

Proceedings of the Thirtieth Annual AOA Research Conference, 1986: Part II

Part I of the Proceedings of the Thirtieth Annual AOA Research Conference was published in the September 1986 issue of JAOA. This included the Introduction, Keynote Address, Louisa Burns Memorial Lecture, highlights of a symposium entitled "Muscle Spindles, Golgi tendon organ, and the neural control of skeletal muscle," and abstracts in the following categories: Burroughs Wellcome fellows, and Osteopathic techniques.

Part II includes abstracts of presentations and poster sessions in Anatomy, Biochemistry, Neurophysiology, Clinical practice, Education, Immunology, Teratology, Physiology, Microbiology, and Pharmacology.

Anatomy

Age-related changes in the optic nerve of the least shrew

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These studies of the optic nerve of the least shrew were undertaken because both the shrew's small size (4-6 gm.) and its phylogenetic placement as a common eutherian progenitor make it a useful animal in which to study fundamental organization of the visual system. The optic nerve is 3.3 mm. in length, with a mean shortest axis diameter of 70 μ m. In twelve optic nerves, electron microscopic examinations of sections taken 1-2 mm. behind the globe indicate that the nerve contains a mean of 2,550 myelinated axons. There are approximately one-third as many unmyelinated axons as myelinated. The shortest axis diameter of myelinated axons (including the myelin) ranges from 0.13 μ m. to 2.17 μ m. The frequency dis-

tribution of the shortest axis diameters is unimodal and positively skewed, with a mode of 0.76 μ m. Preliminary data indicate that mean myelin thickness increases with axon diameter up to axon diameters of approximately 0.64 μ m. Axons of larger diameter have relatively constant mean myelin thickness. The unmyelinated axons range from 0.13 μ m. to 1.91 μ m. and are unimodally distributed with a mode of 0.38 μ m.

Myelination occurs in animals up to 60 days of age. Hypermyelination and axon degeneration is noted in most shrews over 10 months old. In addition to normal age-related changes, retinopathy and basement membrane thickening, attributed to spontaneously occurring maturity onset diabetes, are frequently observed in this species.

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Ovulation in the genetically obese Zucker rat

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Estrous cycles of obese Zucker rats are typically longer in duration (5-6 days) than those of lean rats (4 days), and are frequently separated by extended periods of diestrus. Obese rats also tend to become acyclic at a much earlier age than lean rats.¹ In view of these cycling abnormalities, the question arises whether obese rats ovulate normally with respect to the number of eggs released. Indirect evidence from artificial insemination experiments² suggests that this aspect of ovulation is normal; the present study answers the question directly with actual egg counts. For practical purposes, it was also of interest to determine whether estrous cycles could be induced in the irregularly cycling or prematurely acyclic obese animals.

Obese and lean Zucker rats, 3-9 months of age, maintained on a 14 hr./10 hr. light/dark cycle, were used in this study. For egg counts, cycling rats were sacrificed at the beginning of the dark phase on the

day of vaginal estrus. Oviducts were removed and their contents flushed out into a concavity slide containing a hyaluronidase solution (50 I.U. in 0.5 ml. modified Brinster's media). The mean egg count for lean rats (9.75 ± 1.36 SEM; $n=8$) is not significantly different (student's t test) than that for obese rats (8.89 ± 1.18 ; $n=9$). Thus, our prediction based on insemination studies was confirmed.

Acyclic rats or rats in spontaneously extended diestrous periods of at least two weeks' duration were used in the ovulation induction experiment. A single sub-Q injection of 10 I.U. of pregnant mare's serum (PMS) was given to each animal. All rats (7 obese and 6 lean) had a proestrous vaginal smear by the third day following PMS treatment. Each proestrus was followed by a day of estrus, suggesting that PMS had induced ovulatory cycles.

Our laboratory is engaged in artificial insemination experiments to determine why obese rats cannot maintain pregnancies to term. Since rats must be in proestrus when inseminated, the infrequency of proestrous periods in obese rats poses a frustrating problem. The effectiveness of PMS in inducing estrous cycles in obese rats will be a valuable time-saver in future research. It also provides information that will help to elucidate the mechanism(s) involved in the reproductive dysfunction of these animals.

1. Chelich, A.M., and Edmonds, E.S.: Reproductive function in the genetically obese female Zucker rat. *Anat Rec* 196:264, 1980

2. Chelich, A.M., and Edmonds, E.S.: Reproductive capacity of the obese female Zucker rat. The decidual response and artificial insemination. *JAOA* 83:154, 156, 1983

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Methods for determining intervertebral disk plasticity

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Numerous studies indicate that a sea-

sonal variation exists in the body length of small mammals. The skeletal component of the vertebral column which possesses the plasticity to account for this variation is the intervertebral disk. Before one can study the mechanisms underlying intervertebral disk plasticity, methods of disk measurement must be examined and perfected. This paper examines several modalities of analyzing intervertebral disk size and morphology in the prairie vole.

The most direct and accurate method for determining intervertebral disk size involves the measurement of disk height from a ventral view with the aid of an ocular micrometer in a dissection microscope. There is no significant difference in disk size between fresh and fixed tissue when 10 percent buffered formalin, AFA, or 2 percent glutaraldehyde are used as fixatives. Bouin's solution causes considerable disk shrinkage. Disks are readily observed with visible light, but differential demarcation is improved by ultraviolet illumination.

The caudal four lumbar intervertebral disks represent about one-third of total disk height. One can obtain a fairly accurate determination of disk plasticity in the entire vertebral column by measurement of these four disks.

All measurements require the agreement of two readers. The status of the animals, experimental or control, is not revealed until after the measurements are recorded. Histologic confirmation of disk measurements can be showed by viewing 10 μ m. frozen sections. Decalcified paraffin sections do not accurately reflect disk size. All decalcification agents tend to swell the disks, but the morphology of nucleus pulposus reflects the observed difference. Cells from small disks show equatorial alignment and possess small heterochromatic nuclei with scant cytoplasm.

There is no correlation between age or sex and disk height in adult animals. A relationship exists between disk size and both body length and weight.

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Experimental studies of the effects of vertebral misalignment

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This study is an ongoing investigation to obtain information pertaining to vertebral column changes and misalignment factors causing visceral alterations. Sprague-Dawley rats were used. The first step was production of scoliosis without direct attack on the vertebral column.

This was accomplished by the use of bow stringing with monofilament suture. Very good scoliotic configuration was obtained. For regular periods before sacrifice, both the control and experimental subjects were observed for locomotion attitudes plus grid walking. Both groups performed well. Both groups were weighed and neither exhibited growth interference or weight loss. Prior to sacrifice, animals were infused with glutaraldehyde. The apex of the curve was identified, respective organs were inspected, removed, and stored in fixative. The apex of the vertebral curve showed fibrillary change and there was a partial disruption of this posterior longitudinal ligament. At the disk annulus, the same pathologic state existed in only the three apical vertebrae of the curve. Another local pathologic condition was an increase in general disk height. Associated with the collagen changes were areas in the periosteum of the involved vertebrae of increased vascularity. These areas were specifically located at the junction of the pedicle of the body of the vertebrae. These vessels were of capillary and arteriolar size. Organs examined showed no excessive pathologic change with light microscopy. This very basic type of investigation should be repeated and the internal environment monitored by vital means, immunoassay, and nuclear magnetic resonance as examples.

This project was originally initiated and prepared by Jocelyn Prendergast.

Structural and functional analysis of the anterior cruciate ligament

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In order to evaluate the effects of various casting methods on anterior cruciate ligament strain and to address the question of function within specific areas of this ligament, liquid metal strain gauges were implanted within the anatomic centers of these specific areas in 11, unfixed human anterior cruciate ligaments (ACL). Previous dissections (28) had shown the ACL to be a laminated structure, divisible into two primary laminae: a homogeneous medial lamina or anteromedial bundle; and a more lateral, nonhomogeneous lamina which was further divisible into two components, an anterolateral and posterolateral bundle. The gauge-im-

planted limbs were placed in a testing apparatus and subjected to flexion/extension, anterior-posterior drawer, varus/valgus, and internal/external rotational maneuvers. Following preliminary testing, each limb was placed in a 45° full-leg cast and all appropriate maneuvers repeated. Lastly, the cast was modified to a cast brace by removal of a plaster wedge and the addition of hinged braces to the existing cast, and the limb was again subjected to the above listed maneuvers. The ACL proved to have two functional areas: one anterior, and one posterior. In simple flexion/extension experiments, strains recorded in both functional areas were highest in full extension and decreased with increasing flexion angle. Strains recorded in posterior areas were higher than those recorded in anterior areas. Superimposing all other maneuvers (except posterior drawer) on the basic flexion/extension pattern consistently increased the strain within the ACL at all degrees of flexion tested. Comparisons made between the cast and cast brace showed them to be essentially equal in protecting the ACL from excess strain at 45° of flexion. Information obtained in these experiments suggests that in order to protect the ACL from excess strain, knee immobilization techniques utilize a flexion angle of greater than 55°. This is the first demonstration of simultaneously recorded strain from multiple areas of the ACL and is the first study to quantitatively compare the protective effect of prosthetic devices on the human ACL.

Biochemistry

Plasma glucagon levels in rats bearing the Walker 256 carcinosarcoma

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Male Sprague-Dawley rats (125-150 gm.) were implanted intramuscularly with the Walker 256 carcinosarcoma. After 7 and 10 days, the tumor-bearing rats, along with age-matched, nontumor-bearing rats, were fasted overnight, killed, and plasma levels of glucagon and glucose were assayed. Plasma levels of glucagon in both 7- and 10-day tumor-bearing rats were approximately half the level seen in control rats ($p < 0.01$) even though fasting plasma glucose levels were slightly, but not significantly, less in the tumor-bearing rats. It was of interest, then, to determine if the tumor is responsible for this decrease through degradation of the hormone.

Tumor cells, liberated from freshly excised tumor by homogenization, were in-

cubated at room temperature with ^{125}I -labeled glucagon (0.075 μCi , 2200 Ci/mMol) in 20 mM TRIS-HCl buffer (pH 7.5) with 2.5 percent bovine serum albumin (total volume 750 μl .) at 20 C for 45 minutes. Radiolabeled-glucagon incubated in buffer alone served as control. After incubation, the cells were separated from the incubation medium by centrifugation through N-butylphthalate oil. The cell-free incubation medium was then treated with an equal volume of trichloroacetic acid (TCA, 10 percent) to precipitate protein. After incubation with tumor cells, only about 25 percent of the total radioactivity was TCA-precipitable relative to 97 percent in the control incubations. It also appears that physical contact between the hormone and the tumor cells is unnecessary but, rather, that the cells may release a substance which degrades hormone. When tumor cells are preincubated for 60 minutes at 37 C and then removed from the medium by centrifugation and filtration, the incubation medium contains a factor which degrades subsequently added ^{125}I -glucagon (57 percent TCA-precipitable vs. 98 percent in control incubation). That this factor is a protease is suggested by the results of another experiment in which aprotinin (a protease inhibitor), when added to the second incubation (1 mg./ml.), virtually eliminated degradation of glucagon by a released factor (79 percent TCA-precipitable in the absence of aprotinin vs. 94 percent in its presence and 98 percent in controls).

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Kinetics of p-nitrophenylphosphate hydrolysis by NaK-ATPase

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p-Nitrophenylphosphate (PNPP) is an alternate substrate for NaK-ATPase, the enzyme that serves as the NaK-pump in mammalian cells. The hydrolysis of adenosine triphosphate by this enzyme requires Na, Mg, and K, but the hydrolysis of PNPP requires only Mg and K. Thus, PNPPase proceeds by a relatively simple mechanism which bypasses a Na-dependent step in the reaction sequence. In the present study, we investigated the kinetic mechanisms for activation of PNPPase by Mg and K. The enzyme was prepared as a microsomal fraction from the medulla of dog kidney.

In order to examine the mechanism of Mg activation, we measured PNPP hydro-

lysis as a function of free Mg concentration at various fixed concentrations of PNPP. The K concentration was saturating (10 mM) and did not vary. Kinetic analysis of the data revealed that there is an obligatory order of binding of Mg and PNPP to the enzyme; Mg binding must precede PNPP binding. Therefore, it appears that Mg may activate strictly by allowing PNPP to bind.

To investigate the mechanism of K activation, we varied PNPP concentration at various fixed concentrations of K. The concentration of Mg was saturating (3 mM). In this case, kinetic analysis revealed that K and PNPP bind in random order, but that each ligand reduces the apparent affinity for the other by a factor of about sixfold. Thus, activation by K can be attributed solely to a requirement that both K and PNPP must be bound before hydrolysis of the substrate occurs.

It is known that NaK-ATPase can exist in one of two conformations, E_1 and E_2 . It is also known that Mg and K bind to and stabilize the E_2 conformation. Furthermore, the hydrolysis of PNPP is associated with the E_2 conformation. The requirement of Mg for PNPP binding is consistent with this view; however, the negative binding interaction between K and PNPP suggests that Mg and K may stabilize different subconformations of E_2 which differ in their affinities for PNPP.

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Quantitation of DNA synthesis by the ocular lens in vivo from ^3H -thymidine

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Nonsterol isoprenes formed during cholesterol biosynthesis are reported to be necessary for DNA replication. We are interested in possible connections between cholesterologenesis and cell division and differentiation in the ocular lens. All cells of the ocular lens originate from a monolayer of epithelial cells which cover only the anterior surface. The epithelial cells undergo a terminal differentiation to form fiber cells. Fiber cell formation requires a tremendous increase in formation of a cholesterol-rich plasma membrane. DNA synthesis in the lens is restricted to the epithelial cell layer. As a first step toward understanding the role of cholesterologenesis in regulating epithelial cell division and differentiation, we have devised a method which permits quantitation of DNA synthesis by the lens in vivo.

Two-week-old rats were injected intraperitoneally with 4 $\mu\text{Ci/gm}$. (body weight) of ^3H -thymidine (2 Ci/mMol) dissolved in sterile saline (0.4 mCi/ml.). Rats

were sacrificed at 5 minutes to 5 weeks following injection; the lens were removed and divided into capsule (epithelial cell layer) and lens (minus capsule). Unlabeled DNA carrier (0.25 mg.) was added and the fractions were homogenized in 10 percent trichloroacetic acid (TCA). The precipitates were washed twice with 10 percent TCA and twice with 90 percent ethanol. The resulting pellet and aliquots of the TCA-soluble fraction were assayed for ^3H -radioactivity. The soluble fraction represents ^3H -thymidine available for DNA-synthesis and the pellet is ^3H -labeled DNA. TCA-soluble ^3H was detected in the lens as early as 5 minutes following injection and reached a steady state concentration at between 30 to 60 minutes. TCA-insoluble ^3H was detected in the lens at 30 minutes and reached a steady state concentration by about 3 hours after injection. Essentially all of the TCA-insoluble radioactivity was associated with the epithelial cell layer (capsule).

As epithelial cells differentiate into fiber cells, the location of the ^3H -labeled DNA will shift from capsule to the lens body (to fiber cells). By measuring the rate of change in the ratio of TCA-insoluble ^3H between the capsule and the lens with time after injection, we should be able to directly measure the overall rate of epithelial cell differentiation.

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Covalent and noncovalent factors that affect lectin-erythrocyte interactions

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This study considers noncovalent and covalent factors that altered interactions between erythrocytes and the lectin from *Laburnum alpinum* using the minititer assay, a quantitative agglutination method developed recently. The noncovalent factors studied were pH and the substances lactose, CaCl_2 , MnCl_2 , and EDTA. Inhibition by lactose was identical when phosphate buffer at pH 7.0 was compared with either acetate or citrate buffer at pH 5.6; however, in citrate buffer at pH 5.0, the agglutination was reduced proportionately in both controls and lactose-containing samples, although the percentage of inhibition remained the same.

The fact that the percentage of inhibition remained the same at the three pH values suggests that the lactose inhibition was pH-independent. However, the fact that the lower pH reduced total agglutination implies that there was also a pH-dependent interaction. Studies performed with Ca^{2+} and Mn^{2+} as well as inhibition by EDTA also support this

hypothesis. A number of lectins are known to have binding sites for carbohydrates and metal ions, so these results suggest that the lectin from *Laburnum alpinum* is similar to those lectins.

Covalent interactions were investigated using erythrocytes chemically modified with either glutaraldehyde or the proteinases trypsin or ficin; ficinized cells bound the most lectin and exhibited the strongest agglutination. The treatment of the untreated, trypsinized or ficinized erythrocyte with lectin produced profound changes in the erythrocytes that made them resistant to osmotic shock (hemolysis in deionized water). Although this resistance might be mediated by an ion-transport protein in the membrane, such as the band three protein, it is not known if the binding of the lectin stimulated covalent or noncovalent changes within the erythrocytes.

Role of protein disulfide bonds and protein sulfhydryl groups in protein turnover

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It was postulated that disulfide bonds may stabilize protein structure and that sulfhydryl groups with the potential of forming mixed disulfides may destabilize protein structure. Therefore, this study sought to determine if either disulfide bonds or sulfhydryl groups have an effect on protein half-life, which is partially dependent on protein stability.

A procedure was developed for protein fractionation using various combinations of 0.1 percent sodium dodecyl sulfate, 15 mM N-ethylmaleimide, or 10 mM dithiothreitol (the latter to remove mixed disulfides or to disrupt disulfide bonds), followed by chromatography on thio-propyl-Sepharose 6B. The $^3\text{H}/^{14}\text{C}$ ratio of the proteins from the liver, skeletal muscle, heart, brain, and kidney did not differ significantly when fractionated according to the presence or absence of the following: (1) sulfhydryl groups not involved with mixed disulfides; (2) all sulfhydryl groups; (3) disulfide bonds; and, (4) disulfide bonds and mixed disulfides. Therefore, one can conclude that the presence of sulfhydryl groups, mixed disulfides, or disulfide bonds are apparently not, collectively speaking, of sufficient importance to alter protein turnover. Because most disulfide bonds occur within, rather than between, domains, these data do not rule out the possible importance of domain dissociation as a factor in determining protein half-life.

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Nonmuscle myosin phosphorylation sites for calcium-dependent and calcium-independent protein kinases

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The purpose of these experiments is to investigate the regulation of myosin ATPase activity and filament assembly in nonmuscle cells utilizing purified protein kinases and myosin proteins. Thymus myosin, light chains, and a synthetic peptide (S-S-K-R-A-K-A-K-T-T-K-K-R-P-Z-R-A-T-S-N-V-F-S) corresponding to the N-terminal sequence of smooth muscle myosin light chains were compared as substrates for calcium/calmodulin-dependent protein kinase (MLCK), calcium/phospholipid-dependent protein kinase (PKC), and a MgATP-activated protein kinase (H4PK) from lymphoid cells or human placenta. All protein kinases catalyzed phosphorylation of the three substrates but with differing affinities. MLCK activity was totally dependent on added calcium and calmodulin with each substrate, but the PKC activity required calcium and phospholipids with the peptide substrate only. H4PK showed the highest affinity for the synthetic peptide and isolated light chains. Phosphoamino acid analysis and analysis of thermolysin-derived peptides established that PKC catalyzed phosphorylation of threonine-9 or -10. In addition, PKC and H4PK catalyzed phosphorylation at serine-19, the MLCK site. Collectively, the data support the hypothesis that myosin filament assembly and MgATPase activity in nonmuscle cells may be regulated by a variety of calcium-dependent and calcium-independent protein kinases.

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Activation of factor X by isolated rat hepatocytes

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Hepatocytes, prepared by perfusion of rat liver with collagenase, were incubated in the presence or absence of exogenous factor X at 37°C for up to 4 hours. During this time, the factor X and activated factor X (Xa) activity were determined by clotting assays. The factor X was purified from

human plasma and was present at a concentration of 0.7 $\mu\text{g}/\text{ml}$. (0.24 units/ μg). The incubation volume was 10 ml. and contained 2.5×10^6 cells/ml. Essentially all of the added factor X was converted to factor Xa within the first 5 minutes of the incubation. The incubations without factor X secreted up to 0.06 units of this clotting protein, most of which was present in the activated form (factor Xa). While procoagulant activity has been demonstrated with monocytes and macrophages, this activity would not be expected to be associated with hepatocytes. The physiologic significance of these observations is not clear, and it is possible that the cellular activation of factor X is unmasked during the preparation of hepatocytes by the collagenase procedure.

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Effect of dietary fatty acid manipulation on forskolin-induced lipolysis and cyclic AMP accumulation in adipose tissue in the rat

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Earlier work from this laboratory showed that in animals fed diets high in saturated fatty acids, adipose tissue was virtually unresponsive to the lipolytic action of norepinephrine. The present study extends this observation to include the effects of dietary fatty acid quality on the lipolytic activity of forskolin, an agent that can activate adenylate cyclase in isolated membrane preparations and in intact cells by mechanisms that do not depend upon hormone receptors. Upon attaining a body weight of 80-100 gm., male Sprague-Dawley rats were divided into two groups and placed on diets of known composition containing 16 percent (w/w) fat (2 percent as corn oil to supply essential fatty acids and 14 percent as either coconut oil for saturated fatty acids or safflower oil for polyunsaturated fatty acids). The animals were fed the experimental diet *ad libitum* for a minimum of 4 weeks prior to each experiment.

Following the feeding period, the animals were killed, and the epididymal fat pads were removed, placed in ice-cold saline, and minced with scissors. Minced fat tissue was incubated with forskolin in varying concentrations or vehicle (ethanol) for either 15 minutes in the case of cAMP determinations or 60 minutes for the lipolysis determinations. Lipolysis was determined by the measurement of free fatty acid release by the ^{63}Ni method

of Ho; cAMP was determined by competitive protein binding. In animals fed the safflower oil diet, incubation with forskolin (10^{-6} to 10^{-4} M) produced dose-related increases in both tissue [cAMP] and free fatty acid release. In contrast, forskolin had virtually no effect on these parameters in incubated fat tissue from animals fed the coconut oil-based diet. When fat from the safflower-fed animals was incubated with minimally effective concentrations of forskolin (10^{-6} M) and isoproterenol (10^{-7} M) together, a synergistic effect of these two compounds on cAMP accumulation was noted (628 percent increase relative to basal levels compared to 23 percent and 22 percent with either forskolin or isoproterenol alone, respectively). In contrast, no such synergism was seen in the fat from the coconut oil-fed animals.

The permeabilization of mouse thymocytes by illumination in the presence of erythrosine

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Thymocytes are permeabilized by illumination in the presence of erythrosine B. The percent saturation of quin 2, an organic dye, with calcium following illumination in the presence of erythrosine B can be measured fluorometrically and is ideally suited for following the kinetics of permeabilization. That permeabilization results after illumination has been demonstrated by the increase in fluorescence emission of quin 2, by tritiated quin 2 leakage, by the distribution of quin 2 fluorescence in illuminated versus non-illuminated thymocytes, and by the permeability of the cells to eosin and trypan blue dyes. The rate of the permeabilization reaction is dependent upon the concentration of erythrosine, the time of illumination, the presence of oxygen, and the temperature. Illuminated erythrosine reacts with LDH enzyme. Under certain conditions, the leakage of LDH enzyme can follow a substantial time after the leakage of calcium and quin 2. The stability, as determined under the microscope, of the thymocytes after permeabilization is equal to non-permeabilized cells. This indicates that much of the structure of the membrane

remains intact. Both the gross cellular structure and number of permeabilized lymphocytes remained similar to that of control cells up to 7 hours after permeabilization.

Chicken gizzard desmin-specific calpain

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A calpain I (micromolar calcium-activated cysteine proteinase) specific for the intermediate filament protein desmin has been identified in chicken gizzard smooth muscle. In this study, the purification and subunit structure of the enzyme are reported, along with several properties of a monoclonal antibody to the catalytic subunit. Minced chicken gizzards were subjected to pH 6.2 and pH 4.6 isoelectric precipitations, mild heat treatment, and then chromatographed on Reactive Blue 2-Sepharose CL-6B, DEAE Sephacel, Thiopropyl-Sepharose 6B, and TSK 3000 HPLC. The purified desminase was composed of 83 and 35 kDalton subunits. The 83 kDalton polypeptide was identified as the catalytic subunit by using a tritium-labelled epoxysuccinyl derivative. The desminase was then used in an in vitro immunization and hybridoma fusion using the PEG technique. The monoclonal antibody that was isolated (2G9) was identified as IGM with Kappa light chains. As determined by immunoblotting, 2G9 was specific for the 83 kDalton catalytic subunit of desminase. Furthermore, autolytic degradation products of the catalytic subunit were identified by immunoblotting desminase that had been exposed to 5 mM calcium ion, namely 67 and 50 kDalton polypeptides. Finally, 2G9 was able to bind native hamster skeletal muscle desminase, denatured chicken gizzard wide-specificity calpain II, as well as native and denatured chicken gizzard desminase. These results suggest that the turnover of desmin in smooth muscle is regulated by a specific calpain I, which may in turn be regulated by calcium-mediated autolysis as well as the better characterized interaction with calpain-specific inhibitors (calpastatins). Furthermore, the immunologic cross-reactivity indicates that the desminase has some structural feature(s) not found in the wide-specificity millimolar calcium-requiring calpain found in the same tissue, yet possesses an evolutionarily conserved antigenic determinant to a desminolytic calpain found in mammalian skeletal muscle.

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³¹P Nuclear magnetic resonance studies of phosphorus metabolism in *Biomphalaria glabrata*, the snail vector of the human parasite, *Schistosoma mansoni*

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In our present study, we observed the ³¹P nuclear magnetic resonance (NMR) spectra of intact and hydrolyzed snail eggs during a 10-day incubation period, measuring the phosphonate and phosphate content. The [³²P]phosphoric acid uptake by the snails and its appearance in freshly deposited egg masses as [³²P]2-aminoethylphosphonic acid ([³²P]2-AEP) were also determined.

In the hydrolyzed egg masses, the 2-aminoethylphosphonic acid (2-AEP) concentration decreased from 133.1 ± 3.7 μ g./mg. dry weight to 84.5 ± 5.2 μ g./mg., with a concomitant increase in orthophosphate from 22.1 ± 3.8 μ g./mg. to 66.1 ± 5.2 μ g./mg. Total phosphorus analysis showed that there was no variation in the total phosphorus concentration during the embryonic development period (153.7 ± 7 μ g./mg.). NMR analysis of intact eggs gave four different peaks in the phosphonate region. The major peak at 21.02 δ is free 2-AEP, with smaller peaks at 20.97, 23.37, and 23.83 δ . During embryonic development, 2-AEP decreased from 69.1 to 54.2 percent of the total detected P; the signals at 23.37 and 23.83 δ remained constant, and the signal at 20.87 increased from 14.8 to 16.7 percent of the total observed P. Concomitant with the decrease in phosphonate concentration, the orthophosphate content increased, with high energy phosphates (δ values <1), going from 3.5 to 16.7 percent of detectable P. The two stable phosphonate population at 23 δ may be associated with the egg case and not involved directly in embryo metabolism, while the compound at 20.87 δ may serve as an intermediate in the conversion of phosphonate-P to orthophosphate metabolites.

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SDS polyacrylamide gel electrophoresis of murine cysteine sulfinate decarboxylase

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Cysteine sulfinate decarboxylase (CSAD) is rate-limiting in the metabolic transformation between cysteine and taurine. Taurine occurs in high concentration in adult liver, heart, and muscle, and in fetal liver and brain. With recent postulation that taurine is involved in the regulation of cellular proliferation, we investigated the following: (1) whether or not there is any correlation between CSAD activity and taurine content in normal development; (2) the controlled growth in fetal liver; and, (3) in neoplasia, the uncontrolled growth in murine ascites tumor cells. This report presents our preliminary results on SDS polyacrylamide gel electrophoresis of various murine CSAD preparations. Extracts of ascites tumor cells, host livers, normal livers—at different developmental stages—were prepared by centrifugation of homogenates acidified to pH 5.6. The slab gel, containing 7.5 percent polyacrylamide, received 20 μ l. aliquots of SDS-treated samples per slot. It was electrophoresed in an EC 470 apparatus in tris-glycine buffer (0.04 M, pH 8.3, containing 0.05 percent SDS). Initially, 200 volts were maintained until the front of the tracking dye, pyronin Y, entered the running gel. Then, the voltage was increased to and maintained at 400 volts until the tracking dye had migrated to the bottom of the gel. After electrophoresis, the slab gel was washed with methanol-H₂O, stained with silver nitrate, and fixed with citric acid-formaldehyde with gentle shaking. The electrophoretic pattern showed less CSAD present in the ascites tumor cells, as compared to the host liver. CSAD, although detectable in 16 day-old fetal and neonatal livers, was not fully expressed until 4-6 days after birth. These data agree with our previous findings that CSAD activity was high at birth and that taurine content was also higher in the developing livers than in the adult. These results, although indirect, can be taken as supportive evidence that taurine does, indeed, play a role in cell proliferation.

Neurophysiology

Sexually dimorphic nucleus in the genetically obese Zucker rat

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The brains of Zucker rats were examined by light microscopy to determine whether this strain of rat exhibits sexual dimorphism of the preoptic area. A sexually dimorphic nucleus in this area (SDN-POA) was previously described in at least one strain of rat and was found to be markedly larger in the male when compared to that of the female. Moreover, some studies suggest that the SDN-POA may be a morphologic basis for the sexual differentiation of brain function. Because the obese Zucker rats of both sexes are characterized by reproductive dysfunction, while their lean counterparts are reproductively normal, this study is also a preliminary investigation of brain morphology as it may relate to reproductive dysfunction. The brains of 6 lean and 5 obese male Zucker rats and 5 lean and 5 obese female Zucker rats were perfused and fixed with 10 percent buffered neutral formalin and subsequently prepared for histologic study. For each brain, a single coronal paraffin section (15 μ m.) passing through the most developed region of the SDN-POA was selected for determinations of nuclear area and ratio of nuclear area to brain weight.

Male rats of both the lean and obese groups were found to have significantly larger nuclear areas than their female counterparts. Whether expressed as absolute area or as area relative to brain weight, the size difference is threefold between males and females of the lean group. The size difference between males and females of the obese group is also threefold when expressed as area relative to brain weight, but twofold when expressed as absolute area. No significant differences were found between mean nuclear areas of lean versus obese males, or of lean versus obese females. Failure to demonstrate a significant difference between the SDN-POA areas of the reproductively normal lean rats versus reproductively compromised obese rats does not preclude the possibility that morphologic differences exist. Additional parameters including, for example, nuclear volume, cell density, and cell populations remain to be studied.

In conclusion, the existence of a sexually dimorphic nucleus in the POA of the Zucker rat brain has been demonstrated. Although no significant differences in nuclear areas of the lean versus the obese animals of either sex were found, it is of interest to study the SDN-POA in the ge-

netically obese rat in view of its reproductive abnormalities.

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Pattern electroretinogram: A method to eliminate malingering as the etiology of an abnormal visual-evoked potential

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Visual-evoked potentials (VEP) are abnormal in optic nerve lesions. Reportedly, some patients are capable of volitionally altering their VEP, thereby simulating optic nerve abnormalities. Pattern electroretinograms (P-ERG) allow analysis of retina ganglion cells and can be performed simultaneously with P-VEP. The purpose of this study was to determine if P-ERG, when performed simultaneously with the VEP, could be used to rule out volitional manipulation as the etiology of an abnormal VEP.

On 16 volunteers, first VEP, then VEP simultaneously with P-ERG were performed using 15-, 30-, and 60-minute checks. Subjects were instructed to either cooperate with the test protocol or attempt to alter the test results. All subjects, when cooperative, had normal VEP and normal P-ERG with simultaneous VEP for the 3 check sizes studied when compared to a control population.

Six subjects could volitionally alter their VEP into the abnormal range for at least 1 of the check sizes that were used (range = 118 msec. to completely flat). Fifteen-minute checks could be altered the most consistently, while 60-minute checks were the most difficult to manipulate.

When simultaneous P-VEP and P-ERG were used, these same 6 subjects once again succeeded in volitionally altering the P-VEP into the abnormal range for at least 1 check size. Whenever the VEP could be voluntarily altered, the P-ERG was also altered into the abnormal range. No subject was capable of altering P-VEP without also affecting the P-ERG.

It is concluded that in patients with abnormal P-VEP, the presence of normal, simultaneously recorded P-ERG would be inconsistent with malingering as the etiology of the abnormal P-VEP.

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Modulation of primary afferent neurons by a neuronal central pattern generator

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It has been established that many stereotyped rhythmic motor acts are organized and controlled by neural circuits called central pattern generators (CPGs). In addition, CPGs can function without phasic or tonic input from sensory receptors. However, given the presence of peripheral receptors, there must exist mechanisms for the control of reflex activity in order to appropriately organize these neuronal pathways for the production of a motor behavior. Using an invertebrate model of a CPG, we demonstrated that primary afferent neurons are directly driven by a CPG in order to gate peripheral input to the central nervous system.

Gill ventilation in decapod crustacea is produced by the rhythmic dorsoventral movements of the scaphognathite (SG) or gill bailer of the second maxilla. Movement of the SG is controlled by 5 depressor muscles, which have been classified into 2 functional groups, D1 and D2, and 5 levator muscles, likewise divided into 2 groups, L1 and L2. The movement of the SG can pump water in either of 2 directions, corresponding to forward and reverse ventilation, and is determined by the recruitment sequence of the levator and depressor muscle groups.

The central pattern generator driving the ventilatory rhythm consists of a network of nonspiking interneurons whose interconnection determines the organization of the motor rhythm. The only proprioceptor in the SG is the oval organ, which is innervated by 3 primary afferent fibers. Intracellular recordings from the oval organ afferents in an isolated preparation indicate that these neurons receive a cyclic inhibitory input which in-phase with the motor pattern produced by the ventilatory CPG. This drive therefore serves to modulate the effect of sensory input from these neurons during all phases of the ventilatory cycle.

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Auditory pathways implicated in the "cocktail party effect"

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The auditory system has a remarkable

ability to focus on one voice or signal in the presence of competing noise. A deficit in this ability can be devastating. For example, many students with otherwise normal hearing cannot pick out a teacher's voice in a noisy classroom. This subtle deficit has only recently been recognized as a major contributing factor to learning and behavioral problems.¹

The ability to extract the significant sounds out of a noisy environment (commonly called the "cocktail party effect") has been attributed to the cholinergic part of the brain stem auditory pathway which descends from higher centers to the cochlear nucleus.² In bats, this pathway is unusually concentrated and most easily studied. A combination of neurohistochemical, retrograde and anterograde tracing techniques were used to specifically describe the projections of cholinergic cell groups in the auditory brain stem of the mustache bat, *Pteronotus parnellii*. Reciprocal connections were found between cholinergic cell groups of the superior olive and the cochlear nucleus. These, in turn, could be directly and indirectly related to the large pathway which descends from the brain stem to the cochlea.

Future experiments will explore the influence of these interconnections on specific neural and behavioral responses to auditory signals in noise.

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Effects of cortisol and triiodothyronine on secretion of enzymes and lipids from cultured brain cells

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Cultures of dissociated brain cells from 15-day-old fetal mice were grown in defined media in the presence and absence of 30 nM cortisol, 50 nM triiodothyronine (T3), and 30 nM cortisol plus 50 nM T3. Angiotensin converting enzyme (ACE) activity was increased by both T3 and cortisol with the combination of both hormones increasing ACE levels by 250 percent. About half of the total ACE activity was found in the defined media and there was no evidence of significant cell

death. Significant levels of β -galactosidase, β -glucuronidase, and N-acetylgalactosaminidase were also secreted by these cells. Of these three lysosomal hydrolases, N-acetylgalactosaminidase was the enzyme secreted in highest amount. In contrast, dipeptidyl aminopeptidase (DAP-I) and cathepsin B, 2 lysosomal peptidases, were not excreted. Cultured murine brain cells, primarily astrocytes, and oligodendrocytes, selectively secreted ACE and 3 lysosomal hydrolases while not secreting 2 lysosomal peptidases, when grown in serum-free media. The secreted enzymes may be involved in myelin processing.

Cholesterol was measured in cells and the culture media. Approximately 25 percent of the total cholesterol produced by the cells, grown in the absence of T3 or cortisol, was secreted into the media. Cortisol supplementation modulated the amount of cholesterol secreted into the culture media.

Synaptic contacts on cervical motoneurons

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Motoneurons (MNs) supplying limb musculature are contacted by several morphologic populations of synaptic boutons which may represent different sources of afferent input.¹ However, we know little about synaptic contacts on axial MNs. We have begun to examine this question by characterizing synaptic contacts on cervical MNs in a cryptodryan turtle, *Pseudemys scripta*. Data were taken from spinal segment C3; each MN was sampled at 5 μ m. increments through the soma and horizontally oriented dendrites.

Based on serial reconstruction of ultrathin sections, we have identified at least 7 types of contacts on cervical MNs.² Type S contacts bear clear, round vesicles (31-58 μ m.) and asymmetric membrane densities. The mean length of apposition is 1.3 μ m.; variations of size, vesicle density, and ultrastructural characteristics suggest that there may be more than one morphologic population of S bouton. Type F contacts bear pleiomorphic vesicles (up to 18 $\times$ 36 μ m.) and thin symmetric membrane densities ($\bar{x}$ apposition length = 1.5 μ m.). Sp contacts resemble large S contacts ($\bar{x}$ apposition length = 1.6 μ m.; vesicles 31-50 μ m.) and bear small P boutons on their convex surfaces. T contacts ($\bar{x}$ apposition length = 1.7 μ m.) bear clear, round vesicles and postsynaptic dense bodies. C contacts are large ($\bar{x}$ apposition length = 2 μ m.) with round (CS) or pleiomorphic (CF) vesicles and a subsynaptic cistern. G contacts contain dense

core vesicles (50-77 nm.; x apposition length = 1.3 μ m.).

Most boutons make a single type of contact, but separate C and S type active zones are seen in some boutons. There is a significant positive correlation between maximal apposition length and total size of the active zone. The percent coverage by S and F type contacts is significantly greater than that by other types. There is no difference in the coverage effected by S and F type contacts, unlike the situation reported in cat hindlimb muscles.³ Reconstructions of MNs reveal that for some types of MNs coverage by boutons differs as a function of membrane locus, suggesting that different parts of the cell may receive distinct patterns of synaptic input.

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Clinical practice

A comparative analysis of circuit weight training and aerobic exercise in cardiac patients

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To determine whether a regimen of weight lifting can be safely incorporated into cardiac rehabilitation programs, we compared changes in heart rate (HR), blood pressure (BP), and left ventricular wall motion (LVWM) in patients subjected to both circuit weight training (CWT) and standard aerobic exercise (AE). Thirteen men with documented coronary artery disease were studied. CWT consisted of 2 circuits of 8 upper body exercise stations over a period of 30 to 35 minutes. AE consisted of 35 minutes on a treadmill at 85 percent maximal HR.

In both programs, HR and BP were monitored during exercise. LVWM was measured by two-dimensional echocar-

diography at rest and immediately after each exercise period. Changes in LVWM were analyzed with a Microsonics quad screen analysis system.

With CWT, HR increased 38 ± 20 (SEM) beats per minute (BPM), and BP increased 3 ± 8 mm. Hg. With AE, HR increased 39 ± 10 BPM, and BP increased 20 ± 14 mm. Hg. While the increases in heart rates were similar, AE increased BP significantly more than did CWT ($p < 0.001$). Overall, regional LVWM was not significantly affected by either form of exercise.

We conclude that CWT may not be detrimental to cardiac patients, and suggest a role for the incorporation of CWT into cardiac rehabilitation programs.

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Aging, postural decompensation index, and low back pain

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Static, dynamic decompensation of posture occurs physiologically in aging and has been implicated by several clinicians as an etiologic factor in the low back pain of certain patients. Objective quantification of this postural decline and its relationship to back pain, however, is scarce. Pelvic index (PI), an objective radiographic parameter which can be calculated from postural (standing lumbo-pelvic) radiographs utilizing strict, reproducible protocol, reflects the interrelationship of the sacrum and innominate bones as a ratio that varies with the weight-bearing position of these two bony elements.

In a retrospective study involving the radiographic postural series of 336 patients, PI averages were plotted against age. A gradual decline of pelvic index was documented, averaging $+0.04/\text{decade}$. The PI of 88 patients with documented chronic low back pain (CLBP) was then compared with the pelvic index of 68 control patients having no significant low back pain history. The PI decline of all three groups paralleled one another; however, the average PI in the CLBP group was 30 percent higher than control, suggesting increased pelvic decline. Statistically, PI reflects a difference between age-divided asymptomatic and CLBP patient populations. This study documents a gradual decline of pelvic index with aging in all population groups at a rate of $+0.04/\text{decade}$. CLBP patient groups have PI's statistically higher than in asymptomatic patient groups. PI is shown in this study to be an objective measurement of static, dynamic postural decom-

pensation in age-divided population groups and is capable of differentiating patient populations where chronic low back pain symptomatology is the variable.

Effect of DRGs on the frequency, average length of stay, and rehospitalization rate of patients from a skilled nursing home

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On July 1, 1984, many Illinois hospitals became subject to the DRG-based prospective payment system for providing treatment of Medicare inpatients. At that time, concern was expressed that the elderly patient would be leaving the hospital earlier and this would result in more rehospitalizations. The purpose of this study was to examine the effects DRGs have had on the frequency of admissions, average length of stay, and rate of rehospitalizations within a skilled nursing home patient population.

Patient records of discharges to hospitals and readmission diagnoses as well as the rate of rehospitalizations of patients were examined for a 6-month-period before and after the institution of DRGs. The same 6 months in 2 sequential years were used so that seasonal bias could be avoided. The mean age of both populations was 71.8 and 74.1 years. The total number of hospital admissions for a 6-month-period was 214 and 185 before and after DRGs, respectively. The average length of stay decreased from 12.4 days to 7.9 days, which represents a 36 percent change and is significant ($p < 0.05$). The percentage of rehospitalization for the first 2 weeks after returning to the nursing home was 16 percent and 19 percent before and after DRGs, respectively, which is not significant. The percentage of rehospitalizations for 4 weeks after returning to the nursing home was 28 percent and 38 percent, respectively, which approaches statistical significance ($p < 0.05$). The percentage of rehospitalization for 6 months after returning to the nursing home was increased by 30 percent after the institution of DRGs. Careful analysis of the variety and frequency of admitting diagnoses did not appear to significantly change from one year to the next.

These data indicate that DRGs significantly decreased the average length of

stay in the hospital for our patients with a significant increase in rehospitalizations at 1 and 6 months. However, there appears to be a slight decrease in the total numbers of hospitalizations (14 percent).

Identifying frequency of patterns of somatic dysfunction

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We have previously reported a series of clinical projects in which thermograms of skin temperature (Tsk) on the back of human subjects were recorded in conjunction with osteopathic examination for palpable findings in the thoracic region; acquisition of a medical history followed. In all cases, the examiner was blinded to the details of the medical history, with the exception that the students were studied as a so-called normal healthy group, and the remaining clinic patients were examined without identification of their illness. Throughout the studies, we detailed a method of osteopathic examination and recording in which local segmental responses to standardized mo-

tion tests identified the location of fundamental units of segmental dysfunction. The data about these segmental locations, along with appropriate descriptors of the motion asymmetries present, provided opportunity to classify discrete categories of dysfunction in the somatic system. One category of findings exhibits a characteristic linkage of dysfunction in vertebral and costal columns, in which identical motion asymmetries are present at adjacent vertebral and costal segments at the same spinal level. Records of palpable findings for all subjects were examined for presence of a specific arrangement of dysfunctional linkage characteristics, at vertebra T2 with left rib 2, and at T6 with right rib 6. Data revealed the following frequencies of a pattern in which characteristic linkage occurred at both sites: (1) 33 percent in 86 "healthy young adults"; (2) 33 percent in 21 normotensive adult patients under medical care in a clinical setting; and (3) 72 percent in 69 established hypertensives. Chi square contingency table is shown in Figure 1. Data regarding frequency of linkage characteristics at T4 spinal level dysfunctions in asthmatics were significant when compared to an asthma-free control group (Fig. 2). The methodology used in these studies contributes a data base for longitudinal studies that will provide evidence of the frequency with which stable patterns of somatic dysfunction exist.

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Assessment of renal function in the elderly

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The present study examined the interaction between age and blood urea nitrogen (BUN) values in a group of elderly subjects. Renal function in the elderly cannot be accurately assessed by the measurement of serum creatinine level because its production is decreased. Shapiro et al.¹ noted that 60 percent of geriatric patients had serum creatinine levels that were less than 1 mg./dl. when glomerular filtration rate (GFR) was reduced by as much as 35 percent. Kafery² suggested that BUN may correlate with GFR better than serum creatinine. There is, however, lack of agreement on the interaction between BUN and age in the elderly subject. Caird and Judge³ stated that BUN values of 55-60 mg./dl. were essentially normal. Hale et al.⁴ reported that BUN increased only slightly at age 80.

We undertook a retrospective study of

BUN values in 273 subjects ranging in age from 66 to 103 (mean age = 83.7). Subjects were all residents of the Twin Pines Adult Care Center in Kirksville, Missouri, between 1984 and 1985 under the care of the same attending physician (MAW). We utilized BUN values from general screening profiles measured with a multichannel chemical analyzer (KDA) in the Department of Pathology, Kirksville Osteopathic Hospital. Normal values for BUN on this standardized instrument were 9.6 to 19.6 mg./dl. BUN values in the study population ranged from 6 to 103 mg./dl. (mean = 23.6). Measurements were made without regard to dietary or health status. We were able to identify 38/273 subjects who were suffering from acute illnesses. These patients were dropped, and mean values on the relatively healthy institutionalized subjects were obtained. Subjects in the 65-69 age group had BUN values of 16.6 ± 2.4 , and subjects over 95 years of age had values of 26.8 ± 3.7 (mean $\pm$ S.E.). On the basis of this preliminary study, we conclude that BUN levels in the elderly are not remarkably elevated and that this parameter can be used to provide a reliable assessment of renal function in the geriatric patient.

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Objective monitoring of osteopathic manipulative treatment in tinnitus patients

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Osteopathic literature reports that symptoms of tinnitus, vertigo, and hearing loss can be successfully alleviated using osteopathic manipulative treatment (OMT).

For nearly a decade, researchers and clinicians have been using Auditory Brainstem Response (ABR) tests to document the presence of tinnitus, which is present in 35 percent of the population. By examining ipsilateral and contralateral synchronous neural responses

T ₂ T ₆ Pattern	Hypertension		
	Present	Absent	
Yes	50	35	85
No	19	72	91
	69	107	176

Fig. 1. Retrospective study of T₂, T₆ pattern (patients and students with borderline, unstable, or early hypertension were excluded from this aspect of the retrospective study). Chi square; 1 d.f., $p = 0.001$ ($25.8 > 10.83$).

Linkage T ₄ Vert./Cost. Dys.	History of Asthma		
	Present	Absent	
Yes	5	20	25
No	6	167	173
	11	187	198

Fig. 2. Retrospective study of T₄ linkage of vertebral and costal dysfunction in patients/students with history of asthma. Chi square; 1 d.f., $p = 0.001$ ($11.5 > 10.83$).

to an auditory stimulus, changes in replicability, latency, and amplitude measures of Wave I (auditory nerve), Wave III (superior olivary complex), and Wave V (inferior colliculus), it is possible to differentiate tinnitus from nontinnitus populations and to chart changes with tinnitus patients following medical treatment.

Latency data collected with a Nicolet CA 1000 and analyzed for nontinnitus patients (NT = 16) and tinnitus patients (T = 16), demonstrated significant differences (.01 level) with regard to latencies of Wave I (NT = 1.78; T = 1.93), Wave V (NT = 5.82; T = 6.01), and the interwave interval III-V (NT = 1.97; T = 2.08) using unpaired Students' t-test. All patients (ages 12 to 55 years) had normal acoustic reflex thresholds.

Three ABR-diagnosed tinnitus patients received craniosacral, myofascial release, strain/counterstrain, high velocity-low amplitude thrusting, and soft tissue OMT for tinnitus. Two patients reported reduction in tinnitus; one patient reported no change. The combined latencies of ABR Waves I, III, V and the interwave intervals I-III, III-V, and I-V demonstrated an average .43 msec. difference from pretreatment to posttreatment in changed ears compared to .17 msec. in unchanged ears.

Mean latencies for Waves I, III, V showed a .37 msec. change with tinnitus reduction compared to .13 msec. when no change was reported. There was a .47 msec. interwave interval mean in ears with reduced tinnitus compared to .21 msec. in unchanged ears.

ABR latency changes show promise for demonstrating objective measurement of change in tinnitus resulting from OMT.

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Injury control and anticipatory guidance

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This study was undertaken to determine the attitudes, preventive practices, and mechanisms providing anticipatory guidance for a group of pediatricians and family practitioners.

In December 1983, a random group of 20 pediatricians, 20 family practitioners, 20 pediatric residents, and 10 family practice residents were asked to complete a survey consisting of 4 questions designed to assess their attitudes regarding the importance of various preventive practices and mechanisms utilized in providing an-

tipicatory guidance. Forty-five surveys were adequately completed for analysis (15 pediatric, 16 family practice, 11 pediatric resident, and 3 family practice resident). Immunizations and nutrition were considered to be the most important preventive practice during childhood (71 percent and 69 percent, respectively). Only 24 percent considered accident prevention and injury control as important preventive practices requiring anticipatory guidance. It is interesting to note that none of the attending pediatricians considered accident prevention as the primary preventive practice of importance during infancy and childhood. Attending pediatricians and family practitioners almost exclusively utilized parents' questions and direct recall as stimuli for the provision of anticipatory guidance. Both groups of residents most often utilized standardized lists of preventive practices to stimulate the provision of anticipatory guidance.

These data appear to suggest the following: (1) If a parent does not ask a question regarding injury control or if the physician does not recall (or believe) that injury control should be discussed with parents, it will not be included in the preventive practice protocol of the physician; and (2) anticipatory guidance regarding injury control is not perceived as an important consideration during health maintenance visits. Because of the epidemiologic significance of accidents and injury in the pediatric and young-adult population, appropriate educational interventions at the undergraduate, graduate, and postgraduate levels appear warranted. The importance of injury control and accident prevention must be stressed in pediatric and family practice training.

The infant nutrition behavior study

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The purpose of the infant nutrition behavior study was to evaluate the effects of 4 popular cow milk-based formulas on infant behavior. Sixty-two normal full-term infants were enrolled following parental written and verbal consent. The infants were randomized into 4 groups. Each infant received each formula for 4 weeks. During the 4-week period, parents were asked to observe their infant and record variations in behavior on a daily record sheet. Symptoms such as severe crying, irritability, abdominal distention, colic,

and loose stools were selected because they are commonly attributed to colicky infants. They were assigned a point value so that each infant's behavior could be scored numerically. At the end of 4 weeks, the infants were seen, examined, and the flow sheets collected. Infants who proved allergic to cow's milk protein and infants with nontransient medical conditions such as gastroesophageal reflux, malabsorption syndrome, and pyloric stenosis were excluded from the study.

Four popular iron fortified cow's milk formulas were studied. Three contained whey additives to approximate the 60:40 ratio of whey to casein found in human milk. We believe that the incidence of colicky behavior would be equally distributed among all 4 groups. During the first month of life, the incidence of colicky behavior for those groups fed the whey containing formulas was 25 percent, 62.5 percent, and 50 percent. No colicky behavior was noted in the group fed a standard casein formula.

This study seems to implicate that whey protein additives may contribute to colicky behavior. These findings may represent manifestations of gastrointestinal allergy. Gastrointestinal immaturity, increased antigenicity of formula proteins, and enhanced antigen absorption are all possible etiologic mechanisms.

Our study population was small, attrition rate high, and the range of incidence of colicky behavior in the 3 groups fed whey fortified formulas was very large. Absence of colicky behavior in those infants fed a standard casein formula, therefore, may be coincidental. Larger and more well-controlled studies are needed to substantiate our results.

Supported by the Pediatrics Department, Chicago College of Osteopathic Medicine.

Anticipatory guidance in children hospitalized for ingestion

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We reviewed the medical records of children hospitalized at a major inner-city teaching hospital because of unintentional ingestion of a toxic substance. The study was undertaken to determine the degree of documentation of anticipatory guidance prior to discharge of the patient.

One hundred and one charts were reviewed for the following points in the history or progress notes: (1) By whom the child was found; (2) whether or not the ingestion was observed; (3) from where the substance was obtained; (4) how the

substance was obtained; and (5) container type. Progress notes were reviewed for documentation of discussions with parents regarding the prevention of subsequent ingestions after discharge from the hospital.

Sixty percent of the charts revealed how and/or by whom the patient was found, 20 percent documented where the substance was stored, 19 percent documented the type of container, and none of the 101 charts reviewed had documentation of whether the ingestion was observed. Twenty-one percent of the charts had no information that would prove helpful in providing anticipatory guidance at the end of the hospitalization. In only 9 percent of the records evaluated was there documentation of anticipatory guidance prior to discharge. Of these nine records, the documentation of anticipatory guidance was recorded exclusively by nursing staff. It is interesting to note that 4 of the 9 notes on anticipatory guidance were written by the same nurse.

It can be concluded from this review that there is minimal documentation of anticipatory guidance and preventive practices prior to discharge. If the guidance had been provided by the medical staff, there was minimal documentation of this intervention. Preventive practices and mechanisms for provision of anticipatory guidance to parents of small children at risk for ingestions should be included in all medical education curricula.

Accidental injuries among children in self-care: A prospective analysis

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Specific risk of accidental physical injury is said to exist for "latchkey children" during times of self-care. The data thus far accrued, however, are primarily anecdotal. In order to determine the distribution of children in self-care at the time of a significant accidental injury among children hospitalized for accidental trauma, we studied all admissions to two major teaching hospitals in the Chicago metropolitan area between December 1983 and June 1984. As part of the admitting history, information was obtained regarding the child's caretaker at the time of the injury, the primary caretaker of the child, the type and time of injury, and the employment status of the father and mother. We defined a child in self-care as a child between the ages of 5 and 13 years caring

for themselves for a period of 1 hour or more before or after school or at lunchtime. We also included children less than 5 years old being cared for (for 1 hour or more) by a child less than 13 years of age. Information was entered on data sheets and subjected to analysis.

Eighty-nine children were identified as being hospitalized for accidental injury. Three (3.4 percent) of these children were classifiable as being in self-care at the time of injury. One patient was in self-care and 2 patients were being cared for by a sibling less than 13 years of age. The remainder of patients were being cared for by the primary caretaker (66 percent) or a second caretaker (34 percent). A population based analysis revealed that children in self-care appeared no more likely (and maybe less likely) to be injured significantly enough to require hospitalization than children with a caretaker present.

Pet ownership and attachment patterns in a population of victims of child abuse and neglect

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We studied pet ownership and attachment patterns as the first phase of a multiphase project to determine the effectiveness of pet-facilitated therapy in the prevention and treatment of abused and neglected children. Sixty-two families whose children were admitted to a major inner-city teaching hospital and diagnosed as victims of child abuse and/or neglect were studied. A control group matched for age, sex, race and socioeconomic status consisted of 303 patients admitted to the same hospital with the diagnosis of accidental (unintentional) trauma. Attachment patterns were assessed by analyzing the location of where the pet slept.

No significant differences in pet ownership could be found between the study and control group. Significant differences were identified, however, in attachment patterns. The pet slept with the child or another family member more frequently in the accidental injury group than in the child abuse group ($p < .0001$).

Our data suggest that there is no difference in pet ownership between non-abused children and those who are victims of child abuse and/or neglect. There is, however, a very significant difference in attachment patterns when measured by the location of the pet's

sleeping quarters. Analysis of individual data of the abusive and neglectful families revealed that the pet universally slept outside of the home or in the basement and not with the family. Studies focusing on empathy and bonding with pet animals within households are necessary. This bonding may correlate with bonding between family members. If there proves to be a significant correlation, pets may serve as adjuvants to the development of a nurturing environment and may provide stimulus to the abused/neglected child to develop into an adult capable of nurturing his/her offspring.

Inclusion of Levitor® orthotic device in the management program of refractive low back pain

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The Levitor® orthotic device (LOD) was developed over 50 years ago by M. Jungmann, M.D., a Viennese radiologist, to mechanically counter the postural changes present in patients with low back pain. There are reports in the clinical literature on a reduction of subjective pain ratings in patients with chronic low back pain with the adjunctive use of the Levitor®. This study was undertaken to determine if objective changes parallel the clinical efficacy of the LOD as an adjunct to the conservative treatment programs of patients demonstrating only transitory or minimal response. Our retrospective study involved 110 chronic, posturally aggravated, low back pain patients in which serial postural (standing lumbopelvic) radiographs could be identified. All had only transitory relief of pain while under conservative management (exercise, osteopathic manipulative treatment, medication, and patient education).

In 80 patients (average age, 46.8 years), the LOD was added to the existing program, while 30 patients (average age, 38.6 years) received only conservative management and served as the control group. Linear regression line of the two groups were not statistically different at the first measurement. Changes in pelvic index (PI), an objective radiographic reflector of static dynamic postural decompensation that is known to decline gradually with age, were calculated for both groups. Results demonstrate that conservative management alone showed an average decline of PI of $+0.04$ per 25 month average compared to an actual average improvement of -0.04 per 26 month average in the LOD adjunct group. Individually, further decline of PI was

only seen in 24 percent of the LOD adjunct group as compared to 67 percent in the control group. This study thus demonstrates both individually and as a population, a statistically significant change in PI derived from the addition of the Levitor® orthotic device to patients previously refractive to a total conservative management program. It is suggested that the PI is a useful, objective measurement in evaluating subjective improvement in patients complaining of posturally induced back pain.

Physicians' attitudes on reporting suspected child abuse and neglect

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This study was undertaken to determine attitudes and perceived practices of physicians on the reporting of suspected child abuse and neglect in Illinois.

Surveys were distributed to participants in medical education conferences in Springfield, Champaign-Urbana, and Peoria, Illinois, between July 1, 1984, and December 31, 1984. Instructions on the proper completion of the survey were provided by one of the investigators. A total of 71 surveys were completed. We chose to omit health professionals other than practicing physicians, leaving 20 instruments available for analysis. Data were entered in a file/sort program on an Apple II Plus computer. Demographics of the study population were described. Ninety-five percent of the responding physicians had reported suspected child abuse and/or neglect. Seventy percent had also suspected child abuse/neglect in a child in their practice but were hesitant to file a report. The most significant hesitancy reasons were: (1) uncertainty that the child was abused or neglected; (2) uncertainty that the child should be removed from the home; and (3) lack of faith in the child protection agency. Those factors which were not significant included: (1) the physician didn't know to whom to report; (2) the report would involve too much of the physician's time; (3) the physician was fearful of possible negative legal ramifications; (4) the physicians feared the report would alter how the community viewed the physician; (5) the physician felt he/she could provide the necessary counseling; and (6) reporting the child would do more harm than good.

These data support data collected from other investigators. Many physicians are hesitant to report cases of child abuse/neglect. This hesitancy may be based on

misperceptions of the physician's role in the reporting process, lack of knowledge of the interdisciplinary systems approach to the evaluation of child abuse/neglect victims, limited resources for the protection and treatment of abused and neglected children, and a lack of faith in the system.

Supported by a grant from The Illinois Department of Child and Family Services, and The Child Injury Prevention Study Group.

Education

Multicenter clinical research studies: Organizational strategy

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This study demonstrates a strategy for organizing multicenter clinical research studies. The specific focus is the educational interface among students, interns, residents, and attending physicians in osteopathic teaching hospitals. The objective is to facilitate and enhance the support of distinctive osteopathic medical practice in the educational process.

In 1978, the Ohio University College of Osteopathic Medicine began coordinating clinical research efforts in its affiliated teaching hospitals. A clinician was designated to plan for the design and implementation of protocols to be used in such studies. This clinician served as the Principal Investigator. The primary effort involved establishing teaching programs for the college's students entering their clinical rotations. Clinical research thus became the vehicle for the educational interface. Support for this study was requested and solicited from practicing osteopathic physicians in various specialty areas. The support and cooperation of hospital administration, clinical staff, and nursing staff were also requested and solicited. Selected clinicians were designated as Associate Investigators. Participating students were designated as Student Investigators. Specific training was provided for Student Investigators in each study. Appropriate acknowledgement was made for human subjects' safety. Studies implemented under this arrangement dealt with pneumonia and acute myocardial infarction.

In 1982, a modification of this approach was implemented for the use of residents involved in clinical research studies. The role of the Principal Investigator changed to that of a consultant. In this capacity, service was provided in the preparation of

the osteopathic diagnosis and treatment component of the study specified by the residency trainer and conducted by the resident. Bronchiolitis was the first subject studied under this format. Studies are pending in asthma and neonatal craniofacial asymmetry.

The cumulative experiences indicate that this strategy is a cohesive force for stimulating and maintaining interest in distinctive osteopathic medical practice.

Spatial abilities and performance in anatomy

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The objective of this study is to explore the relationship between the spatial abilities of medical students and their performance in anatomy. The overall goal is to determine whether information about spatial abilities can be used to improve student performance by leading to changes in how anatomy is taught or by identifying students likely to have difficulty and providing assistance.

At the beginning of the 1985 Fall quarter, incoming students at Ohio University College of Osteopathic Medicine (OU-COM) took five tests of their spatial abilities. The tests represented spatial abilities that might contribute to anatomy learning, including perceptual speed, visual memory, disembedding of hidden figures, and three-dimensional mental manipulation. Additional background information was obtained, including incoming GPAs (science, nonscience, and overall), MCAT scores, age, degrees earned, and majors. With the spatial test scores, these comprise a potential set of independent variables that could be used to predict anatomy performance. Dependent variables include final anatomy grade and anatomy test scores.

Factor analysis of the spatial tests identified three factors: mental manipulation in three dimensions, perceptual speed, and visual memory. These factors correlate with the reading and quantitative MCAT subtests, but not other background variables. All MCAT subtests are highly intercorrelated, but could not be reduced to fewer factors. GPA and age did not correlate with other variables.

Regressions run on the first written and laboratory tests suggest that the current set of predictors may have limited practical significance: the equations accounted for 10 percent and 17 percent of the variance in the laboratory and written tests, respectively. In addition, some of the spatial factors and MCAT scores appear with negative weights, suggesting

that further analysis of relationships is needed.

While our initial results are not as clear-cut as we had hoped, all relevant data have not yet been collected. In addition to studying upcoming test results and final grades, we plan to explore non-linear relationships in the data. Results to date provide useful information on the structure of spatial factors, relationships with background variables, and possible relationships to anatomy performance.

Funding provided by the Ohio University College of Osteopathic Medicine.

A database management system for the tracking of applicants seeking admission to medical school

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The Kirkville College of Osteopathic Medicine currently receives multiple applications for admission for each available position in any school year. With the massive amounts of information generated by these applications and the increased number of reports required by various agencies, the need was present to develop a microcomputer-based database system to keep track of each applicant and to generate the required reports.

The system that was designed is implemented on an IBM PC-XT with a 512 K memory and a 10 M byte hard disk. The program was developed using the DBASE III language from Ashton-Tate. The database is composed of 44 items which are normally included in each application. Data are added to the database by a two-page screen-generated form. Once in the database, any record can be edited by an edit routine which allows editing single or multiple items. Following either an addition or editing of a record, a copy of the most recent version of the current record is printed.

Data can be accessed for editing by use of either student name, social security number, or an office-assigned file number. Any record requested for editing may simply be viewed without changing data. Once the data have been entered and edited, a series of reports may be generated. For example, total number of applicants, total number accepted, list of accepted applicants by state, age, marital status, college(s) attended, D.O. relative, etc. A total of 13 different reports can be generated. The use of a word processor with a merge facility is used to develop lists of applicants all of whom, for example, are ac-

cepted together with addresses for the generation of correspondence.

A final feature is an automatic back-up of the current database, which limits the possibility of the open file being destroyed causing a loss of all data.

A comparison of attitudes of osteopathic medical students and experts in osteopathic manipulative treatment (OMT) toward OMT

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A distinctive characteristic of an osteopathic physician is the knowledge of osteopathic manipulative treatment (OMT). However, there seems to be some variation in the extent to which osteopathic physicians use OMT in practice. Some general practitioners use it extensively to treat a wide variety of patient problems; however, some subspecialists use it rarely. The extent to which osteopathic physicians use OMT depends upon a variety of factors, including the nature of their practice, their patient population, their level of OMT skill, and their attitude toward OMT. With respect to the last factor, it seems reasonable to suggest that if all other factors are equal, then physicians who have a more positive attitude toward OMT will be more likely to use it than those who have a less positive attitude.

A primary responsibility of the colleges of osteopathic medicine is to provide instruction in the theory and practice of OMT. This is accomplished through lectures, reading assignments, demonstrations, practicals, role modeling, and other educational activities. Along with the cognitive objectives related to instruction in osteopathic philosophy, principles, and practices, there are explicit and "hidden" affective objectives that relate to the attitudes that students form about the use of OMT in clinical practice. Ideally, the cognitive and affective objectives should reinforce each other in that positive growth in cognitive objectives should facilitate positive growth in affective objectives. The capability to assess such a relationship depends, however, on having valid and reliable measures of both kinds of objectives. Fortunately, there are ample measures of the cognitive objectives; unfortunately, there are very few valid and reliable measures of affective objectives, such as attitudes toward OMT. Given this, it is difficult to ascertain whether a relationship exists between the two kinds of objectives. Moreover, it is difficult to determine what kinds of educational activities designed to facilitate

cognitive objectives have the greatest impact upon the affective ones. And, finally, it is impossible to discover a "growth curve" (if one even exists) in the acquisition of affective objectives related to OMT. For all of these reasons, a project was implemented to investigate whether a valid and reliable instrument could be developed to measure attitudes toward OMT.

The development of the instrument and results from the first study are reported elsewhere.¹ This second study compares the responses to the Attitudes About Osteopathic Medicine survey of two different groups within the osteopathic profession: preclinical students at an osteopathic college and experts in the use of OMT. The responses of the two groups (students and experts) to the 30-item survey were plotted and analyzed by rank order correlation. The results were highly significant ($p < .001$), which implies that whatever variable is being defined by the items of the survey, it is defined in the same way for the two groups. The items tend to retain their relative order for students and experts, and this means that students and experts respond the same way to the items—a measure of the instrument's reliability.

Although there is a very high correlation between students and experts with respect to these items, the correlation is not perfect; therefore, students "agree" with some items more often than the experts and the experts "agree" with some items more often than students. For example, students more often agree with the statement that "OMT should be considered an adjunctive rather than standard treatment." However, experts more often agree with the statement that "OMT can be beneficial in essentially any patient problem." These differences in levels of agreement, as well as several others, may imply a valid difference in the attitude toward OMT between students and experts. If these differences have this implication, then this suggests that the instrument may be valid as well as reliable. However, that cannot be determined from these data. What can be determined from these data is that the survey items are very stable with respect to the way two very different groups of people respond to them.

The results of this study encourage further research to establish the validity and reliability of the survey with a wide variety of groups within the profession. This is important, for if the validity and reliability of this instrument can be established, then it may facilitate the identification of educational activities that produce osteopathic physicians who have positive attitudes toward the use of OMT in clinical practice.

1. Walton, F.C., and Draba, R.E.: Report on osteopathic attitudes survey responses of osteopathic medical students. *JAOA* 85:671-2, Oct 85

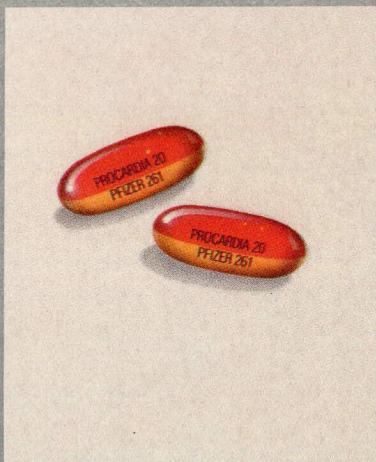
Supported by a grant from the Chicago College of Osteopathic Medicine.

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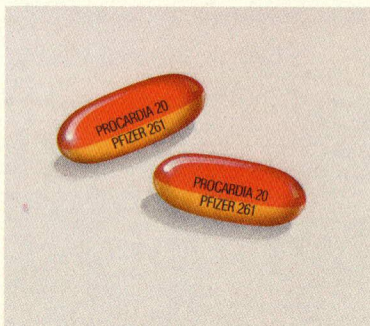
PROCARDIA[®]
(NIFEDIPINE)

NEW **20**mg
Capsules



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Please see PROCARDIA[®] (nifedipine) brief summary on next page.



PROCARDIA[®] NEW 20mg Capsules

(NIFEDIPINE)

Providing greater convenience and improved compliance for your angina patients

Excellent control through simplified titration

To achieve control,

- Initiate therapy with 10 mg *t.i.d.*
- If necessary, titrate to 20 mg *t.i.d.*
- The usual effective range is 30 to 60 mg per day
- More than 180 mg/day is not recommended
- Monitor the patient's blood pressure for signs or symptoms of excessive peripheral vasodilation

Brief Summary

PROCARDIA[®] (nifedipine) Capsules

INDICATIONS AND USAGE: I. **Vasospastic Angina:** PROCARDIA (nifedipine) is indicated for the management of vasospastic angina confirmed by any of the following criteria: 1) classical pattern of angina at rest accompanied by ST segment elevation, 2) angina or coronary artery spasm provoked by ergonovine, or 3) angiographically demonstrated coronary artery spasm. In those patients who have had angiography, the presence of significant fixed obstructive disease is not incompatible with the diagnosis of vasospastic angina, provided that the above criteria are satisfied. PROCARDIA may also be used where the clinical presentation suggests a possible vasospastic component but where vasospasm has not been confirmed, e.g., where pain has a variable threshold on exertion or in unstable angina where electrocardiographic findings are compatible with intermittent vasospasm, or when angina is refractory to nitrates and/or adequate doses of beta blockers.

II. **Chronic Stable Angina (Classical Effort-Associated Angina):** PROCARDIA is indicated for the management of chronic stable angina (effort-associated angina) without evidence of vasospasm in patients who remain symptomatic despite adequate doses of beta blockers and/or organic nitrates or who cannot tolerate those agents.

In chronic stable angina (effort-associated angina) PROCARDIA has been effective in controlled trials of up to eight weeks duration in reducing angina frequency and increasing exercise tolerance, but confirmation of sustained effectiveness and evaluation of long-term safety in these patients are incomplete.

Controlled studies in small numbers of patients suggest concomitant use of PROCARDIA and beta-blocking agents may be beneficial in patients with chronic stable angina, but available information is not sufficient to predict with confidence the effects of concurrent treatment, especially in patients with compromised left ventricular function or cardiac conduction abnormalities. When introducing such concomitant therapy, care must be taken to monitor blood pressure closely since severe hypotension can occur from the combined effects of the drugs. (See WARNINGS.)

CONTRAINDICATIONS: Known hypersensitivity reaction to PROCARDIA.

WARNINGS: Excessive Hypotension: Although in most patients, the hypotensive effect of PROCARDIA is modest and well tolerated, occasional patients have had excessive and poorly tolerated hypotension. These responses have usually occurred during initial titration or at the time of subsequent upward dosage adjustment, and may be more likely in patients on concomitant beta blockers.

Severe hypotension and/or increased fluid volume requirements have been reported in patients receiving PROCARDIA together with a beta blocking agent who underwent coronary artery bypass surgery using high dose fentanyl anesthesia. The interaction with high dose fentanyl appears to be due to the combination of PROCARDIA and a beta blocker, but the possibility that it may occur with PROCARDIA alone, with low doses of fentanyl, in other surgical procedures, or with other narcotic analgesics cannot be ruled out. In PROCARDIA treated patients where surgery using high dose fentanyl anesthesia is contemplated, the physician should be aware of these potential problems and, if the patient's condition permits, sufficient time (at least 36 hours) should be allowed for PROCARDIA to be washed out of the body prior to surgery.

Increased Angina and/or Myocardial Infarction: Rarely, patients, particularly those who have severe obstructive coronary artery disease, have developed well documented increased frequency, duration and/or severity of angina or acute myocardial infarction on starting PROCARDIA or at the time of dosage increase. The mechanism of this effect is not established.

Beta Blocker Withdrawal: Patients recently withdrawn from beta blockers may develop a withdrawal syndrome with increased angina, probably related to increased sensitivity to catecholamines. Initiation of PROCARDIA treatment will not prevent this occurrence and might be expected to exacerbate it by provoking reflex catecholamine release. There have been occasional reports of increased angina in a setting of beta blocker withdrawal and PROCARDIA initiation. It is important to taper beta blockers if possible, rather than stopping them abruptly before beginning PROCARDIA.

Congestive Heart Failure: Rarely, patients, usually receiving a beta blocker, have developed heart failure after beginning PROCARDIA. Patients with tight aortic stenosis may be at greater risk for such an event.

PRECAUTIONS: General: Hypotension: Because PROCARDIA decreases peripheral vascular resistance, careful monitoring of blood pressure during the initial administration and titration of PROCARDIA is suggested. Close observation is especially recommended for patients already taking medications that are known to lower blood pressure. (See WARNINGS.)

Peripheral edema: Mild to moderate peripheral edema, typically associated with arterial vasodilation and not due to left ventricular dysfunction, occurs in about one in ten patients treated with PROCARDIA. This edema occurs primarily in the lower extremities and usually responds to diuretic therapy. With patients whose angina is complicated by congestive heart failure, care should be taken to differentiate this peripheral edema from the effects of increasing left ventricular dysfunction.

Laboratory tests: Rare, usually transient, but occasionally significant elevations of enzymes such as alkaline phosphatase, CPK, LDH, SGOT and SGPT have been noted. The relationship to PROCARDIA therapy is uncertain in most cases, but probable in some. These laboratory abnormalities have rarely been associated with

clinical symptoms, however, cholestasis with or without jaundice has been reported. Rare instances of allergic hepatitis have been reported.

Limited clinical studies have demonstrated a moderate but statistically significant decrease in platelet aggregation and increase in bleeding time in some PROCARDIA (nifedipine) patients. No clinical significance for these findings has been demonstrated.

Positive direct Coombs test with/without hemolytic anemia has been reported.

Although PROCARDIA has been used safely in patients with renal dysfunction and has been reported to exert a beneficial effect in certain cases, rare, reversible elevations in BUN and serum creatinine have been reported in patients with pre-existing chronic renal insufficiency. The relationship to PROCARDIA therapy is uncertain in most cases but probable in some.

Drug interactions: Beta-adrenergic blocking agents: (See Indications and Warnings.) Experience in over 1400 patients in a non-comparative clinical trial has shown that concomitant administration of PROCARDIA and beta-blocking agents is usually well tolerated, but there have been occasional literature reports suggesting that the combination may increase the likelihood of congestive heart failure, severe hypotension or exacerbation of angina.

Long-acting nitrates: PROCARDIA may be safely co-administered with nitrates, but there have been no controlled studies to evaluate the antianginal effectiveness of this combination.

Digitalis: Administration of PROCARDIA with digoxin increased digoxin levels in nine of twelve normal volunteers. The average increase was 45%. Another investigator found no increase in digoxin levels in thirteen patients with coronary artery disease. In an uncontrolled study of over two hundred patients with congestive heart failure during which digoxin blood levels were not measured, digitalis toxicity was not observed. Since there have been isolated reports of patients with elevated digoxin levels, it is recommended that digoxin levels be monitored when initiating, adjusting, and discontinuing PROCARDIA to avoid possible over- or under-digitalization.

Coumarin anticoagulants: There have been rare reports of increased prothrombin time in patients taking coumarin anticoagulants to whom PROCARDIA was administered.

Cimetidine: A study in six healthy volunteers has shown a significant increase in peak nifedipine plasma levels (80%) and area-under-the-curve (74%) after a one week course of cimetidine at 1000 mg per day and nifedipine at 40 mg per day. Ranitidine produced smaller, non-significant increases. If nifedipine therapy is initiated in a patient currently receiving cimetidine, cautious titration is advised.

Carcinogenesis, mutagenesis, impairment of fertility: Nifedipine was administered orally to rats for two years and was not shown to be carcinogenic. When given to rats prior to mating, nifedipine caused reduced fertility at a dose approximately 30 times the maximum recommended human dose. *In vivo* mutagenicity studies were negative.

Pregnancy: Pregnancy Category C. Nifedipine has been shown to be teratogenic in rats and embryotoxic in rats, mice and rabbits. There are no adequate and well controlled studies in pregnant women. PROCARDIA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

ADVERSE REACTIONS: The most common adverse events include dizziness or lightheadedness, peripheral edema, nausea, weakness, headache and flushing, each occurring in about 10% of patients, transient hypotension in about 5%, palpitation in about 2% and syncope in about 0.5%. Syncopal episodes did not recur with reduction in the dose of PROCARDIA or concomitant antihypertensive medication. Additionally, the following have been reported: muscle cramps, nervousness, dyspnea, nasal and chest congestion, shortness of breath, diarrhea, constipation, gastrointestinal cramps, flatulence, inflammation, joint stiffness, shakiness, jitteriness, sleep disturbances, blurred vision, difficulties in balance, dermatitis, pruritus, urticaria, fever, sweating, chills, sexual difficulties, thrombocytopenia, anemia, leukopenia, purpura, allergic hepatitis, gingival hyperplasia, erythromelalgia, depression, paranoid syndrome, transient blindness at the peak of plasma level, and arthritis with ANA (+). Very rarely, introduction of PROCARDIA therapy was associated with an increase in anginal pain, possibly due to associated hypotension.

In addition, more serious adverse events were observed, not readily distinguishable from the natural history of the disease in these patients. It remains possible, however, that some or many of these events were drug related. Myocardial infarction occurred in about 4% of patients and congestive heart failure or pulmonary edema in about 2%. Ventricular arrhythmias or conduction disturbances each occurred in fewer than 0.5% of patients.

HOW SUPPLIED: PROCARDIA soft gelatin capsules are supplied in:

Bottles of 10: 10 mg (NDC 0069-2600-66) orange #260; 20 mg (NDC 0069-2610-66) orange and light brown #261. Bottles of 30: 10 mg (NDC 0069-2600-72) orange #260; 20 mg (NDC 0069-2610-72) orange and light brown #261. Unit dose packages of 10: 10 mg (NDC 0069-2600-41) orange #260; 20 mg (NDC 0069-2610-41) orange and light brown #261.

The capsules should be protected from light and moisture and stored at controlled room temperature 59° to 77°F (15° to 25°C) in the manufacturer's original container.

More detailed professional information available on request.

Revised June 1986

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How to use computers to improve medical education

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This study evaluates the use of computers to manage basic science lecture information for first- and second-year medical students and suggests additional applications for managing information and saving time and money. Specifically, the use of hired typists to transcribe lecture material was compared to that of students using Apple computers with simple word processing software.

Students were asked by questionnaire to compare information content, mechanical errors, and legibility. Notetakers were surveyed to determine change in time and effort required to process information. Also, turnover time, operating costs, and projected costs were compared to previous years.

Results indicate the following: (1) A significant reduction in turnover time, especially during critical pre-examination periods; (2) slight increase in student time/lecture hour ratio necessary to process and distribute information; (3) a 50-65 percent reduction in operating costs; and (4) maintenance of good quality.

This study demonstrates that this application of computer word processing for information management by medical students is both time efficient and cost effective. Furthermore, observations made during this study have suggested several other potential applications for using personal computers to improve medical education.

This project was supported by the UHS-COM class of 1988.

The relationship between interview rankings and performance in writing orders for applicants to osteopathic internship training

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Because of an increase in the number of graduates from colleges of osteopathic medicine and the pressures on all hospi-

tals to reduce the number of beds and cut costs, competition for an internship in an approved osteopathic setting has increased over the last several years. Although there currently exists an approximate balance between the number of graduates and the number of funded internship positions, there are many more applicants than funded positions for those internships that are perceived as being highly desirable by graduates of osteopathic colleges. At the Chicago College of Osteopathic Medicine (CCOM), there are about 4 applicants for each of its 62 internship positions. Even though it is not difficult to make decisions about the extremes in this applicant pool, there nevertheless remains a large number of applicants for the number of positions after the strongest applicants have been accepted and the weakest ones rejected. Hence, the problem for intern training programs like the one at CCOM is to decide who among the many applicants occupying the middle ground will be selected for training.

It is not uncommon to use performance in the intern interview as a significant criterion of selection. Typically, an applicant is interviewed by several attending physicians in a single group session or in several individual sessions. The applicant is usually asked a wide variety of questions, including questions related to clinical situations or problems. Attending physicians use some type of form to evaluate the applicant, and these evaluations are scored. The interview score along with other data—letters of evaluation, prior clinical performance, Board scores, and the like—are used to compare the applicant to others. But, at CCOM, performance in the intern interview, which emphasizes clinical questions, has been and continues to be the single most important selection criterion.

Given the emphasis CCOM places on performance in the intern interview, a study was undertaken to assess the predictive validity of the interview.¹ Performance in the intern interview was compared to performance as a CCOM intern. The study indicated that there was no significant relationship between interview performance and performance as an intern. Although there are factors which help to explain this result, it was decided to undertake an exploration of additional intern selection criteria which might be significantly associated with performance as an intern.

In this regard, it was thought that the ability to write orders for clinical cases would be the kind of selection criterion that would have a high level of predictive validity with respect to performance in the intern interview and as an intern. Consequently, applicants to the 1986-87 intern class were asked to write orders for one mandatory clinical case and one of two optional cases. The quality of these written orders were evaluated by two internal medicine residents and two general practice residents. Two methods of evaluation were used. On the mandatory

case, a norm-referenced method was used in that the orders written by one applicant were compared to the orders written by other applicants, and then, the orders were ranked from highest to lowest. On the optional case, a criterion-referenced method was used in that an ideal set of orders was established for both cases, and then, the orders of the applicants were evaluated in terms of that ideal.

Performance on the task of writing orders was compared with intern interview performance. Results from these data indicate that there was no significant relationship ($p < .05$) between an applicant's performance in the interview and his/her performance on either set of orders, mandatory or optional. Moreover, it was found that there was no significant relationship between performance on the mandatory and optional set of orders. These results were somewhat unexpected, for it was thought that since the interview rank at CCOM is so strongly associated with the attending physicians' perception of the clinical knowledge of the applicant, performance on an explicit test of clinical knowledge, such as writing orders, would be strongly associated with the interview rank. This follows from the observation that similar measures of a cognitive trait are quite often significantly associated with each other. But this was not the case with "similar" measures from the interview and orders, and this was not the case with the "similar" measures from the mandatory and optional clinical cases.

Because writing orders is manifestly related to the role of an intern, it is possible that, unlike interview performance,¹ performance in writing orders on either the mandatory or optional case will be significantly associated with performance as an intern. This, however, cannot be determined at this point because the subjects of this study have not completed the intern year. When they do, the relationship between these measures of performance will be determined and reported. However, for now, in preparation for the next group of applicants to CCOM's internship program, four steps will be taken to improve the methods of this current study: (1) Cases for writing orders will be improved and will require more detailed answers; (2) there will be two mandatory cases and no optional one; (3) only one method of evaluation will be used; and (4) the intern interview as well as the interview evaluation form will be more structured to enhance reliability. These changes in methods may reveal what seems to be intuitively true, namely, that there is a significant relationship between measures of clinical ability obtained from an interview and a set of written orders.

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Computer-assisted nutritional instruction for the pregnant teenager

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Computer-assisted instruction is often viewed as an impersonal approach to information dissemination. However, this medium can be utilized to provide confidential, individualized instruction to persons who may find it difficult to acquire knowledge by conventional methods. This is often the case for pregnant teenagers.

The primary objective of this project is to create a microcomputer software package that can be easily utilized in a variety of settings to provide health and nutrition information to pregnant teenagers. The focus of these packages is the nutritional needs of the mother and child during both pregnancy and the early postnatal period. Each software package has two sections. The initial section is a nutritional evaluation quiz in which the nutritional habits of the subject are evaluated and quantitated. Feedback in terms of an overall evaluation of the nutritional habits is then returned to the user. The second section is a series of nutrition information units that emphasize the principles of good nutrition and health care during pregnancy. These units are designed to provide information relevant to a teenage population, such as smoking, alcohol, "fast foods". The ease of distribution and use of these packages will make nutritional education readily available to a frequently neglected group of women.

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Immunology

DTPA conjugation and Indium-111 labeling of T-101 monoclonal antibody for immunoscintigraphy

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With the increased availability of mono-

clonal antibody (MoAb) preparations directed against tumor antigens, the development of radioimmunoimaging systems for cancer detection has become feasible. Improvements in labeling and conjugation techniques permit proteins to be labeled with metallic radionuclides such as In-111, Ga-67, and Tc-99m. Using MoAb T-101* as a model, we evaluated the effects of DTPA conjugation on MoAb immunoreactivity and, in addition, have established a set of favorable conditions for Indium-111 labeling of DTPA-T-101.

Briefly, concentrated antibody solution (8 mg./ml.) and solid cyclic DTPA anhydride (molar ratio 1:10) were incubated for 30 minutes at 24 C, according to the Krejcarek procedure.¹ Conjugated products were separated on a Sephadex G-50 column and the protein fraction was then mixed with equal volumes of In-111 Acetate at a final pH of 5.5. Radiochemical purity of each preparation was determined by ITLC Silica Gel chromatography which yielded values of between 80 and 95 percent labeling efficiency. Immunoreactivity was measured by comparing the binding capacity of DTPA conjugated MoAb and unconjugated MoAb to five serial dilutions of malignant T-8402 lymphocyte cells* grown in culture.

In summary, our results indicate that the MoAb T-101 can be conjugated with DTPA at a molar ratio of 1:10 without significantly altering the immunoreactivity. The final specific activity of In-111-DTPA-T-101 was 1 μ Ci/ μ g. protein with greater than 85 percent retained immunoreactivity. These results are ideal for an immunoscintigraphic system.

*Provided by Hybritech, Inc., San Diego, California.

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Biophysical properties of immune complexes using Ga-67 and In-111 labeled ferritin and monoclonal anti-ferritin

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Binding characteristics of monoclonal anti-ferritin (QCI-054) antibody (MoAb) and human spleen ferritin (Ag)* were studied. To ascertain which molar ratio of MoAb:Ag would be optimal for preparation of useful immune complexes, varying

proportions of each were reacted in phosphate buffered saline, pH 6.8. MoAb concentrations in the free and antigen-bound phases were determined using polyacrylamide gradient gel electrophoresis or polyethylene glycol precipitation. Ferritin:anti-ferritin complexes precipitate when the MoAb:Ag ratio exceeds 4:1. The precipitated 4:1 complexes will not bind additional MoAb. Incorporation of Ga-67 into the ferritin¹ does not alter its antigenicity towards the MoAb. A precipitate forms at 4:1, just as with unlabeled ferritin. The 4:1 precipitates are insoluble in saline, phosphate buffered saline at pH 6.8, and in TRIS-Borate buffer at pH 8.4. In rats, injection of a suspension of the Indium-111 DTPA labeled precipitate showed liver and spleen uptake with no trapping by the lungs. Monoclonal antibodies to ferritin can be useful for determining the structural, conformational, and immunological relationships of MoAb (individually, and in combination) and ferritins from various tissues and their sub-units. This relatively simple procedure is useful as an "in vitro" screening procedure for determining the optimum combination of MoAb and tumor associated antigen. Subsequently, this optimal combination can be used as an in vitro screen for the presence of tumor antigens.

*341482 Calbiochem, La Jolla, California.

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Teratology

Teratogenic potential of hydromorphone administered via a miniature implantable pump in mice

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Recent biomedical advances, such as the development of novel delivery systems for drug administration, have opened new vistas in evaluating teratogenic potentials of therapeutic agents, such as the

narcotic analgesics. In this study, single injections (5 mg./kg.) and continuous infusion of hydromorphone (HDM, Dilaudid), administered by a micro-osmotic pump at lower concentrations, have been shown to produce birth defects in the offspring of CF-1 mice. The subcutaneous injections of HDM from days 7-12 of gestation produced both skeletal and soft-tissue anomalies similar to those produced by other narcotic agents, such as morphine. Dose-response studies with the pump showed that more fetal malformations were produced by the higher than lower concentrations of HDM. In addition, animals implanted with pumps earlier in pregnancy displayed fetuses with more abnormalities than those implanted later; however, the overall teratogenic effects produced by continuous delivery of HDM differed from those observed in single-dose studies. From these data, it is apparent that continuous delivery of a drug produces teratogenic effects different from those observed in single-dose studies and, therefore, continuous infusion systems, such as the micro-osmotic pump, should be valuable tools in experimental teratology. Not only will the miniature implantable pump provide a broader view of how narcotic analgesics, such as hydromorphone, affect pregnancy, but also how other drugs and conditions could impair the fetal environment and endanger perinatal development and survival.

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Physiology

Endogenous opiate actions in coronary and femoral arteries

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Experiments were conducted to evaluate participation by endogenous opiates in coronary and peripheral vascular responses in the dog. Two model systems were utilized: isolated coronary arteries and an isolated auto-perfused hindlimb. To test that hypothesis that opiate receptors modify adrenergic activity locally in canine coronary arteries, segments of ventricular branches of the circumflex coronary artery (4 mm. long) were used. Vessels mounted in tissue baths, equilibrated at 1.5 gm., and contracted with PGF α (2-5 μ M), were then relaxed by cumulative addition of norepinephrine (NE) from 1.0 pM to 100 μ M. After wash-

ing, 10 μ M naloxone (NX) was added to the baths and NE responses were repeated. The post-NX responses to NE provided a clear rightward shift in the relaxation curve and a significant ($p < 0.05$) increase in the ED $_{50}$ for NE. These experiments show that the responses of coronary artery segments to NE are altered in the presence of opiate receptor blockade. This observation provides evidence for the presence of opiate peptides in proximity to vascular autonomic nerve terminals and suggests a possible physiologic role for opiates as local modulators of adrenergic activity in the coronary circulation.

Systemically administered methionine enkephalin (ME) can provoke a precipitous fall in blood pressure. We measured blood flow and determined vascular resistance in the hindlimb during acute hypotensive responses to ME. We found an increase in femoral arterial blood flow and a decrease in hindlimb vascular resistance during the acute decline in systemic blood pressure. This observation suggests that the fall in systemic pressure and the rise in hindlimb flow are, in part, secondary to a decline in peripheral vascular resistance. When the same doses of ME are administered locally into the femoral artery, the changes in both systemic and hindlimb hemodynamics are reduced and delayed in time. These data indicate that the peripheral vasculature participates in the acute hypotensive responses to administered opiates and the observed responses are likely mediated through sites outside the hindlimb.

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Effect of testosterone on the citrate production by the prostate epithelial cells *in vitro*

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The *in vivo* prostate cells are responsive to testosterone and demonstrate the unique property of citrate production. The purpose of the present investigation was to determine if these characteristics are maintained *in vitro*. Pig prostate was chosen for this study because unlike human prostate it contains large amounts of glandular epithelium. Monolayer cultures were established from collagenase (0.25 percent in HBSS) dispersed acini and cultured in minimum essential medium supplemented with

bovine serum (5 percent). After the cultures were established (7 days), they were treated with testosterone in the presence or absence of actinomycin D (AMD). Spent media was collected on alternate days for citrate analysis and cells stained with Giemsa for morphologic analysis and for aspartate aminotransferase activity by the lead sulphide precipitate method. Initial epithelial cell growth was observed within 24 hours and by 5-6 days 80-90 percent confluent cultures were obtained. Intensive, formalin resistant acid phosphatase activity was present in the cells and the level of citrate accumulation, measured by fluoroenzymatic technique, was significantly higher in cells cultured in the presence of testosterone (0.2 μ g./ml.) than in cells cultured in medium containing androgen extracted serum. Aspartate aminotransferase activity was also stimulated by the presence of testosterone and inhibited by AMD. These results indicate that aspartate transamination could be a possible metabolic site for the regulation of citrate production by prostate cells.

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Impaired recovery of cardiac function in reperfused low-flow ischemic rat hearts exposed to elevated free fatty acids

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It has been hypothesized that high levels of fatty acids may be harmful to the ischemic myocardium. The purpose of this study was to determine the effects of elevated perfusion medium free fatty acid (FFA) concentration on the recovery of cardiac output after 90 min. low-flow ischemia followed by 15 min. of reperfusion in isolated perfused rat hearts. The recovery of cardiac output was correlated to the intracellular accumulation of fatty acid intermediates: long chain acyl carnitine and coenzyme A esters. Hearts perfused with 0.4 mM palmitate and 5.5 mM glucose recovered 61 percent of their initial cardiac output after 90 min. of ischemia and 15 min. of reperfusion while hearts perfused 1.2 mM palmitate and 5.5 mM glucose recovered only 23 percent of their initial cardiac output. The accumulation of fatty acid intermediates during ischemia was significantly greater in ischemic hearts perfused with elevated FFA. These data show that excess exogenous FFA are detrimental to the ischemic myocardium. Although not conclusive, the data suggests that the accumulation of fatty acid intermediates may be contributing factors to the de-

velopment of irreversible ischemic damage.

Prototype analogue to digital software package for cardiovascular signal processing

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It is difficult to obtain accurate areas or time derivatives of cardiovascular signals (pressures, flows, cardiac dimensions) using manual analysis of strip chart recordings. We therefore developed a computer software package to record and analyze cardiovascular data. We regarded the following specifications as essential: (1) that the system be maximally flexible with respect to type and number of data channels recorded so that it remained useful as laboratory protocols changed; and (2) that the investigator be able to scan the data for analysis. Software was developed on a 56k Apple II computer with a 16 channel A/D converter made by Interactive Systems. A data acquisition program and a data manipulation program were written in Applesoft Basic utilizing assembly language subroutines. The data acquisition program samples up to 16 channels of strip chart data at a sample rate of 100 Hz. Data are saved on floppy disks in labeled files whose maximum real time length is inversely proportional to the number of data channels recorded (22s when 4 channels are recorded). No limit is placed on the number of files per experiment. The data manipulation program allows the cardiovascular signal of any channel to be scanned in 2s intervals. The time derivative of any cardiovascular signal can also be displayed. A list of times associated with different given events (eg., end diastole, beginning ejection, end systole, etc.) can be stored to allow later data access by user written programs. The user written programs can utilize assembly language subroutines included with the software package to average, integrate, or find maxima or minima of the cardiovascular signals between the various event markers. Early experience suggests that this software package is both simple and flexible enough to be useful in analyzing a variety of cardiovascular signals collected under a variety of experimental conditions.

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Fluoride sensitivity of the myocardium

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Fluoridation of public drinking water has been practiced for approximately 40 years. A majority of the epidemiologic studies of public fluoridation fail to support any correlation in cancer or general mortality rates between the inhabitants of fluoridated and nonfluoridated communities. Studies which focus on potentially susceptible segments of the community, such as those with myocardial disease, are sparse. Fluoride is an inhibitor of glycolysis, specifically of associated enzymes such as enolase (E.C. 4.2.1.11), phosphoglucosyltransferase (E.C. 2.7.5.1), glucose-6-phosphatase (E.C. 3.1.3.9), phosphoglycerol mutase (E.C. 3.1.3.11), and pyruvate kinase (E.C. 2.7.1.40). The myocardium of patients with chronic obstructive heart disease converts from aerobic to the less effective anaerobic metabolism via the glycolytic pathway, which in many cases is adequate to meet the basal metabolic needs of certain jeopardized cells and permit their survival.

This study seeks to determine whether fluoride concentrations comparable to those found in fluoridated water supplies will produce detectable changes in isolated ventricular cells which, unlike the heart *in situ*, routinely shift to anaerobiosis even in the absence of tissue hypoxia. Preliminary morphometric analytic data indicate changes in mitochondrial integrity and lysosomal aerial fractions in response to fluoride treatment. The physiologic significance of direct cytotoxic effects of fluoride at concentrations encountered by the general population must be evaluated separately for those patients who have compromised cardiac function.

Effect of moderate cooling on the affinity of alpha-adrenergic antagonists in the canine tracheal smooth muscle

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This *in vitro* study was undertaken to investigate whether moderate cooling

affects the affinity of α -adrenergic antagonists in the tracheal smooth muscle. The adult dog, weighing approximately 15 kg., was anesthetized with pentobarbital sodium and the trachea was obtained and placed into chilled Krebs solution. The trachealis muscle was separated as described by Stephens,¹ and suspended in separate tissue baths containing Krebs solution aerated with 95 percent oxygen and 5 percent carbon dioxide. The tissue baths were maintained either at 38 C or 24 C. Not more than 8 tracheal muscle strips were utilized from each trachea. Passive tension required for optimal responsiveness was initially determined. All tracheal muscle strips were exposed to propranolol, cocaine, and normetanephrine for at least 40 minutes in order to block β -adrenoceptors, and neuronal and extraneuronal uptake processes, respectively. The cumulative dose-response curve (DRC) of norepinephrine (NE) was subsequently determined. After thorough washings, the DRC of NE was determined again in the presence of all previous blockers plus selective α -adrenergic antagonist, prazosin or yohimbine. The affinity of the competitive antagonists, prazosin and yohimbine, as reflected by the pA_2 values were determined at 24 C and 38 C and compared. The pA_2 values of prazosin and yohimbine determined at 24 C were significantly greater than those determined at 38 C. The observations of this study shows that the affinity of α -antagonists is significantly increased by moderate cooling of the tracheal muscle.

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Evidence that prostaglandins are not involved in platelet aggregation in normotensive and hypertensive rats

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The present study was conducted to examine the role of prostaglandins (PG) in rat platelet activation using normal Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Up to 30 μ M adenosine diphosphate (ADP) did not induce secondary phase of platelet aggregation in rat platelet-rich plasma (PRP), and up to 30 μ M epinephrine did not produce any response in rat PRP. In other

experiments, ADP (1.0 μ M) and epinephrine (2.0 μ M) induced typical biphasic aggregation responses in human PRP. Up to 20 μ M U46619, a stable analogue of PGH₂, did not induce platelet aggregation in rat PRP. In contrast, 2.0 μ M U46619 caused maximal aggregation in human PRP. Arachidonic acid (1.5-2.0 mM) induced aggregation in washed rat platelets. However, this was associated with excessive (67 percent and 94 percent) loss of cytoplasmic LDH. The low concentrations of thrombin (0.04 and 0.05 U/ml.), induced two- to three-fold increases in aggregation response in SHR platelets as compared to WKY platelets. Higher concentrations of thrombin (0.1 and 0.3 U/ml.) induced similar aggregation responses in SHR and WKY platelets. Thrombin (0.04-0.3 U/ml.) induced serotonin secretion in a concentration-dependent manner. The extent of secretion was the same in SHR and WKY platelets at all concentrations. Thrombin-induced synthesis of thromboxane A₂ (TXA₂) in WKY and SHR platelets was quantified using a radioimmunoassay for TXB₂. Thrombin (0.04-0.3 U/ml.) produced TXB₂ in WKY and SHR platelets in a concentration-dependent manner. The SHR platelets produced significantly larger amounts of TXB₂ as compared to WKY platelets. In other experiments, aspirin (500 μ M) inhibited thrombin (0.05 U/ml.) induced TXB₂ synthesis by 75 percent in both WKY and SHR platelets but failed to inhibit aggregation or secretion in either WKY or SHR platelets. Based on these data, it is suggested that PG are not involved in platelet aggregation in either normotensive or hypertensive rats.

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Effects of semi-starvation and sleep deprivation on exercise performance

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Shift workers often are semi-starved (SS) and sleep-deprived (SD). These perturbations may alter cardiopulmonary fitness (CPF) and exercise performance. A 1-min-

TABLE 1. RESULTS OF SEMI-STARVATION AND SLEEP DEPRIVATION ON A PATIENT.

	$\dot{V}O_2$	$\dot{V}CO_2$	$\dot{V}_E$	fc	O ₂ P	AT	Watts
BL	4.0	4.4	170	164	28.4	2.8	275
SD	4.0	5.1	161	160	25.3	2.0	275
% Δ	0	+14.0	-5.4	-5.3	-10.9	-29.8	0
SS	3.5	4.1	126	160	22.1	2.2	240
% Δ	-12.4	-5.6	-25.9	-5.3	-22.2	-21.2	-12.7
SS/SD	3.8	3.9	119	147	26.0	2.5	240
% Δ	-4.5	-11.3	-30.0	-13.0	-8.4	-10.8	-12.7

Key: $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$ and AT measured in liters/min.; fc measured in beats/min.; and O₂P in cc/beat.

ute, incremental-cycle ergometry test will be used to study the effects of SS, SD, and combined SS and SD on CPF. Noninvasively, maximum oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), heart rate (fc), oxygen pulse (O₂P), and anaerobic threshold (AT) will be determined before (BL) and after 30 hours of SD, 8 days of SS, and combined SD and SS on the ninth day. The results of one patient are shown in Table 1.

Metabolic rate decreases with SS and maximum external work decreases. A corresponding reduction in $\dot{V}_E$ occurs. O₂P decreases with an earlier development of AT. SD decreased O₂P and AT occurred earlier. The combined perturbations of SS and SD produced no additional change.

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Thyrotropin-releasing hormone (TRH): Immunohistochemical localization in skin of larval and adult *Rana pipiens* and *Rana catesbeiana*

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Thyrotropin-releasing hormone (pGlu-His-ProNH₂) was first isolated and identified by Guillemin and Schally in 1969. It was originally named for its regulatory role in the mammalian pituitary-thyroid axis. Yet, in amphibians, the primary function of this hormone is not well un-

derstood. In addition to its localization in neural tissues, radioimmunoassays have demonstrated TRH in high concentrations in skin of adult frogs. However, its more precise location within the skin has not been extensively investigated. A few immunohistochemical studies have implicated serous glands as the major site of cutaneous TRH, but, to our knowledge, localization of TRH within skin of larval amphibians has not been reported. Therefore, the present investigation was undertaken to demonstrate the distribution of TRH within dorsal skin of larvae and adults of two amphibian species, *Rana pipiens* and *Rana catesbeiana*.

Samples of dorsal skin from animals in representative developmental stages were fixed in acrolein and embedded in a glutaraldehyde gelatin mixture. Thick sections were cut on a vibratome and stained by an indirect immunoperoxidase technique and a well characterized antibody (gift of Dr. I.M.D. Jackson). Included among the many controls was the use of antibody preabsorbed with synthetic TRH.

Results indicated that in frog skin immunoreactive TRH (IR-TRH) was located primarily in the serous glands; these are concentrated in the dermal plicae of *R. pipiens* and are more generally distributed in the skin of *R. catesbeiana*. In larval skin, positive staining for TRH was detected within such glands as soon as they developed. In contrast, mucous glands were TRH-negative in both species of frogs and tadpoles. Some indications of IR-TRH were also evident in the connective tissue of the dermis of all stages of both species and in the epidermis of *R. pipiens* but not *R. catesbeiana*. The latter suggests a possible species difference in TRH distribution.

In conclusion, the present immunohistochemical study demonstrates serous glands as the major sites of IR-TRH in dorsal skin of both larval and adult *R. pipiens* and *R. catesbeiana*.

Comparison of graded and rapid increases in exercise work load on heart rate

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The influences of work load and duration of exercise on cardiac performance were studied in 11 healthy male athletes, age 16 to 25 years. Each was subjected by chance order to 2 different treadmill exercise protocols given 2 or 3 days apart. ECG recordings were taken continuously from chest leads. In one procedure, the work load was increased in increments (Bruce protocol). The subject was on the treadmill for 4 consecutive periods of 3 minutes each, without intervening periods of rest: (1) 1.7 mph, 10 percent grade; (2) 2.5 mph, 12 percent grade; (3) 3.4 mph, 14 percent grade; and (4) 4.0 mph, 16 percent grade. In the acute physical stress protocol developed by us and reported elsewhere, the subjects stepped onto the treadmill at 20 percent grade and the speed immediately increased to, and maintained at, 4 mph for 1 minute and 20 seconds. The present study reports on the pattern of recovery of the heart rate following exercise. Heart rate was determined by measurements of RR intervals between consecutive beats. In the Bruce protocol, the heart rate in 10 of the 11 subjects returned at a steady regression pace to pre-exercise levels within 3 minutes; few distortions were seen. In all men subjected to the acute stress, maximum minute heart rate was 5 to 12 beats lower at end of exercise than in graded stress. When minute rate fell to 130-115 range, oscillations in rate patterns occurred as previously reported. Drops in minute rate of 50 to 55 beats were seen frequently to occur between two consecutive beats. The last half of period 4 of the Bruce protocol was essentially similar to the whole period of the acute stress protocol. Stages 1 and 2 of the Bruce protocol actually constituted "warm-up" periods to the final 3 minutes of exercise at 4 mph at 16 percent grade, but no "warm-up" period preceded the 1 minute and 20 seconds of acute exercise stress at 4 mph at 20 percent grade. Suggested explanation: coronary artery perfusion of myocardium is greatest during ventricular diastole. At rates above 160, the total time per minute that the ventricle is in diastole is greatly diminished and ventricular perfusion is reduced. When work load is increased in stages adjustments are made (hormonal?). When work load is increased

rapidly, there are brief reflex periods of cardiac asystole ("vagal recapture") which provide time for adequate coronary flow.

A comparison of pulmonary gas exchange during exercise in patients with pulmonary hypertension: Chronic obstructive pulmonary disease versus obliterative pulmonary hypertension

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Seven patients with obliterative pulmonary hypertension (OPH) were compared with 7 chronic obstructive pulmonary disease (COPD) pulmonary hypertensive patients at rest and exercise to determine and compare the mechanism of exertional hypoxemia. The multiple inert gas technique was used to determine the distribution of lung ventilation-perfusion ($\dot{V}_A/\dot{Q}$) inequality. Resting and exercise oxygen consumption were similar for both groups. As expected, because of parenchymal lung disease, COPD patients had a lower mean $\dot{V}_A/\dot{Q}$ and more $\dot{V}_A/\dot{Q}$ inequality (log SD) than OPH patients. During exercise, both OPH and COPD patients had a fall in arterial PO_2 (PaO_2) and mixed venous PO_2 (PvO_2), and no increase in $\dot{V}_A/\dot{Q}$ inequality. Differing from OPH, COPD patients did not increase the mean $\dot{V}_A/\dot{Q}$ and consequently the arterial pCO_2 ($PaCO_2$) rose (Table 1).

In conclusion, exercise in both COPD with pulmonary hypertension and OPH patients does not worsen the degree of $\dot{V}_A/\dot{Q}$ inequality. The progressive hypoxemia in both groups is due to the fall in PvO_2 . This fall is mitigated by an increase in the mean $\dot{V}_A/\dot{Q}$ in the OPH

group but is accentuated by the insufficient ventilatory response and subsequent hypercapnia in the COPD group.

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Age-related reactivity of α -adrenergic receptor subtypes of canine tracheal smooth muscle

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This *in vitro* study was undertaken to investigate whether the subtypes of α -adrenergic receptors in the tracheal smooth muscle exists at birth. It was also intended to examine whether there is any change in the proportion and reactivity of these receptor subtypes, especially in the early postnatal life as compared to that observed in adult dogs. The animals were divided into four groups according to age: Group I, 1-3 days; Group II, 15-20 days; Group III, 30-40 days; and Group IV, adults (15 kg., appx.). Isolated tracheal rings were prepared from young animals (Groups I-III) according to the method described by Hooker et al.¹ Four to 8 tracheal rings, obtained from the middle part of the trachea, were suspended in separate isolated tissue baths. The adult tracheal muscle strips were prepared according to the method described by Stephens.² Passive tension required for optimal responsiveness was initially determined. All tracheal muscle preparations were exposed to propranolol, cocaine, and normetanephrine for at least 40 minutes in order to block β -adrenoreceptors, and neuronal and extra-neuronal uptake process, respectively. The cumulative dose-response curves (DRC) of norepinephrine (NE) was then determined. After thorough washings, the DRC of NE was again determined in the presence of all previous

TABLE 1. COMPARISON OF PULMONARY GAS EXCHANGE DURING EXERCISE AND REST.

	n	$\dot{V}_E$ L./min.	$\dot{Q}_T$ L./min.	PaO_2 mm. Hg	$PaCO_2$ mm. Hg	PvO_2 mm. Hg	$\dot{V}_A/\dot{Q}$ log DS	Mean
OPH	7 R	8.5	4.9	64	31	32	.53	1.53
	E	29.6*	7.5*	56*	30	23*	.45	3.91*
COPD	7 R	7.4	4.9	76	56	38	.73	.76
	E	15.2*	9.0*	63*	62*	32*	.73	.78

* $p < .05$; $\dot{V}_E$ = minute ventilation; $\dot{Q}_T$ = cardiac output.

blockers plus selective α -adrenoceptor antagonist, prazosin or yohimbine. In all age groups, yohimbine shifted the DRC of NE to the right more effectively than prazosin; the individual KB values of prazosin and yohimbine were closely similar in all age groups. These findings suggest that there is a greater proportion of α -2 than α -1 adrenoceptors in the tracheal muscle in all age groups, and they largely contribute to contraction in response to α -agonists.

1. Hooker, C.S., Calkins, P.J., and Fleisch, J.H.: On the measurement of vascular and respiratory smooth muscle responses in vitro. *Blood Vessels* 14:1-11, 1977

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Ventilatory response during progressive exercise: A comparison of patients with primary pulmonary hypertension and normal subjects

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Patients with primary pulmonary hypertension (PPH) have severe exercise limitation because of cardiovascular factors; however, an exaggerated ventilatory response to exercise has been recognized. Eleven PPH patients were studied by progressive, upright cycle exercise testing and compared to 11 matched, sedentary control subjects (C) for their ventilatory response below anaerobic threshold. Minute ventilation ($\dot{V}_E$), at any level of carbon dioxide production during exercise was significantly higher in the PPH group as compared to C. Additionally, the rate of ventilatory rise (the response slope) was also greater in the PPH group. At rest, the PPH group had a chronic respiratory alkalosis with a mean arterial $p\text{CO}_2$ (PaCO_2) of 32 mm. Hg and during exercise they maintained their hypocapnia ($\text{PaCO}_2 = 29$ mm. Hg). Similarly, the ventilatory dead space ratio ($\dot{V}_D/\dot{V}_T$) did not

TABLE 1. PHENYLEPHRINE NITROPRUSSIDE, AND BILATERAL CAROTID ARTERY OCCLUSION.

	PH	NP	BCO
UT	3.8 ± 2.8	2.6 ± 0.7	-1.2 ± 0.4
T	2.7 ± 0.5	2.9 ± 0.7	-1.2 ± 0.4

change significantly from resting value (Fig. 1).

In conclusion, PPH patients have an exaggerated ventilatory response at rest and exercise. The high resting $\dot{V}_D/\dot{V}_T$ and the lack of that $\dot{V}_D/\dot{V}_T$ to fall with exercise partially explains this observation. The resting PaCO_2 value, as a "set point" value, also contributes to the ventilatory response regulation.

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Baroreceptor sensitivity in untrained and endurance-trained, anesthetized dogs

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Exercise training is known to alter auton-

omic neural activity to the heart; parasympathetic efferent activity increases and sympathetic efferent activity decreases. The present study was designed to investigate whether 10 weeks of daily exercise will alter the baroreceptor response to increases and decreases in systemic arterial pressure, and to bilateral carotid artery occlusions in healthy pentobarbital-anesthetized dogs. Studies were performed in 8 untrained dogs (UT) and in 6 dogs which were endurance-exercise trained (T) for 10 weeks prior to the studies. Parameters measured included systolic arterial blood pressure (AP), ECG, and heart rate (HR). The baroreflex was activated by three means: (1) bolus intravenous injections of $5 \mu\text{g}/\text{kg}$. phenylephrine (PH) to raise AP 30-50 mm. Hg; (2) $20 \mu\text{g}/\text{kg}$. nitroprusside (NP) to reduce AP 20-40 mm. Hg; and (3) with a 30-second bilateral carotid artery occlusion (BCO). The slope (msec./mm. Hg) of the RR interval plotted against AP was calculated and used as an index of baroreceptor sensitivity. The values are given in Table 1.

No significant differences in AP, HR, RR interval, or in the slope of the RR interval versus AP were noted in UT and T dogs. These data suggest that 10 weeks

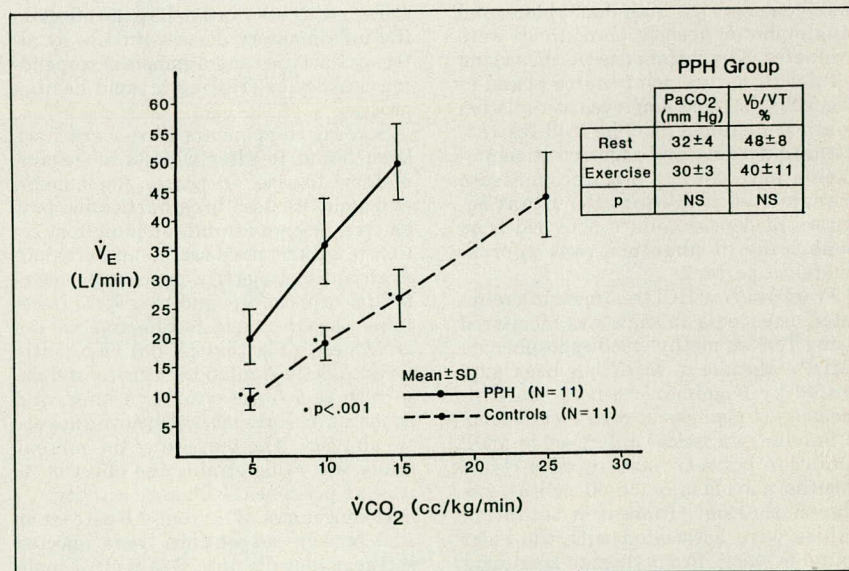


Fig. 1.

of daily exercise does not alter baroreceptor sensitivity to increases or decreases in AP in anesthetized dogs.

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Calcium loss and changes in bone metabolism: New approaches in detection and treatment of osteopenia

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Calcium loss and changes in bone metabolism are areas of concern in many branches of medicine. Our purpose in these studies was to attempt to improve upon current methods used to evaluate and treat osteopenia, because most current techniques are controversial and have questionable risk/benefit ratios.

Employing a conventional scintillation camera and computer, we developed a transmission-imaging technique to evaluate bone density. Both dual-photon and single-photon absorption methods were evaluated. The system used a converging collimator with a point source placed at the focus. Isotopes employed as radiation sources included Tc-99m and Xe-133. Field-uniformity and edge-detection programs were used to precisely measure transmitted radiation. Resultant accuracy of density values obtained from bone mineral phantoms was approximately 2 percent.

Promethazine HCl treatment of age-related osteopenia in mice was monitored using Tc-99m methylene diphosphonate (MDP). Uptake of MDP has been suggested by Fogelman¹ to be a sensitive measure of changes in bone metabolism. A baseline statistical difference in MDP retention between mature mice (14-16 months) and old mice (36-40 months) was shown to exist. Increased retention values were associated with the older group of mice. Promethazine treatment resulted in decreased retention values.

Values increased to baseline values when treatment was stopped.

Overall results of our investigations suggest the following: (1) Transmission imaging using conventional nuclear medicine equipment is an accurate and reproducible method of detecting conditions of osteopenia; (2) Tc-99m MDP retention is a sensitive method of following changes in bone metabolism; (3) promethazine HCl suppresses whole body retention of Tc-99m MDP in mice; and (4) as in humans, there is a correlation in mice between bone loss and whole body retention of Tc-99m MDP.

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Microbiology

Effect of *Aloe vera* on mouse peritoneal cell populations

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Aloe vera has been effective in treating cuts, burns, arthritis, and benign tumor growth. It is assumed to have an effect on the inflammatory disease process by altering the types and numbers of responding leukocytes creating a rapid healing process.

Several components of *A. vera* have been found to alter aspects of inflammatory disease. Of these, aloe-emodin and aloin are used in cathartics and purgatives; aloenin exhibits an inhibitory action in gastric juice secretion; barbaloin is an antibiotic active against *Mycobacterium tuberculosis* and ringworm infections, succinic acid is effective in the treatment of arthritis and rheumatic fever; Aloctin A inhibits arthritis and the growth of a fibrosarcoma in mice, and saponins are present which have antiseptic abilities. The purpose of the present study was to determine the effect of *A. vera* on peritoneal leukocyte activity.

Homogenates of *A. vera*, 1.0 percent or 10.0 percent suspensions, were injected intraperitoneally into BALB/cByJ male mice and the numbers and types of leuko-

cytes were determined at 24, 48, and 72 hours. Mice injected with 1 ml. of 1.0 percent *A. vera* showed an increase of neutrophils at 24 hours which switched to an increase in lymphocytes by 72 hours. The total peritoneal cell response to 1.0 percent *A. vera* also increased significantly by 72 hours. The effect of 10.0 percent *A. vera* on mouse peritoneal cell types was found to be an increase in neutrophils at 24 hours. Other cell types, including the total number of cells present in the peritoneal cavities of the mice, were not altered by the injection of 10.0 percent *A. vera*. Modulation of the response of peritoneal leukocytes by *A. vera* serves as a model to study aloe's effect on inflammatory processes.

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The protective role of polymorphonuclear leukocytes, T-lymphocytes, and natural killer cells in *Candida albicans*-challenged gnotobiotic mice

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The precise nature of protection by cellular immune mechanisms directed against the opportunistic pathogen *Candida albicans* is controversial and complicated because the organism reportedly possesses immunomodulatory potential that may suppress host defense against infection. Using polymorphonuclear leukocytes (PMN)/natural killer (NK) defective C57BL/6 beige (bg/bg) and T cell-deficient athymic (nu/nu) BALB/C recipient mice, we investigated resistance to *C. albicans* challenge following adoptive transfer of PMNs or enriched subpopulations of T cells. To evaluate the role of NK cells, we monitored NK cell activity in relation to systemic infection. Colony-forming units (CFU) of *C. albicans* obtained from homogenates of internal organs were used as an index of systemic infection in mice challenged with 2×10^4 yeast cells.

Conventional (CV) beige mice infused with PMNs elicited from thioglycollate-injected germfree (GF) or CV C57BL/6 (+/bg) donor mice had significantly fewer CFU in kidneys, livers, and spleens, compared with controls. In addition, GF beige

mice given PMNs from GF black mice had fewer CFU than either GF or CV recipients of CV black PMNs.

T cell subpopulations were obtained from the spleens of *C. albicans*-immunized BALB/C mice using lymphocyte panning techniques. Recipient T cell-deficient mice were injected with 2×10^7 Lyt 1+, 2-, Lyt 1-, 2+ or unseparated T cells prior to challenge with *C. albicans*; Lyt 1+, 2- T cells were effective in reducing CFU in kidneys, livers, and spleens.

Splenic NK cell activity was markedly enhanced in athymic mice challenged intravenously with *C. albicans*; in marked contrast, challenge of euthymic mice resulted in a significant reduction in NK cell activity. These divergent levels of NK cell activity did not correlate with *Candida* CFU, however, suggesting that while *Candida* modulated NK cell activity nonspecifically, NK cells may not provide protective immunity against *Candida* infection.

Collectively, our studies suggest that multiple cellular defense mechanisms, including PMNs and specific T cell subpopulations, operate to provide immunity to systemic candidiasis.

A comparison of procedures for the isolation of plasmid DNA from *Mycobacterium* spp

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Three documented procedures for plasmid isolation were modified and compared to determine a reliable screening method for mycobacteria. Procedure I involved lysing cells suspended in 20 percent sucrose with lysozyme and sodium dodecyl sulfate (SDS), and pelleting cellular debris by centrifugation. Procedure II included lysing cells with alkaline SDS (pH 11.5), salting out chromosomal material with sodium chloride, and precipitating plasmid DNA with cold isopropyl alcohol. Procedure III also employed alkaline SDS to lyse cells, but at an elevated temperature (65 C). Protein and cellular debris were removed by extraction with phenol-chloroform. Plasmid detection in lysates from all 3 procedures was performed by agarose gel electrophoresis.

Eight environmental isolates presumptively identified as *Mycobacterium* spp., 2 known strains of mycobacteria, *M. rhodochrous* and *M. smegmatis*, and 6 strains of *Escherichia coli*, each containing a previously characterized plasmid, were employed in the study.

Results from both procedures I and II proved unsatisfactory. Lysates obtained using procedure I were often contaminated with large amounts of chromosomal DNA, which obscured detection of plasmid DNA. Procedure II produced lysates with low concentrations of both chromosomal and plasmid DNA. No plasmids were observed in any of the mycobacteria lysed using I or II methods. Procedure III, however, permitted visualization of plasmid DNA from all of the *E. coli* strains and 2 of the mycobacteria strains. A plasmid with an estimated size of 80 Mdal was observed in an environmental isolate, and 2 plasmids with estimated sizes of 40 and 60 Mdal were observed in *M. rhodochrous*. Procedure III appeared to be a suitable protocol for the selective isolation of plasmid DNA in mycobacteria.

Supported by the Southeastern College of Osteopathic Medicine.

Methods for activation of macrophage fungicidal activity for pathogenic yeasts

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Previous studies from my laboratory have demonstrated that lymphokines can be used to enhance growth inhibition of *Cryptococcus neoformans* by cultured murine macrophages. More recent studies have revealed that under certain culture conditions, murine macrophages can be induced to kill this yeast. These conditions include depriving the macrophages of colony-stimulating-factor for a period of time before assay (1-4 weeks), followed by a lymphokine priming and a lipopolysaccharide (LPS) triggering sequence. Lymphokines were obtained from the supernatants of murine spleen cells cultured in medium containing 3.0 µg./ml. of concanavalin A. Optimal priming of the macrophages for fungicidal activity for *C. neoformans* occurred when lymphokines were added to the culture medium 4 hours prior to or simultaneous with the addition of 10.0 ng./ml. of LPS and fungus challenge. Addition of priming lymphokines for too long a period prior to fungus challenge (24 hours prior to assay), only enhanced growth inhibition of the yeast, and no killing was ob-

served. The effects of lymphokine priming on macrophage fungicidal activation is titratable, and has no effect on the rate of uptake of the yeast. Future studies with this model system of host-parasite interactions will be used to examine the effects of cryptococcal polysaccharide and cryptococcal polysaccharide-anticryptococcal polysaccharide antigen-antibody complexes on this yeast-macrophage interaction.

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Immunopotentiating effects of clindamycin

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Clindamycin (Cleocin) is an antibiotic which is concentrated intracellularly by host phagocytic cells, and which has been demonstrated to enhance phagocytic and bactericidal activity of polymorphonuclear leukocytes at subinhibitory concentrations. In addition, previous studies from my laboratory have demonstrated that clindamycin enhances both macrophage and polymorphonuclear leukocyte fungicidal activity for *Candida* species. Current investigations have focused on the ability of clindamycin to enhance murine contact sensitivity (CS) responses to dinitrofluorobenzene (DNFB), as determined by an ear-thickness assay. Enhancement of CS occurred only when clindamycin was administered simultaneously with DNFB skin painting on days 0 and 1 of the assay, and not when the drug was administered prior to DNFB exposure (day -1 and -2 or day -3 and -4 or day -6 and -7). However, unlike studies conducted with other drugs such as amphotericin B (Fungizone), clindamycin does not reverse tolerance mediated by intravenous injection of 2,4-dinitrobenzene-1-sulfonate (DNBSO₃), and it does not appear to effect humoral immune response. Clindamycin's effects appear to be limited to T lymphocytes, and future studies will be directed toward defining the particular subset(s) of T lymphocytes responsible for mediating enhanced CS.

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Effects of thoracic pump on guinea pigs infected with *Streptococcus pneumoniae*

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The immunoglobulin levels of infected pumped and nonpumped guinea pigs were determined over time. IgG, IgM, and IgA levels were determined quantitatively each day for 7 days and at death of the animal. Fifty animals were in each group. It was found that peak Ig activity in pumped animals took place on day 5 of infection in pumped animals, while the Ig level was highest initially in nonpumped animals and never returned to the initial level during the entire infection. Pumped animals survived on the average twice as long as nonpumped animals.

A study of peritoneal macrophages indicated by direct carbon uptake and by a measure of both beta-galactosidase and beta-glucosaminidase activities that macrophages from infected and pumped animals were substantially more active than macrophages from other groups of animals.

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The role of nicotinamide deamidase in the binding and uptake of nicotinamide by *Escherichia coli*

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Wildtype *Escherichia coli* contains a protein on the cell envelope which binds nicotinamide. Osmotically shocked cells release this protein into the shock fluid. This suggests the presence of an active

transport system for nicotinamide in *E. coli*. In order to prove this hypothesis, we have shown that the uptake of nicotinamide by cold-starved, resting cell cultures is dependent on the addition of glucose to the reaction mixture. The time course of nicotinamide accumulation was linear for 25 minutes, after which it began to level off, and steady state was reached at 40 minutes of incubation. Substrate saturation occurred at a concentration of 2.5×10^{-5} M nicotinamide. The K_m for the reaction is 1.35 mM and the V_{max} is 0.153 nmoles nicotinamide per minute per mg. of dry weight. Unlabelled nicotinamide successfully competes with labelled nicotinamide for transport. Inhibitors of glycolysis and uncouplers of the adenosine triphosphate (ATP) driven transport inhibit uptake. An *unc* mutant is unable to utilize lactate to energize this transport of nicotinamide, although glucose is a suitable source of energy for the energized transport.

Mutants of *E. coli* resistant to the action of 6-aminonicotinamide lack an active nicotinamide deamidase. These *pncA* mutants are unable to convert nicotinamide to nicotinic acid and cannot actively transport nicotinamide. The *pncA* gene maps at minute 39 on the chromosome.

These data indicate that the transport of nicotinamide by *E. coli* is an active process mediated by the product of the *pncA* gene and that the cell discriminates the source of energy in transporting nicotinamide.

Ascaris suum activation of peritoneal leukocyte lysophospholipase

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The role immunity plays with regard to lysophospholipase activity (LPL-A) has been studied with bacterial, viral, protozoal, and several helminth models of infection. LPL-A in tissues of immunized animals develops faster and reaches higher levels than LPL-A in nonimmunized animals. This change in LPL-A varies between infection models, differs due to increasing worm burdens, and is dependent on T-lymphocyte activity. The basic components of these infectious agents responsible for cellular LPL-A have not been studied. The purpose of this

research was to partially characterize the LPL activating components of one of the helminth infections, *Ascaris suum*.

Experiments were designed to demonstrate which parts of *A. suum* induce LPL-A, *in vitro*, in mouse peritoneal leukocytes. BALB/cByJ mice were infected, *per os*, with *A. suum* eggs, challenged intraperitoneally 6 weeks later with varying extracts of adult worm body parts, and the peritoneal leukocytes were collected 24 hours after challenge. Five repetitions of 1×10^6 washed cells were stimulated with 1.0 mg. quantities of *A. suum* components including whole worm extract (WEE), ascaris cuticle (AC), digestive tract (DT), peritoneal fluid (PF), or ascaris muscle (AS). WEE was found to increase LPL-A in the presence of zymosan-activated mouse complement (ZAC); AC itself increased LPL-A, DT did not alter LPL-A, PF decreased LPL activation by ZAC, and AM decreased both the effects of challenge and ZAC on LPL-A. In conclusion, this study demonstrated that different *A. suum* body parts have compounds present that are directly involved in LPL-A associated with mouse leukocytes.

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Age-related changes in pyridine nucleotide metabolism of *Paramecium tetraurelia*

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The eukaryotic protozoan *Paramecium tetraurelia* is able to exhibit the phenomenon of aging in terms roughly comparable to that of mammals. The purpose of this study was to continue investigating the relationship between the aging and the metabolism of pyridine nucleotides via the pyridine nucleotide cycle (pnc).

The major methods used consisted of high pressure liquid chromatography, thin layer chromatography, and enzyme kinetics. Several results tend to support the original hypothesis: (1) Evidence for a *de novo* pathway of nicotinamide adenine dinucleotide arising from tryptophan has been demonstrated; (2) there is a change in the ratio of NADP/NAD by cells exiting autogamy; and (3) variations in the specific enzymes NAD synthetase and NAD kinase have been found which seem to be correlated with age.

The results obtained above seem to in-

dicates that there is indeed a metabolic shift which occurs in *P. tetraurelia* as it ages. Thus, as the cells exit autogamy and ve immaturity, there is a shift in the ratio of NADP/NAD as well as other compounds involved in pyridine nucleotide metabolism. Mixtures of cells containing immature and adolescent members exhibit the presence of perhaps isozymic forms of the two enzymes NAD synthetase and NAD kinase.

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Pharmacology

Alteration of Na-K pump activity in supersensitive rat caudal artery following 6-hydroxydopamine treatment

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Contractile response and the Na-K pump activity, measured as ouabain-sensitive ^{86}Rb -uptake, were determined in caudal artery strips of rats pretreated with 6-hydroxydopamine. At 6-7 days after 6-hydroxydopamine treatment, the potencies of norepinephrine and serotonin in causing contraction of rat caudal artery were significantly increased by 2.3- and 1.7-fold, respectively. There was, however, no change in maximum contractile response to either agent. Treatment with 6-hydroxydopamine also reduced endogenous catecholamine content of the caudal artery to 7 percent of the control. Analysis of ouabain-inhibitable ^{86}Rb -uptake of rat caudal artery by the double-reciprocal plots showed that both the rate of ^{86}Rb -uptake and the affinity for rubidium were depressed after 6-hydroxydopamine treatment. The results indicate that 6-hydroxydopamine-induced supersensitivity in the rat caudal artery is associated with a decrease in the Na-K pump activity. These data provide additional support to the concept that inhibition of the Na-K pump may result in partial depolarization of the cell membrane which leads to supersensitivity of smooth muscle to excitatory drugs.

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Differential activation and inhibition of calmodulin-sensitive enzymes by phenothiazine-calmodulin adducts

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Calmodulin is a widely distributed Ca^{2+} -binding protein that modulates the activities of a variety of Ca^{2+} -sensitive enzymes. Upon irradiation with ultraviolet light, chlorpromazine binds irreversibly to calmodulin. The resulting drug-calmodulin adduct is unable to activate calmodulin-sensitive phosphodiesterase but can inhibit the activation of the enzyme by native calmodulin, apparently by competing with calmodulin for regulatory sites on the enzyme. These preliminary findings suggest that it might be possible to develop a new class of calmodulin antagonists that act at calmodulin-recognition sites on calmodulin-sensitive enzymes; such agents might display greater selectivity than antagonists that interact with calmodulin itself. The present studies were undertaken to determine whether other phenothiazine-calmodulin adducts might also inhibit calmodulin and whether these agents could differentially affect various calmodulin-sensitive enzymes.

Three different phenothiazine-calmodulin adducts (abbreviated UV-CPZ-CaM, HRP-CPZ-CaM, and FNM-CaM) were studied. UV-CPZ-CaM was prepared by irradiating solutions of calmodulin in the presence of chlorpromazine; HRP-CPZ-CaM was made by treating solutions of chlorpromazine and calmodulin with horseradish peroxidase and H_2O_2 ; and FNM-CaM was prepared by incubating solutions of calmodulin with fluphenazine nitrogen mustard, an alkylating phenothiazine. The effect of these adducts on the basal and calmodulin-stimulated activities of purified calmodulin-sensitive phosphodiesterase and erythrocyte membrane Ca^{2+} -ATPase were then examined. None of the adducts displayed calmodulin activity, as evidenced by the fact that they failed to activate either enzyme. In addition, the HRP-CPZ-CaM and the FNM-CaM complexes had no effect on the calmodulin-induced activation of either enzyme. By contrast, the UV-CPZ-CaM complex differentially affected the two enzymes; it inhibited the calmodulin-induced activation of phosphodiesterase but had no effect on the activation of the ATPase by calmodulin. These findings indicate that various phenothiazine calmodulin ad-

ducts may exhibit different pharmacologic properties and that some of these agents may selectively inhibit one calmodulin sensitive enzyme but not another.

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The effects of MRZ-2663BR (naltrexone methobromide) on blood pressure responses to leu- and met-enkephalin

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The purpose of this project is to help elucidate the mechanism(s) through which the two endogenous pentapeptide opioids, leucine- (leu) and methionine- (met) enkephalin, alter blood pressure. Previous studies in our laboratory have shown that the intravenous administration of both leu- and met-enkephalin produced a dose-dependent decline in blood pressure without significantly changing heart rate. The drop in blood pressure due to leu-enkephalin was antagonized by naltrexone and diphenhydramine; whereas, met-enkephalin was more efficiently antagonized by naltrexone. Both of the antagonists have both central and peripheral effects. A series of studies utilizing MRZ-2663BR (naltrexone methobromide) was conducted to determine if the effects of the enkephalins on blood pressure are due to a direct effect on vascular tissue rather than a central effect. MRZ-2663BR, supplied by Boehringer Ingelheim, is a naltrexone analogue that is a quaternary opioid antagonist with selective peripheral activity. Chloralose-anesthetized cats were administered the enkephalins in doses of 0.32 to 100 $\mu\text{g}/\text{kg}$, intravenously, to establish dose-effect curves. MRZ-2663BR was then given in a dose of 1 mg./kg., intravenously. The enkephalins were again administered. The administration of MRZ-2663BR itself produced a decline in blood pressure that persisted for approximately 7 minutes. The drops in blood pressure seen with doses of 0.32 and 32 $\mu\text{g}/\text{kg}$. of both leu- and met-enkephalin were completely blocked by MRZ-2663BR. The higher doses of the enkephalins were significantly antagonized. The antagonism of the enkephalins by MRZ-2663BR was significantly greater than an equivalent dose of naltrexone. The results of this study indicate that the decrease in blood pressure following intravenous administration of

the enkephalins is due, at least primarily, to a direct effect on opioid receptors on vascular tissue.

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Bioassay of blood pressure elevation induced by methoxamine using a behavioral procedure in the rat

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Drug discrimination paradigms have been used extensively to study the subjective effects of psychoactive drugs. Recently, the discrimination paradigm was applied to study subjective effects of drugs affecting the autonomic nervous system. We have demonstrated that the ability of spontaneously hypertensive rats to detect clonidine is quantitatively dependent upon blood pressure.¹ The purpose of the present study was to determine if internal cues produced by blood pressure elevation predict rat behavior which correctly detects the hypertensive agent, methoxamine. Initially, male hooded rats were food-deprived and trained to lever press on a schedule in which food was delivered following every 10 presses. The ability to detect the methoxamine action was trained in subsequent sessions in which presses on only 1 of 2 levers yielded food. During these sessions the correct lever was predetermined according to whether methoxamine or saline had been injected prior to the session. The rats learned the methoxamine-saline discrimination to a criterion of appropriate lever selection on 10 consecutive tests in an average of 39 training sessions. In order to determine if successful detection of methoxamine was related to the hypertension produced by this drug, blood pressure and methoxamine-appropriate lever selection were compared following experimental variations of dose and injection-test interval. Systolic blood pressure was measured by a tail-cuff occlusion method separately. Selection of the methoxamine-appropriate lever increased as a function of dose and varied as an inverted U-shaped function of time following injection. Systolic blood pressure changes closely paralleled dose- and time-dependent changes in methoxamine-appropriate lever selection, indicating that elevation of blood pressure was a sufficient explanation for successful detection

of this drug. The findings suggest that rats can be trained to detect and use internal cues related to hypertension.

I. Lal, H., and Yaden, S.: Discriminative stimuli produced by clonidine in spontaneously hypertensive rats. Generalization to antihypertensive drugs with different mechanisms of action. *J Pharmacol Exp Ther* 232:33-9, Jan 85

Supported by American Osteopathic Association grant no. 85-11-175, and HHS grant no. NHLBI HL07465.

Age-related differences in the effects of naltrexone on stress-induced hypertension

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Several studies have demonstrated the activation of endogenous CNS opioid pathways in response to a variety of stresses. The function served by the opioids has been the subject of speculation and has included a role in the activation of the cardiovascular system to respond to the stress. This study evaluated the contribution of the endogenous opioids in stress-induced hypertension. Four groups of rats were housed in a chamber designed to permit exposure to an established environmental stress paradigm. At the beginning of the experiment, 2 of the groups in the chamber were composed of young rats. All young rats were less than 4-weeks-old (body wt. < 100 gm.). The other two groups were rats that were greater than 8-weeks-old (body wt. > 250 gm.). One group in each age category received implants of sustained release preparations of naltrexone, an opioid antagonist, one day prior to the initiation of the stress paradigm. All rats were subjected to the same stress paradigm, and their systolic blood pressure was monitored for a period of 12 weeks. Naltrexone administration did not significantly alter the onset or magnitude of the stress-induced hypertension in the older rat group. However, the drug did potentiate the magnitude of the hypertension in the young rat population. These data suggest that the endogenous opioids play a role in minimizing the effects of chronic stress in young animals. Additionally, this protective role of the

endogenous opioids is lost as the animal ages.

Supported by American Osteopathic Association grant no. 84-04-305.

Effect of ANAPP₃ on pelvic ganglionic transmission in the cat

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Recent work indicating the presence of P₂ receptors on pelvic parasympathetic ganglia which mediate depolarization prompted us to study the effects of arylazido aminopropionyl ATP (ANAPP₃) on pelvic ganglionic transmission. This study was to determine if ANAPP₃, a P₂ receptor antagonist in bladder smooth muscle, had any effect on the inhibition of ganglionic transmission by purine analogues, such as adenosine (ADS) or ATP. ADS and ATP inhibit ganglionic transmission in the pelvic ganglia, as well as other autonomic ganglia, such as the superior cervical ganglia. This inhibition is antagonized by theophylline and other methylxanthines. However, with the reports of P₂ receptors on this ganglia, we felt that ANAPP₃ should be tested on these responses.

The urinary tract of naive anesthetized cats was exposed via a midline incision. Preganglionic and postganglionic pelvic nerves were prepared for stimulating and recording, respectively, using silver bipolar electrodes. The preganglionic nerves were stimulated at 5 Hz, 0.05 ms duration at varying intensities. Drugs were administered intra-arterially via an abdominal aorta cannula.

ANAPP₃ had a mixed effect on the inhibition of ganglionic transmission. It consistently antagonized inhibition induced by β , γ -methylene ATP. ADS and ATP inhibition was antagonized only in part of the experiments. ANAPP₃ decreased the magnitude of the ganglionic action potential produced by pelvic nerve stimulation. The results suggest that there may be P₂ pelvic ganglia receptors involved in inhibition of ganglionic transmission. Previous reports indicate the existence of P₂ receptors which mediate excitation. These pelvic ganglionic receptors may be the P₂ inhibitory type.

Supported by NIH grant no. AM21465.

Occurrence of senescence-like deterioration of learning ability in autoimmune mice

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Cognitive disorders occur in association with normal aging, Alzheimer's disease, and certain forms of autoimmune disease, such as systemic lupus erythematosus. An increase in the reactivity of serum with CNS constituents is associated with each of these conditions, implicating the formation of brain-reactive antibodies (BRA) in their etiologies. To test the hypothesis that autoimmune- and senescence-related cognitive disorders involve similar etiologies, age-related deterioration of learning abilities in senescent mice was compared to that exhibited by inbred, autoimmune-prone mice. Young (1.5- and 3-month-old), mature (6-month-old), and senescent (26-month-old) C57BL/6 mice were tested for learning and memory abilities using an active avoidance testing procedure. Three autoimmune-prone strains, New Zealand Black (NZB), MRL/1, and BXSB were similarly tested at ages before and after the clinical manifestations of their autoimmune disorders (1.5 and 3 months). It was expected that learning or memory abilities of the autoimmune strains would undergo deterioration between 1.5 and 3 months, whereas similar deterioration would not occur until 26 months in C57BL/6 mice. Although there were differences in learning abilities among the strains at 1.5 months, relative declines occurred in all autoimmune strains by 3 months in accordance with prediction. C57BL/6 mice showed no deterioration during this period, but exhibited comparable decline of ability between 6 and 26 months of age. Comparisons among autoimmune and senescent mice suggested that declines in test performance involved a common defect in acquisition. The findings indicate that autoimmune and senescent mice share the characteristic of abnormal avoidance learning. The appearance of this abnormality with advancing age is accelerated in the autoimmune mice, as are certain other behavioral, morphologic, and pharmacologic abnormalities associated with senescence. Hence, the study of autoimmune strains may provide important clues to the immunologic/genetic determinants of aging in the CNS.

Supported by U.S.P.H.S. grant no. AGO3263,

and a research grant from Miles Institute for Clinical Pharmacology.

Effects of short-term food deprivation on ethanol's ability to deplete rat tissue glutathione (GSH)

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The important role which GSH (γ -glutamylcysteinylglycine) plays in tissue detoxification reactions is well established. It has been reported that tissue GSH content undergoes diurnal variation and that starvation decreases hepatic GSH content and abolishes the diurnal rhythm. It is also known that rat sensitivity to the lethal and hepatotoxic effects of drugs can be augmented by decreased hepatic GSH levels. Age-related alterations in hepatic oxidative drug-metabolizing capabilities have been reported.

The object of this study was to evaluate age-related effects of both short-term food deprivation on tissue GSH levels as well as the ability of the short-term food deprivation to alter tissue sensitivity to the GSH depleting action of ethanol.

Male Sprague-Dawley rats of 3, 4, 8, and 12 weeks of age were used throughout this study. Fed animals were allowed free access to food and water; 6 hours before sacrifice, the food was removed. Food-deprived animals had food withdrawn 21 hours prior to sacrifice. Ethanol (4.0 gm./kg., intraperitoneally) or saline was administered 6 hours before animals were sacrificed. Animals were sacrificed by decapitation; heart, kidneys, and liver were rapidly removed and refrigerated at -80°C . Animals were always sacrificed between noon and 1:00 p.m. to avoid diurnal differences in tissue GSH levels. Tissue samples were assayed for GSH using the method of Ellman.¹

Short-term food deprivation (21 hours) significantly decreased hepatic GSH levels in 12-week-old rats but had no effect on 3, 4, or 8-week-old rats. Ethanol significantly decreased hepatic GSH in rats from all age groups. However, 12-week-old rats, both fed and food-deprived, were most sensitive to the hepatic GSH depleting effects of ethanol (fed 42 percent, food-deprived 47 percent), while 3-week-old rats were the least sensitive (fed 22 percent, food-deprived 15 percent).

We conclude that hepatic GSH level is

age-related and that both age and food deprivation influences ethanol's ability to deplete hepatic glutathione.

I. Ellman, G.L.: Tissue sulfhydryl groups. *Archas Biochem Biophys* 82:70-7, 1959

Supported by a grant from the University of Osteopathic Medicine and Health Sciences Faculty Research Fund.

Subjective effects of nicotine withdrawal: Evidence for role of GABAergic system

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Interoceptive stimuli produced by withdrawal from several dependence-producing drugs are detected by the pentylenetetrazol (PTZ) discrimination paradigm. The purpose of this study was to test whether perturbing the GABA/benzodiazepine system would alter the PTZ-like stimulus produced by nicotine withdrawal. Diazepam (5 mg./kg., intraperitoneally) was used to increase GABA activity, and isoniazid (200 mg./kg., intraperitoneally), a GABA-synthesis inhibitor, was used to decrease GABA activity. Food-deprived rats were trained, in a 2-lever task, to press 1 lever after PTZ, 20 mg./kg., intraperitoneally, and the other lever after saline. Ten presses on the correct lever were rewarded with a food pellet. Well-trained rats were given nicotine, 1.25 mg./kg., subcutaneously, tid, for a minimum of 2 weeks. Withdrawal was assayed by determining the extent to which these rats detected a PTZ-like stimulus after saline, at various intervals after the last nicotine injection. Prior to chronic nicotine, saline produced no (0 percent) and isoniazid produced little (33 percent) PTZ-lever selection. After chronic nicotine, a small but consistent selection of the PTZ-appropriate lever, (35 percent) occurred following saline injections, and this withdrawal effect was blocked by diazepam. PTZ-lever selection at 5 days after nicotine was increased 75 percent by isoniazid. The effect of isoniazid during withdrawal was also blocked by diazepam. These results demonstrate that nicotine withdrawal can be detected by

drug discrimination methodology, and it can be modulated by treatments affecting GABA activity. Thus, the GABA/benzodiazepine system in the CNS may be involved in the subjective symptoms of nicotine withdrawal.

Supported by American Osteopathic Association grant no. 82-11-045.

Failure of a cardiotoxic dose of Adriamycin to stimulate cardiac lipid peroxidation in the mouse

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Evidence has accumulated suggesting

that the cardiotoxicity of the cancer chemotherapeutic agent, Adriamycin, is related to the production of semiquinone free radical metabolites and reactive oxygen radicals. The mechanism of the tissue injury produced following Adriamycin administration is thought to be related to free radical induced lipid peroxidation. In order to further evaluate this hypothesis, we utilized a refined method of estimating *in vivo* lipid peroxidation that depends upon the measurement of malonaldehyde, a breakdown product of lipid peroxide formation.

Adult female mice (ICR Cox strain) were given a single cardiotoxic dose of Adriamycin, 20 mg./kg., or saline, intraperitoneally, 4 days prior to sacrifice for the collection of serum, liver, and cardiac tissue samples. The serum samples were used for the determination of SGOT and SGPT used as parameters of hepatic injury. Malonaldehyde levels were determined in heart and liver samples and are

reported as nanomoles of malonaldehyde per gram of wet weight of tissue. SGPT and SGOT were significantly ($p < 0.05$) elevated (3- to 4-fold) by Adriamycin treatment, indicating that Adriamycin has a significant potential to produce hepatic injury. Malonaldehyde levels were significantly ($p < 0.05$) elevated in liver samples from Adriamycin-treated mice, whereas, malonaldehyde levels in heart samples were not significantly different from controls.

In these preliminary studies, Adriamycin failed to produce evidence of lipid peroxidation reported by other investigators. The failure of Adriamycin to induce cardiac lipid peroxidation may be due to strain or sex differences of the mice in these investigations. Further investigation of sex and strain differences are being conducted.

Supported in part by a grant from the Chicago College of Osteopathic Medicine.

Books

continued from page 624/28

ating from medical school in 1931, she had to battle the chauvinistic attitudes of the male-controlled surgical residency programs. Unfortunately, this type of prejudice is still very much alive. If every medical student and director of educational training read this book, perhaps it would minimize the trauma we put our younger female physicians through. These barriers do not have to exist. Throughout the book, one is left with the unmistakable impression that once given the opportunity, female physicians have performed far above the standards set for them by their male counterparts. Even in today's society, the female physician is in a unique position. She is expected to play the roles of housewife, mother, companion, and educator, as well as to fulfill all other expectations of a physician. We often marvel at the excellence with which most of our female physician acquaintances have fulfilled these tasks.

It is interesting that other ideas have not changed much through the centuries. The book includes an excerpt from a *JAMA* editorial that first appeared in 1901: "The multiplication of doctor factories has gone far enough in this country. It is not a dignified comparison, that of the medical graduates to the output of a machine shop, but the same principles of political economy apply in a measure to both. Overproduction in either has its bad effects and we have not the recourse of foreign markets enjoyed by the ordinary manufacturer." This sentiment is true even in this day and age, and it is quite appropriate to remember that similar problems existed in the early 1900s.

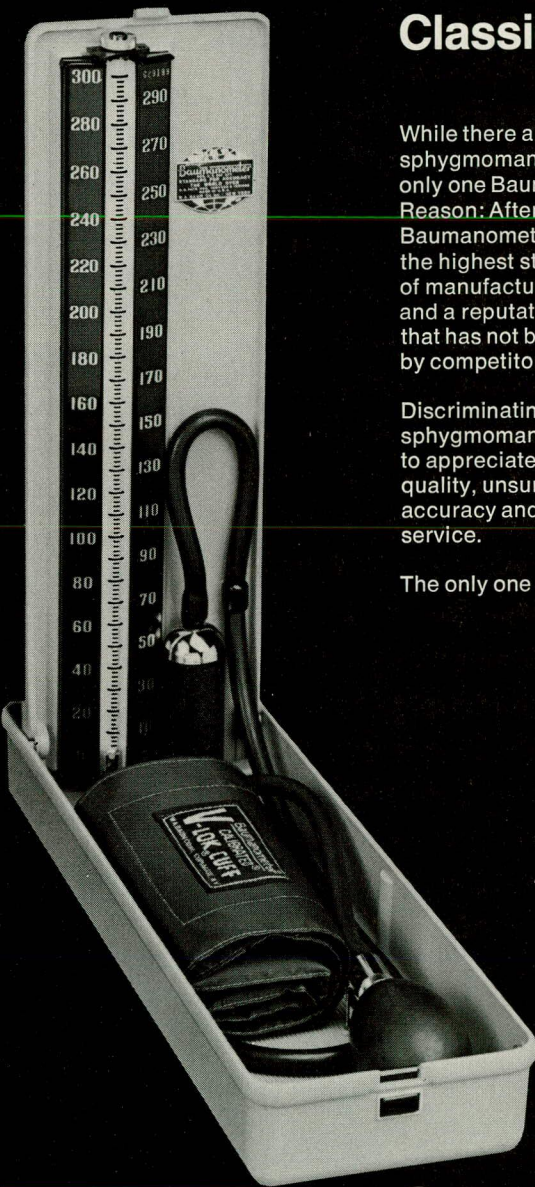
Being graduates of an osteopathic school, we have noticed that while the book clearly refers to homeopathy and water cure (hydropathy), there is no mention of osteopathy. Presumably, this is due to the author's lack of knowledge about osteopathic medicine. She should be made aware of the significant contributions of female osteopathic medical graduates. Women have always been enrolled in osteopathic colleges, although they did not comprise 10 percent of the student body until 1975. The percentage of women en-

rolled at osteopathic schools rose to over 20 percent in 1983, and it is expected to reach more than 25 percent in 1989. Perhaps a chapter in a future edition will be devoted to the pioneers in the osteopathic profession. It could be dedicated to the female physicians who have undertaken the exciting and rough road to medicine and surgery.

The book has been interesting and relaxing reading. It has not gone into great detail about the emotional as-

pects of being a female physician. Neither does it touch upon many of the artificial barriers and obstacles—raised by male peers—that women entering the profession must overcome. Nevertheless, we recommend the book to medical school libraries. It should be required reading for the study of interpersonal relationships and medical sociology.

N.K. PANDEYA, D.O.
SUSAN BECK, D.O.
Des Moines, Iowa



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Advances in vascular surgery

Edited by John S. Najarian and John P. Delaney. Pp. 530, with illus. Year Book Medical Publishers, Inc., 35 East Wacker Drive, Chicago 60601, 1983, \$65.00.

Advances in vascular surgery was compiled from the 46th Annual Continuation Course in Surgery, which was held at the University of Minnesota Medical School in Minneapolis. The list of contributing authors reads like a Who's Who in contemporary vascular surgery. The subject material is contrived not as a reference text, but as a panel discussion that covers controversial topics. The material ranges from carotid endarterectomy (the most commonly

performed peripheral vascular procedure) to aneurysmectomy and arterial suturing techniques. Various nonsurgical topics include pulmonary thromboembolism, noninvasive vascular testing, and new concepts of anticoagulation. The reading is easy and without extensive rhetoric.

The book is an excellent review source for the postgraduate surgeon or for any physician who practices vascular surgery. It can also help one to prepare for the board examination review. The panel discussions perform the most valuable service of the book, in that they render insight into the minds of some of the most experienced vascular surgeons of our time.

WILLIAM STANLEY, D.O., FACOS
Des Moines, Iowa

Arthroscopy of the knee: A diagnostic color atlas

By David Dandy. Pp. 159, with illus. Distributed by Lea and Febiger, 600 Washington Square, Philadelphia 19106-9982, 1984, \$44.00.

Arthroscopy of the knee: A diagnostic color atlas is an excellent visual journey through the knee joint via the arthroscope. This volume is divided into seven sections: normal knee anatomy; normal knee arthroscopic anatomy; synovial disorders of the knee; lesions of bone and articular cartilage; patellofemoral joint and loose bodies; lesions of ligaments; and pathology of the medial and lateral menisci.

Dr. Dandy, a renowned knee surgeon from England, has produced a worthwhile volume. It features beautiful, clear arthroscopic photographs that depict normal arthroscopic knee anatomy as well as pathology. Alongside these photographs are well-labeled illustrations of the various normal and abnormal structures.

This book does not touch upon the various causes or treatments of the pathologic conditions shown, but that is really not within the scope or intent of the text. The purpose was to provide the reader with an atlas of the normal and abnormal structures of the knee joint, and this goal was accomplished admirably.

I recommend this book to any orthopedic surgeon who performs diagnostic and/or operative arthroscopy of the knee. It is also highly appropriate for residents who are being trained in this surgical procedure. The book is not suitable for those who do not routinely perform arthroscopy, or for the reader who is interested in the causes and treatments of various pathologic knee conditions.

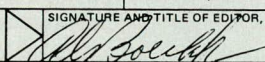
DENNIS B. KOLARIK, D.O.
Massillon, Ohio

Bronchodilator therapy

Edited by T.J.H. Clark and C.M. Cochrane. Pp. 230, with illus. Adis Press Limited, Auckland, New Zealand, 1984, \$62.00.

Bronchodilator therapy covers much of the basic treatment of asthma and

continued on page 692/109

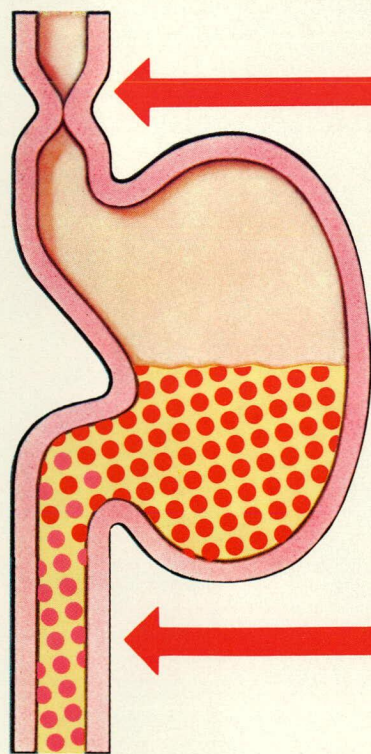
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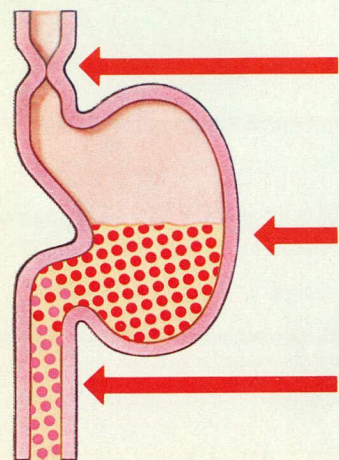
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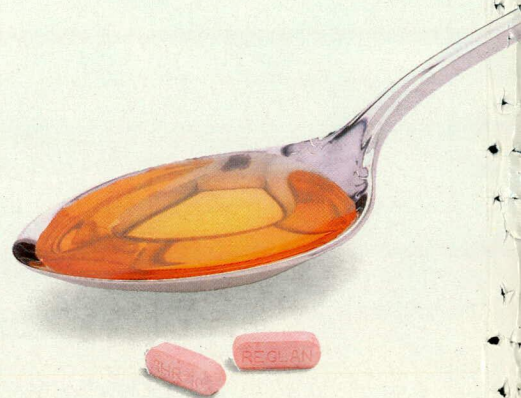
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Indications and Usage: **Symptomatic gastroesophageal reflux:** Reglan Tablets are indicated as short-term (4 to 12 weeks) therapy for adults with symptomatic, documented gastroesophageal reflux who fail to respond to conventional therapy.

The principal effect of metoclopramide is on symptoms of postprandial and daytime heartburn with less observed effect on nocturnal symptoms. If symptoms are confined to particular situations, such as following the evening meal, use of metoclopramide as single doses prior to the provocative situation should be considered, rather than using the drug throughout the day. Healing of esophageal ulcers and erosions has been endoscopically demonstrated at the end of a 12-week trial using doses of 15 mg q.i.d. As there is no documented correlation between symptoms and healing of esophageal lesions, patients with documented lesions should be monitored endoscopically.

Contraindications: Metoclopramide should not be used whenever stimulation of gastrointestinal motility might be dangerous, e.g., in the presence of gastrointestinal hemorrhage, mechanical obstruction, or perforation.

Metoclopramide is contraindicated in patients with pheochromocytoma because the drug may cause a hypertensive crisis, probably due to release of catecholamines from the tumor. Such hypertensive crises may be controlled by phentolamine.

Metoclopramide is contraindicated in patients with known sensitivity or intolerance to the drug.

Metoclopramide should not be used in epileptics or patients receiving other drugs which are likely to cause extrapyramidal reactions, since the frequency and severity of seizures or extrapyramidal reactions may be increased.

Warnings: Depression bearing a temporal relationship to Reglan administration has been reported.

Extrapyramidal symptoms, manifested primarily as acute dystonic reactions, occur in approximately 1 in 500 patients treated with the usual adult dosages of 30–40 mg/day of metoclopramide. These usually are seen during the first 24–48 hours of treatment with metoclopramide, occur more frequently in children and young adults, and are even more frequent at the higher doses used in prophylaxis of vomiting due to cancer chemotherapy. These symptoms may include involuntary movements of limbs and facial grimacing, torticollis, oculogyric crisis, rhythmic protrusion of tongue, bulbar type of speech, trismus, or dystonic reactions resembling tetanus. Rarely, dystonic reactions may present as stridor and dyspnea, possibly due to laryngospasm. If these symptoms should occur, inject 50 mg Benadryl® (diphenhydramine hydrochloride) intramuscularly, and they usually will subside. Cogentin® (benztropine mesylate), 1 to 2 mg intramuscularly, may also be used to reverse these reactions.

Parkinsonian-like symptoms have occurred, more commonly within the first 6 months after beginning treatment with metoclopramide, but occasionally after longer periods. These symptoms generally subside within 2–3 months following discontinuance of metoclopramide.

Tardive Dyskinesia: Tardive dyskinesia, a syndrome consisting of potentially irreversible, involuntary, dyskinetic movements may develop in patients treated with metoclopramide. Although the prevalence of the syndrome appears to be highest among the elderly, especially elderly women, it is impossible to predict which patients are likely to develop the syndrome. Both the risk of developing the syndrome and the likelihood that it will become irreversible are believed to increase with the duration of treatment and the total cumulative dose.

Less commonly, the syndrome can develop after relatively brief treatment periods at low doses; in these cases, symptoms appear more likely to be reversible.

There is no known treatment for established cases of tardive dyskinesia although the syndrome may remit, partially or completely, within several weeks-to-months after metoclopramide is withdrawn. Metoclopramide itself, however, may suppress (or partially suppress) the signs of tardive dyskinesia, thereby masking the underlying disease process. The effect of this symptomatic suppression upon the long-term course of the syndrome is unknown. Therefore, the use of metoclopramide for the symptomatic control of tardive dyskinesia is not recommended.

Precautions: **General:** Metoclopramide may impair the mental and/or physical abilities required for the performance of hazardous tasks such as operating machinery or driving a motor vehicle. The ambulatory patient should be cautioned accordingly.

In one study in hypertensive patients, intravenously administered metoclopramide was shown to release catecholamines; hence, caution should be exercised when metoclopramide is used in patients with hypertension.

Drug Interactions: The effects of metoclopramide on gastrointestinal motility are antagonized by anticholinergic drugs and narcotic analgesics. Additive sedative effects can occur when metoclopramide is given with alcohol, sedatives, hypnotics, narcotics or tranquilizers.

The finding that metoclopramide releases catecholamines in patients with essential hypertension suggests that it should be used cautiously, if at all, in patients receiving monoamine oxidase inhibitors.

Absorption of drugs from the stomach may be diminished (e.g., digoxin) by metoclopramide, whereas absorption of drugs from the small bowel may be accelerated (e.g., acetaminophen, tetracycline, levodopa, ethanol).

Gastroparesis (gastric stasis) may be responsible for poor diabetic control in some patients. Exogenously administered insulin may begin to act before food has left the stomach and lead to hypoglycemia. Because the action of metoclopramide will influence the delivery of food to the intestines and thus the rate of absorption, insulin dosage or timing of dosage may require adjustment.

Carcinogenesis, Mutagenesis, Impairment of Fertility: A 77-week study was conducted in rats in oral doses up to about 40 times the maximum recommended human daily dose. Metoclopramide elevates prolactin levels and the elevation persists during chronic administration. Tissue culture experiments indicate that approximately one-third of human breast cancers are prolactin-dependent *in vitro*, a factor of potential importance if the prescription of metoclopramide is con-

templated in a patient with previously detected breast cancer. Although disturbances such as galactorrhea, amenorrhea, gynecomastric, and impotence have been reported with prolactin-elevating drugs, the clinical significance of elevated serum prolactin levels is unknown for most patients. An increase in mammary neoplasms has been found in rodents after chronic administration of prolactin-stimulating neuroleptic drugs and metoclopramide. Neither clinical studies nor epidemiologic studies conducted to date, however, have shown an association between chronic administration of these drugs and mammary tumorigenesis; the available evidence is too limited to be conclusive at this time.

An Ames mutagenicity test performed on metoclopramide was negative.

Pregnancy Category B. Reproduction studies performed in rats, mice, and rabbits by the i.v., i.m., s.c. and oral routes at maximum levels ranging from 12 to 250 times the human dose have demonstrated no impairment of fertility or significant harm to the fetus due to metoclopramide. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers. Metoclopramide is excreted in human milk. Caution should be exercised when metoclopramide is administered to a nursing mother.

Adverse Reactions: In general, the incidence of adverse reactions correlates with the dose and duration of metoclopramide administration. The following reactions have been reported, although in most instances, data do not permit an estimate of frequency.

CNS Effects. Restlessness, drowsiness, fatigue and lassitude occur in approximately 10% of patients receiving the most commonly prescribed dosage of 10 mg q.i.d. (see Precautions). Insomnia, headache, confusion, dizziness or depression (see Warnings) occur less frequently. In cancer chemotherapy patients being treated with 1–2 mg/kg per dose, incidence of drowsiness is about 70%. There are isolated reports of convulsive seizures without clearcut relationship to metoclopramide. Rarely, hallucinations have been reported.

Extrapyramidal Reactions (EPS). Acute dystonic reactions, the most common type of EPS associated with metoclopramide, occur in approximately 0.2% of patients (1 in 500) treated with 30 to 40 mg of metoclopramide per day. In cancer chemotherapy patients receiving 1–2 mg/kg per dose, the incidence is 2% in patients over the ages of 30–35, and 25% or higher in children and young adults who have not had prophylactic administration of diphenhydramine. Symptoms include involuntary movements of limbs, facial grimacing, torticollis, oculogyric crisis, rhythmic protrusion of tongue, bulbar type of speech, trismus, opisthotonus (tetanus-like reactions) and rarely, stridor and dyspnea possibly due to laryngospasm; ordinarily these symptoms are readily reversed by diphenhydramine (see Warnings).

Parkinsonian-like symptoms may include bradykinesia, tremor, cogwheel rigidity, mask-like faces (see Warnings). Tardive dyskinesia most frequently is characterized by involuntary movements of the tongue, face, mouth or jaw, and sometimes by involuntary movements of the trunk and/or extremities (see Warnings).

Motor restlessness (akathisia) may consist of feelings of anxiety, agitation, jitteriness, and insomnia, as well as inability to sit still, pacing, foot-tapping. These symptoms may disappear spontaneously or respond to a reduction in dosage.

Endocrine Disturbances. Galactorrhea, amenorrhea, gynecomastric, impotence secondary to hyperprolactinemia (see Precautions). Fluid retention secondary to transient elevation of aldosterone (see Clinical Pharmacology).

Cardiovascular. Hypotension, hypertension and a single instance of supraventricular tachycardia (see Contraindications, Precautions).

Gastrointestinal. Nausea and bowel disturbances, primarily diarrhea.

Renal, Urinary frequency and incontinence.

Hematologic. A few cases of neutropenia, leukopenia, or agranulocytosis, generally without clearcut relationship to metoclopramide.

Allergic Reactions. A few cases of rash, urticaria, or bronchospasm, especially in patients with a history of asthma. Rarely, angioneurotic edema, including glossal or laryngeal edema.

Miscellaneous. Visual disturbances. Porphyria. Rare occurrences of neuroleptic malignant syndrome (NMS) have been reported. This potentially fatal syndrome is comprised of the symptom complex of hyperthermia, altered consciousness, muscular rigidity and autonomic dysfunction.

Transient flushing of the face and upper body, without alterations in vital signs, following high doses intravenously.

Overdosage: Symptoms of overdosage may include drowsiness, disorientation and extrapyramidal reactions. Anticholinergic or antiparkinson drugs or antihistamines with anticholinergic properties may be helpful in controlling the extrapyramidal reactions. Symptoms are self-limiting and usually disappear within 24 hours.

Hemodialysis removes relatively little metoclopramide, probably because of the small amount of the drug in blood relative to tissues. Similarly, continuous ambulatory peritoneal dialysis does not remove significant amounts of drug. It is unlikely that dosage would need to be adjusted to compensate for losses through dialysis. Dialysis is not likely to be an effective method of drug removal in overdose situations.

Literature reports describe methemoglobinemia in premature and full term neonates who were given metoclopramide intramuscularly, 1–2 mg/kg/day for 3 or more days. Methemoglobinemia has not been reported in similar infants treated with 0.5 mg/kg/day in divided doses. Methemoglobinemia can be reversed by the intravenous administration of methylene blue.

Dosage and Administration: **For the relief of symptomatic gastroesophageal reflux:** Administer from 10 mg (1 tablet) to 15 mg (1 1/2 tablets) i.d. 3 or 4 times daily, with or without meals and at bedtime, depending upon symptoms being treated and clinical response (see Clinical Pharmacology and Indications). If symptoms occur only intermittently or at specific times of the day, use of metoclopramide in single doses up to 20 mg prior to the provoking situation may be preferred rather than continuous treatment.

Experience with esophageal erosions and ulcerations is limited, but healing has thus far been documented in one controlled trial using q.i.d. therapy at 15 mg/dose, and this regimen should be used when lesions are present, so long as it is tolerated (see Adverse Reactions). Because of the poor correlation between symptoms and endoscopic appearance of the esophagus, therapy directed at esophageal lesions is best guided by endoscopic evaluation.

Therapy longer than 12 weeks has not been evaluated and cannot be recommended.

A-H-ROBINS

Pharmaceutical Division
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Rev. May 1986

chronic obstructive airway disease. The therapy discussed is mostly utilized in England; the use of inhaled bronchodilators is emphasized and that of xanthines is downplayed.

The early chapters present the basic pathophysiology of bronchoconstriction and the pharmacology of bronchodilators. This is accomplished in a very clear and excellent manner. The drawings are well prepared and very useful, and the many color photographs add to the beauty of the book. The next few chapters set a plan for the general management of asthma, including patient education techniques to improve compliance. Drug therapy approaches to the prevention of asthma attacks are also described.

Chapter 6, which discusses the use of bronchodilators in childhood asthma, is particularly well done. Again, the illustrations are very legible and aid in clarifying the text. Bronchodilator use in specific situations, including exercise-induced asthma, nocturnal asthma, obstructive airway disease in the elderly, and severe acute asthma, is addressed next. Another chapter specifically covers the use of bronchodilators for chronic bronchitis and emphysema.

Three helpful appendices summarize information on structure-activity relationships, dose equivalents of bronchodilator drugs, and inhalation devices. These data add to the usefulness of this volume.

The book is authored by several authorities on respiratory diseases, all of whom are European, which gives a distinctive flavor to their ideas and tends to broaden American readers' perceptions on the use of bronchodilators.

Considering the number of color illustrations, *Bronchodilator therapy* is fairly priced at \$62.00. It is probably most useful to pulmonologists, residents, and fellows, and I highly recommend it to such readers. The book would also be an excellent resource for medical students who are studying the pharmacology of bronchodilators and the pathophysiology of bronchoconstriction.

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Dayton, Ohio

The diagnosis and treatment of epilepsy

By Richard Lechtenberg. Pp. 239. Macmillan Publishing Company, 866 Third Avenue, New York 10022, 1985, \$34.95.

This book provides physicians with the basic strategies for diagnosing and treating patients with epilepsy. It is written for the neurologist, family physician, gynecologist, pediatrician, general surgeon, internist, or any physician who is responsible for the patient with seizures. Dr. Lechtenberg, the author, is an associate professor of clinical neurology at State University of New York Downstate Medical School in Brooklyn.

The 15 chapters that comprise *The diagnosis and treatment of epilepsy* include information on the origin of seizures, the risk of inheritance, and the causes and problems of epilepsy from infancy to adulthood. Treatment using antiepileptic drugs as well as nonpharmaceutical approaches are discussed. Several chapters focus on epilepsy and repro-

duction; topics include birth defects, the rate of miscarriage, possible toxic complications of drug therapy on mother and fetus, and the hereditary role of epilepsy in the father. There is also useful information on employment of epileptics, hospitalization frequency and its effect upon the patient, and coping with coincidental problems such as paralysis, vulnerability to other diseases, sexual activity, cosmetic effects of anti-epileptic treatment, genetic counseling, prognosis, rehabilitation, and mortality. A section on breast feeding while the nursing mother is taking more than one antiepileptic drug is very informative. The chapter on treatment principles provides an excellent review of drugs of choice, alternatives for the various seizure types, and the protocol for discontinuing treatment for seizure-free patients who have been on long-term medication.

The diagnosis and treatment of the patient with seizures can be tricky because the complex of neurologic
continued on page 693/112



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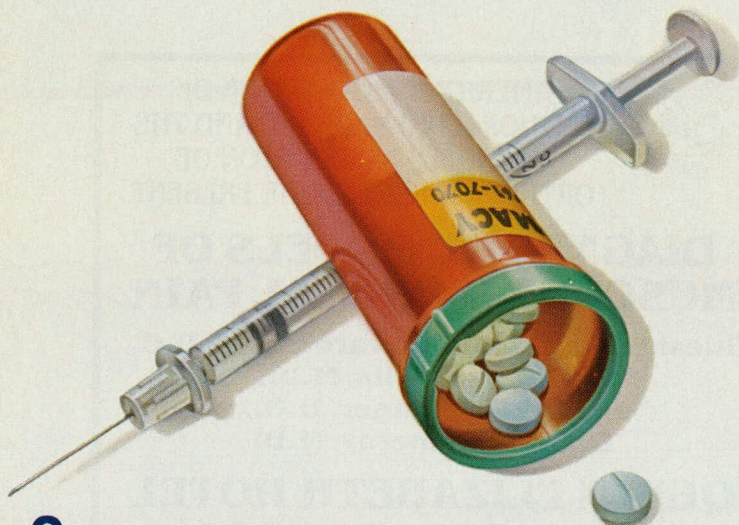
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1. Micronase—a rational choice in type II diabetes

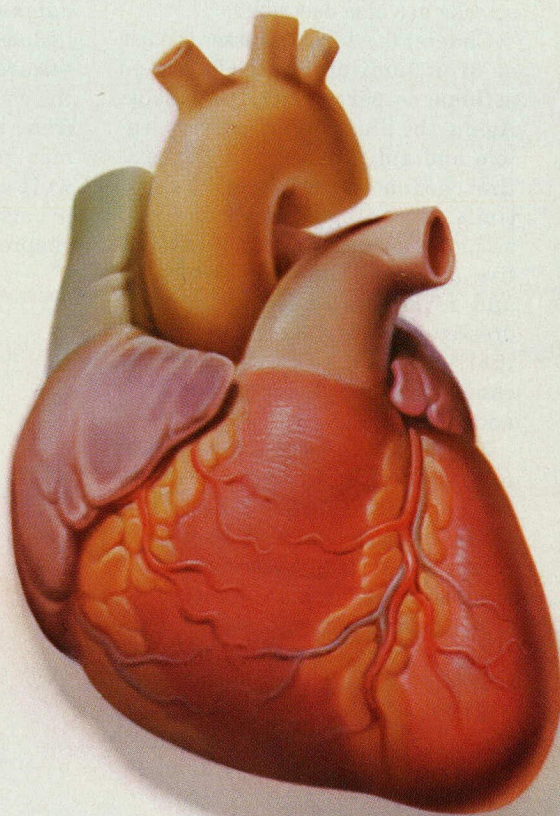
Insulin levels are normal or elevated in most patients with type II diabetes, although insulin action is markedly impaired. MICRONASE helps normalize the tissue response to endogenous insulin.

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4. Micronase—an important consideration in the type II diabetic patient with renal impairment: Control plus unique dual excretion... 50% urine, 50% bile

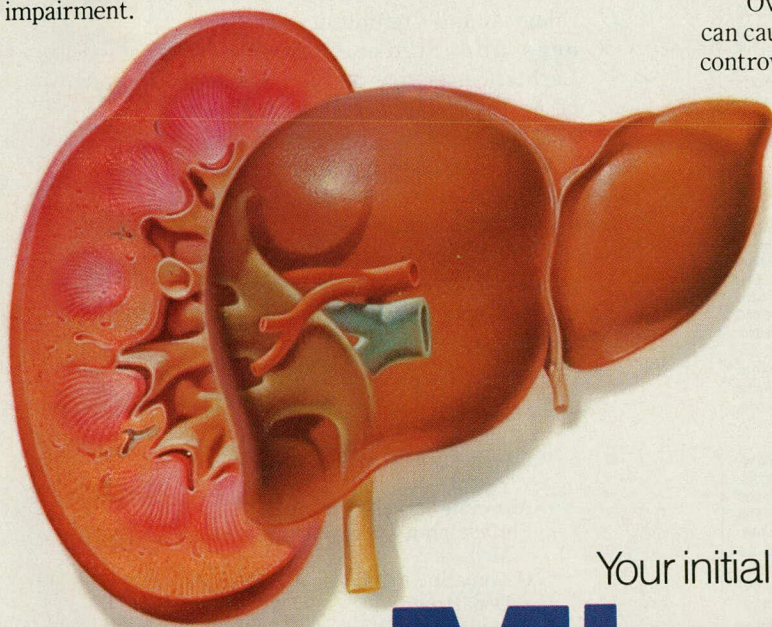
Elimination of MICRONASE equally in bile and urine reduces the risk of drug accumulation, which may result in hypoglycemia. MICRONASE should be used with caution in patients with renal impairment; however, in a single-dose study, plasma clearance of MICRONASE was prolonged only in patients with severe renal impairment.

5. Micronase—for the patient who fails on other diabetic therapy: Potency and dosage flexibility

MICRONASE may prove effective when other drugs fail. Five mg of MICRONASE is approximately equivalent to 250 mg of chlorpropamide or 500 mg of acetohexamide in its ability to lower blood glucose. The dosage range of MICRONASE allows for greater dosage flexibility than other agents.

Overdosage of sulfonylureas, including MICRONASE, can cause hypoglycemia. Although the interpretations are controversial, the UGDP study reported in 1970 that the use of tolbutamide, an oral hypoglycemic drug, was associated with increased cardiovascular mortality.

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Micronase® An advance in diabetes management

Dosage Guide*

Although relatively rare, hypoglycemia may occur during the conversion to MICRONASE from other therapy.

Prior therapy or condition	Considerations before starting therapy	Initial MICRONASE dose (mg/day)
Dietary therapy ineffective	No priming necessary	1.25 to 5.0 mg
Oral therapy	Discontinue oral hypoglycemic*	2.5 to 5.0 mg
Insulin therapy (< 40 units/day)	Completely discontinue insulin injections under medical supervision	2.5 to 5.0 mg
Insulin therapy (> 40 units/day)	Gradually discontinue insulin injections under close medical observation or hospitalization	5.0 mg

*See complete prescribing information.

*See package insert for special precautions when transferring patients from chlorpropamide.

Micronase Tablets (brand of glyburide tablets)

INDICATIONS AND USAGE MICRONASE Tablets are indicated as an adjunct to diet to lower the blood glucose in patients with non-insulin-dependent diabetes mellitus (type II) whose hyperglycemia cannot be satisfactorily controlled by diet alone.

CONTRAINDICATIONS MICRONASE Tablets are contraindicated in patients with: 1. Known hypersensitivity or allergy to the drug. 2. Diabetic ketoacidosis, with or without coma. This condition should be treated with insulin. 3. Type I diabetes mellitus, as sole therapy.

SPECIAL WARNING ON INCREASED RISK OF CARDIOVASCULAR MORTALITY. The administration of oral hypoglycemic drugs has been reported to be associated with increased cardiovascular mortality as compared to treatment with diet alone or diet plus insulin. This warning is based on the study conducted by the University Group Diabetes Program (UGDP), a long-term prospective clinical trial designed to evaluate the effectiveness of glucose-lowering drugs in preventing or delaying vascular complications in patients with non-insulin-dependent diabetes. The study involved 823 patients who were randomly assigned to one of four treatment groups (*Diabetes*, 19 (Suppl 2):747-830, 1970).

UGDP reported that patients treated for 5 to 8 years with diet plus a fixed dose of tolbutamide (1.5 grams per day) had a rate of cardiovascular mortality approximately 2½ times that of patients treated with diet alone. A significant increase in total mortality was not observed, but the use of tolbutamide was discontinued based on the increase in cardiovascular mortality, thus limiting the opportunity for the study to show an increase in overall mortality. Despite controversy regarding the interpretation of these results, the findings of the UGDP study provide an adequate basis for this warning. The patient should be informed of the potential risks and advantages of MICRONASE and of alternative modes of therapy.

Although only one drug in the sulfonylurea class (tolbutamide) was included in this study, it is prudent from a safety standpoint to consider that this warning may apply to other oral hypoglycemic drugs in this class, in view of their close similarities in mode of action and chemical structure.

PRECAUTIONS General Hypoglycemia: All sulfonylureas are capable of producing severe hypoglycemia. Proper patient selection and dosage and instructions are important to avoid hypoglycemic episodes. Renal or hepatic insufficiency may increase the risk of serious hypoglycemic reactions. Elderly, debilitated or malnourished patients, and those with adrenal or pituitary insufficiency, are particularly susceptible to the hypoglycemic action of glucose-lowering drugs. Hypoglycemia may be difficult to recognize in the elderly and in people who are taking beta-adrenergic blocking drugs. Hypoglycemia is more likely to occur when caloric intake is deficient, after severe or prolonged exercise, when alcohol is ingested, or when more than one glucose lowering drug is used.

Loss of Control of Blood Glucose: In diabetic patients exposed to stress such as fever, trauma, infection or surgery, a loss of control may occur. It may then be necessary to discontinue MICRONASE and administer insulin. Adequate adjustment of dose and adherence to diet should be assessed before classifying a patient as a secondary failure.

Information for Patients: Patients should be informed of the potential risks and advantages of MICRONASE and of alternative modes of therapy. They also should be informed about the importance of adherence to dietary instructions, of a regular exercise program, and of regular testing of urine and/or blood glucose. The risks of hypoglycemia, its symptoms and treatment, and conditions that predispose to its development should be explained to patients and responsible family members. Primary and secondary failure should also be explained. **Laboratory Tests** Response to MICRONASE Tablets should be monitored by frequent urine glucose tests and periodic blood glucose tests. Measurement of glycosylated hemoglobin levels may be helpful in some patients. **Drug Interactions** The hypoglycemic action of sulfonylureas may be potentiated by certain drugs including nonsteroidal anti-inflammatory agents and other drugs that are highly protein bound, salicylates, sulfonamides, chloramphenicol, probenecid, coumarins, monoamine oxidase inhibitors, and beta-adrenergic blocking agents. Certain drugs tend to produce hyperglycemia and may lead to loss of control. These drugs include the thiazides and other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blocking drugs, and isoniazid. **Carcinogenesis, Mutagenesis, and Impairment of Fertility** Studies in rats at doses up to 300 mg/kg/day for 18 months showed no carcinogenic effects. Glyburide is nonmutagenic when studied in the Salmonella microsome test (Ames test) and in the DNA damage/alkaline elution assay.

Pregnancy Teratogenic Effects: Pregnancy Category B. Reproduction studies in rats and rabbits have revealed no evidence of impaired fertility or harm to the fetus due to glyburide. There are no adequate and well controlled studies in pregnant women. This drug should be used during pregnancy only if clearly needed. Insulin should be used during pregnancy to maintain blood glucose as close to normal as possible. **Nonteratogenic Effects:** Prolonged severe hypoglycemia (4 to 10 days) has been reported in neonates born to mothers who were receiving a sulfonylurea drug at the time of delivery. MICRONASE should be discontinued at least two weeks before the expected delivery date. **Nursing Mothers** Some sulfonylurea drugs are known to be excreted in human milk. Insulin therapy should be considered. **Pediatric Use** Safety and effectiveness in children have not been established.

ADVERSE REACTIONS Hypoglycemia: See Precautions and Overdosage sections. **Gastrointestinal Reactions:** Cholestatic jaundice may occur rarely; MICRONASE Tablets should be discontinued if this occurs. Gastrointestinal disturbances, e.g., nausea, epigastric fullness, and heartburn are the most common reactions, having occurred in 1.8% of treated patients during clinical trials. They tend to be dose related and may disappear when dosage is reduced. **Dermatologic Reactions:** Allergic skin reactions, e.g., pruritis, erythema, urticaria, and morbilliform or maculopapular eruptions occurred in 1.5% of treated patients during clinical trials. These may be transient and may disappear despite continued use of MICRONASE; if skin reactions persist, the drug should be discontinued. **Porphyria cutanea tarda** and photosensitivity reactions have been reported with sulfonylureas. **Hematologic Reactions:** Leukopenia, agranulocytosis, thrombocytopenia, hemolytic anemia, aplastic anemia, and pancytopenia have been reported with sulfonylureas. **Metabolic Reactions:** Hepatic porphyria and disulfiram-like reactions have been reported with sulfonylureas; however, hepatic porphyria has not been reported with MICRONASE and disulfiram-like reactions have been reported very rarely.

OVERDOSAGE Overdosage of sulfonylureas, including MICRONASE Tablets, can produce hypoglycemia. If hypoglycemic coma is diagnosed or suspected, the patient should be given a rapid intravenous injection of concentrated (50%) glucose solution. This should be followed by a continuous infusion of a more dilute (10%) glucose solution at a rate which will maintain the blood glucose at a level above 100 mg/dL. Patients should be closely monitored for a minimum of 24 to 48 hours, since hypoglycemia may recur after apparent clinical recovery.

DOSAGE AND ADMINISTRATION There is no fixed dosage regimen for the management of diabetes mellitus with MICRONASE Tablets. **Usual Starting Dose** The usual starting dose is 2.5 to 5.0 mg daily, administered with breakfast or the first main meal. Those patients who may be more sensitive to hypoglycemic drugs should be started at 1.25 mg daily. (See Precautions Section for patients at increased risk.) **Maximum Dose** Daily doses of more than 20 mg are not recommended. **Dosage Interval** Once-a-day therapy is usually satisfactory. Some patients, particularly those receiving more than 10 mg daily, may have a more satisfactory response with twice-a-day dosage.

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problems often have overlapping characteristics. The section on diagnosis is, therefore, very helpful and informative. Investigative techniques such as electroencephalography, computed tomography of the head, digital subtraction angiography, and positron emission tomography are discussed in brief fashion to give perspective to the physician who does not use these techniques regularly.

I would recommend this helpful reference text to all physicians who treat seizures. It gives a broad overview of the diagnosis and practical management of epilepsy, and there are abundant, up-to-date references at the end of each chapter if more information is desired. Several figures and tables are used throughout the book for clarification. The text is rich in useful clinical information that can be applied to patient care, and it will aid the physician in arriving at an accurate diagnosis without ordering extraneous tests. I highly welcome *The diagnosis and treatment of epilepsy* as an additional source in my library.

As a side note, a recent statement by the Chief of the Epilepsy Branch of the National Institute of Neurological and Communicative Disorders and Stroke,¹ echoes Dr. Lechtenberg's view on the treatment of epilepsy. James J. Cereghino, M.D., remarked, "Physicians often start patients on drugs but stop treatment as soon as they get nervous about side effects. These findings, however, confirm that it is important to give a drug an appropriate trial despite the side effects, which usually go away with time."

1. Epilepsy Rx gets easier to take. *Medical World News* 27:46, 9 Jun 86

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Breast carcinoma: Current diagnosis and treatment

By Stephen A. Feig and Robert McLelland. Pp. 653, with illus. Year Book Medical Publishers, Inc., 35 East Wacker Drive, Chicago 60601, 1983, \$95.00.

Breast carcinoma is second only to heart disease as the leading cause of death of American women. Approximately 1 in 11 women will develop breast carcinoma during their lifetime. Over 1,000,000 breast cancers are reported worldwide each year, with more than 100,000 cases in the United States alone. The significance of the breast cancer problem is overwhelming, and Drs. Feig and McLelland have published a complete text dealing with this subject. The 653-page book is a compendium of the most significant reports presented at the 19th and 20th National Conferences on Breast Cancer, which were organized by the American College of Radiology and cosponsored by the American Cancer Society. The years covered extend from 1981 through 1982.

An outstanding international faculty covers just about every facet of the constantly changing armamentarium for the detection, diagnosis, and management of breast cancer. They expound upon the etiology, risk factors, psychologic aspects, and nat-

ural history of breast carcinoma, as well as the various diagnostic imaging modalities and treatments available. The text concludes with a look at future directions in management, such as biologic markers for the detection of breast cancer.

Although Drs. Feig and McLelland are both radiologists, they seem to emphasize the imaging modalities of breast cancer detection. Mammography, xeromammography, ultrasonography, thermography, computed tomography, magnetic resonance imaging, diaphanography, and light scanning are featured in great detail. Additional chapters discuss nonpalpable lesions, various breast cancer detection programs, and mass screening techniques. The favorable results of breast self examination are mentioned as an important adjunct to imaging studies.

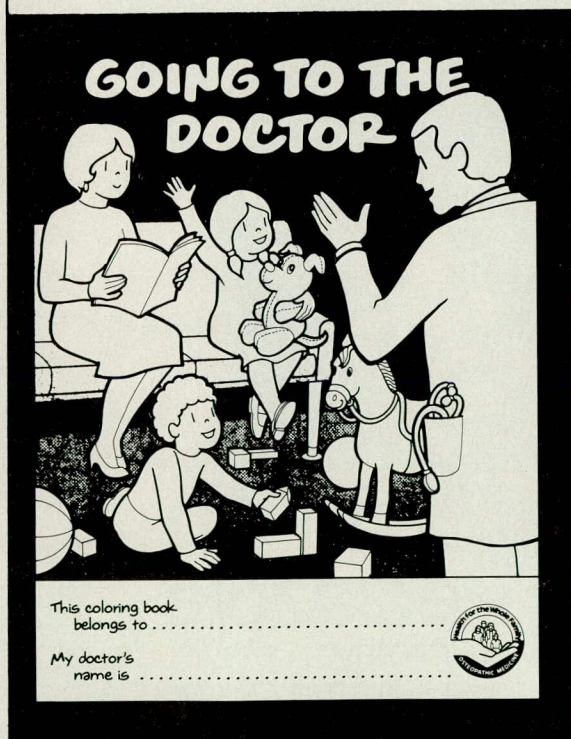
Numerous well-respected surgical authorities discuss the changing concepts in the surgical management of breast cancer, from simple wedge excisions (lumpectomy) to the more radical procedures. The risks and

benefits of each type of surgery are considered. The role of adjuvant radiotherapy is presented, and chapters are devoted to hormone considerations and chemotherapy. Further chapters discuss the emotional problems of the postmastectomy patient, as well as changing ideas about breast reconstruction following mastectomy. Additionally, I enjoyed Dr. Bragg's recommendations and considerations on the radiologic detection of breast metastases. This chapter presents a most conservative approach, while utilizing all of the current imaging modalities.

Overall, the book is probably the most inclusive text on breast cancer available on the market today. I can wholeheartedly say that all physicians who deal with any aspect of breast carcinoma can rely on *Breast carcinoma: Current diagnosis and treatment* for state-of-the-art information on diagnostic imaging procedures and treatments.

STEVEN L. EDELL, D.O.
Chairman, Department of Radiology
Riverside Hospital
Wilmington, Delaware

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Contraindications

Known hypersensitivity to either erythromycin or sulfonamides.

Infants less than 2 months of age.

Pregnancy at term and during the nursing period, because sulfonamides pass into the placental circulation and are excreted in human breast milk and may cause kernicterus in the infant.

Warnings

Usage in Pregnancy (SEE ALSO: CONTRAINDICATIONS): The safe use of erythromycin or sulfonamides in pregnancy has not been established. The teratogenic potential of most sulfonamides has not been thoroughly investigated in either animals or humans. However, a significant increase in the incidence of cleft palate and other bony abnormalities of offspring has been observed when certain sulfonamides of the short, intermediate and long-acting types were given to pregnant rats and mice at high oral doses (7 to 25 times the human therapeutic dose).

Reports of deaths have been associated with sulfonamide administration from hypersensitivity reactions, agranulocytosis, aplastic anemia and other blood dyscrasias. The presence of clinical signs such as sore throat, fever, pallor, purpura or jaundice may be early indications of serious blood disorders. Complete blood counts should be done frequently in patients receiving sulfonamides.

The frequency of renal complications is considerably lower in patients receiving the most soluble sulfonamides such as sulfisoxazole. Urinalysis with careful microscopic examination should be obtained frequently in patients receiving sulfonamides.

Precautions

Erythromycin is principally excreted by the liver. Caution should be exercised in administering the antibiotic to patients with impaired hepatic function. There have been reports of hepatic dysfunction, with or without jaundice occurring in patients receiving oral erythromycin products.

Recent data from studies of erythromycin reveal that its use in patients who are receiving high doses of theophylline may be associated with an increase of serum theophylline levels and potential theophylline toxicity. In case of theophylline toxicity and/or elevated serum theophylline levels, the dose of theophylline should be reduced while the patient is receiving concomitant erythromycin therapy.

Surgical procedures should be performed when indicated.

Sulfonamide therapy should be given with caution to patients with impaired renal or hepatic function and in those patients with a history of severe allergy or bronchial asthma. In the presence of a deficiency in the enzyme glucose-6-phosphate dehydrogenase, hemolysis may occur. This reaction is frequently dose-related. Adequate fluid intake must be maintained in order to prevent crystalluria and renal stone formation.

Adverse Reactions

The most frequent side effects of oral erythromycin preparations are gastrointestinal, such as abdominal cramping and discomfort, and are dose-related. Nausea, vomiting and diarrhea occur infrequently with usual oral doses. During prolonged or repeated therapy, there is a possibility of overgrowth of nonsusceptible bacteria or fungi. If such infections occur, the drug should be discontinued and appropriate therapy instituted. The overall incidence of these latter side effects reported for the combined administration of erythromycin and a sulfonamide is comparable to those observed in patients given erythromycin alone. Mild allergic reactions such as urticaria and other skin rashes have occurred. Serious allergic reactions, including anaphylaxis, have been reported with erythromycin.

The following untoward effects have been associated with the use of sulfonamides:

Blood dyscrasias: Agranulocytosis, aplastic anemia, thrombocytopenia, leukopenia, hemolytic anemia, purpura, hypoprothrombinemia and methemoglobinemia.

Allergic reactions: Erythema multiforme (Stevens-Johnson syndrome), generalized skin eruptions, epidermal necrolysis, urticaria, serum sickness, pruritus, exfoliative dermatitis, anaphylactoid reactions, periorbital edema, conjunctival and scleral injection, photosensitization, arthralgia and allergic myocarditis.

Gastrointestinal reactions: Nausea, emesis, abdominal pains, hepatitis, diarrhea, anorexia, pancreatitis and stomatitis.

C.N.S. reactions: Headache, peripheral neuritis, mental depression, convulsions, ataxia, hallucinations, tinnitus, vertigo and insomnia.

Miscellaneous reactions: Drug fever, chills and toxic nephrosis with oliguria or anuria. Periarthritis nodosa and L.E. phenomenon have occurred.

The sulfonamides bear certain chemical similarities to some goitrogens, diuretics (acetazolamide and the thiazides) and oral hypoglycemic agents. Goiter production, diuresis and hypoglycemia have occurred rarely in patients receiving sulfonamides. Cross-sensitivity may exist with these agents.

Rats appear to be especially susceptible to the goitrogenic effects of sulfonamides, and long-term administration has produced thyroid malignancies in the species.

Dosage and Administration

PEDIAZOLE SHOULD NOT BE ADMINISTERED TO INFANTS UNDER 2 MONTHS OF AGE BECAUSE OF CONTRAINDICATIONS OF SYSTEMIC SULFONAMIDES IN THIS AGE GROUP.

For Acute Otitis Media in Children: The dose of Pediazole can be calculated based on the erythromycin component (50 mg/kg/day) or the sulfisoxazole component (150 mg/kg/day to a maximum of 6 g/day). Pediazole should be administered in equally divided doses four times a day for 10 days. It may be administered without regard to meals.

The following approximate dosage schedule is recommended for using Pediazole:

Children: Two months of age or older.

Weight	Dose—every 6 hours
Less than 8 kg (less than 18 lb)	Adjust dosage by body weight
8 kg (18 lb)	1/2 teaspoonful (2.5 ml)
16 kg (35 lb)	1 teaspoonful (5 ml)
24 kg (53 lb)	1 1/2 teaspoonfuls (7.5 ml)
Over 45 kg (over 100 lb)	2 teaspoonfuls (10 ml)

How Supplied

Pediazole Suspension is available for teaspoon dosage in 100 ml (NDC 0074-8030-13) and 200-ml (NDC 0074-8030-53) bottles, in the form of granules to be reconstituted with water. The suspension provides erythromycin ethylsuccinate equivalent to 200 mg erythromycin activity and sulfisoxazole acetyl equivalent to 600 mg sulfisoxazole per teaspoonful (5 ml).

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Books received

New books received by the Andrew Taylor Still Memorial Library are acknowledged below. Those of greatest interest to readers will be reviewed later.

Hamilton Bailey's Demonstrations of physical signs in clinical surgery. Edited by Allen Clain; ed. 17, p. 622, with illus.; PSG Publishing Co., Inc., 545 Great Road, Littleton, MA 01460, 1986, \$37.50 (paper).

Selfassessment questions and answers on clinical surgery. (Based on the seventeenth edition of Hamilton Bailey's Demonstrations of physical signs in clinical surgery.) By Allan Clain; ed. 2, pp. 227; PSG Publishing Co., Inc., 545 Great Road, Littleton, MA 01460, 1986, \$12.50 (paper).

Casarett and Doull's Toxicology. The basic science of poisons. Edited by Curtis D. Klaassen, Mary O. Amdur, and John Doull; Macmillan Publishing Co., 866 Third Ave., New York 10022, \$49.95.

Clinical hypertension. By Norman M. Kaplan; ed. 4, pp. 492, with illus.; Williams & Wilkins, 428 E. Preston St., Baltimore 21202, 1986, \$54.95.

Respiratory failure. (Written to provide clinicians with information to manage respiratory failure in patients of all age groups.) By Robert R. Kirby and Robert W. Taylor; pp. 665, with illus.; Year Book Medical Publishers, Inc., 35 E. Wacker, Chicago 60601, 1986, \$75.00.

A clinician's companion: A study guide for effective and humane patient care. (Directed primarily to young physicians, medical students, and house officers.) By Joseph S. Alpert and Stephen M. Wittenberg; pp. 207, with illus.; Little, Brown, and Co., 34 Beacon Street, Boston 02106, 1986, \$16.00 (paper).

1986 Year book of medicine. Edited by Rogers, et al.; pp. 839, with illus.; Year Book Medical Publishers, Inc., 35 E. Wacker, Chicago 60601, 1986, \$44.95.

Vertebral manipulation. (Discusses how to use the techniques of mobilization and other manipulative procedures on the different intervertebral levels; by an Australian physiotherapist.) By G.D. Maitland; ed. 5, pp. 390, with illus.; Butterworth Publishers, 80 Montvale Ave., Stoneham, MA 02180, 1986, \$24.95 (paper).

1986 Year book of plastic and reconstructive surgery. Edited by Frederick J. McCoy; pp. 349, with illus.; Year Book Medical Publishers, Inc., 35 E. Wacker, Chicago 60601, 1986, \$46.95