levels of education, inability to read and write, and lower incomes than did the study population as a whole.

There was insufficient evidence to confirm the hypotheses. However, correlation coefficient results showed a significant relationship existed among all three categories of information requested on the interview schedule.

## Enhanced Toxicity of Combinations of Bacterial Endotoxin with Antitumor Drugs

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*Department of Microbiology*

Combinations of bacterial endotoxin with antitumor drugs resulted in an enhanced toxicity for mice. Sublethal doses of either pactamycin or sparsomycin given simultaneously to three strains of mice (BALB/sy, RFW, N.Y.S.R.) with sublethal doses of endotoxin killed a substantial proportion of the animals so treated.

The antihistamine chlorpheniramine and the non-steroidal anti-inflammatory agent, phenylbutazone, were unsuccessful in alleviating the pactamycin-endotoxin synergistic toxicity. Heparin, an anti-coagulant, was likewise found ineffective in reducing the toxicity associated with the sparsomycin-endotoxin synergy. Methyl-prednisolone, a potent anti-inflammatory steroid, was found to be successful in reducing the death associated with both sparsomycin-endotoxin and pactamycin-endotoxin synergies. It has been suggested that both sparsomycin and pactamycin be re-evaluated in combination therapy with anti-inflammatory steroids.

Pactamycin was found to be composed of more than one constituent when developed on silicic acid thin layer chromatographic strips in toluene-isopropanol-ethyl acetate (60:10:30). Presumptive evidence has been presented indicating a reduced clearance and/or detoxification of pactamycin when the drug is given in combination with endotoxin.

Death due to daunomycin was found to be a delayed process. When endotoxin was given to mice that had four days earlier been administered daunomycin, an increase in the death rate, indicative of a synergistic interaction, was observed.

## Changing Personnel Requirements of a Modern Clinical Laboratory in the Face of Automation and Computerization

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The introduction of automated and semi-automated equipment into the clinical laboratory within the past few years has had a great impact upon its staffing requirements. The more recent introduction of computerized laboratory systems has created even greater demands for more highly specialized personnel. In order to determine what these demands are and how they affect the Medical College of Virginia, a study was made of the future plans of companies marketing this equipment and of the degree of automation presently established in the different sections of the laboratory. Another study was also made to determine how greatly automation has affected the laboratory workload from 1947 to the present time.

Since problems of personnel requirements are concentrated primarily around the registered medical technologist, the educational background and preparation of these persons was studied by surveying 872 catalogues from colleges offering baccalaureate degrees with a major in medical technology. The cur-

riculums were compared as to courses required and recommended. Affiliation and non-affiliation of the colleges with schools of medical technology were noted. Performance of the students graduating from the School of Medical Technology, Medical College of Virginia, from 1953-1968 was surveyed as to the grades they achieved in college compared to those achieved in training on a year-to-year basis. Finally, a survey was made of current recruitment literature distributed by the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists to determine whether or not the information contained therein was an accurate description of the personnel requirements of the clinical laboratory.

The following conclusions were drawn from the data presented in this study:

(1) Automated equipment for use in the clinical laboratory is becoming too complicated to be efficiently operated by persons now being trained in schools of medical technology. As a result, laboratories will soon need persons trained especially in the maintenance and operation of automated and computerized equipment.

(2) The impact of automation on the clinical laboratory at the Medical College of Virginia is not easily assessed since accurate records of the number of tests run by automated equipment and manual methods are not available. Also, the increase in workload cannot be studied for the same reason.

(3) An analysis of the data from the records of students graduating from the School of Medical Technology, Medical College of Virginia, from 1953-1968 reveals that there has been essentially no change in the ability of the students to achieve good grades, nor has there been any trend toward better or poorer grades from year to year.

(4) A survey of recruitment literature shows that most publications do not accurately describe the jobs performed by medical technologists in the modern clinical laboratory. As a result, persons who may be of great value in the laboratory, such as men who have an interest in electronic equipment, are not being attracted into the field of medical technology.

A proposed solution to the problem of providing well-trained personnel for the clinical laboratory is the extension of Whitehead's "hot" and "cold" laboratory concept which is currently being used at the Queen Elizabeth Hospital, Birmingham, Alabama. The "cold" laboratory consists of automated equipment and computers and is concerned mainly with routine laboratory testing. The "hot" laboratory employs more highly trained personnel who are concerned with manual testing procedures, "stat" procedures, and research. The establishment of "hot" and "cold" schools of medical technology would solve the manpower needs of both the small, 50-bed hospitals and the large,

1000-bed hospitals. "Hot" schools would be concerned with teaching good laboratory techniques and the theory behind each procedure taught. "Cold" schools would teach maintenance and servicing of automated equipment and computers and the theory behind their operation.

The future clinical laboratory will be a sophisticated set-up of both automated-computerized and manual methods of testing. Therefore, personnel of varied educational levels and training will be required to meet its demands.

### Relationship of Antibodies to Cytomegalovirus Between Normal Blood Donors and Open-Heart Surgery Patients

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In a serological study of Cytomegalovirus (CMV) infection in the Richmond area, sera samples from 247 blood donors were tested for the presence of cytomegalovirus complement-fixing antibodies. Ages of the donors ranged from 19 to 61 years.

While 25% of the donors over 35 years of age had positive serum, the maximum incidence was found in the group composed of persons 54 to 61 years old (42%). Forty-three of the 247 samples of sera contained demonstrable complement-fixing antibodies (17.5%).

Thirteen patients who had open-heart surgery and received multiple fresh blood transfusions were studied. A four- and five-fold significant postoperative rise in the titer of complement-fixing antibodies to cytomegalovirus was found in two of the twelve surviving patients (16%). A two-fold rise in titer was found in a third patient, whose preoperative titer had been low. The cytomegalovirus was not isolated from blood or urine of the patients. None of the patients was reported to have any clinical manifestation of a cytomegaloviral disease, and no post-transfusion mononucleosis was recorded. The transmission of the infection via large amounts of transfused fresh blood and the possible reactivation of a latent or past infection are discussed.

### A Nonequilibrium Thermodynamic Model of ION Transport in a Three-Compartment System

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A physical analog of steady-state sodium and potassium transport in a two-membrane, three-compartment system was studied utilizing the principles of nonequilibrium thermodynamics. This physical system is analogous to physiological systems where one compartment consisting of a cell monolayer separates two other compartments, such as the interstitial fluid and the renal tubule lumen in the kidney. The membranes in the model system serve only to localize the chemical potential gradients between compartments. The phenomenological equations relating the flows through the membranes to the chemical potential gradients were developed from the equation for energy dissipation within each membrane. The flows defined both the nonsteady-state rates of change of concentrations within each compartment and the steady-state transport across each membrane.

Ion transport due to chemical convection was studied by adding water to the "cell" compartment and removing it from the "interstitial" compartment. The "lumen" compartment was left as a strictly passive compartment. The  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  concentrations were measured periodically until a steady-state was reached.

In further experiments the concentrations of components in the "lumen" compartment were held constant by a constant flow of  $\text{NaCl-KCl-H}_2\text{O}$  solution through the compartment. The constant flow of water into the "cell" compartment distributed itself among both the "lumen" and "interstitial" compartments according to the mechanical filtration properties of each membrane. In initial experiments, the flows were unidirectional into the "interstitial" compartment. In later experiments the flow was distributed to both the "interstitial" and "lumen" compartment. After the system had reached a steady-state, the concentration of components, the flows in and out of the "lumen" compartment and the flow of water into the "cell" compartment were measured. The magnitudes and directions of the steady-state transport of components were determined.

The nonsteady-state experiments demonstrated a transient transport of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  ions from the "lumen" compartment to the "interstitial" compartment against a concentration gradient. At low solvent fluxes the ion transport occurs with the concentration gradient. At intermediate solvent fluxes,  $\text{K}^+$  and  $\text{Na}^+$

are transported in opposite directions;  $K^+$  is transported down a concentration gradient while  $Na^+$  is transported against an equal or larger gradient.

Steady-state transport of  $Na^+$  and  $K^+$  from the "lumen" compartment to the "interstitial" compartment may be maintained by a solvent flux in the direction of transport. The magnitude of this transport is greatest when the concentrations of components in the two compartments are equal, and decreases as the concentration ratio of components in the "interstitial" compartment to those in the "lumen" increases. For the combinations of solvent fluxes and component concentrations investigated, the transport of  $K^+$  was usually greater than the transport of  $Na^+$ .

### The Effects of Alcohol on Three Levels of Complex Human Behavior

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The effects of alcohol on three levels of complex human behavior were studied in twelve male subjects between the ages of 21 and 35 using the LRC Complex Coordinator. Each level of complexity contained an increasing component indicative of cognitive behavior. The motor component of all three levels was maintained approximately constant. The blood alcohol concentrations studied were 0.000, 0.010, 0.050, and 0.100 percent, as determined with the Breathalyzer<sup>®</sup>. Alcohol was administered in the form of 50 percent ethanol mixed with frozen orange juice concentrate. All blood alcohol concentrations were studied in the same subject during one test session. The study was replicated. The study was repeated twice without alcohol.

Analysis of variance was performed on the data for ten subjects using as dependent variables the time to perform 100 problems and the total errors for all four limbs for 100 problems. The variability between subjects was significant for both the alcohol test sessions and the control sessions. The variability due to blood alcohol concentrations was significant only for the test sessions during which the subjects received alcohol. The variability due to complexity of the task was significant for both the alcohol and control test sessions. There was a component of variability which indicated that the subjects responded differently to the increasing complexity. When the time to perform 100 problems was analyzed, there was an indication that as the task became more complex the alcohol effect became more pronounced but this did not hold true when the total errors per 100 problems was analyzed. When the time to perform 100 problems was ana-

lyzed, there was a component of variability that indicated that all subjects responded in the same direction to increasing blood alcohol concentrations but this relation did not hold when the total errors was analyzed.

Data are presented which indicate that cognitive processes were not affected by these blood alcohol concentrations. Performance on all three tasks was affected significantly; however, the effect of the alcohol appeared to be on the subject's ability to make precision positioning movements of the limbs.

### Exposure of Man to a Simulated Lunar Magnetic Environment, Physiological and Central Nervous System Effects

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The lunar magnetic field intensity at the surface was measured by Apollo 12 to be between 30 and 40 gamma. The geomagnetic field is approximately 0.5 gauss or about one thousand times more intense than the lunar field. The basic element of this study is the search for some aspect of human physiology which is coupled to the normal geomagnetic field and which would be significantly changed in the low intensity lunar magnetic field.

In previous experiments, six human subjects were exposed for ten days to a simulated lunar magnetic field generated by a coil system and two subjects were exposed for five days to a simulated lunar magnetic field created by a high permeability shield. Extensive physiological and psychological tests were conducted on these subjects. Only one test, scotopic critical flicker frequency (SCFF), indicated a possible physiological effect of the low field environment. The present study is a continuation of this previous work.

The coil system and shielded facilities were used to study a total of 18 subjects. The results indicate that psychological stress may be induced in some subjects by the necessary confinement associated with the experimental procedure; however, there is no indication that any physiological stress is induced by the low magnetic field environment. In conclusion, humans may be safely exposed to the lunar magnetic environment for up to 14 days; however, in view of previous studies by other authors in which a generalized hyperplasia was found in chronically exposed mice, the safety of humans is still in question for exposure periods exceeding 14 days.

SCFF apparatus was developed with the following

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significant features: 1) a discontinuous exposure to the flickering stimulus without changing the size or intensity of the test area, thus permitting the subject to compare a flicker and fused condition continually throughout the measurement period, 2) the end point is reached when the subject sees no change in the test area even though the presentation is still discontinuous, 3) a constant rate of frequency advance, and 4) an active response by the subject for each flickering stimulus period.

A reaction time apparatus was developed which can provide a histogram in approximately ten minutes. The first sustained rise of the histogram was much more stable than the mean. A theoretical analysis of these histogram parameters indicates that the position of the first sustained rise is a function of the homeostatic state of the neuromuscular system and that the position of the mean relative to the first sustained rise is a function of the psychophysiological state of the central nervous system. This analysis is useful for chronic studies of these systems. It is also useful for studying the direct physiological influence and the indirect psychophysiological influence of a particular sensory input on the central nervous system.

### Evaluation of the Essentiality of Dextran in the Dental Caries Process

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Dextran is an extracellular polysaccharide, an *alpha* 1,6' polyglucose, synthesized from sucrose by some bacteria. Dextran has been postulated as a fundamental component in the dental caries process. The proposed role of dextran is that of an adhesive, extracellular agent which gives rise to bacterial tooth colonization. This hypothesis that dextran is an essential factor in the dental caries process has been evaluated experimentally.

A diet containing 10% high molecular weight dextran was fed to microbe-bearing rats to test the dextran hypothesis.

*Lactobacillus casei* 4646, an organism which induced dental caries in sucrose-fed, gnotobiotic rats but caused no dental plaque, was examined for an extracellular dextranase enzyme. Cell-free, extracellular preparations from *L. casei* 4646 culture fluids were assayed for dextranase by two methods:

1. A modified-Somogyi assay was employed to estimate the release of reducing sugar from sucrose by the extracellular, cell-free preparations.

2. A coupled-enzyme, spectrophotometric assay was developed to estimate fructose release from sucrose

and to differentiate glucose from fructose release by the cell-free, extracellular preparations.

Sucrose-grown *Lactobacillus casei* 4646 cells were examined for extracellular dextran. *L. casei* 4646 cells, growing in a sucrose-containing, *in vitro* system, were tested for the ability to form visible, adherent layers of cells on a stainless-steel wire.

Dextran-fed rats did not experience tooth decay. Although cariogenic, *Lactobacillus casei* 4646 possesses no extracellular dextranase, does not synthesize extracellular dextran, and does not form visible, adherent layers of cells on the stainless-steel wire. From these and other considerations, it was concluded that dextran was not essential in the dental caries process.

### Methylurea—Its Intermediary Role in the Physiological Disposition of Methylamine

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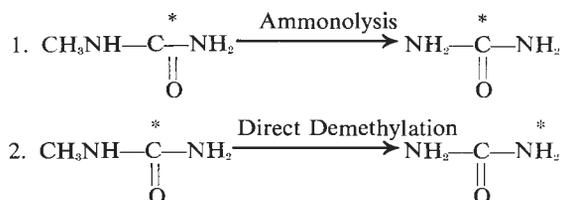
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Evidence has been presented to show that the metabolism of the compound leads to the formation and excretion of methylurea, a metabolite suspected but never conclusively shown to exist by previous investigators.

Urinary methylurea, after the administration of methyl-<sup>14</sup>C-amine, was shown to have <sup>14</sup>C-activity at both the carbonyl and methyl portions (groups) of the molecule. The presence of the <sup>14</sup>C-activity in the carbonyl group of methylurea was derived from the same metabolic pool of carbon dioxide that is used in the formation of urinary urea and respiratory carbon dioxide. Administration of methyl-<sup>14</sup>C-amine led to the formation of respiratory CO<sub>2</sub>-<sup>14</sup>C, as noted by various investigators, and it was shown that during a 24-hour period the ratio of specific activity, urea/respiratory CO<sub>2</sub>, approached one, in agreement with previous data reported by Mackenzie and du Vigneaud presented for the rat after administration of L-methionine-methyl-<sup>14</sup>C.

In order to quantitatively investigate some aspects of metabolism of methylurea a procedure was developed for the convenient synthesis of N-methyl-<sup>14</sup>C-urea and N-methylurea-<sup>14</sup>C-carbonyl. Here advantage was taken of the Wurtz reaction, utilizing methyl-<sup>14</sup>C-amine and potassium cyanate-<sup>14</sup>C respectively. The reaction mixture was then passed through cationic and anionic exchange resins to obtain a virtually pure product.

In a series of experiments in which N-methylurea-<sup>14</sup>C-carbonyl and N-methyl-<sup>14</sup>C-urea were injected intraperitoneally, the urinary urea of the treated animals was examined for radioactivity. Limited amounts of radioactivity were found in the carbonyl group of urea following administration of N-methyl-<sup>14</sup>C-urea. The appearance here of <sup>14</sup>C-activity is attributed to the enrichment of the carbon dioxide pool with <sup>14</sup>C through oxidation of the <sup>14</sup>C-methyl group. After administration of N-methylurea-<sup>14</sup>C-carbonyl, urinary urea contained a 200-fold excess of <sup>14</sup>C-activity, based upon the measurement of the specific activity of the respiratory carbon dioxide. These data indicate a metabolism of methylurea to urea which is accomplished in such a manner as to maintain the integrity of one or more of the nitrogen carbon bonds of methylurea.



\* isotopic

The data presently available do not permit exclusion of either route as a participating factor in the demethylation of methylurea, and point to the necessity for additional studies for complete elucidation of the mechanisms involved.

In addition to providing new routes for the mammalian metabolism of methylamine through a proposed methylurea cycle, this dissertation provides indirect evidence to demonstrate that the effect of iproniazid as an inhibitor of methylamine oxidation is mediated through enzyme systems separate from monoamine oxidase systems which have been invoked as major contributors to metabolism of methylamine by other investigators.

## The Adrenergic and Cholinergic Innervation of the Autotransplanted Canine Kidney

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Until recent years, it was assumed that severing the renal blood vessels and ureter during transplantation would, in effect, denervate the kidney. According to some investigators, transplantation may not produce total denervation, for some intact renal reflex arcs may remain; several small hilar and intrarenal ganglia

might be distal to the line of vascular division and suture.

No studies using modern histochemical methods have been performed on transplanted kidneys; rather, the technique of stripping the renal vessels and ureter has been used. This "denervating" method has been the subject of criticism due to the possibilities of an incomplete stripping procedure.

In addition to the lack of information regarding the innervation of renal transplants, there exists an abundance of controversial descriptions of normal renal innervation. Knowledge regarding the intrinsic innervation of the kidney was based for years on experiments using various standard nerve staining techniques, particularly silver impregnation and methylene blue. Recently, there have been reports on the application of newer histochemical methods to the study of renal innervation.

Due to the existence of controversial descriptions of renal innervation and the paucity of information regarding the innervation of transplanted organs, this investigation was undertaken. This study directly concerned the normal intrinsic distribution of the renal nerves and what effects are imposed on these nerves by autotransplantation. The following points were investigated: (1) the normal distribution of intrarenal neuronal elements using modern histochemical techniques, (2) if total degeneration of adrenergic and cholinergic nerves does indeed occur following transplantation, (3) if and when regeneration of adrenergic and cholinergic fibers occurs, (4) if the acetylcholinesterase (AChE) activity in the glomerulus is due to nervous or non-nervous elements, and (5) if discrete hilar ganglia exist.

Nineteen mongrel dogs were used in transplantation studies in which one kidney was autotransplanted to the ipsilateral iliac fossa. Every animal was anesthetized with 30mg/kg sodium pentobarbital administered intravenously. The dogs were all females which varied in weight from 15 to 18 kilograms. The severed renal vessels were anastomosed to the common iliac artery and vein. One operation was performed in which identical surgical procedures were followed with the exception of actually transplanting the kidney. This sham-operated kidney, as well as the left kidney in each dog, served as controls.

To investigate the existence of autonomic ganglia in the hilum, 7 normal kidneys were removed from normal dogs. The hilar regions of each kidney was removed and was examined using the following histological techniques: hematoxylin and eosin, luxol fast blue—P.A.S., Holmes' silver impregnation, and the Koelle thiocholine method. The catecholamine levels in two 2-week post-transplant kidneys were also determined. Catecholamine assays were run in duplicate on renal cortex samples of approximately 5g. Normal kidneys served as controls.

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The evaluation of experimental results regarding transplanted kidneys was accomplished by using two specific histological techniques. To determine the presence of adrenergic nerve fibers, the fluorescence technique of Falck was employed. Cholinergic fibers were identified using a modification of the thiocholine technique of Koelle.

Both adrenergic and cholinergic nerve fibers were observed only in relation to the preglomerular arterial vessels. The nerves were seen to form a dense plexus immediately outside the tunica media of the vessels. In addition, larger nerve bundles were present in the connective tissue sheaths of the renal, interlobar, and arcuate arteries. Adrenergic and cholinergic nerves were also seen coursing with the vasa recta. Only adrenergic fibers could be observed in relation to the large veins.

Following transplantation there was a total degeneration of adrenergic fibers within one week. Catecholamine levels fell to negligible amounts post-transplant. In only one case was regeneration of a few adrenergic fibers noted. Conversely, total degeneration of cholinergic fibers was observed in only 3 of 11 transplants; the other 8 showed variable degrees of degeneration, from almost total to very little. AChE activity was apparent in glomeruli but was greatly diminished or abolished by renal perfusion with normal saline before normal histologic procedures were begun. Positively-stained erythrocytes were regarded as being responsible for the staining seen. Discrete autonomic ganglia which were intensely AChE-positive were seen in the renal hila. It is believed these AChE-positive ganglion cells (also found scattered along hilar nerve bundles) are the source of the nerve fibers which remain intact and viable following vascular division during transplantation. Therefore, it is suggested that "total denervation" should refer to intrinsic as well as extrinsic innervation.

### Studies on the Carboxyl-Terminal Tetrapeptide Sequence of Bovine Serum Albumin

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Although bovine serum albumin (BSA) has been used widely in scientific investigations, little is known about its amino-acid sequence. Only the dipeptide sequence in the carboxyl-terminal region has been determined, while the amino acid composition through the hexapeptide has been reported. This composition

and sequence were determined by digestion of BSA with the hydrolytic enzyme, carboxypeptidase, and were reported as (Ser, Val)-(Thr, Ala)-Leu-Ala. In addition, peptide fragments from BSA have been hydrolyzed with carboxypeptidase and the sequence of one of these fragments has been related to that of the in-tact protein.

Based upon the evidence cited above, it was surmised that small peptides with the same amino-acid composition as that in the carboxyl-terminal region of BSA might be employed advantageously to further enhance our knowledge of this C-terminal sequence of BSA. These peptides could be hydrolyzed with carboxypeptidase and the rates of release of the amino acids could be determined and compared with the release rates obtained for the amino acids from the carboxyl-terminal region of BSA when both were digested under identical conditions.

Three hexapeptides, each with a sequence that might match that of the carboxyl-terminal hexapeptide sequence of BSA, were prepared by the solid-phase method of peptide synthesis, which was developed by R. B. Merrifield at the Rockefeller University. The three synthetic peptides contained an alanyl residue at the carboxyl-terminus because this residue was assigned that position in BSA by several investigators. These peptides were prepared in yields of 65-72% and were then isolated and purified by gel-filtration chromatography. Yields of the purified peptides were 46-49%.

These synthetic hexapeptides were hydrolyzed with carboxypeptidase as was the protein, BSA. The free amino acids in each of the hydrolyzates were determined periodically over a 29-hour period. These data were then plotted as the ratios of alanine to leucine and of alanine to threonine as a function of time, for each of the peptides, and for albumin. Comparison of the curves and other available data leads to the conclusion that the carboxyl-terminal tetrapeptide sequence of BSA is Thr-Ala-Leu-Ala.

In addition to the hexapeptides, three pentapeptides and three tetrapeptides were synthesized. All of the synthetic peptides were studied instrumentally, with infrared, nuclear magnetic resonance, optical rotatory dispersion, and circular dichroic spectroscopy, to determine what conformation these peptides assume in the solid state and in solution. It was concluded from the infrared spectral studies that all of the peptides in the solid state assume an extended form which is characterized by strong intermolecular hydrogen bonding. Optical rotatory dispersion data lead to the conclusion that in water solution the peptides also assume an extended conformation. No evidence was obtained to suggest an onset of helix formation. The optical rotatory dispersion spectra of the peptides were characterized by negative plain curves. The dispersion constants for most of the peptides investigated were in the

range of  $216 \pm 2$  nm. The circular dichroic spectra of the peptides were characterized generally by negative minima in the range of 220–229 nm.

Nuclear magnetic resonance spectroscopic data could not be interpreted reliably in terms of peptide conformations, but they did contain information relative to exchangeable protons in the peptide structures. In addition, these data provided information concerning hydrogen-hydrogen interactions within the various peptide structures.

## Substituent Effects on Cholinergic Activity of Substituted Benzyltrimethylammonium Salts

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In recent years, physico-chemical methods of determining structure-activity relationships of biological agents have supplemented classical chemical and pharmacological methods. Physico-chemical methodology has previously been applied to problems concerning the muscarinic and nicotinic receptors of the cholinergic portion of the autonomic nervous system. However, the nature of the structure of many muscarinic and nicotinic agents has limited the application of physico-chemical methods. Thus the study of the nature of cholinergic receptors has been hindered by a technical incompatibility in methodology. With this problem in mind, two series of cholinergic agents have been designed to permit the simple determination of some of their physico-chemical properties. These properties would possibly be correlatable to the pharmacological activity of the compounds. Two series of substituted benzyltrimethylammonium salts have been designed to fulfill the "five-atom rule" of muscarinic activity.

Of the compounds prepared and tested as muscarinic agonists on rat jejunum, only two demonstrated muscarinic activity. Seven of the compounds tested possessed curariform activity as measured by rat diaphragm paralysis. The low order of activity of these compounds precluded any correlation of biological activity with physico-chemical parameters. It was noted that the "five-atom rule" does not appear to be valid for this particular series of compounds.

## The Role of the Reticuloendothelial System in Host Defense

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The studies reported here examine the organism's defenses to better understand the RES and its participation in protection of the host to the external environment. The RES was studied by using agents which modify the phagocytic activities of the fixed macrophages of the liver, lung and spleen and by evaluating the intravascular clearance, organ uptake, immunologic response and host defense to microorganisms, tumors and drugs during various functional phagocytic states of the RES.

Inbred male albino mice (NYLAR) were used throughout the studies. *S. aureus*, *D. pneumoniae*, *C. neoformans* and *T. duttoni* were the microorganisms used and Ehrlich adenocarcinoma was the tumor studied. The metabolism of hexobarbital was used to measure the microsomal oxidase status during RES modification.

Vascular clearance and organ uptake of  $^{51}\text{Cr}$  sRBC and  $^{131}\text{I}$  "RE Test Lipid Emulsion" showed similar clearance patterns as colloidal carbon but showed different organ distribution patterns. Pyran copolymer, a synthetic polyanion and a known antitumor and interferon inducing agent was the RES modifying agent studied. The functional state of the RES during the biphasic response (blockade of particulate uptake followed by stimulation of uptake) produced by pyran copolymer pertained only to vascular clearance and hepatic uptake of the injected particulates. The localization of sRBC and the "RE Test Lipid Emulsion" into the spleen and lungs was independent of the biphasic response. This indicates that vascular clearance and organ uptake of more than one size of particulate is necessary to more fully evaluate the functional status of the RES.

The 19S immunoglobulin response to sRBC, as measured by hemolysin titer and Jerne plaque technique, correlated most closely with the amount of sRBC phagocytized by the spleen and was not related to the phagocytic status of the liver. Stimulation of hepatic and splenic phagocytosis was associated with increased host defense against *D. pneumoniae*, *S. aureus* and *T. duttoni*. The pulmonary macrophages may also have played an important role in the resistance to *C. neoformans*. The development of the Ehrlich adenocarcinoma was markedly decreased in mice undergoing RES stimulation and this tumor inhibition may be the result of an enhanced immune response.

In regard to drug metabolism, modification of the

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RES whether depressed or stimulated resulted in a marked inhibition in the metabolism of hexobarbital. This inhibition appears to be noncompetitive in nature. Stimulation and depression of the metabolism of hexobarbital by SKF 525A and chlorcyclizine produced no changes in the functional status of the RES. Chlorcyclizine given in a protocol to enhance drug metabolism reverses the depressant effects of zymosan on hexobarbital metabolism. SKF 525A given in a protocol to inhibit drug metabolism summates with pyran copolymer on the inhibition of hexobarbital metabolism. This additive effect may be the summation of the competitive action of SKF 525A and the noncompetitive activity by pyran copolymer. The noncompetitive activity is thought to be mediated through or by the Kupffer cell.

The RES is a multicompartimented system made up of various cells in those organs whose function includes monitoring and removing from the internal environment substances from within, such as old or damaged erythrocytes, tumor cells, etc. or substances derived from the exterior, such as bacteria, fungi and viruses. Although all macrophages have the ability to phagocytize, it appears that there is a division of labor in regard to other functions ascribed to the RES. For example, the liver represents a highly efficient filtering system of macrophages which can remove foreign materials from the circulation and in most cases catabolizes and excretes the phagocytized material. While alveolar macrophages patrol the pulmonary tissue for inhaled particulates and carry these to the lymphatic system for removal or antibody processing.

The splenic and lymph node macrophages are primarily concerned with antibody processing. After phagocytizing the antigen, the macrophage is believed to form an RNA or RNA-antigen complex which is passed on to the lymphocytic type cells which make antibody. Antibody, acting as an opsonin, unites all phagocytic cells in their ability to remove, destroy and excrete foreign antigens.

### The Application of Absorbents and of Complexing Agents in Urolithiasis

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In a review of the literature on the causes of calcium oxalate urolithiasis the concept of a critical cluster has been used as a unifying principle in stone formation. Matrix materials could promote the formation of such critical clusters and (or) prevent their disintegration.

There is no established satisfactory treatment for calcium oxalate stone formers. The control of urinary pH is neither practical nor effective. The use of low oxalate diets, low and high phosphate diets, the administration of magnesium salts, and the irrigation of catheterized patients with citrate buffers and salts of EDTA have proven to be of marginal help in preventing or slowing down the growth of oxalate stones.

The foreign body technique for producing experimental urolithiasis in rats is suitable for magnesium ammonium phosphate. Less consistent results were obtained in calcium oxalate lithiasis produced by the administration of small doses of ethylene glycol. Attempts to produce larger amounts of calcium oxalate deposits on foreign bodies failed. It was found that deposition of oxalate crystals may occur both on a foreign body in the bladder and in the tissue of the kidney. Therefore, a more suitable model of calcium oxalate lithiasis was developed with the use of histopathological evaluation of kidney slices for tissue damage and the amount of calcium oxalate crystals.

Methylene blue or Vitamin C had little, if any, effect on the growth rate of deposit of calcium oxalate. A combination of these two compounds showed a synergic effect. Results with Azure A and New methylene blue and the *in vitro* adsorption studies of the alphazurin series suggests favorable results in inhibition of deposits by increased size of substituents on the amino nitrogen of methylene blue.

The tetramethyl ester of EGTA showed promise in the treatment of oxalate urolithiasis. Experiments using larger doses of the ester in which the EGTA/Ca ratio in urine can become greater than one may have a significant inhibiting effect in oxalate urolithiasis.

The administration of magnesium salts can be quite disastrous to the test animal. Magnesium can protect the animal in preventing calcium oxalate lithiasis but unfortunately large amounts of magnesium ammonium phosphate are produced.