NEW YORK HEART ASSOCIATION

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*The Mechanism of New Sarcomere Production in the Human Myocardial Cell**

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How the myocardial cell achieves normal growth in preadult life, how it repairs or replaces deteriorated contractile units, and how it hypertrophies in response to volume or pressure overloads are subjects of special interest.

The Z band, which delimits the sarcomere (the contractile unit of the myocardial cell) has always been thought to perform a purely mechanical function in cardiac tissue, i.e., to serve as an attachment site for thin filaments in the sarcomere.

When myocardial tissue obtained at the time of open heart surgery from 22 patients with ASHD, CHD, or RHD was examined in the electron miscroscope, it became apparent that Z substance is probably involved in the production of new sarcomeric units. Accumulation or hypertrophy of Z substance was a frequent finding in human myocardial cells, and in some of these areas the accumulated Z material had differentiated into a homógeneous mass of fine filaments embedded in an amorphous matrix. Fully differentiated thick and thin filaments were being spun off this mass of primary myofilaments and were being laid down in an orderly fashion to produce new sarcomeric units.

To support the thesis that Z-band material is the source of new sarcomeres in the cell, we examined the pattern of myofilament production in rat ventricular cells grown in tissue culture. In this preparation, configurations exactly analagous to those seen in human myocardium, in which accumulated Z substance was being transformed into myofilaments, were abundant. Twenty-four-hour old and presumably actively growing dog myocardium, which bridged the gap between the primitive cell grown in tissue culture and the adult myofiber, showed exactly similar patterns.

Z substance, then, is probably essential for the production of new sarcomeres in the cardiac cell and is the source of new contractile units in situations of myocardial growth, maintenance, and repair. *(Supported by a grant-in-aid from the New York Heart Association.)*

*This paper was selected by the Committee on the Scientific Session on Research of the New York Heart Association to receive the association's second annual J. Murray Steele Award, established in honor of the late J. Murray Steele, M.D., a director of the New York Heart Association, 1951-1964, for the best presentation before the Scientific Session on Research.

Ischemic Cardiac Metabolism in Man

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Patients with coronary artery disease were subjected to an ischemic stress by means of right ventricular pacing. Simultaneous sampling from the coronary sinus and left ventricle permitted evaluation of myocardial metabolism. Hemodynamic, EKG, and biochemical monitoring was performed during a resting (control) and paced (ischemic stress) state to the point of angina, or to a heart rate of 150-160. During constant infusion of ^{14}C-1-palmitate the concentrations of total FFA, ^{14}C-FFA, triglyceride, glucose, lactate, and $^{14}CO_2$ (arterial = A, coronary sinus = CS) were measured and expressed as % extraction $\left(\frac{\text{A-CS}}{\text{A}} \times 100\right)$ of % production.

The heart rate was increased from 10% to 100% (mean: 35%) and was associated with a 32% decrease in stroke-work index. The cardiac index, power index, and "effort" index increased with pacing in almost all patients, indicating increased cardiac external work and increased oxygen demand.

Pacing caused an immediate, significant increase in lactate production which was related to the severity of coronary artery disease as evaluated clinically (symptoms, EKG, coronary arteriogram, hemodynamic and symptomatic response to pacing). The enhanced lactate production persisted throughout the 15 to 20 minute stress period with an apparent balance between the increase in glucose extraction and lactate production. Free fatty acid (FFA) extraction and oxidation occurred at a normal rate during the control, resting state.

In the early stress period there was a significant mean decrease in FFA extraction of 33% and concomitant increase in ^{14}C-FFA oxidation of 25%, an effect opposite to that seen in experimental animals, suggesting that pacing in humans results in augmented oxidation of intracardiac ^{14}C-1-palmitate with a simultaneous reduction of extraction of exogenous palmitate. However, in the late stress period, the rate of FFA extraction and oxidation returned to normal, in contrast to lactate production, suggesting different regulatory controls for hypoxic human carbohydrate and lipid metabolism. Increased myocardial lactate production was the most sensitive metabolic measure of oxygen lack.

During infusion of labeled FFA the serum triglyceride fraction accumulated up to 26% of the ^{14}C, indicating endogenous esterification of circulating ^{14}C-FFA. This process permitted estimation of triglyceride extraction by the myocardium to a varying degree and suggests that triglyceride may serve as a significant, albeit limited, source of human cardiac substrate. *(Supported by the New York Heart Association, the National Institutes of Health [Research Grant HE-06216 from the National Heart and Lung Institute and Contract PH-43-67-1439] and the Muscular Dystrophy Associations of America, Inc.)*

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Hemodynamic Studies After Open Heart Surgery Utilizing Thermal Dilution

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The ability to monitor cardiac output continuously and easily is essential to the successful treatment of patients with a wide variety of serious conditions. At present all methods for measurements of cardiac output enjoying widespread use are cumbersome in comparison to thermal dilution, which provides a simple, accurate, and rapid technique.

A balloon-tipped 5 French double-lumen thermistor-tipped catheter (recently developed by myself) has now been passed, at the bedside, both percutaneously and via peripheral vein cut down in 10 patients undergoing a valve operation. Inflating the balloon with 0.5 cc. of air carries the tip of the catheter into the right ventricle and into the pulmonary artery without need for fluoroscopy. Continuous moni pressure curves indicates the] catheter. Coupled with this small portable computer whi direct readout of cardiac o minations were made using 7 t temperature saline as the indi as 3× per minute. Reproduci cellent; the difference betwe less than 200 cc. By obtainin ary artery and right ventric it was possible to calculate p cular resistance, right vent stroke volume, and stroke i have had catheters in positio hours without arrhythmias or due to the catheters.

The Mode of Action of the Natural Mitral Valve

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A technique has been developed for studying the natural mitral valve *in vivo*. In blocked and normal animals, phasic left atrial, left ventricular, and aortic pressures and phasic flows across mitral and aortic valves are recorded while filming movements of the mitral valve cusps, papillary muscles, and atrioventricular ring, made visible by small subendothelial injections of barium or radiopaque threads. By a synchronizing device, each frame of cine-film is related to a precise point on the pressure-flow records.

Separation of anterior and] and forward flow through t instantaneously with cross-ov lar and atrial pressures at th diastole. The atrioventricular opening of the valve reach early and together. Flow due increases after this time and mum when the gradient is m cusps are already closing. W traction, extra flow to 25% c

curs and the cusps reopen briefly. The cusps stay open, with forward flow continuing, after ventricular contraction starts. Closure occurs with minimal insufficiency just before aortic flow starts. Atrial, papillary muscle, and ring contraction did not seem critical to proper valve function. *(Supported by P.H.S. Research Grant HE11565 of the National Heart and Lung Institute, Bethesda, Md.)*

A Diagnostic Pattern of Nocturnal Solute Excretion in Pyelonephritis

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The development of a reliable method for quantitative bacterial study of the urine led to the discovery that bacteriuria is a widespread phenomenon in the asymptomatic population. Asymptomatic bacteriuria, detected on routine culture, is commonly encountered in school children, young women, pregnant women, patients with urologic anomalies, geriatric patients, and less commonly in others without specific complaints pointing to urinary infection. A crucial diagnostic problem in such cases is the differentiation of a lower urinary tract infection from a silent renal infection that may progress to chronic pyelonephritis. The ability to detect pyelonephritis at an early and possibly treatable stage could prevent the evolution of a chronic disease in those bacteriuric individuals harboring a silent renal infection.

The present study is aimed at the development of a simple test for the presence of pyelonephritis in the patient without the characteristic clinical, laboratory, and radiologic evidence seen in chronic infections with renal failure.

Daily solute-excretion patterns were studied in the urine of 7 biopsy or radiologically proved pyelonephritic patients without renal failure and 13 age- and sex-matched controls. Aliquots of each voiding were collected over a 24-hour period in otherwise uncontrolled subjects. Analysis of the electrolyte concentration and total osmolarity were plotted against time after awakening. The percentage of sodium concentration to osmolarity predictably fell during the last 6 sleeping hours in the control group as a result of the decrease in the glomerular filtration rate and increased aldosterone secretion. The mean fall was —1.306% per hour. However, the pyelonephritic group demonstrated a consistent increase during the same hours by a mean rise in the ratio of +1.059% per hour. The difference between overnight changes in the percentage ratio of sodium to osmolarity for the two groups is significant at the level $p < 0.001$. These results suggest a diminished tubular responsiveness to physiologic stimuli in the pyelonephritic group.

This marked difference from normal controls can be used as a very sensitive test in uncovering silent pyelonephritis. From the data a table has been constructed which enables the clinician to determine whether a given rate of overnight change in the percentage ratio of sodium to osmolarity is pyelonephritic or normal. A group of bacteriuric patients without clinical evidence of pyelonephritis is presently being studied. Fifty per cent of these patients are diagnosed as pyelonephritic according to the parameters derived from this study. *(Supported by Research and Development Fund No. 14-3040 of the Maimonides Medical Center, New York, N. Y.)*

Replication Rate of DNA in Reovirus-Infected Cells

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Reovirus, a cytocidal RNA virus, inhibits cellular DNA synthesis in tissue-culture cells without demonstrably blocking precursor synthesis or affecting the final size of newly-replicated DNA segments. This suggests that initiation or rate of replication may be inhibited by viral infection. In the present investigation we have examined the rate of replication of cellular DNA in reovirus-infected cells to determine whether this might be the mechanism of inhibition.

We infected mouse L cells in suspension culture with reovirus at a multiplicity of 200 PFU/cell. Seven hours after infection, we added 5-fluoro-2′-deoxyuridine (FUdr) to both infected and uninfected cultures to exhaust endogenous thymidine pools. One hour later, cells were pulse-labeled for 30 minutes with ^{3}H-thymidine. The labeled thymidine was chased with unlabeled thymidine for 10 or 20 minutes. Incorporation of exogenous thymidine during the pulse was inhibited over 70% in infected cells. We then isolated strands of cellular DNA for autoradiographic analysis. Measurement of linear segments of labeled DNA indicated that DNA chains were replicated at a rate of 0.6 micron/minute in both infected and uninfected cells during the pulse. DNA chains appeared to be replicated during the chase period at a rate of 1.0 micron/minute in both infected and uninfected cells. The difference in replication rates during the pulse and chase periods may reflect an *in vivo* difference due to the transient FUdr block, or it may be an artifact as the method of analysis during the pulse may select strands replicating at a slower rate. In either case, there is no difference between the rates of DNA replication in reovirus-infected and uninfected cells. Taken with evidence from earlier studies, our results suggest that reovirus infection inhibits cellular DNA synthesis by interfering with multifocal initiation of replication on chromosomal DNA. *(Supported by P.H.S. Research Grant A I-03445 of the National Institute of Allergy and Infectious Diseases, Bethesda, Md.)*

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Serum Digoxin Studies in Infants and Children

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It is well known that requirements for digoxin dosage are greater, on a mg./kg. basis, in infants than in older children or adults receiving this drug. To determine whether the increased dosage is reflected in increased serum levels, serum digoxin concentrations were studied by the radioimmunoassay method. Forty-three blood samples were obtained from 25 infants age 1 month to 11 months, and 40 blood specimens were collected from 24 children 2 to 14 years of age receiving digoxin therapy. Levels were obtained 6 to 24 hours after administration of a maintenance dose of

digoxin. Five of the infants and 6 of the older children displayed clinical evidence of digitalis toxicity.

Over 80% of the infants receiving standard maintenance therapy (0.015 to 0.028 mg./kg.) had digoxin levels >2.0 mμg./ml., a range which is commonly associated with toxicity in adults, whereas under 15% of the older children on routine daily dosage (0.01 to 0.02 mg./kg.) had serum concentrations above this value. The mean serum digoxin concentration in the nontoxic infants was 2.7 ± 1.2 mμg./ml., a value significantly greater than that obtained in the older children or adults ($P < 0.01$). The mean serum digoxin concentration in nontoxic children (1.4 ± 0.5 mμg./ml.) was identical to that observed in adults. Mean serum digoxin levels in the digitalis-toxic infants was 4.5 ± 2.5 mμg./ml. and in the toxic children, 2.9 ± 1.8 mμg./ml.

The data suggest that the infant not only tolerates a higher serum digoxin concentration without toxicity, but may require more digoxin for optimal inotropic effect. *(Supported by a grant-in-aid from the New York and American Heart Associations and by grants from the U.S. Public Health Service.)*

Orthotopic Transplantation of the Rat Heart and Lungs

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The authors have developed the first successful technique for the orthotopic transplantation of the rat heart and lungs. Cardiopulmonary bypass is not required; instead, hypothermia is utilized to provide the necessary 1 hour of cerebral anoxic time. Donor preparations are maintained as autoperfusing preparations and transplanted into cold-narcotized recipient rats. Microsurgical and microvascular surgical techniques are utilized for the operations and will be documented by a short film and appropriate slides.

Over 300 transplants were performed until a survivor was obtained. After fully awakening, 10 recipients have succumbed at 2½ hours to cerebral edema. A series of 25 hypothermia experiments has determined that steroids and cooling only to 15 C. will prevent the development of cerebral edema. On the respirator, recipients have now survived over 12 hours; spontaneous respirations are in the range of 20-30/min. In order to obtain long-term survivors in the face of this hypoventilation, we are now utilizing a radiofrequency electrophrenic nerve stimulator.

We anticipate utilizing this "micromodel," with its well defined genetics, to study the role of the chemical mediators of immediate hypersensitivity in allografted organ rejection. Isologous transplants will serve as controls for these investigations. *(Supported by NIH General Research Support Grant, Cornell University Medical College; Ethicon, Inc.—Microsurgery Research Grant; Carl Zeiss, Inc.—Dissecting Microscope.)*

Potassium Flux in the Heart and its Relation to Magnesium Iron

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Inhibition of potassium-induced vasoconstriction by magnesium has been demonstrated in peripheral vessels. To find a similar antagonism in potassium-related responses in the myocardium of dogs, ventricular fibrillation thresholds were determined for bolus injections of potassium (mEq.), volts of alternating current shock and duration (min.) of circumflex coronary occlusion alone and with simultaneous magnesium infusions (1.6 mEq./min.). Magnesium raised ventricular fibrillation thresholds an average of 3.4 mEq. for potassium injections, 4.25 volts for alternating current shock, and 7.75 min. for circumflex coronary occlusion ($P < 0.01$ in 12 exp.). Ventricular premature contractions and ventricular tachycardia caused by ouabain overdosage (1.25 to 2.15 mg.) were promptly reversed by intravenous magnesium (av. 15.75 mEq. in 4 exp.) to either nodal or sinus potassium. In 22 dogs coronary sinus-blood potassium was serially determined during alternating current shock, circumflex coronary occlusion, and vagus stimulation, and during graded administration of ouabain, epinephrine, and norepinephrine (1 ug./kg./min.), isoproterenol (1 ug./kg./min.) and magnesium. Alternating current shock, circumflex coronary occlusion, ouabain, epinephrine, and norepinephrine were associated with prompt coronary sinus-potassium increases (range 0.75 to 4.0 mEq./l. ($P < 0.001$ in 15 exp.). Infusions of magnesium (1.6 mEq./min.) with circumflex coronary occlusion, isoproterenol, and vagus stimulation caused immediate reductions in coronary sinus blood potassium 0.3 to 2.0 mEq./l. ($P < 0.001$ in 12 exp.). Coronary sinus-potassum values ranging from 5.0 to 8.0 mEq./l. associated with ouabain-induced ventricular premature contractions and ventricular tachycardia were rapidly lowered by magnesium infusions 1.75 to 3.5 mEq./l. approaching control potassium values. Simultaneous conversion to sinus or supraventricular rhythms was noted.

These data suggest that myocardial potassium efflux occurs in response to adrenergic stimulation and is a factor in the genesis of ventricular fibrillation due to myocardial ischemia, electroshock, and glycoside intoxication. Magnesium appears to reduce extracellular potassium and the development of ventricular premature contractions, tachycardia, and fibrillation. Cholinergic and beta stimulation also cause an intracellular $K+$ shift. *(Supported by P.H.S. Research Grant 301-FR-00503 from the Division of Research Facilities and Resources, Bethesda, Md. and a grant from G. D. Searle and Company.)*

Hypersensitivity Reactions of the Heart: Reduction of Anaphylactic Crisis by Theophylline or Glucagon

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Hypersensitivity reactions of the human heart can lead to arrhythmias and acute failure. As an experimental model, acute anaphylactic crises of graded intensity were induced in the isolated guinea pig heart. Impairment of cardiac function was correlated with the amounts of histamine released by the heart. Theophylline is known to inhibit the immunologic release of histamine from human leukocytes. The ability of theophylline to inhibit the immunologic release of histamine from cardiac tissue was studied with this model. Theophylline did not prevent the occurrence of the anaphylactic crisis, but it did diminish its intensity in a dose-dependent fashion. Increasing concentrations of theophylline counteracted the anaphylactic decrease in coronary flow and reduced the incidence and duration of arrhythmias of conduction. These changes were paralleled by a reduction in release of histamine from the heart. The possibility that these effects were mediated by increased intracellular levels of cyclic AMP (3′,5′-cyclic adenosin-monophosphate) due to inhibition of its metabolism by theophylline, was indirectly tested by comparison with glucagon, which is also known to increase intracellular cyclic AMP in cardiac cells, through activation of the adenylcyclase system. Glucagon also inhibited the immunologic release of histamine in a dose-dependent fashion, improved coronary flow, and reduced the incidence of arrhythmias. On the basis of these experiments I suggest that cyclic AMP modulates the immunologic release of histamine from heart mast cells. These observations should prove useful in the management of patients with cardiac failure and arrhythmias due to hypersensitivity reactions. *(Supported by the New York Heart Association.)*

Physiologic Evaluation of the Renal Cadaver Allograft

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Short-term kidney preservation for subsequent transplantation after tissue typing and recipient selection is now clinically possible by hypothermic perfusion with type-specific cryoprecipitated human plasma after the method of Belzer. While on the preservation system, physiological evaluation of allograft quality is also possible by monitoring alterations in flow, pressure, and *p*H as well as by determining changes in the potassium concentrations or enzyme levels in the perfusate.

Twenty human kidneys collected from 11 cadaver donors have been studied for 4 to 72 hours while on the perfusion system at a pulse rate of 60 and a systolic pressure initially set at 60 mm. Hg. Flow was thus dependent on kidney size and on renal vascular resistance. In all instances of a viable organ, the pressure fell, with a widening pulse pressure within 1 to 2 hours after placement in the preservation unit. Flow usually increased as the pressure fell.

When flow was less than 50 cc. per minute from a kidney on the preservation unit the *p*H usually fell, and could not be corrected by adding bicarbonate to the perfusate,

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which is indicative of anaerobic metabolism and lactic acidosis. Likewise SGOT and LDH levels as well as K^+ rise under such circumstances.

By using these parameters for a viable organ, only 15 of the 20 kidneys perfused were considered satisfactory physiologically for subsequent transplantation; an additional 2 were not used because there was no matched recipient. The 5 considered unsatisfactory for transplantation were irretrievably damaged by the agonal state secondary to hypovolemia, oliguria, use of vasopressors, or to excessively long warm ischemic time (from cardiac arrest to removal of the kidneys and placement in iced saline solution).

Of the 13 kidneys selected for transplantation, all but one subsequently regained normal renal function; 6 exhibited a period of acute renal failure predictable on the basis of flow-pressure relation. Six more showed prompt return of renal function in that posttransplant dialysis was not necessary. One failed to function for reasons not clear but probably not related to the preservation. One was considered only a fair result because of complications related either to the preservation or to the problems of reimplantation.

In considering the short-term results (6 to 9 months), 12 of 13 patients are alive (92%); 10 of 13 kidneys are functioning and sustaining life (77%). One patient succumbed to infection with a functioning allograft for a graft survival rate of 85% at the present time. *(This investigation was supported by an anonymous grant to the hospital.)*

Serial Serum Digitalis Levels in States of Altered Myocardial Metabolism

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Digitalis toxicity may be due to excessive myocardial concentrations of the drug or to changes in the myocardial toxic threshold secondary to disease or altered myocardial metabolism. To assess the possible role of digitalis in the etiology of arrhythmias, serial serum-digoxin or digitoxin levels were measured by specific radioimmunoassays (standard deviations $\pm 5\%$). Studies in patients with presumed normal myocardial metabolism established toxic thresholds. Thus in 27 control subjects with digitoxin-induced arrhythmias mean serum digitoxin level was 58 ng./ml. (range: 41 to 99 ng./ml.). In 54 control subjects with digoxin-induced arrhythmias the mean serum-digoxin level was 3.2 ng./ml. (range: 2.2 to 6.0 ng./ml.).

Twenty patients receiving maintenance digoxin who underwent cardiopulmonary bypass for a variety of cardiac surgical procedures were studied. Eight developed arrhythmias 2-18 hours following bypass. Mean serum digoxin level was 1.3 ng./ml. (range: 0.9-1.5 ng./ml.). Twelve experienced no arrhythmias, mean serum-digoxin level was 0.7 ng./ml. (range: 0.3-1.4 ng./ml.) ($P < 0.001$). Of 17 patients who were hospitalized with acute myocardial infarction and who were receiving maintenance digoxin at the time of onset, 11 developed arrhythmias in the first 24 hours. Mean serum-digoxin level was 1.2 ng./ml. (range: 1.0 to 1.7 ng./ml.). Six patients experienced no arrhythmias and mean serum digoxin was 0.5 ng./ml. (range: 0.3 to 0.6 ng./ml.) ($P < 0.001$). In 14 patients receiving maintenance digitoxin, 10 developed arrhythmias in the first 24 hours after onset of acute myocardial infarction when mean serum-digitoxin level was 29 ng./ml. (range: 23-34 ng./ml.). Four patients had no arrhythmias, mean serum digitoxin level was 15 ng./ml. (range: 12 to 18 ng./ml.)

It is concluded that alterations in myocardial response in the early hours after cardiopulmonary bypass or acute myocardial infarction may lower the toxic threshold to digitalis. *(Supported by P.H.S. MIRU Contract PH 43-67-1439 and N.I.H. Fellowship 5 F03 HE 44354-02.)*

Interaction of Sarcomere Length and Excitation-Contraction Coupling in Ventricular Myocardium

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Mechanisms governing the triggering and duration of the active state in ventricular muscle have been considered to vary independently of those responsible for the length-tension relation. Recently attention has been called to the fact that the duration of ventricular contraction does, in fact, increase, together with force development, when muscle length is altered. In 15 experiments on excised frog ventricle strips, progressive increase in length of muscles and contractile force resulted in a progressive increase in time-to-peak of contraction at a bath temperature of 27°C. The magnitude of the increase in time-to-peak was between 20 and 50% on the ascending portion of the length-tension curve. In 8 experiments, transmembrane action potentials were recorded after graded changes in muscle length. A small degree of stretch produced a moderate change in time-to-peak (+14%), with a slight change in 90% repolarization time (+11%). Optimal amounts of stretch resulted in larger increases in time-to-peak (+33%), with a comparable lengthening in action potential duration (+50%). Preliminary observations in cat papillary muscle (29°C.) showed only a slight increase in 90% repolarization time (+10%), when the increase in time-to-peak was very much greater (+46%). The precise mechanism of this "back-coupling" between the contractile process and excitation of membranes is not yet clear. The effect on the mammalian ventricular action potential is dramatically smaller than that on the amphibian one. This finding is compatible with the present view that intracellular stored calcium plays a larger role in determining the time course of contraction in mammalian than in amphibian ventricular muscle, which has a poorly developed sarcotubular system. *(Supported by P.H.S. Research Grant 5 R01 HE 12774 from the National Heart and Lung Institute, Bethesda, Md.)*

A Comparison of the Effects of Temperature on the Metabolic Activities of the Isolated, Perfused Dog Kidney at 10° and 37°C.

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At present many systems of renal preservation employ hypothermic perfusion to insure viability of the organs. In order to investigate the factors influenced by hypothermia, the metabolism of isolated dog kidneys was studied when perfused at 10° or at 37° C. on the Belzer Organ Preservation System. Hourly samples of plasma perfusate were analyzed for significant changes in carbohydrate and lipid products, as well as the enzyme lactic dehydrogenase, electrolyes, and *p*H during the course of the 6-hour perfusion.

We have observed the following to occur during perfusion at 37°. There is a marked increase in lactic acid (~ 170 mg.%) together with a decrease in glucose levels (~ 145 mg.%). The *p*H drops rapidly to as low as 7.0, and this is difficult to control. Perfusate levels of the intracellular enzyme, lactic dehydrogenase, increase markedly (> 500 International Units). Potas-

sium levels in the perfusate also show a steady increase from 4.8 to 6.6 mEq./l. All of these parameters are without significant change at 10°. Pyruvate levels are not different at the two temperatures.

At 37°, total nonesterified free fatty acid levels show a slow but steady rise (> 140 μmoles/l.). However, the utilization of labeled palmitic acid-1-C^{14} is rapid and the radioactivity decreases by greater than 75%. Both free fatty acid and palmitic acid levels are stable at 10°. Triglyceride levels are not different at the two temperatures.

These observed differences must reflect a more active metabolic state at 37° C. Energy demands exceed the constant oxygen content dissolved in the plasma perfusate, resulting in anaerobic metabolism. The nonvolatile lactic acid cannot be removed by the Belzer Apparatus, leading to acidosis, disruption of cell metabolism, and release of LDH and K^+.

These studies suggest the possibility that hypothermic organ preservation may not require processed plasma perfusate as is currently employed in cadaver-organ procurement, and further suggest that future study in the evaluation of anaerobic metabolities, as well as intracellular enzymes, may prove to be accurate parameters of organ viability. *(Supported by P.H.S. Training grant FR-05396-09 from the Division of Research Facilities and Resources.)*

Digitalis Intoxication Studied with Microelectrode Techniques

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Considerable information concerning digitalis glycosides has been obtained through standard microelectrode studies of isolated canine Purkinje fibers (PF) utilizing physiologic salt solutions as a perfusate. It is uncertain to what extent results obtained by these methods reflect the *in vivo* effects of digitalis. A recently developed technique permits isolated PF to be perfused in a tissue bath with arterial blood from heparinized donor dogs. The blood is then returned to the donor. The effects of intravenously administered drugs on donor and PF may thus be studied simultaneously. This technique was used to evaluate the effects of ouabain upon electrophysiologic properties of PF. While isolated PF were stimulated at rates varying from 60 to 150 beats/minute, ouabain was infused into normokalemic donors until electrocardiographic evidence of toxicity developed. At slower rates of stimulus, small changes in electrophysiologic properties of the PF were noted. At stimulus rates approximating donor heart rate (120 to 150/minute), these changes, including loss of action potential (AP) amplitude and resting membrane potential, shortening of AP duration and phase-2 repolarization, and decrease in rate of rise of phase 0 of the AP were more marked. The conduction time increased. Significant automaticity developed infrequently. In other experiments, hearts from ouabain-toxic donors were removed when ventricular premature contractions or ventricular tachycardia occurred and their PF were studied in modified Tyrode solution. Changes in AP characteristics, as described above, were noted. Disturbances of conduction occurred, but there was no significant automaticity. These experiments suggest: 1) rate of development of digitalis toxicity in PF is related to cardiac rate, 2) ouabain levels causing ventricular arrhythmias in intact normokalemic dogs are associated with changes in AP characteristics and conduction, and less frequently with altered automaticity in PF. *(Supported in part by grant from the New York Heart Association.)*

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Renal Mechanism for DOCA Escape in Man

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To determine the renal mechanism responsible for increased sodium excretion during "DOCA escape" in man, proximal and distal reabsorption of sodium were estimated during maximal water diuresis. Fractional reabsorption of sodium in the proximal tubule was calculated from the filtered load of sodium and the rate of delivery to the distal portion of the nephron. The amount of sodium delivered to the distal tubule and its fractional reabsorption were estimated from the excretion of free water, sodium, potassium, and hydrogen ions. Four normotensive males acting as their own controls were studied prior to the administration of DOCA and during "escape." In each of four paired studies, free water clearance increased above control values (31%, 45%, 60%, 130%). The filtered load of sodium was unchanged in one and increased in three (11%, 17%, 29%). There was a marked increase in distal delivery of sodium (50%, 54%, 56%, 172%). The absolute proximal reabsorption of sodium was increased somewhat in two studies, but fractional reabsorption decreased in all. Absolute reabsorption of sodium in the distal nephron increased markedly while distal fractional reabsorption decreased in all. Absolute reabsorption of sodium in the distal nephron increased markedly while distal fractional reabsorption decreased in all four patients. The data suggest that increased distal delivery of sodium is responsible for DOCA escape, although it is not possible to choose between increased filtered load or decreased relative proximal reabsorption as the primary mechanism. Paradoxically, distal sodium reabsorption is increased during DOCA escape, as evidenced by the marked increase in free water clearance. *(Supported by P.H.S. Research Grant 5-R01-HE-03272 from the National Heart and Lung Institute, Bethesda, Md.)*

Slow Conduction, Reentry, and the Mechanism of Ventricular Arrhythmias in Myocardial Infarction

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Myocardial infarction deprives cardiac tissue of adequate perfusion, thus depressing excitability. Reentrant arrhythmias were therefore studied by deliberately impairing excitability in bundles and networks of canine Purkinje fibers. Action potentials were recorded by intracellular microelectrodes. Excitability was depressed by encasing part of a bundle or network in agar containing high K^+, by exposing part of a bundle to 12 to 16 mM K^+ in the middle chamber of a three-chamber bath,

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or by exposing an entire bundle or network to high K^+ and epinephrine. Reentry was shown to depend upon slow conduction and one-way block in depressed areas. In slow conduction the normal action potential is replaced by a fundamentally different electrical response. In one type of reentry, which we call reflection, the impulse reaching a depressed area turns back in the direction from which it came. Reflection causes coupled beats identical with the ventricular extrasystoles of myocardial infarction and with echo beats or return extrasystoles. Another type of reentry occurs when a bundle terminates in a closed loop containing a site of slow conduction at one end and a site of entry block at the other end, permitting a true circus movement around the loop and back into the bundle. This circus occurs in a loop less than 1 cm. long because of the greatly lowered conduction velocity. Multiple circuits around a loop, or reflection alternating with a circus movement gave rise to sustained tachycardias in which a single impulse was followed by over 100 reentrant impulses. The results prove that reentrant arrhythmias arise from slow conduction and provide a powerful tool for study of the origin and treatment of reentrant arrhythmias. *(Supported by the New York Heart Association and P.H.S. Research Grant HE 119904-03 from the National Heart and Lung Institute, Bethesda, Md.)*

Effects of Hypertension and Its Reversal on the Female Rat Aorta

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The effects of hypertension and its reversal on the wall of the thoracic aorta of the female rat were studied. Hypertension was induced by clipping a renal artery and reversed by opening the clip. Hypertension of 10-weeks' duration resulted in increased aortic diameter, wall thickness, tangential tension, and medial area compared to normotensive vessels. Absolute amounts of elastin, collagen, and noncollagenous, alkali-soluble protein were significantly increased in the walls of hypertensive vessels. In association with the decrease of calculated tension and wall stress to normotensive levels for an additional 10-week period after reversal of hypertension, thickness of the wall and medial area returned fully to control values. Absolute amounts of noncollagenous, alkali-soluble protein also returned to control values after reversal of hypertension, but elastin and collagen persisted in elevated amounts; this resulted in an above-normal concentration of these fibrous elements in the wall. Comparison of amounts of fibrous components in vessels subjected to sustained and interrupted hypertension indicated a close relation between duration of hypertension and duration of accelerated fibrous protein synthesis and accumulation. On the basis of this finding, the reduction of blood pressure might be expected to influence favorably the progression of arteriosclerotic cardiovascular disease but not to reverse disease already established. *(Supported by P.H.S. Research Grant HE 12766 from the National Heart and Lung Institute, Bethesda, Md. and Grant U-2041 of the New York Health Research Council.)*