Abstracts of Theses for Graduate Degrees

Medical College of Virginia, June, 1969

Anticholinergic Agents Based on Ariens' Dual Receptor Site Theory

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In the classical receptor theory of competitive antagonism, both the agonist and antagonist are presumed to have an affinity for the same receptor site. However, changes in the structure of cholinergics and competitive anticholinergics do not result in a parallel change in activity. If affinity is the property altered by the structural modification, then classical receptor theory offers no explanation for this. Ariens has suggested that competitive antagonists may be occupying an area that overlaps only a portion of the agonist area and gain affinity through attraction to "additional receptor parts" not utilized by the agonists. After considering these proposed sites at postganglionic parasympathetic synapses and reviewing the chemical structures of potent cholinergic and anticholinergic drugs, a new series of compounds has been designed. These compounds may possess potent anticholinergic properties if Ariens' dual receptor site theory is valid. They contain a cationic head joining two groups—one resembling well-known, potent antagonists and the other resembling classical, potent agonists. The rationale for this structural design is that such compounds may bind to both cholinergic and anticholinergic sites of the receptor simultaneously. Because they should bind to both receptor sites, these compounds may possess a greater affinity than known, potent anticholinergic drugs not containing an agonist moiety and binding to only one receptor site.

The joining of the agonist and antagonist moieties was done by alkylation of basic amines to form the desired quaternary compounds. Seven quaternary compounds were obtained in pure condition for pharmacological testing. They are 2-[(2-acetoxyethyl) methylamino] ethyl benzilate methobromide, 3-[(2-acetoxyethyl) methylamino] propyl benzilate methobromide, 2-[(2-carbamyloxyethyl) methylamino] ethyl benzilate methochloride, 3-[(2-carbamyloxyethyl) methylamino] propyl benzilate methochloride, 2-[(2-carbamyloxyethyl) methylamino] ethyl diphenylacetate methochloride, 2-[(5-methylfurfuryl) methylamino] ethyl benzilate methiodide, and 3-[(5-methylfurfuryl) methylamino] propyl benzilate methiodide.

These seven potential anticholinergics were evaluated by means of the pA_2 described originally by Schild. Isolated segments of guinea pig ileum and rat jejunum were used as test systems. Both acetylcholine and carbachol were used as agonists. Four or five dose-response curves were determined on each isolated tissue preparation, and a fresh segment was used for each agonist-antagonist combination. The compounds behaved as true competitive antagonists and were highly specific for the cholinergic receptor.

The pA_2 values were obtained by linear regression analysis. There was no significant difference between the pA_2 values obtained with acetylcholine and carbachol. However, differences between antagonists were significant.

Three basic alterations of the lachesine molecule were made. The ethyl group on the onium nitrogen was replaced by acetoxyethyl, carbamyloxyethyl, and 5-methylfurfuryl. All of these substitutions decreased the anticholinergic activity compared to lachesine. The anticholinergic activities were in the following order: acetoxyethyl > 5-methylfurfuryl > carbamyloxyethyl. The distance between the onium nitrogen and the ester group in the benzilate moiety of the molecule was increased from two carbon atoms to three carbon atoms. All of the compounds with a two carbon chain in this moiety possessed greater anticholinergic activity than their homologues containing a three carbon chain. When the hydroxyl group of the benzilate moiety was eliminated to give the diphenylacetate group, anticholinergic activity was drastically decreased. Thus, all alterations to the lachesine molecule decreased anticholinergic activity. These new compounds would have to possess greater affinity and, therefore, greater anticholinergic activity than lachesine to allow any positive conclusions to be made concerning the general validity of Ariens' dual receptor site theory.

One possible explanation of these results may be that Ariens' dual receptor site theory is not valid for the cholinergic receptor. However, several alternative explanations might be advanced for the lack of higher pA_2 values. (1) The lower affinity might be only apparent due to a lowered ability of the compound to reach the site of action. This seems very improbable with the compounds of this study for the isolated organ systems employed. (2) The receptor sites may not be arranged in a linear fashion. In such a case, the agonist and antagonist moieties of the dual-ended molecule may not bend into a conformation which permits binding with their respective sites simultaneously, thus decreasing their potential affinity. (3) Ariens' dual receptor site theory may not be applicable to the ester-type antagonists chosen for inclusion in the present series of compounds. However, Ariens' theory may be applicable to other compounds such as the well-known, non-ester anticholinergic drugs.

Although no conclusions concerning the general validity of Ariens' dual receptor site theory can be presented, further investigation of carefully chosen molecules may provide enough information to do so in the future.

The Relationship Between Emotionality and Behavioral Performance in a Random Population of Male Charles River Rats

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The capacity of a rat to adapt to the stress of repeated exposure to an open field is the basis for the determination of individual emotionality in a random population of animals. The emotionality of male CD rats was determined by exposing each animal to twelve daily, two minute sessions in a modified Hall open field. During these sessions individual spontaneous activity, rearing, and number of boluses were determined and used as indices of adaptation to this novel stimulus. The relationships between these parameters of emotionality and learning, curiosity and pituitary-adrenal activation were then studied by specific experimentation. The design of these experiments was such that correlations were made possible between open field performance and other behavioral parameters.

Animals exposed to the open field showed adaptation in defecation rate after eight days. Equilibration of activity was apparent following the sixth day. The number of days defecating and mean spontaneous activity during the last four days (9–12) were used for individual emotionality classification. Negative correlations between spontaneous activity and defecation were observed in all experimental groups during this period. Analysis of an intercorrelational matrix of these emotionality parameters further suggested that activity and defecation were two different forms of emotionality. In general, the high emotional rat could be characterized as being a high defecator with low spontaneous activity.

Maze experimentation indicated that all rats tested could learn a double T maze, but running times varied greatly. Correlations between defecation rate (days 9–12) and maze running time alluded to a relationship between emotionality and maze learning. All six of the first six ranked maze learners of Experiment I and five of the first seven in Experiment II were classified as low emotional by defecation scores during days 9–12. It was suggested that in reality, learning was not estimated, but the fear evoked by the maze.

In a second experiment designed to test individual rat curiosity, low defecating rats were also observed to be more active in areas with high illumination. High defecators, on the other hand, were more often found in less illuminated areas, suggesting these latter animals to be more fearful. Correlations between open field performance and curiosity again revealed that a rat which is a high defecator may also be an extremely fearful animal. Food deprivation appeared to reduce these correlations with less of an effect occuring in high emotionality animals. From these data, it was suggested that a second exposure to this test situation may have unmasked a latent curiosity in the emotional rat which surpassed that in the non-emotional animal.

The retention of a one trial avoidance procedure appeared to be greater in the high emotionality rat. This was true 24 and 48 hours after the initial learning session. The fear evoked by a negative reinforcement prevented the high emotional rat from returning to a darkened chamber in which he was previously shocked for one minute. These results supported the hypothesis that high emotionality rats learn passive avoidance procedures more rapidly than non-emotional animals.

High emotional rats exhibited a 50 per cent increase over resting plasma steroid levels in response to an acute psychological stress, while the non-emotional group showed a 27 per cent increase to the same auditory stimulus. Correlations between emotionality factors and plasma levels were not significant. However, positive correlations between defecation and plasma steroid levels further suggested that high emotionality animals were more responsive to this stress. Adrenal steroid levels did not reflect these changes and correlations between emotionality parameters and these endocrine levels were also inconclusive.

Fetal Development and Functional Significance of the Epiphysis Cerebri in Rats and Hamsters: A Light and Electron Microscopic Investigation.

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Reports regarding the significance of the mammalian epiphysis cerebri or pineal organ have for some time presented conflicting or inconclusive data. Out of the confusion arose the concept that this structure in mammals is merely a functionaless vestige of a third eye found in some lower vertebrates.

Two significant findings within the last ten years have led to a resurgence of interest in the pineal organ. (1) A previously unknown compound, melatonin (N-acetyl-5-methoxytryptamine) was isolated from bovine pineals and was found to be synthesized only in pineal tissue. (2) Ultrastructural studies revealed photoreceptor cells in the pineals of numerous submammalian vertebrates. It has subsequently been found that the activity of certain pineal substances, including melatonin, is influenced by environmental lighting and that the pineal organs of certain mammals subjected to light deprivation are capable of inducing gonadal atrophy. Information about environmental lighting is transmitted to the pineal in such animals via the lateral eyes and autonomic nerves: the mammalian pineal cells appear to lack the photoreceptive capacity demonstrated by pineal cells of certain submammalian vertebrates. It appears thus that two functional pineal systems have evolved: a photoreceptor system and a secretory or endocrine system. It has been suggested that the cells constituting these two systems may be homologous.

In light of this hypothesis, the present investigation was designed to examine the nature of the mammalian pineal cell which has been variously described as neural, neuroglial, and endocrine. Since light deprivation has been shown to result in pineal-induced gonadal atrophy in rats and hamsters, these rodents were chosen for this study which consisted of two parts. (1) Morphogenesis and cytogenesis of pineal tissue were studied at the light and electron microscopic levels in 16–20 day fetal rats and in 12–16 day fetal hamsters. The pineals in both animal differentiate from ependyma of the dorsal diencephalon and in both, appear to be composed primarily of one type of cell. The resemblance of apical cytoplasmic structures in these cells to certain features of known photoreceptor cells suggests that the former may at least possess the potential to develop into a photoreceptor type of cell. (2) Adult rats and hamsters were subjected to blinding (bilateral orbital enucleation) and to blinding combined with pinealectomy, and were sacrificed 6 weeks post-operative. Pineals of blinded rats and of normal and blinded hamsters were studied at the ultrastructural level. In the rat the testicular, seminal vesicle, and adrenal weights did not vary significantly among the two operated groups and the control group. Likewise, no obvious ultrastructural differences were detected between pineal tissues of blinded and normal rats. Possible explanations for the negative results are considered. In the hamster, as previously reported, blinding resulted in atrophy of the testes and seminal vesicles in the male and of the ovaries and uteri in the female; blinding combined with pinealectomy did not affect the weights of these organs. As further evidence for a pineal-gonadal relationship in the hamster, it was found that pinealectomy of previously blinded, sham-pinealectomized animals resulted in a rebound by the atrophic gonads towards normal testicular weight. Additionally it was found that pineal antigonadal properties in blinded animals were sufficient not only to counteract the unusual gonadal hypertrophy in the remaining testis produced by unilateral castration but to induce atrophy of the remaining testis. Pineal tissue from blinded, sham-pinealectomized hamsters differed from that of normal animals by its greater content of vesicles and granules in pineal cell processes and especially by the frequent appearance in the former of membranous "lamellar whorls."

Speculation regarding the functional and morphological nature of the mammalian pineal cell based on these and other findings is discussed.

Separation and Partial Characterization of Components Derived from Human Erythrocyte Membranes

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Considerable attention has been focused on the problem of the protein composition of red cell membranes. Evidence has been cited to support the hypothesis that a single protein accounts for the major part of the membrane. To test this hypothesis, a method has been devised of separating the membrane components and analyzing the components for phospholipid and protein composition.

The red cell membrane was isolated by buffer and water washes coupled with homogenization and centrifugation. The alteration in the chemical composition of the membrane during preparation was studied by amino acid, carbohydrate and phospholipid analysis. The final white membrane was similarly characterized and also analysed for hemoglobin and ATPase activity to ensure purity.

The membrane was solubilized in phenol:urea:acetic acid:water (2:1. 2:1:1, W/W/V/V) and subjected to analytical disc gel electrophoresis. After establishing the optimum parameters for separation of the membrane components, the system was scaled up to preparative disc gel electrophoresis. The components were eluted from the preparative acrylamide electrophoresis columns with 50 per cent acetic acid. The effluent was collected in 6.0 ml fractions; a minimum of 400 fractions were collected.

The fractions were analyzed for protein, following hydrolysis in 6N HCl, by the ninhydrin reaction using a Technicon Autoanalyzer. An aliquot of each fraction was also analyzed for phospholipid by phosphorus analysis following perchloric acid digestion. A total of 22 ninhydrin positive peaks and 26 phospholipid components were obtained. Complete amino acid analyses were done on the ten major protein components.

The preparation of the membrane resulted in a loss of protein without concommittant loss of lipid or carbohydrate. This protein must be loosely bound to the membrane since simple water washes removed it. Although this protein may be important to the function of the intact membrane, the classical trilaminar image was preserved as viewed by electron microscopy. The chemical composition of the membrane was: 50 per cent protein, 38 per cent lipid and 12 per cent carbohydrate. Ten per cent of the lipid of the membrane was cholesterol and the remainder was phospholipid. The carbohydrate consisted of 8 per cent neutral sugars, 2 per cent sugar amines and a small amount of sialic acids and fucose. The distribution of amino acids and the chemical composition of the membrane was found to be similar to that reported by other investigators.

The results of the preparative disc electrophoresis show that the membrane was composed of a large number of components. These components were found to be of three types: lipoprotein, protein and lipid. The amino acid analyses of the major components showed that each component was different to the other and to the whole membrane, demonstrating that there were at least 10 different proteins in the human red cell membrane. The per cent of protein of these major components varied from 5 per cent to 17 per cent of the total protein analyzed. Other components contained protein but in quantities too small to obtain accurate analyses. Therefore, 17 per cent was found to be the upper limit for any one component.

It has been postulated that the protein of the membrane consists of a family of proteins. This data leads to a new hypothesis, ie, there are different families of protein in cell membranes. Some of these proteins have an affinity for lipid while others occur as protein alone.

Free Amino Acid Release from Isolated Rat Liver Cells

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The purposes of this thesis were (1) to investigate the amino acid permeability of rat liver cells dispersed with tetraphenylboron (TPB), (2) to investigate the effects of thyroxin and testosterone on the amino acid permeability of TPB dispersed cells, and (3) to present evidence which would serve as proof that the cells are viable.

To determine the amino acid permeability of isolated rat liver cells, measurement of the intracellular and extracellular amino acid concentrations was taken as a function of time. Thyroxin or testosterone were added, in large doses, to demonstrate their effect, if any, on the amino acid permeability of the cells. Respiratory studies were carried out and pictures of the cells were taken to demonstrate two aspects of cell viability.

TPB dispersed rat liver cells were found to maintain a free amino acid ratio across their membrane during incubation *in vitro*. Addition of testosterone or thyroxin in large doses does not seem to effect this ratio. The cells respire at rates comparable to rates previously seen for liver cells, and the cells are demonstrated to maintain normal gross morphology during the course of the experiments.

*Passer Domesticus**

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Study of the house sparrow revealed that there was a shift in predominant lactate dehydrogenase (LDH) isozymes for ventricle, but not for either breast muscle or cerebrum during development, a pattern of differentiation unlike that of chicken heart and breast muscle. Values of pyruvate concentration giving optimal LDH velocity for sparrow ventricle and pectoralis muscle were similar to published values for these two chicken tissues. The absolute pyruvate concentration giving optimal velocity of LDH isozymes in ventricle decreased during development. However, these concentrations of pyruvate failed to differ significantly among five age groups. The same relationship existed for developing cerebrum. There was a gradually increased inhibition of LDH activity produced by high pyruvate concentrations for ventricle, but not for cerebrum. These catalytic properties coincided with the isozymic changes observed in these tissues during morphogenesis. The observed pattern of inhabition resulted in a higher $NADH_L/NADH_H$ ratio for adult ventricle than for adult cerebrum or pectoralis muscle. The total LDH activity of cerebrum reached a minimum just before hatching and then it increased as development progressed.

* Presented June, 1968.

The Investigation of the Bradypnea Response in Dogs Following Left Atrial Distention

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The observation of striking decrease in respiratory rate after sudden inflation of a balloon in the left atrium of a dog anesthetized with chloralose warranted further investigation. Respiratory rate, left atrial pressure, and usually tidal volume and femoral and pulmonary arterial pressures were measured in 93 experiments before, during and after left atrial balloon inflation in three groups of dogs: 1) 14 dogs anesthetized with chloralose, 2) 4 dogs anesthetized with sodium pentobarbital, and 3) 10 dogs unanesthetized (mildly sedated in some experiments). Balloon placement was by retrograde catheterization in the chloralose and pentobarbital groups and by surgical implantation in the unanesthetized group. Marked, moderate and slight bradypnea was observed in 7 of 11 chloralosed dogs (3 of 14 disqualified), in 4 of 10 unanesthetized dogs and in none of the pentobarbital group. Alternating bradypnea and tachypnea following inflation, and bradypnea or deflation were two other responses commonly observed in all groups. Tachypnea, seen in only three experiments of the chloralose group, was predominant in the pentobarbital group and occurred in 50 per cent of experiments without anesthesia. The detailed results suggest that two opposing reflex mechanisms are operating during left atrial distention. Tachypnea may result from sensitization of pulmonary stretch receptors secondary to pulmonary vascular engorgement. The hitherto undescribed bradypnea response might be mediated by left atrial receptors, known to exist. Failure to observe bradypnea in chloralosed dogs, in which the left atrial appendage and a single pulmonary vein near its atrial junction were distended by implanted balloons suggests either that these regions of the left atrium are not the sites of the postulated receptors or that an insufficient area was stimulated. The marked braypnea response in one dog in which the balloon slipped from the atrial appendage on inflation and became lodged in the mitral orifice suggests that receptors mediating the bradypnea response might be situated in tissues surrounding the mitral ring. Assuming this, it may be conjectured that in those animals in which striking bradypnea was repeatedly observed, the inflated balloon was low enough within the atrium to produce adequate distention of the mitral area.

Glucose Dehydrogenase Activity of a Sweet Sensitive Protein From Bovine Tongues

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We have prepared a fraction of the homogenate of bovine tongue which has the ability to combine with sugars. The relative strengths of the complexes formed are in close agreement with previously published data. With a suitable acceptor present, this protein will catalyze the dehydrogenation of D-glucose. The reaction is quite specific for D-glucose, other reducing sugars being oxidized more slowly.

The material was assayed by measuring its rate of dehydrogenase activity with reducing sugars, using NAD as an acceptor. The Michaelis constants were calculated and were compared with the relative sweetness of the sugars tested.

We speculate that what we have isolated from the bovine taste bud is a glucose dehydrogenase, the sugar binding properties which have been exploited evolutionarily to form the receptor molecule of sweet taste perception. It is hoped that further research will be able to link this protein directly to taste.

Hematology Quality Control in a Large Medical Center

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An evaluation of the needs for hematology quality control in a large medical center with multiple laboratories has been conducted. The areas of greatest need have been identified and procedures have been presented for testing the accuracy and precision of the eight laboratories included in the study.

The historical development of quality control with a synopsis of methods currently being used is given. Both general methods as well as those specifically related to hematology are considered.

Laboratory error usually can be traced to one of four sources. Each one of these was studied in preliminary experiments and showed:

1. collection of the specimen—One tenth of the total weekly samples collected contained less than 3 ml of blood. If hematocrit determinations had been ordered on all of these, this constitutes a possible error of 10 per cent. Small blood or fibrin clots were detected in 3 per cent, constituting a second possible source of error in samples.
2. technical ability of the staff—There was a definite difference between the technologist and the technician.
3. the instruments or methods used—Standard deviation target values of 0.3 per cent for hemoglobin and 400 cells per cubic millimeter for white blood cells were established. Determinations from all of the participating laboratories were two or three times values.
4. transcription of results—Errors due to faulty transcription of results were found in less than one percent of the total week's work. Only three of the eight laboratories were studied.

Expansion of the preliminary investigation into definitive studies focused on the instruments, the methods and the reagents used throughout all of the laboratories. These were without uniformity in individual laboratories. In addition, there was no homogeneous pattern of technical skill represented.

The definitive investigation included performance semi-weekly of hemoglobin and white cell determinations. These were performed in each of the eight laboratories on aliquots of the same samples. The data collected after three months were subjected to statistical analyses which included an analysis of variance (ANOVA) and a Duncan's Range Test. The ANOVA showed a significant difference exists between the results reported from different laboratories and due to a day-by-day laboratory interaction. The Duncan's Range Test grouped the laboratories according to which ones produced similar results. Results of these tests showed that each laboratory maintained a level of performance that resulted in acceptable precision, but poor accuracy. The wide range of results is believed to be due to the variety of instruments and methods used.

Based on these findings, it is possible to conclude that quality control in a large center with a multiplicity of laboratories, methods, and personnel is difficult to achieve. However, quality control with striving to achieve accuracy is the quintessence of a good laboratory.

A Kinetic Study of the Homogeneous Catalytic Hydrogenation of 2-Butyne-1, 4-Diol and *cis*-2-Butene-1, 4-Diol

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A review of the chemical literature has shown that certain coordination complexes of the transition metals, especially those in the platinum groups, successfully catalyze the addition of molecular hydrogen to unsaturated hydrocarbons and their derivatives in a homogeneous system. The employment of tris (triphenylphosphine)-chlororhodium (I) in homogeneous catalytic hydrogenation studies has been reported by Wilkinson. However, the studies which have been reported were made using hydrogen at pressures of less than one atmosphere.

It was the objective of this study to demonstrate the use of tris (triphenylphosphine) chlororhodium (I) as a catalyst in the Parr hydrogenation system, since this is the system most frequently found in chemical laboratories. Many kinetic investigations carried out with a heterogeneous catalyst in the Parr system have been reported in the literature, and it was of interest to extend these studies, employing a homogeneous catalyst. 2-Butyne-1, 4-diol and *cis*-2-butene-1, 4-diol were selected as the organic substrates to be hydrogenated.

It was found the hydrogenation of olefins and acetylenes in the Parr system was first-order with respect to hydrogen concentration and zero-order with respect to organic substrate concentration, provided that the initial hydrogen pressure is at least 35 psig, and the minimum amount of substrate used is 0.5 gram. Butynediol was found to hydrogenate first to the butenediol by taking up one mole of hydrogen. Reaction continued and another two-thirds of a mole of hydrogen was taken up before the reaction ceased. It is thought that complete hydrogenation to butanediol was not observed due to formation of water in the system, resulting from the cyclization of butanediol, the hydrogenation product. The water thus formed moved in to occupy the vacant site in the rhodium complex normally held by a solvent molecule, and a "poisoning effect" resulted. It was found that the hydrogenation of *cis*-2-butene-1, 4-diol was poisoned much earlier than the 2-butyne-1, 4-diol, due to the fact that butanediol was formed much earlier in this hydrogenation.

The hydrogenations were carried out at four different temperatures, 30°, 35°, 40°, and 45°C. Applying the Arrhenius equation to the rate constants obtained at these temperatures, activation energies for the hydrogenations of 2-butyne-1,4-diol and *cis*-2-butene-1,4-diol were calculated. From a consideration of the temperature dependence of these reactions in light of the absolute reaction rate theory, it was possible to calculate enthalpies and entropies of activation for each of the reactions. The marked increase in the entropy of activation of the *cis*-2-butene-1,4-diol over the 2-butyne-1,4-diol supports the belief that the olefin is in fact bonded to the coordination complex in the transition state of the reaction.

Structure-Activity Relationships of Tetracyclines: Cell Division, Protein Synthesis and Nucleic Acid Synthesis in *Escherichia coli* W.

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The kinetics of inhibition of *E. coli* in a peptone broth as a function of the concentration of 18 tetracyclines have been determined. These experiments were designed to obtain antibiotic activities suitable for use in structural activity correlations. Viable and total cell count methods were used to measure rates of cell division. Rates of protein and nucleic acid synthesis were determined simultaneously by a membrane filter technique in conjunction with the Folin-Lowry Assay and the orcinol reaction, respectively. The relationship of the experimentally obtained rate constants to the antibiotic concentrations served as an estimate of antibiotic activity under the test conditions. *(These activities have been successfully employed by Dr. Peradejordi (another member of the Pharmacy Department) to obtain correlations of electronic properties of the tetracyclines calculated by quantum mechanical methods with their antibiotic activity.)* The rate constants obtained in the presence of any given concentration of tetracycline were essentially the same regardless of the methods used to obtain them, ie, rates of protein and nucleic acid synthesis and rates of cell division were equal in inhibited as well as uninhibited cultures. This observation indicates that inhibited cultures are probably growing in balanced growth and invalidates previously proposed mathematical models of tetracycline inhibition. Attempts to formulate new models consistent with all the reported experimental results were not entirely successful. Different times before onset of inhibition of protein synthesis, cell division and nucleic acid synthesis were observed in some kinetic experiments. These differences, if real, can be interpreted as consistent with the theory that the mode of action of these drugs is a primary inhibition of protein synthesis. Changes in broth pH also caused changes in these times of onset of inhibitions under certain conditions. These broth induced changes can be explained as due to the existence of a finite time of tetracycline permeation in *E. coli* W cultures in a high peptone media.

A Time Course Study of *in Vivo* Effects of Hydrazine on Rat Liver Protein and Nucleic Acid Content

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Hydrazine and its derivatives are used as pharmaceuticals, insecticides and rocket propellants. Exposure to hydrazine by many routes of administration results in variable physiological effects, ranging from minor skin irritations to convulsions. The outstanding pathological finding is lipid accumulation in the liver, which is generally accompanied by depletion of liver glycogen and elevation of liver protein.

A time course study was undertaken to determine the effect of a single sub-convulsive dose of neutralized, anhydrous hydrazine administered intraperitoneally to rats. Saline- and hydrazine-injected animals were sacrificed at 4, 12, 24, 36 and 48 hr periods after treatment. The results indicated that *in vivo* alterations in liver protein metabolism were greatest from 24 to 48 hr after treatment. Significant elevations in liver wet weight, total protein and RNA contents, and the uptake of C^{14} leucine into liver protein were demonstrated in hydrazine-treated animals compared to control animals, starting at 24 hr. Prior to 24 hr, significant, hydrazine-induced alterations in all these parameters were not observed. Hydrazine did not produce changes in liver total DNA content during the experimental period. When protein, RNA and C^{14} leucine uptake were expressed on a DNA basis, the effects of hydrazine on these cellular constituents were described.

Considered in terms of the total liver or the liver cell, the data were consistent with an hydrazine-induced net stimulation of hepatic protein biosynthesis which was maximal at 24 hr. These results parallel ultrastructural changes in the nucleolus and rough endoplasmic reticulum reported by others. The action of hydrazine appeared to involve a "degenerative" phase over the initial 4 hr period followed by a "regenerative" phase from 4 through 24 hr and, finally, the leveling off to a new steady state.

Ionic Contribution to the Genesis of the Frog Skin Potential

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Although the problem has been extensively studied, there is at present no completely satisfactory explanation of the genesis of the potential difference which exists across frog skin.

In the present study the electrochemical basis of the skin potential has been approached from three points of view. First, the recently proposed "diffusion delay theory" to explain the electrical response of the epidermis to Na^+ has been tested. Second, the potentiometric response of the skin to several anions and the mode of handling of the anions by the skin has been investigated. Third, the potential profile of the frog skin has been determined using a carefully designed microelectrode experiment.

1. To test the "diffusion delay theory" net Na^+ fluxes were measured isotopically in skins bathed on the epidermal side in solutions of varying Na^+ concentration and containing as an anion the impermeable $SO_4^=$ ion. The net fluxes found satisfied the equation $1/J_n = 6.75 + 30(1/[Na^+]_o)$. The net flux to permeability ratio (J_n/P_D) necessary to explain the electrical response of the epidermis to Na^+ was found to be in excellent agreement with data extracted from recently published kinetic studies on Na^+ transport in frog skin.

In addition to flux measurements, the theory was tested potentiometrically by determining the potential response of the epidermis to varying Na^+ concentrations in $NaHCO_3$ solutions. The "diffusion delay theory" was found to adequately explain the results obtained.

2. The potentiometric response of the frog skin to anions was determined by monitoring the skin potential during changes in the solutions bathing either the epidermal or corium side of the skin. The anions utilized Cl^-, HCO_3^-, $SO_4^=$ and NO_3^-. The order of permeability of the skin to these anions was $Cl^- \gg HCO_3^- > SO_4^=$ and NO_3^-. In addition it was found that when solutions containing Cl^- were substituted on the epidermal side of the skin for solutions containing any of the other anions there was an occasional reversal of the sign of the potential difference across the skin. No effect on the skin potential was found with replacement on the corium side of the skin.

To determine the mode of handling of Cl^- and HCO_3^- by the skin, net fluxes of the anions were measured isotopically and chemically in open-circuited and short-circuited skins, respectively. It was found that under conditions of low Cl^- (12 mEq/1) at the corium side of the skin there was a net inward active transport of Cl^-. The HCO_3^- ion was handled passively by the skin.

3. The potential profile of skins was determined by monitoring the potential difference between a glass microelectrode which was driven through the skin and a macroelectrode in the bathing solution at the epidermal or corium side of the skin. The skin was bathed in Cl^--Ringer's solution on both sides. Two discrete potential steps were found as the skin was penetrated, increasingly positive in the direction epidermis → corium. The steps were separated by a distance of 10 μ and were considered to occur at the outer and inner borders of the stratum germinativum.

The potential profile of skins bathed in low Cl^- solutions (12 mEq/1) at the corium side were also determined. Two discrete potential steps were found, increasingly negative in the direction epidermis → corium. These steps were separated by a distance of about 10 μ and were considered to occur at the outer and inner borders of the stratum germinativum.

Inheritance and Characterization of a Type A Blood Subgroup

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Blood group A subtypes were examined in 198 Caucasians and 78 Negroes. The frequencies of A_1 (A intermediate) and A_2 subtypes were much higher for Negroes than for Caucasians.

The A antigen strength was quantitatively measured by an immunohemolytic test and by lectins *Dolichos biflorous* and *Phaseolus limensis*. The H antigen was quantitated by the lectin *Ulex europaeus*. The Caucasian distribution of antigenic strength was discontinuous with clear separation between A_1 and A_2 subtypes. Negroes exhibited a continuous distribution in antigenic strength, the mean of A_1 samples falling between the means for A_1 and A_2. However, individual A_1 samples overlapped considerably with A_1 and A_2 samples.

The A_1 samples showed a distinct agglutination pattern with lectins; high titers were found with both *Ulex* and *Dolichos*. Family studies indicated A_1 type to be inherited by one or more alleles. A_1, therefore, appears to be a distinct inheritable subtype with unique lectin agglutinations and antigenic strength.

A Scheme for the Rapid Identification of the Enterobacteriaceae

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There is great need for an inexpensive and rapid technique for the identification of microorganisms. The present system employed in most laboratories involves the use of standard size tube cultures requiring from 18 to 24 or more hours of incubation. Usually, after primary isolation, a step by step procedure in the selection of biochemical tests is used until the organism is identified.

Many attempts to circumvent this time barrier have been tried. Three types of medium systems have been reported: the Multitest, the Microtest, and the Minitest. The multitest system consists of media in which two or more reactions can be observed simultaneously. The microtest system decreases the volume of media and utilizes a heavy inoculum. The minitest system utilizes the reagents and sometimes media contained in tablets or inpregnated paper.

Two different kinds of microtest have been developed; both are rapid when compared with standard methods, and in almost all cases, the results correlate with them. The two kinds use different principles; in the first, a heavy inoculum is grown in a small volume of medium, previously warmed to 37 C. This type of test can show both preformed and induced enzymes. In the second kind, living suspensions in water, saline or buffer are added to the test substrate. Multiplication does not occur, and the test reveals only those enzymes that are prefound. For certain tests, it is necessary to grow the organism on special medium (to induce enzyme formation) before making the suspension. The present study describes the application of the first type of microtest system for the rapid identification of the Enterobacteriaceae. A comparative study was made on each organism using both the micro and standard methods.

Most of the organisms in this study were fresh human isolates grown from urine, sputum, wounds and feces. A few were obtained from the Virginia State Health Department Laboratories. A total of 455 organisms from different genera were studied. The criteria used to identify the organism were the same as those described by Edwards and Ewing.

The media in this study were dehydrated Difco products made according to the directions given on the bottle. The media selected were those presently used in the blood culture section of the microbiology laboratory at the Medical College of Virginia. The inoculum used was a heavy suspension of the organism in saline and the techniques involved were essentially

those already employed in a routine microbiology laboratory.

There was good correlation in the results obtained by both methods. To test the efficacy of the microtest system under diagnostic laboratory conditions, a blind study was conducted using routine blood cultures from the diagnostic laboratory. These results also correlated well.

The microtest system, as used in this study, has been most useful in the identification of enteric gram-negative organisms as found in a diagnostic clinical laboratory. This system can be converted to routine laboratory use with little difficulty. It is a time-saving system which decreases the operational expenses. Its greatest feature will be the speed and accuracy with which the laboratory will be able to assist the physician in the patient's care.

Transient Electro-optic Kerr Effect in Spheroidal-like Particles

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The transient electro-optic Kerr effect has been calculated for ellipsoids of revolution. The derived expression consists of a double exponential function of time, in which the exponential parameters are linearly related to the corresponding rotational diffusion constants. This is to be compared with the single exponential equation derived by Benoit for an ellipsoid of revolution.

The types of molecular information available from the experimental techniques of the transient electro-optic Kerr effect, depolarization of fluorescence, and dielectric dispersion are discussed and compared and it is shown that, heretofore, the Kerr effect has provided only half of the information available from the other two techniques. The equation derived in this thesis predicts that for the case of an ellipsoid of revolution the Kerr effect should provide the same amount of information as the other two effects. In fact, when the limiting behavior of this equation is examined for the case of spherical as well as for long rod shaped particles the number of time exponentials is reduced from two to one, in agreement with the equation of Benoit. It would appear, therefore, that Benoit's equation is applicable only to a sphere and not to an ellipsoid of revolution as originally intended.

Experimental results on several molecular species can be accounted for in terms of 2 relaxation times as predicted by the double exponential for solute particles which are ellipsoids of revolution. This increase in information with respect to ellipsoids of revolution permits the particle dimensions to be obtained, by a computer analysis, without the requirement of additional information concerning the size of the particle.

Studies of Potentially Useful Agents in Urolithiasis

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In a review of the literature on urolithiasis the following successful chemical treatments of this disease found: treatment of uric acid and cystine stones with allopurinol and d-penicillamine respectively, treatment of less than six months old calcium oxalate stones by irrigation of the kidney with ion chelating solutions of EDTA or of less effective citrate, and finally treatment of non-infected cases with methylene blue.

The use of an agent which has the ability to chelate calcium more specifically than magnesium would be desirable. Such an agent, ethyleneglycol bis (betaamino-ethylether)- N,N,N′,N′-tetraacetic acid, EGTA, was evaluated as to its acute and subacute toxicity, and for its absorption and excretion properties. The toxicology studies required a feasible method for determining calcium, magnesium, and chelating agents in biological fluids. Such a procedure was developed and calcium was determined using a solution of Na_4EGTA as the titrant for calcium at an initial pH of 8.0, followed by titration of magnesium at the same pH with a solution of Na_4EDTA. Chelating agents were determined using Na_4EGTA in a back titration after addition of an excess of cadmium nitrate to the sample containing chelates at an initial pH of 4.0-4.5. A sharp break in the pH plot indicated the equivalence point in these methods.

In toxicological evaluation the oral LD_{50} for Na_2H_2EGTA in male Holtzman rats was found to be 9.43 mmole/kg. compared with 6.25 mmoles/kg. reported for Na_2H_2EDTA. In subacute studies EGTA was compared directly with EDTA by feeding up to 10 per cent by weight of the disodium salt of each agent in the regular chow to rats for 90 days. Both EDTA and EGTA were absorbed to less than 5 per cent of the subacute dose and recovery from the urine and feces ruled out significant metabolism. The proportion of calcium not bound to chelate decreased as the amount of dietary chelate increased. EGTA was better tolerated in the diet and was less irritating than EDTA. Because EGTA appeared to be much less toxic and more palatable than EDTA, further study of the use of this agent and its derivatives as potentially effective agents in urolithiasis or in elimination of heavy metals should be made.

Clinical study of organic dyes has been limited to methylene blue. This agent caused both reduction of stone size and prevention of recurrent stone formation in patients producing the common calcium oxalate and phosphate stones.

Experimental studies indicated that analogs of methylene blue containing fewer N-methyl groups were less effective in inhibiting deposition on implanted zinc pellets in the bladder. *In vitro* studies of the Alphazurin series of dyes indicate that larger N-alkyl substituents increase adsorption of these dyes on crystals that are common constituents of kidney stones. No analogs of methylene blue which contained higher N-alkyl substituents were available and three of such compounds were synthesized. These compounds, 3,7-bis(diethylamino)-phenazathionium bromide, 3,7-bis(methylbenzylamino)-phenazathionium bromide, and 3,7-bis (ethylbenzylamino)-phenazathionium bromide, were made by addition of bromine to phenothiazine, both in ethyl ether solution, followed by addition of the appropriate secondary amine. The compounds were obtained in pure form by recrystallization from ethyl acetate, water, and mixtures of ethyl acetate and methanol, respectively. The structures were confirmed by elementary analyses and infrared and NMR spectra.