INFLUENCE OF THE AUTONOMIC NERVOUS SYSTEM AND CARDIAC DRUGS UPON MYOCARDIAL GLYCOGEN METABOLISM

by

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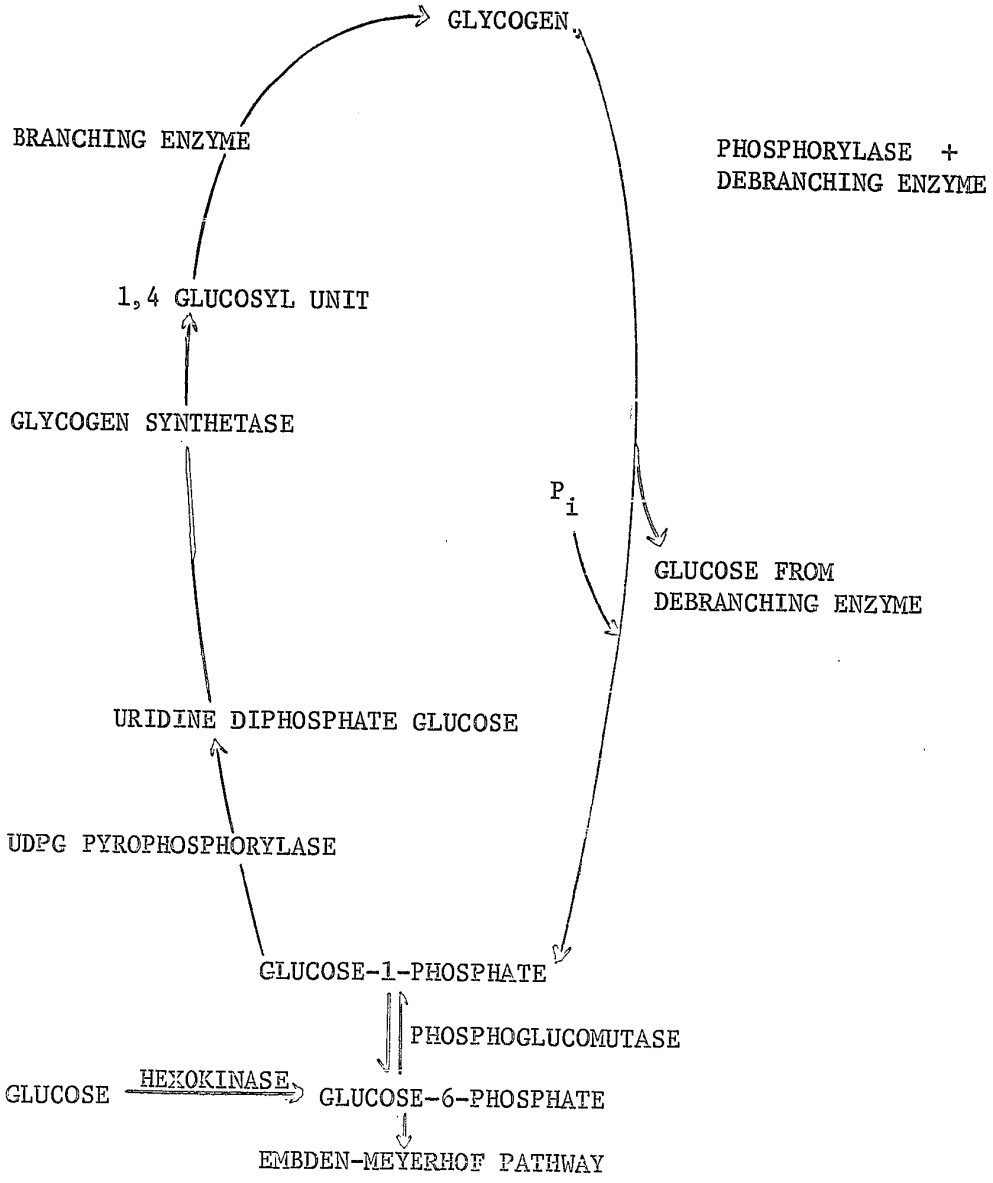
LIST OF ABBREVIATIONS

kg	kilogram
mg	milligram
ml	milliliter
min	minute
µg	microgram
ECG	electrocardiogram
mm	millimeter
sec	second
Hg	mercury
UTP	uridine triphosphate
G-1-P	glucose-1-phosphate
PHOS	Phosphorylase
UDPG	uridine diphosphate glucose
PP	pyrophosphate
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
M	molarity
R.P.M.	revolutions per minute
hr	hour
U-P-P^{32}-P^{32}	radioactively-labelled uridine triphosphate
GAL-1-P	galactose-1-phosphate

Gly. Syn.	Glycogen synthetase
g	gravitational force
Tris	Tris-hydroxymethyl-aminomethane
mM	millimolar
mμ	millimicrons
UDP	uridine diphosphate
PEP	phosphoenolpyruvate
PY	pyruvate
DNPH	dinitrophenylhydrazine
Na_2EDTA	Disodium Ethylenediamine Tetraacetate
N	normality
P_i	inorganic phosphate
fig.	figure
SA	sino-atrial
A-V	atrio-ventricular
CKTS	centistokes, (viscosity index)
V	volts
CPS	cycles per second
msec	millisecond
gm	gram
P R	Prosthetic group removing
D C I	dichloroisoproterenol
ATP	adenosine triphosphate
ADP	adenosine diphosphate

I INTRODUCTION

Glycogen is the principal storage form of carbohydrate in animal tissue and constitutes a major source of energy in the myocardium. The process of cardiac glycogen metabolism may be summarized thus:



Phosphorylase is the rate-limiting enzyme in glycogenolysis. Under normal conditions, the enzyme is present in two forms; an active form, designated phosphorylase a, and an inactive form, designated phosphorylase b (Fischer and Krebs, 1955; Rall et al, 1956). The activity of the a form increases during enhanced glycogen degradation. Similarly, glycogen synthetase, the rate-limiting enzyme involved in glycogenesis, exists in an active I and an inactive D form (Villar-Palasi and Larner, 1960b: Roselle-Perez et al, 1962). The enzyme uridine diphosphate glucose pyrophosphorylase is also involved in glycogenesis but is not rate-limiting (Villar-Palasi and Larner, 1960a; Williams and Mayer, 1966).

The demonstration by Green and Cori (1943) that increased contractility of skeletal muscle resulted in enhanced phosphorylase activity has created interest in the relationship between levels of enzyme activity and augmented muscle contractility. In the myocardium, catecholamine administration or sympathetic nerve stimulation have been reported to increase phosphorylase a activity, (Mayer and Moran, 1960). However, the influence of cardiac sympathetic nerve excitation upon the activities of glycogen synthetase and uridine diphosphate glucose pyrophosphorylase has not been previously described in the literature. Thus, in the first series of experiments, changes in glycogen metabolism and total cardiac glycogen levels in response to electrical stimulation of cardiac sympathetic

nerves were investigated. In order to correlate these changes with alterations in cardiovascular functions, concurrent changes in heart rate, contractile force, blood pressure and electrocardiograms were also studied.

The parasympathetic nervous system is believed to oppose the action of the sympathetic nervous system in the myocardium. Hess et al (1961a, 1961b, 1962b) have reported that temporary cardiac arrest induced by either acetylcholine administration or vagal excitation depressed phosphorylase a activity. The influence of this procedure upon concurrent alterations in the glycogen synthesizing enzymes and total cardiac glycogen concentrations of the myocardium has not been previously examined. In the second series of experiments, the resultant effects of cardiac slowing and temporary arrest upon glycogen metabolism and associated cardiovascular parameters were investigated.

The precise mechanism by which cardiac glycosides exert their positive inotropic and toxic actions is not known. Increases, decreases as well as no change in cardiac glycogen metabolism have been reported following their administration (Kimura, 1948; Belford and Feinlieb, 1959; Mayer and Moran, 1960; Yusuf and Gans, 1966; Kypson et al, 1968). The prevention and treatment of cardiac glycoside intoxication is a major problem in medicine. Consequently, any agent which supresses the vulnerability of the heart to digitalis-induced arrhythmias is of interest for potential

clinical use. Lidocaine, a local anesthetic agent, has gained prominence as a potentially useful agent in the management of cardiac dysrhythmias induced by cardiac glycosides (Katz and Zitnik, 1966; Morrow and Bosomworth, 1967). Its mechanism of antiarrhythmic action does not appear to be related to a myocardial depressant action (Mendez and Kabela, 1970). In the final series of experiments, alterations in cardiac glycogen metabolism induced by ouabain and lidocaine were determined. The influence of lidocaine administration upon ouabain-induced changes in cardiac glycogen and dysrhythmias was also assessed.

It was hoped that the results obtained might yield additional knowledge on the regulation of cardiac glycogen metabolism and the relationship between myocardial glycogen levels and cardiac glycoside action.

II HISTORICAL REVIEW

A. Studies on Uridine Diphosphate Glucose Pyrophosphorylase Activity

The presence of uridine diphosphate glucose pyrophosphorylase (UDPG pyrophosphorylase) was first demonstrated in brewer's yeast by Trucco (1951). Munch-Petersen et al (1953), observed that when uridine diphosphate glucose, inorganic pyrophosphate, Tris buffer (pH 8.0), magnesium chloride and Zwischenferment (glucose-6-phosphate dehydrogenase) were incubated, glucose-1-phosphate and uridine triphosphate resulted. Similarly, the incubation of glucose-1-phosphate, Tris buffer (pH 8.0), magnesium chloride and radioactive-labelled uridine triphosphate ($U\text{-}P\text{-}P^{32}\text{-}P^{32}$) resulted in the formation of uridine diphosphate glucose and radioactive pyrophosphate, according to the following reaction:

$$UTP + G\text{-}1\text{-}P \xrightleftharpoons{\text{UDPG Pyrophosphorylase}} UDPG + PP$$

It was demonstrated by Strominger et al (1957) that the incubation of uridine diphosphate glucose with glycine buffer (pH 8.7), nicotinamide adenine dinucleotide and a liver extract resulted in the formation of uridine diphosphate glucose glucuronic acid, hydrogen ions and reduced nicotinamide adenine dinucleotide, according to the reaction:

$$\text{UDPG} + \text{NAD} \xrightarrow{\text{UDPG Dehydrogenase}} \text{NADH} + \text{H}^{+} + \text{UDPG Glucuronic acid}$$

This reaction is catalyzed by the enzyme uridine diphosphate glucose dehydrogenase and can be measured spectrophotometrically at 340 mμ. It is the method in current use for the enzymatic assay of UDPG pyrophosphorylase.

The presence of UDPG pyrophosphorylase in cardiac muscle and other tissues such as liver, diaphragm, brain and skeletal muscle was demonstrated by Villar-Palasi and Larner (1958, 1960a, 1961). When the supernatant, obtained after centrifugation of homogenates at 100,000 x g for 60 min, was incubated with a mixture of uridine triphosphate, magnesium chloride, nicotinamide adenine dinucleotide, glucose-1-phosphate, glucose-6-phosphate and glycylglycine buffer (pH 7.4), there was a resultant formation of pyrophosphate. These observations were confirmed by Kalckar and Maxwell (1958) and Williams and Mayer (1966).

It was observed that the activity of UDPG pyrophosphorylase was 10 - 20 times greater than glycogen synthetase activity (Villar-Palasi and Larner, 1960a). This was also confirmed by Williams and Mayer (1966) who demonstrated that when epinephrine (10 μg/kg) was administered intravenously to open-chest rats, and the heart removed within 15 sec after drug administration, the activity of UDPG pyrophosphorylase was greater than glycogen synthetase activity. It would appear from these observations that UDPG pyrophosphorylase is not a rate-limiting enzyme in glycogen synthesis.

B. Glycogen Synthetase Activity and Its Alterations by Catecholamines

The presence of the enzyme glycogen synthetase was first demonstrated by Leloir and Cardini (1957). By employing a technique which determined the net synthesis of glycogen as well as the spectrophotometric formation of uridine diphosphate from the substrate uridine diphosphate glucose, it was demonstrated that when a rat liver extract was incubated with uridine diphosphate glucose, glycogen, tris-hydroxymethyl-aminomethane buffer (pH 7.4), and ethylenediamine tetraacetate (at 37°C for 45 min), there was a resultant formation of uridine diphosphate and net glycogen, according to the following reaction:

$$\text{UDPG} + \text{Glycogen} \xrightarrow{\text{Glycogen Synthetase}} \text{UDP} + (\text{Glycogen} + 1)$$

This reaction occurred only when glycogen was present in the incubation mixture (Steiner et al, 1961).

Glycogen synthetase is present in the brain (Breckenridge and Crawford, 1960; Basu and Bachhawat, 1961); myocardium (Hauk and Brown, 1959); and skeletal muscle (Villar-Palasi and Larner, 1958; Robbins et al, 1959; Hauk and Brown, 1959; Kornfeld and Brown, 1962).

The activity of glycogen synthetase; i.e., the ability to form glycogen from the substrate uridine diphosphate glucose, is

enhanced in the presence of glucose-6-phosphate. Thus, Steiner et al, (1961) demonstrated that when glucose-6-phosphate was added to an incubation mixture containing a homogenate of rat liver or heart, there was an enhanced synthesis of glycogen. When an excess of nicotinamide adenine dinucleotide was added to promote a rapid oxidation of glucose-6-phosphate, no net synthesis of glycogen occurred. It was therefore suggested that glucose-6-phosphate may be a necessary cofactor for glycogen synthetase activity.

The enzyme exists in two forms, i.e. an active and an inactive form. This was first demonstrated using a rat diaphragm preparation by Villar-Palasi and Larner (1960b). These investigators observed that when insulin was added to an incubation mixture lacking glucose-6-phosphate, enhanced glycogen synthetase activity, as well as glucose-6-phosphate concentrations, resulted. When the enzyme was precipitated, and its activity measured in the absence of glucose-6-phosphate, enhanced activity without concurrent formation of glucose-6-phosphate was observed. It appeared that whereas insulin could increase the activity of a form of glycogen synthetase, which did not require glucose-6-phosphate, a form which requires glucose-6-phosphate for its activity was not influenced by insulin. From these observations, it was suggested that glycogen synthetase might exist in two forms, and an interconversion between the two forms might occur. The presence and differentiation

of these two forms of glycogen synthetase was clearly demonstrated by Roselle-Perez et al, (1962) using rat skeletal muscle. The tissue was homogenized followed by centrifugation (8000 x g for 40 min), precipitated with ammonium sulphate, dialyzed against Versene, lyophilized to a powder and then incubated with mercaptoethanol (30°C for 60 min), to produce one form of the enzyme. When this form was incubated in the absence of glucose-6-phosphate, an increase in its activity was observed. The other form of the enzyme, requiring glucose-6-phosphate as a cofactor, was obtained when frozen muscle tissue was stored for 12 weeks in a deep freeze, homogenized and then centrifuged (100,000 x g). The form not requiring glucose-6-phosphate as a cofactor was designated as glycogen synthetase independent or I form, while the form requiring glucose-6-phosphate was designated glycogen synthetase dependent or D form.

Glycogen synthetase I can be converted to glycogen synthetase D and vice-versa. This was demonstrated by Friedman and Larner (1963). Glycogen synthetase I, obtained from rat skeletal muscle, when incubated with ATP and magnesium chloride (at 30°C for 20 min), was inactive in the absence of glucose-6-phosphate. When P^{32}-labelled ATP was incubated with glycogen synthetase I, the radioactivity was associated with a protein possessing glycogen synthetase D activity. If the purified enzyme (purified 800-fold by a phosphocellulose column) was incubated with ATP and magnesium

chloride, its activity was present in the absence of glucose-1-phosphate, suggesting that a phosphoprotein kinase, present in the crude form, was necessary for its conversion to glycogen synthetase D. The interconversion of glycogen synthetase D to glycogen synthetase I was also studied. When P^{32}-labelled glycogen synthetase D was incubated with a crude extract of rat skeletal muscle (30°C for 25 min) in the absence of glucose-6-phosphate, increased activity resulted, suggesting a conversion from the D to the I form. The radioactive phosphate released could be precipitated as inorganic phosphate using magnesia mixture. This indicated that a dephosphorylation had occurred. The reactions involved are summarized in the following equations:

$$(1) \quad \text{Gly. Syn. I} + n\text{ATP} \xrightarrow[\text{Transferase Kinase}]{Mg^{++}} \text{Gly. Syn. D} + n\text{ADP}$$

$$(2) \quad \text{Gly. Syn. D} \xrightarrow{\text{transferase I phosphatase}} \text{Gly. Syn. I} + nP_i$$

Catecholamines influence glycogen synthetase activity. This was first demonstrated by Belocopitow (1961). When a homogenate of hemidiaphragm from a fasted (3 hr) rat was incubated with epinephrine (8.2×10^{-5}M at 37°C for 30 min), uridine diphosphate glucose and glucose-6-phosphate, there was a resultant decrease in both glycogen and uridine diphosphate concentrations; i.e., total

glycogen synthetase activity was lowered. The effects of epinephrine were enhanced, when ATP, magnesium chloride and cyclic 3',5'-AMP were included in the incubation mixture.

Craig and Larner (1964) using rat hemidiaphragm preparations observed that epinephrine (6.0×10^{-5}M) decreased glycogen synthetase I as well as total glycogen synthetase (I + D) activity. With a concentration of 5.5×10^{-6}M epinephrine, glycogen synthetase I activity was reduced while total glycogen synthetase activity remained unchanged. It was concluded that at high concentrations of epinephrine, the activity of both forms of the enzyme were decreased, while at lower concentrations, a conversion of the active to the inactive form occurred.

Williams and Mayer (1965, 1966, 1967) studied the effects of epinephrine upon glycogen synthetase activity in skeletal muscle "in vivo". When a rapid intravenous injection of epinephrine (1, 2.5 or 10 µg/kg) was administered to rats and the gastrocnemius muscle rapidly frozen (15 - 20 sec after injection) "in situ" using modified Wollenberger tongs cooled in liquid nitrogen, there was a significant decrease in the percentage of glycogen synthetase I activity, while the total glycogen synthetase level was unchanged. It was suggested that epinephrine induced a conversion from glycogen synthetase I to glycogen synthetase D.

Catecholamines also influence cardiac glycogen synthetase activity. In the investigations of Robison et al, (1965)

norepinephrine (20 μg) was administered to isolated, perfused rat hearts. Maximal increases in contractile force (40%) occurred within 20 sec after drug administration but no associated alterations in glycogen synthetase activity could be demonstrated. Huijing (1966) using an identical procedure confirmed these observations.

Williams and Mayer (1965) studied the effects of epinephrine upon glycogen synthetase activity in the open-chest rat heart preparation. It was observed that epinephrine (2.5 or 10 μg/kg) induced maximal increases in arterial pressure associated with a marked increase in glycogen synthetase I activity, but no change in total glycogen synthetase (I + D) activity. It was concluded that epinephrine induced a conversion from the D to the I form in the intact rat heart preparation.

In a continuation of these studies Williams and Mayer (1966), determined the duration of the epinephrine-induced increases in glycogen synthetase I activity by means of continuous infusion studies. Cardiac samples were taken at various intervals ranging from 30 sec to 10 min during the infusion. When epinephrine (1.0 μg/kg/min) was infused, glycogen synthetase I increased from 22% to 32% within 2 min, but decreased to control values within 10 minutes. Samples of skeletal muscle taken concurrently demonstrated a decrease from 22% to 8% in glycogen synthetase I activity within 2 min, and this level was maintained throughout the

infusion. A larger dose (2.5 μg/kg/min) induced an initial increase in cardiac glycogen synthetase I activity (from 21% to 32% within 1 min), followed by a decrease to below control values (13%) within 10 minutes. Pretreatment with pronethalol (20 μg/kg) prevented the epinephrine-induced changes in glycogen synthetase I activity. Williamson (1966) confirmed the observation that epinephrine (0.05 μg/ml) could produce a biphasic response in glycogen synthetase activity, i.e. an initial increase followed by a decrease below control levels, in the perfused working rat heart. In addition, Belford and Cunningham (1968) demonstrated that all three catecholamines increased glycogen synthetase I activity without affecting total glycogen synthetase (I + D) activity in the open-chest cat heart preparation.

In summary then, it has been reported that (1) glycogen synthetase is present in mammalian tissue, (2) the enzyme exists in two forms, both of which can be interconverted, and (3) in skeletal muscle, catecholamines decrease the level of glycogen synthetase I by causing its conversion to glycogen synthetase D, while in cardiac muscle catecholamine administration increases the level of glycogen synthetase I activity.

C. Alterations in Glycogen Phosphorylase Activity Induced by Catecholamine Administration and Autonomic Nervous System Excitation

The presence of phosphorylase in mammalian skeletal muscle was first demonstrated by Cori and Cori (1936). When rat skeletal muscle was incubated in a phosphate buffer (pH 7.4 at 20°C for 60 min), to which adenylic acid was added, a new phosphorylated ester resulted. The ester was later identified as glucose-1-phosphoric acid. It was suggested that adenylic acid was an essential mediator in the transfer of inorganic phosphate to the carbohydrate molecule in this reaction. More extensive investigations led Cori et al (1939) to the discovery that skeletal muscle, heart, brain and liver, contained an enzyme which was capable of forming glucose-1-phosphate from glycogen and inorganic phosphate. When a homogenate of rabbit tissue was incubated with phosphate buffer (pH 7.2), glycogen, adenylic acid and magnesium chloride at 25°C for 60 min, there was a resultant disappearance of glycogen with a concurrent increase in glucose-1-phosphate. The enzyme was designated glycogen phosphorylase, and was thought to be involved in the following reaction:

$$\text{Glycogen} + P_i \xrightarrow[\text{adenylic acid}]{\text{phosphorylase}} \text{G-1-P}$$

These results were confirmed by Shapiro and Wertheimer (1943) who demonstrated that the enzyme was also present in adipose tissue, skin, lung, testes and the placenta of animals. Glycogen phosphorylase activity was measured as the amount of inorganic phosphate liberated when a tissue, containing the enzyme, is incubated with glucose-1-phosphate and glycogen at 25°C for several incubation periods; i.e., phosphorylase activity was measured in the direction of glycogen synthesis in the following reaction:

$$\text{Glycogen}_N + \text{G-1-P} \rightleftharpoons P_i + \text{Glycogen}_{N+1}$$

Adenylic acid was a necessary cofactor and markedly enhanced the activity of phosphorylase.

Green and Cori (1943) using an extract of rabbit skeletal muscle, produced crystalline phosphorylase which was found to possess activity in the absence of adenylic acid. It was designated active phosphorylase or phosphorylase *a*. However, these investigators also observed that skeletal muscle tissue possessed a more soluble, amorphous protein, which, although it could not be crystallized, had the same activity as phosphorylase *a* in the presence of adenylic acid. It was therefore suggested that phosphorylase existed in two forms in skeletal muscle tissue; an active form which does not require adenylic acid (phosphorylase *a*), as well as an inactive form requiring

adenylic acid and designated phosphorylase b. Phosphorylase b was later crystallized by Cori and Cori (1945), as well as by Fischer and Krebs (1958).

Phosphorylase a can be interconverted to phosphorylase b by phosphorylase phosphatase. Cori and Green (1943) found that when phosphorylase a, obtained by direct ammonium sulphate precipitation of rabbit skeletal muscle tissue, was left standing at room temperature, enzymatic activity was present only in the presence of adenylic acid. It was also demonstrated that an enzyme, designated the prosthetic group-removing (PR) enzyme, was necessary for the conversion. By means of P^{32}-labelled phosphorylase a, Fischer et al (1957), and Krebs and Fischer (1960) demonstrated that the PR enzyme produced a phosphopeptide, which resembled phosphorylase b in molecular weight and activity, as well as inorganic radioactive phosphorous. The PR enzyme was therefore renamed phosphorylase phosphatase. The reaction involved may be summarized thus:

$$P^{32}\text{-phos. a} + 4H_2O \xrightarrow[\text{phosphatase}]{\text{phosphorylase}} 2 \text{ phos. b} + 4 \text{ inorganic } P^{32}$$

Similar observations were reported by Rall et al (1956) using cardiac tissue.

Phosphorylase b can also be converted to the a form by the enzyme phosphorylase b kinase (Fischer and Krebs, 1955; Krebs and

Fischer, 1956; Rall et al, 1956; Krebs and Fischer, 1960) according to the following reaction:

$$2 \text{ phos. } \underline{b} + 4\text{ATP} \xrightarrow[\text{phos. } \underline{b} \text{ kinase}]{Mg^{++}} \text{phos. } \underline{a} + 4 \text{ ADP}$$

A mechanism of phosphorylase interconversion which involves calcium has also been suggested to occur during excitation-contraction coupling according to the following reaction:

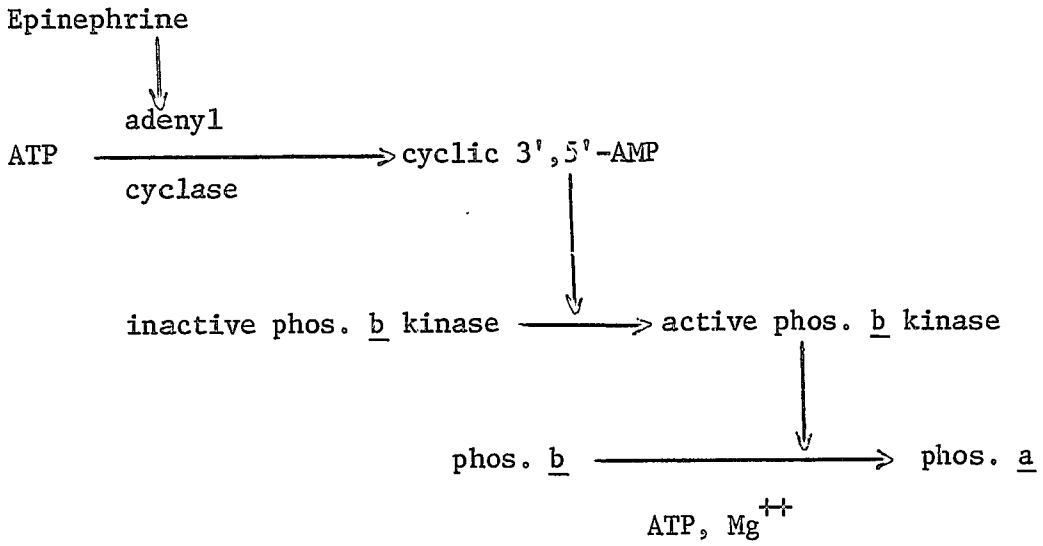
$$\text{phos. } \underline{b} \xrightarrow[Ca^{++}\text{-activating factor}]{Ca^{++}} \text{phos. } \underline{a}$$

(Krebs et al, 1959; Meyer et al, 1964; Caputto et al, 1967). Since this form of phosphorylase a cannot be converted to the b form, and total glycogen depletion has not been demonstrated to occur with repetitive stimulation of skeletal muscle (Staneloni and Piras, 1969), this mechanism seems unlikely.

The effects of exogenous epinephrine administration upon phosphorylase activity have been extensively studied. Sutherland (1951) demonstrated that when rat diaphragms were incubated at 37°C for 20 min in a phosphate buffer, and epinephrine (0.3 µg) was added to the incubation medium, an increase in phosphorylase activity in the absence of adenylic acid resulted. However, the total

phosphorylase (phos. a + phos. b) activity, measured in the presence of adenylic acid, remained unchanged indicating that epinephrine not only caused an increase in phosphorylase a activity, but also caused a conversion of phosphorylase b to phosphorylase a. This observation was confirmed in the myocardium by Williamson and Kreiberg (1963).

The mechanism by which epinephrine increases phosphorylase a activity was elucidated by Rall and Sutherland (1958, 1960). They observed that when a liver homogenate was incubated for 10 min at 37°C with epinephrine (5.5 µg/ml), there was an increase in phosphorylase a activity as well as a concurrent increase in the level of the nucleotide cyclic 3',5'-adenosine monophosphate. Posner et al (1962) demonstrated that epinephrine (10 µg/kg) administered to rats, followed by removal of the gastrocnemius muscle, induced increases in the activity of phosphorylase b kinase and phosphorylase a, as well as in the level of cyclic 3',5'-adenosine monophosphate. From these observations it was concluded that epinephrine increased phosphorylase activity through an indirect mechanism according to the following scheme:



This mechanism was shown in the liver by Haynes et al (1960), in skeletal muscle by Krebs and Fischer (1962) and Bowness (1966), and in cardiac tissue by Øye et al (1964).

Kukovetz et al (1959) demonstrated that administration of either epinephrine, norepinephrine, or isoproterenol in doses ranging from 1 µg to 6 µg to isolated, perfused rat hearts, increased both phosphorylase a activity and cardiac contractile force. Methoxamine administration induced no alteration in either contractile force or phosphorylase a activity. The order of potency in augmenting contractile force as well as phosphorylase a activity were isoproterenol, norepinephrine and epinephrine respectively. These observations have been confirmed by the investigations of Hornbrook and Brody (1963) and Hess et al (1958, 1959, 1962a).

On the other hand, Mayer and Moran (1960) could not demonstrate any correlation between positive inotropism and elevated

phosphorylase a levels in the open-chest dog heart preparation following catecholamine administration. Administrations of epinephrine, norepinephrine and isoproterenol (5 µg/kg of each) produced a significant increase in contractile force without concurrent alterations in phosphorylase a activity. When epinephrine (10 µg/kg/min) was administered in a continuous infusion (Mayer et al, 1961, 1963), the increase in contractile force was maintained while phosphorylase a activity rose initially (within 1 min) and then fell to control values before the end of the infusion.

Similarly, Robison et al (1965) using isolated, perfused rat hearts demonstrated that epinephrine (20 µg) increased contractile force maximally (37% above control) within 20 sec, while phosphorylase a activity reached its maximum (from 18% to 66%) 45 sec after epinephrine administration. The study of Belford and Feinlieb (1959) supports the concept that there is a dissociation between the influence of epinephrine upon contractile force and phosphorylase a activity. When sodium pentobarbital (50 - 100 µg/ml) was introduced into a perfusion chamber containing a guinea-pig auricle, the contractility of the atria was depressed (-72%) while phosphorylase a activity remained unchanged.

Phosphorylase isolated from skeletal and cardiac muscle responds differently to epinephrine administration. Williams and Mayer (1966), using an open-chest rat heart preparation,

demonstrated that a rapid intravenous administration of epinephrine (10 μg/kg) resulted in a marked activation of phosphorylase *a* in skeletal muscle but had no significant effect upon cardiac muscle phosphorylase *a*. When epinephrine (1.0 μg/kg/min) was infused and biopsies were taken at periods ranging from 0.5-10 min during the infusion, phosphorylase *a* activity was found to be increased (from 27% to 62%) within 1 min in skeletal muscle, and remained at the maximal level throughout the infusion. On the other hand, heart muscle phosphorylase *a* increased (from 9% to 34%) within 1 min and returned to control values within 5 minutes. This was later confirmed by Kennedy and Ellis (1969) using a similar technique.

Alterations in the activity of the autonomic nervous system profoundly influence cardiac glycogen metabolism. Mayer and Moran (1960), using open-chest dogs, demonstrated that stimulation of postganglionic cardiac sympathetic nerves induced maximal increases in contractile force (90% above control) and phosphorylase *a* activity (from 20% to 60%) within 45 sec after the onset of the stimulus.

Similarly, Hess *et al* (1961a, 1961b, 1962b), using open-chest rat heart preparations, demonstrated a decrease in phosphorylase *a* activity following acetylcholine (50 μg/kg) administration, as well as following temporary cardiac arrest induced by vagal stimulation.

Summarizing the literature reviewed, it appears that phosphorylase, similar to glycogen synthetase, exists in two forms, an active a and an inactive b form. Catecholamine administration as well as excitation of the cardiac autonomic nervous system influences myocardial phosphorylase activity. Since concurrent alterations in the glycogen synthesizing enzymes were not reported, a more systematic evaluation of the influence of cardiac sympathetic and parasympathetic nerve excitation upon myocardial glycogen metabolism is desirable.

D. Cardiac Drugs and Myocardial Glycogen Metabolism

The mechanism by which cardiac glycosides exert their positive inotropic, as well as toxic action, is not well understood. Libert (1946) and Levy et al (1946) demonstrated that when either ouabain or digitalis (50-100 γ) was incubated with guinea pig or rat heart slices in a Ringer solution (pH 7.2), there was an increase in oxygen consumption (oxygen consumed mm^3/mg/hr). Wollenberger (1947) also observed an increase in oxygen consumption when guinea-pig ventricular slices were incubated with ouabain (2×10^{-7}M) in the presence of glucose. It was concluded that cardiac glycosides enhanced the utilization of glucose.

There is no agreement on the influence of cardiac glycoside administration upon cardiac glycogen levels. Cherkes and Angarskaya (1939) observed that strophanthin administration to open-chest rats increased the glycogen content in the heart. However, when a toxic dose was administered, glycogen depletion occurred. Kypson et al (1968) demonstrated that incubation of ouabain (10^{-4}M) or digitoxin (10^{-5}M) with rat diaphragm muscle, increased the glycogen concentration. It was also observed in this study that the cardiac glycosides were capable of inhibiting epinephrine (10^{-5}M) and cyclic 3',5'-adenosine monophosphate (10^{-3}M) induced glycogenolysis. In contrast, Kimura (1948) observed that in fasted rats, digitoxin administration (12 mg/kg for 3 days) produced no significant change

in cardiac glycogen levels, while Yusuf and Gans (1966) demonstrated that incubation of dog apical slices with ouabain (2.5×10^{-9} moles/ml) resulted in a decrease in the glycogen content as compared to control non-treated animals.

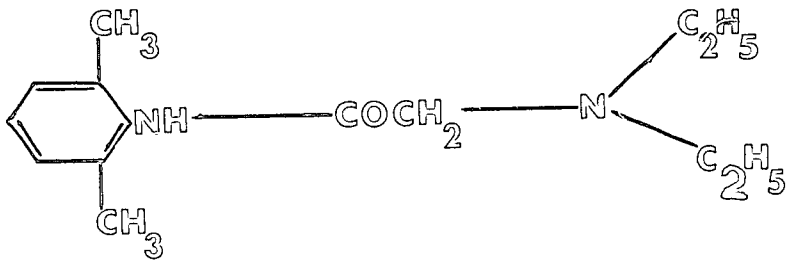
Belford and Feinlieb (1959) demonstrated that K-strophanthin administration ($1.5 - 2.5 \times 10^{-6}$M) to isolated, perfused auricles induced an increase in both contractile force (110% above control) and phosphorylase *a* activity (from 18% to 36%). A larger dose ($2.0 - 3.5 \times 10^{-6}$M), decreased contractile force (-70%) but increased phosphorylase *a* activity (from 5% to 23% above control level). In another series of experiments digitoxin (0.5 mg/kg every 5 min) was administered to open-chest cats until signs of cardiac arrhythmias occurred. An increase in phosphorylase *a* activity (from 48% to 55%) was observed. On the other hand, Mayer and Moran (1960) administered ouabain (0.075 mg/kg) over a 5 - 10 min period to open-chest dogs until signs of arrhythmias occurred. It was observed that ouabain elevated cardiac contractile force (30% above control), while phosphorylase *a* activity remained unchanged.

Arese *et al* (1967) suggested that ouabain may produce its action at the enzymatic level. When ouabain (1 ml of 1% solution) was administered via the inferior vena cava to frogs, and the hearts frozen in liquid nitrogen within 50 sec, there was a decrease in the substrate glucose-1-phosphate, while the concentration of the metabolite glucose-6-phosphate was elevated. It was suggested

that glucose-1-phosphate, derived from glycogen, was being converted to glucose-6-phosphate by phosphoglucomutase, indicating that ouabain might be enhancing phosphorylase a activity.

In summary, it is seen from a review of the literature that there is no agreement on the influence of cardiac glycosides upon cardiac glycogen content. While most investigators favour a decrease, especially following a toxic dose, increases as well as no change have also been reported.

Lidocaine (2-diethylamino-2',6'-acetoxylidide), a synthetic local anesthetic with the following structure:



has gained popularity as an antiarrhythmic agent, and has its greatest use in the treatment of acute arrhythmias (Southward, 1950; Carden and Steinhaus, 1956; Hitchcock and Keown, 1959; Weiss, 1960). It is at present a choice drug in antagonizing digitalis intoxication (Mendez and Kabela, 1970). Katz and Zitnick (1966) demonstrated that lidocaine (2 mg/kg) converted ouabain-induced ventricular tachycardia to a normal sinus rhythm in dogs. In addition, Morrow and Bosomworth (1967) demonstrated that when ouabain

(36 mg/kg/hr) was administered to dogs, ventricular tachycardia occurred within 109 minutes. Lidocaine (1 mg/kg) administration, following 1-2 min of sustained ventricular tachycardia, resulted in the emergence of a nodal rhythm within 20 seconds. These results were confirmed in human studies by Grossman et al (1968), who observed that arrhythmias induced by digitalis (0.4 mg/kg) could be converted to normal sinus rhythm by lidocaine (1.5 mg/kg) administration. Beiliveau and Covino (1968) studied the effects of lidocaine administration upon carbohydrate metabolism. When rat ventricular slices were incubated with lidocaine (25 µg/ml), a resultant decrease in glycogen content was demonstrated after a 10 min incubation period. Whether or not this is related to its antiarrhythmic action is not known.

More extensive investigations on the influence of this agent, upon the cardiac glycogen metabolizing enzymes, are therefore necessary before a final assessment of the underlying mechanisms by which it antagonizes digitalis intoxication can be made.

III. METHODS AND MATERIALS

A. Techniques for Recording Blood Pressure and Heart Contractions and for Sampling Cardiac Muscle

All experiments were performed on mongrel dogs of either sex weighing 10-20 kg. The animals were anesthetized with pentobarbital sodium (Nembutal 30 mg/kg) and artificially respired with room air, (20 ml/kg at a rate of 20 strokes/min) using a Harvard (model 607A) respiratory pump. After cannulation of a femoral artery and vein, the chest was opened medially through a sternal splitting incision and excessive bleeding was controlled by electrocoagulation using a National (200 watt) cautery. The rib cage was retracted and the heart was suspended in a pericardial cradle. A Walton-Brodie strain guage arch was sutured to the apex of the left ventricle for recording changes in cardiac contractile force. The muscle segment was stretched 30-50% of its normal length, or with a tension of 10 grams, by pulling the movable arm of the strain guage arch. This procedure reduced the relative importance of local bulging of the muscle segment, introduced by changes in the venous return and arterial pressure. The electrocardiogram (ECG, Lead II) was recorded in each experiment and used to determine the heart rate as well as to determine any disturbances in the normal cardiac rhythm. Arterial blood pressure was recorded from the cannulated femoral artery by the use of a catheter (PE 205) attached to a Stratham (model P23AC) transducer. The catheter was filled with heparinized saline

(10 units/ml) and maintained free of blood. Concurrently, blood pressure, ECG and cardiac contractile force were recorded either on a Grass (model 5D) or a Sanborn (model 964) 4 channel polygraph, at a paper speed of 25 mm/second.

Cardiac incisional biopsies (approximately 25 mg/samples) were taken from the right ventricle by means of modified Wollenberger tongs and immediately placed in liquid nitrogen. These samples were assayed at the end of each experiment for the presence of cardiac glycogen metabolizing enzymes (see Section B).

B. Technique for Assaying Glycogen Metabolizing Enzymes and Glycogen

1. Assay for Uridine Diphosphate Glucose Pyrophosphorylase

The principle of the assay for UDPG pyrophosphorylase is based on the method of Williams and Mayer (1966). This enzyme catalyzes the reaction:

$$UTP + G\text{-}1\text{-}P \rightleftharpoons UDPG + PP$$

The UDPG thus formed acts as a substrate in a coupled reaction in which uridine diphosphate glucose dehydrogenase oxidizes UDPG in the presence of the cofactor nicotinamide adenine dinucleotide (NAD). The amount of reduced nicotinamide adenine dinucleotide formed is a measure of the enzymatic activity according to Bergmeyer (1963). The formation of UDPG is expressed in terms of the number of micromoles of UDPG/gm tissue/minute.

To each cardiac sample, potassium chloride (0.15M at pH 7.4) was added in a volume of 10 ml/25 mg of tissue. This was followed by homogenization at 2°C for 1 min using a Fisher homogenizer at a speed of 1200 R.P.M. The homogenate was centrifuged at 2400 x g for 10 min, using an International Clinical Centrifuge. A volume of 0.2 ml of the supernatant was added to a mixture of Tris-hydroxymethyl-aminomethane (Tris) buffer (0.4M at pH 7.4), glucose-1-phosphate (8 mM), magnesium chloride (50 mM), potassium

fluoride (70 mM), uridine triphosphate (8 mM) and glass distilled water to make a final volume of 1 milliliter. The mixture was incubated for 20 min at 30°C, and the reaction was terminated by placing the incubate in boiling water for 90 sec, and was then cooled in ice for 1 minute. The tubes containing the incubate were then centrifuged at 2400 x g and 2°C for 10 minutes. A volume of 0.5 ml of the resultant supernatant was added to a tube containing 0.47 ml Tris buffer (0.1M at pH 8.7), 0.02 ml nicotinamide adenine dinucleotide (21 mg/ml solution), and 0.01 ml uridine diphosphate glucose dehydrogenase (25,000 units/ml) to make a final volume of 1 milliliter. The reaction:

$$\text{UDPG} + 2\text{NAD} \xrightarrow{\text{UDPG Dehydrogenase}} 2\text{NADH} + 2\text{H}^{+} + \text{UDPG Glucuronic acid}$$

was allowed to proceed for 10 min following which time the optical density was read at a wavelength of 340 mμ in a Unicam Spectrophotometer. Blanks were treated in an identical manner, except that the supernatant was added after the incubation period.

2. Assay for Glycogen Synthetase

The principle of the assay for glycogen synthetase is based on the methods of Leloir and Goldemberg (1960), Basu and Bachhawat (1961), and Danforth (1965). This enzyme catalyzes the reaction:

$$\text{UDPG} + \text{Glycogen} \longrightarrow \text{UDP} + (\text{Glycogen} + 1)$$

The UDP formed can be reacted with phosphoenolpyruvate (PEP) in the presence of pyruvate kinase to yield pyruvate (PY), which is then reacted with 2,4-dinitrophenylhydrazine (DNPH) to form an orange red hydrazone of pyruvate. The amount of hydrazone formed is representative of the amount of UDPG converted into glycogen/gm tissue/hour. The essentials of this reaction can be expressed thus:

(a) $$\text{UDP} + \text{PEP} \xrightarrow{\text{pyruvate kinase}} \text{PY} + \text{UTP}$$

(b) $$\text{PY} + \text{DNPH} \longrightarrow \text{hydrazone of pyruvate}$$

Under normal conditions in the tissue, only a portion of the total glycogen synthetase is active. This is usually designated as glycogen synthetase I.

To each sample of cardiac tissue was added 1 ml/25 mg tissue of a solution containing Tris buffer (50 mM at pH 7.5), 2 mM disodium ethylenediamine tetraacetate (Na_2EDTA) and sodium fluoride (20 mM). The mixture was homogenized at 1200 R.P.M. and $2^{\circ}C$ for 90 seconds. The homogenate was then centrifuged at 1800 x g and $2^{\circ}C$ for 15 minutes. The resultant supernatant was utilized in the assay of glycogen synthetase I, total glycogen synthetase (I + D)

as well as in preparing blank samples.

To determine glycogen synthetase I, 0.05 ml of the supernatant was added to a tube containing Tris buffer (150 mM at pH 7.5), magnesium chloride (37.5 mM), disodium ethylenediamine tetraacetate (30 mM), 3.0% purified glycogen, cysteine base (2.5 mM), uridine diphosphate glucose (9.0 mM) and glass distilled water to make a final volume of .13 ml. In assaying for total glycogen synthetase a volume of 0.05 ml of supernatant was added to a tube containing the same reagents as for glycogen synthetase I except that in addition glucose-6-phosphate (10 mM) was included. Mixtures to be assayed for glycogen synthetase I and total glycogen synthetase were incubated at 37°C for 30 minutes. The enzymatic reaction was stopped by placing the tubes in boiling water for 2 minutes. Blanks were treated in an identical manner except that UDPG and glucose-6-phosphate were omitted from the incubation mixture and the supernatant was added to the mixture after the incubation period. All tubes were cooled in ice for 1 min, and phosphoenolpyruvate (1.0 mM) in potassium chloride (1.5 M) solution as well as pyruvate kinase (0.5 mg protein/ml) were added. The mixture was incubated for 15 min at 37°C and the reaction was stopped by adding 0.15 ml of cold 0.1% 2,4-dinitrophenylhydrazine in 1N hydrochloric acid containing 0.5N trichloracetic acid. After 5 min, the solutions were made alkaline by adding 0.2 ml sodium hydroxide (10 N). To each

sample was then added 1 ml of 95% ethyl alcohol, the mixture shaken, and then centrifuged at 3000 R.P.M. for 15 min using a Sorvall centrifuge. The optical density of the supernatant solution was read in a Unicam Spectrophotometer at a wavelength of 520 mμ. The percentage of glycogen synthetase I activity was calculated thus:

$$\frac{\text{glycogen synthetase I}}{\text{total glycogen synthetase}} \times 100 = \% \text{ glycogen synthetase I}$$

where % glycogen synthetase I refers to the percentage of the total glycogen synthetase which is active.

3. Assay for Glycogen Phosphorylase

The principle of the assay of glycogen phosphorylase is based on the method of Mayer et al (1963). The basic reaction is:

$$\text{G-1-P} + \text{Glycogen}_N \rightleftharpoons \text{Glycogen}_{N+1} + P_i$$

The liberation of inorganic phosphate (P_i) is indicative of glycogen phosphorylase activity which may be calculated as the number of micromoles of P_i released/gm tissue/minute. In the presence of excess G-1-P at pH 6.8, the formation of glycogen is favoured.

The enzyme was assayed in the direction of glycogen synthesis. Under normal physiological conditions only a fraction of the total phosphorylase is active. This is usually designated as phosphorylase a.

The frozen heart tissue was homogenized at 1200 R.P.M. in a solution of sodium fluoride (0.5M), and Na_2EDTA (1.0M) at pH 6.7 and at a concentration of 25 mg tissue/10 milliliters. The homogenate was centrifuged at 2400 x g and 2°C for 15 minutes. Both phosphorylase a and total phosphorylase were assayed. To determine phosphorylase a, the assay mixture consisted of purified G-1-P and purified glycogen at pH 6.1. Total phosphorylase contained, in addition to the above substrates, adenosine monophosphate (AMP) at pH 6.1. A volume of 0.05 ml of tissue supernatant was added to both phosphorylase a and total phosphorylase mixtures followed by incubation at 30°C for 10 minutes.

The reaction was stopped by adding 1 ml of cold phosphate reagent (2.5% ammonium molybdate, 1% copper sulphate, Buell's* reducing agent and glass distilled water). Blanks were prepared in an identical manner as the samples for total phosphorylase except that the phosphate reagent was added after 10 min of incubation following which time the supernatant was added to the

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* Buell's reducing agents consist of 95% sodium bisulphite, 5.4% sodium sulphite and 0.5% 1-Amino-2-Napthol-4-Sulphonic Acid.

mixture. After the incubation was stopped the tubes were shaken and kept at 22°C for 20 minutes. The optical density of the solution was then read in a Unicam Spectrophotometer at a wavelength of 700 mμ. The reference solution was glass distilled water to which was added phosphate reagent. The percent of phosphorylase a activity was calculated thus:

$$\frac{\text{phosphorylase a}}{\text{total phosphorylase}} \times 100 = \% \text{ phosphorylase } \underline{a}$$

where % phosphorylase a refers to the percentage of the total phosphorylase which is active.

4. Assay for Glycogen

Cardiac biopsies were immediately placed in hot 30% (100°C) KOH solution, until the tissue was dissolved. The solution was cooled, and glycogen was precipitated with 0.05 ml saturated Na_2SO_4, followed by 1.5 ml of ethanol (95%). The precipitate was then warmed (75°C) for 10 min followed by centrifugation (4000 R.P.M. for 30 min) using a Sorvall (model RC2-B centrifuge). The resultant precipitate was assayed for glycogen according to the anthrone method of Steifer et al (1950). The value of glycogen was calculated as mg/gm of tissue.

C. Technique for Electrical Stimulation of the Cardiac Autonomic Nerves

The autonomic nerve supply to the dog's heart is illustrated in Fig. 1. The major sympathetic and parasympathetic nerves arise as postganglionic fibres beyond the level of the stellate ganglion. The cranial cardiosympathetic (cardioaccelerator) nerve innervates the sinoatrial (SA) node and possibly the atrioventricular (AV)node. Its stimulation increases heart rate and AV conduction without influencing cardiac contractile force. The middle cardiosympathetic (cardioaugmentor) nerve is distributed almost exclusively to the ventricles. When stimulated, cardiac contractile force increases while heart rate and AV conduction are unaffected. The cardiovagal nerve supplies the atria and SA node. Stimulation of postganglionic branches of this nerve induces cardiac slowing, or temporary cardiac arrest.

In each experiment the nerve was isolated and desheathed by blunt dissection and then placed on bipolar platinum electrodes. To prevent spread of electrical current during stimulation, this preparation was placed in a pocket formed from the surrounding fascial tissue, and covered with Mineral Oil (Light mineral oil, 40 CKTS). Electrical stimulation was effected by the use of a Grass (model S5) stimulator. The stimulus characteristics chosen were: 5V, 5 cps frequency, and 2 msec duration for 20 seconds. At this voltage temporary cardiac arrest occurred during cardiovagal excitation. In order to produce cardiac slowing the stimulus

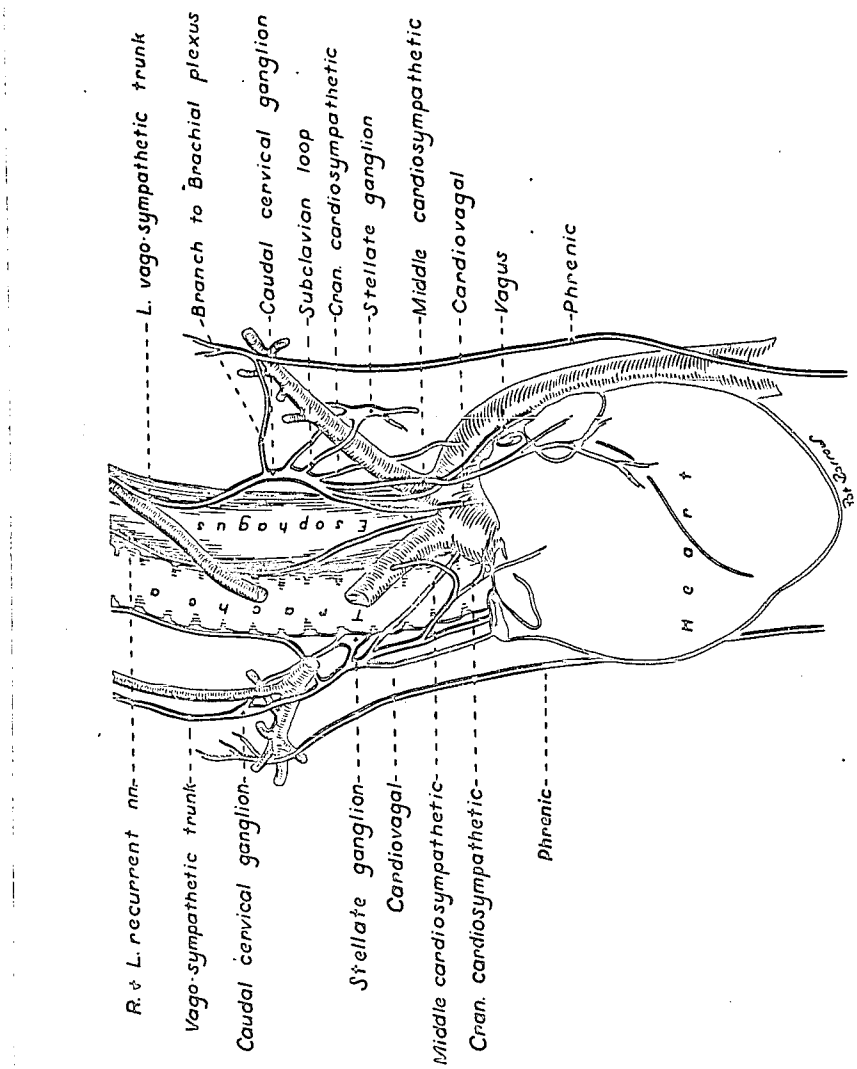

Fig. 1 The Autonomic Innervation of the Dog's Heart.

intensity was reduced until a decrease in the heart rate of 40-50 beats/min occurred (1-2V). When necessary, the stimulus was repeated at 20 min intervals.

D. Reagents and Drugs Used

1. Potassium chloride supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.
2. TRIS - hydroxymethyl-aminomethane (Trizma(R) Base) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.
3. Magnesium Chloride supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.
4. Uridine 5' - Triphosphate (sodium salt) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.
5. Potassium Fluoride supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.
6. α-D-Glucose-1-Phosphate (dipotassium salt) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.
7. β-Diphosphopyridine Nucleotide (β-DPN: β-NAD) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.
8. Uridine 5'-Diphosphoglucose Dehydrogenase was supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.
10. Disodium Ethylenediamine Tetraacetate supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.
11. Sodium Fluoride supplied by Nichols Chemical Co. Ltd., Montreal, Canada.
12. Cysteine supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.

13. Uridine 5'-Diphosphoglucose (sodium salt) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.

14. D-Glucose-6-Phosphate (disodium salt) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.

15. Phospho (Enol) Pyruvic Acid (trisodium salt) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.

16. Pyruvate Kinase (crystalline suspension in $(NH_4)_2SO_4$) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.

17. 2,4-Dinitrophenylhydrazine supplied by Eastman Organic Chemicals, Rochester, New York, U.S.A.

18. Hydrochloric Acid supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.

19. Trichloroacetic Acid supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.

20. Sodium Hydroxide (pellets) supplied by Nichols Chemical Co. Ltd., Montreal, Canada.

21. Adenosine 5'-Phosphoric Acid (AMP) supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.

22. Ammonium Molybdate supplied by J.T. Baker Chemical Co., Phillipsburg, New Jersey, U.S.A.

23. Cupric Sulphate supplied by Nichols Chemical Co. Ltd., Montreal, Canada.

24. Sodium Bisulphite supplied by Mallinckrodt Chemical Works, Montreal, Canada.

25. Sodium Sulphite supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.

26. 1-Amino-2-Naphthol-4-Sulphonic Acid supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.

27. Mineral Oil (Light USP) supplied by Noco Drugs Ltd., Toronto, Canada.

28. Potassium Hydroxide supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.

29. Sodium Sulphate supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.

30. Anthrone supplied by Fisher Scientific Co., Fair Lawn, New Jersey, U.S.A.

31. Oubain supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

32. Lidocaine Hydrochloride supplied by K and K Laboratories, Incorporated, Plainview, New York, U.S.A.

33. Heparin (sodium) supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

34. Pentobarbital sodium (Nembutal$^{(R)}$) supplied by Abbot Laboratories Ltd., Montreal, Canada.

Each drug investigated was freshly prepared in physiological (0.9%) saline and was administered intravenously as a single injection, and washed in with a constant volume of 3 ml of saline.

Statistical procedures employed are according to Steel and Torrie (1960). Results were considered significant if they exceeded the 95% probability level ($P < 0.05$), where an asterisk denotes significance. In each series of experiments the data presented represents the mean values $\pm$ the standard errors of 3 to 6 experiments.

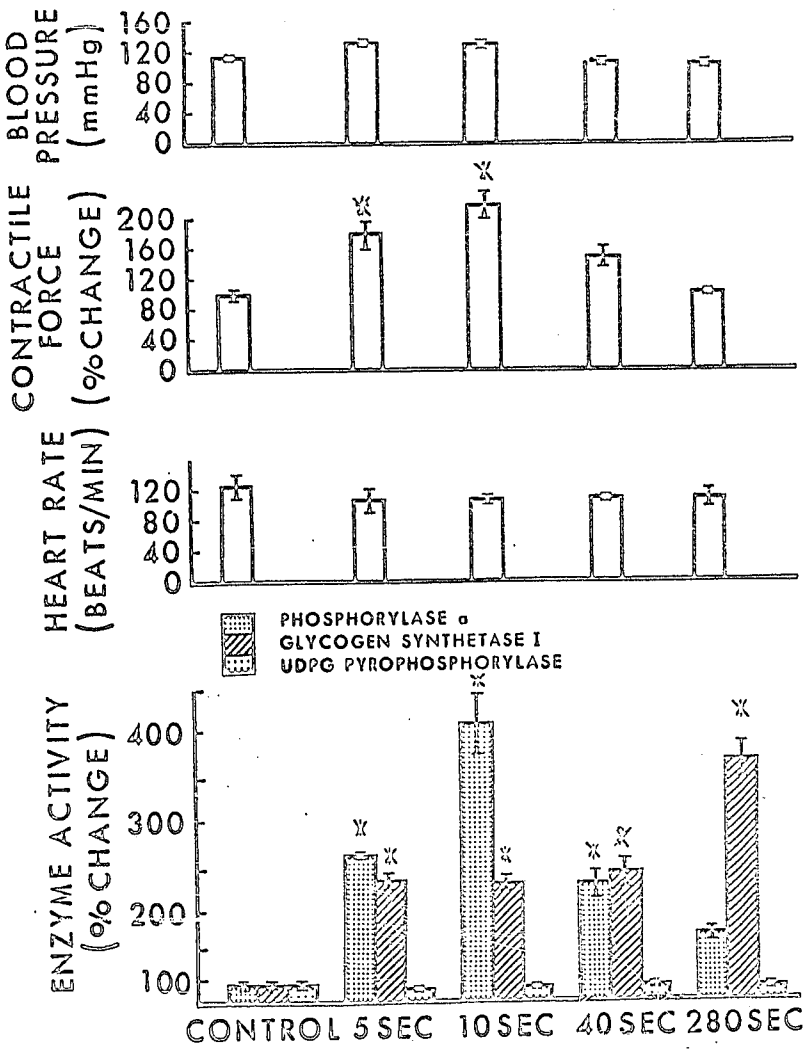

Fig. 2 Concurrent changes in phosphorylase a, glycogen synthetase I, UDPG pyrophosphorylase, heart rate, contractile force and blood pressure induced by medial cardiosympathetic nerve stimulation. Values shown at 5 and 10 sec were taken during stimulation, while values shown at 40 and 280 sec were taken after cessation of the stimulus. Control values of contractile force and enzyme activities were taken as 100%. Values are mean ± S.E. of 6 experiments and * denotes significance at the 95% confidence level.

IV RESULTS

A. Influence of Cardiosympathetic Nerve Stimulation Upon Cardiac Glycogen Metabolism, Blood Pressure, Contractile Force, Heart Rate and ECG

1. Influence of Medial Cardiosympathetic Nerve Excitation

The medial cardiosympathetic nerve primarily innervates the left ventricle (Miller, 1962). In the first series of experiments, this nerve was stimulated for 20 sec and biopsies were taken during stimulation (at 5 sec and 10 sec), and at periods of 40 and 280 sec after cessation of the stimulus. The results obtained are summarized in Figure 2.

Within 5 sec after the onset of stimulation, there was a significant increase in the activity of phosphorylase a (275 $\pm$4%), which reached a maximum value of 451 $\pm$ 44% within 10 seconds. This was followed by a decline in activity as stimulation was discontinued. Samples of cardiac tissue taken 40 and 280 sec after cessation of the stimulus yielded values of 242 $\pm$ 19% and 186 $\pm$ 8% respectively.

Concomitant with the changes in phosphorylase a activity, the level of glycogen synthetase I activity was also dramatically altered by electrical stimulation. It can be readily observed that within 5 sec after the onset of stimulation, the activity of this enzyme increased to a maximum of 238 $\pm$ 13%. This increased

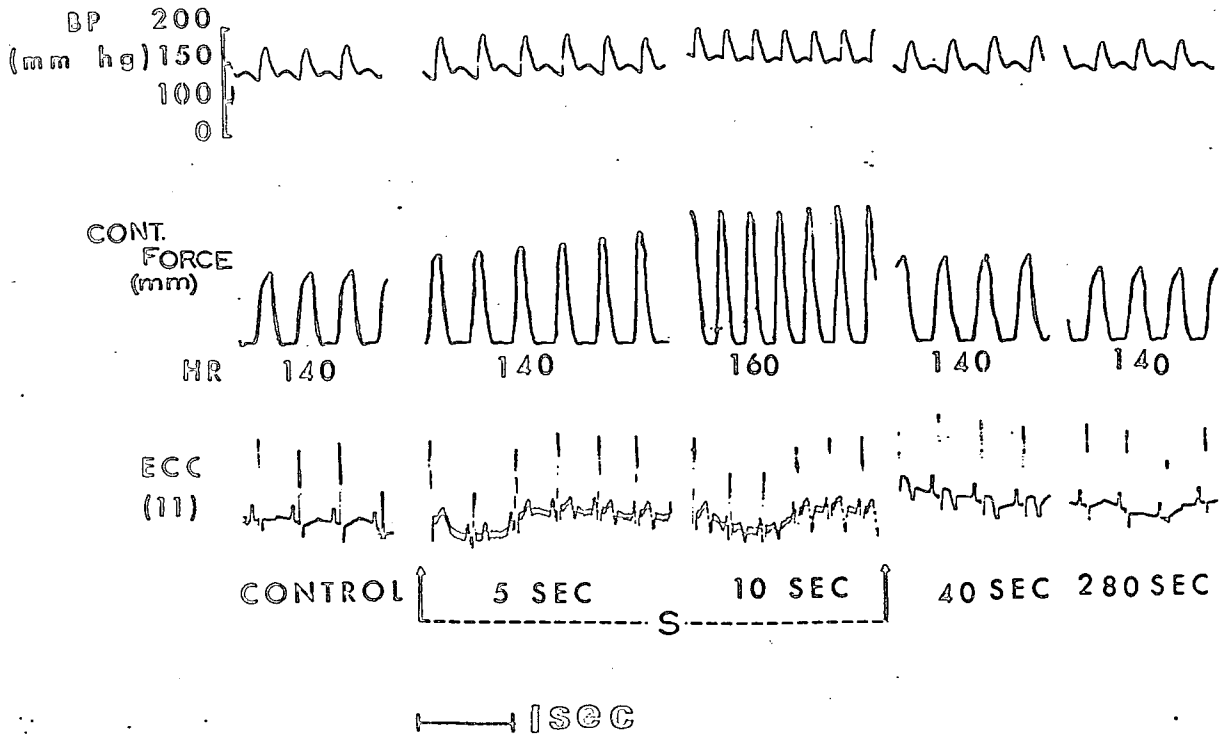

Fig. 3 Illustrating the influence of medial cardiosympathetic nerve stimulation upon ECG, heart rate, contractile force and blood pressure. S indicates the onset of stimulation.

activity was maintained during stimulation and, on cessation of the stimulus, rose gradually to a value of 400 ± 23%, 280 sec later.

The level of activity of UDPG pyrophosphorylase, the stable enzyme, was not affected by stimulation as can be observed.

Concurrent changes in heart rate, cardiac contractile force and blood pressure are also shown. No significant increases in heart rate or blood pressure were induced by electrical stimulation. However, cardiac contractile force was profoundly influenced. Maximal changes 220 ± 20% occurred within 10 sec and were followed by a decline to the control value within 280 seconds.

Records of an experiment are illustrated in Figure 3. It can be observed that while blood pressure and heart rate were only slightly altered, contractile force increased maximally within 10 sec and declined to control values within 280 seconds. Pronounced inversion of the T wave of the ECG was also observed during, and immediately following, the stimulus. The control ECG pattern was restored within 280 seconds.

Since both phosphorylase _a_ and glycogen synthetase I activities were influenced by stimulation, in the next series of experiments the stimulus was repeated at 20 min intervals for 5 consecutive stimuli. In each of these experiments biopsies were taken during the period of maximal increases in

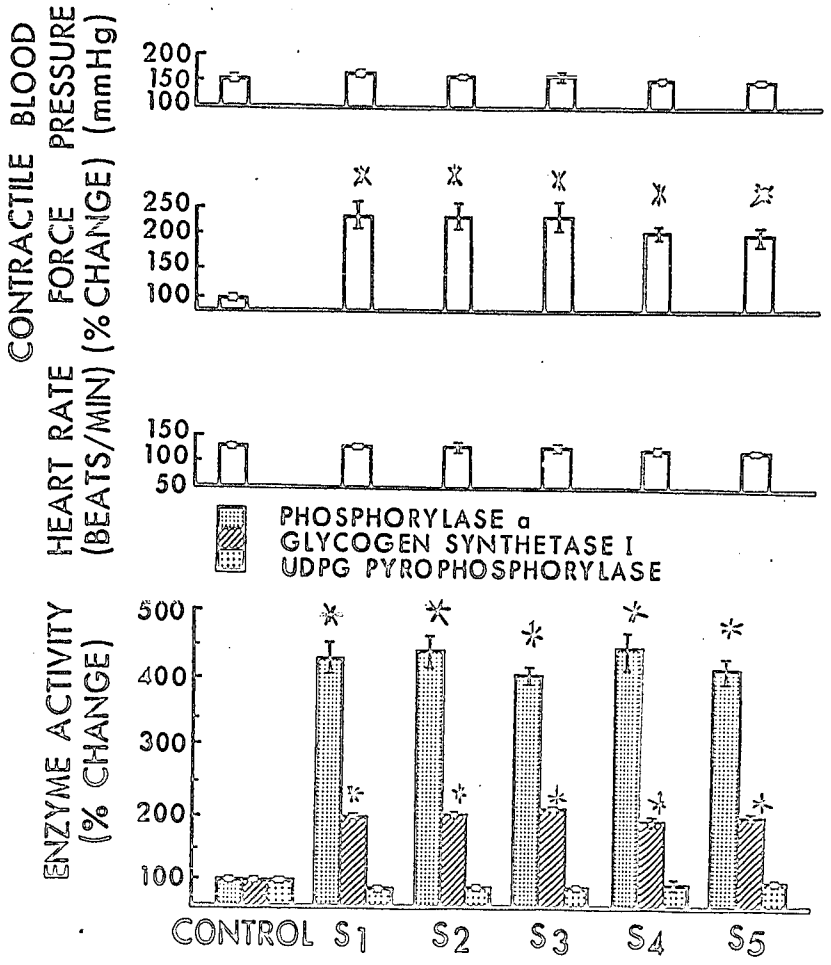

Fig. 4 Changes in phosphorylase a, glycogen synthetase I, UDPG pyrophosphorylase, heart rate, contractile force and blood pressure induced by repeated medial cardiosympathetic nerve excitation at 20 min intervals. Control values of enzyme activities and contractile force were taken as 100%. S_1 denotes first stimulus. Values are mean ± S.E. of 6 experiments and * denotes significance at the 95% confidence level.

contractile force (within 10 sec after the onset of the stimulus). Figure 4 summarizes comparative changes induced by 5 consecutive stimuli at 20 min intervals. Phosphorylase *a* activity increased to a maximum value of 448 ± 23% during the first stimulus. The activity of glycogen synthetase I also increased (200 ± 4%), while UDPG pyrophosphorylase activity decreased slightly (84 ± 5%). While heart rate and blood pressure were moderately altered, cardiac contractile force increased to a maximum of 230 ± 20%. These values were readily repeatable at 20 min intervals for up to 5 consecutive stimuli, as shown in the figure. In order to correlate the observed changes in phosphorylase *a*, glycogen synthetase I, and UDPG pyrophosphorylase activity with alterations in myocardial glycogen content, determinations of the level of total cardiac glycogen were conducted in similar experiments. It was consistently observed that myocardial glycogen content was reduced to a value of 70 ± 5% below the control level of 100% during repeated medial cardiosympathetic nerve stimulation.

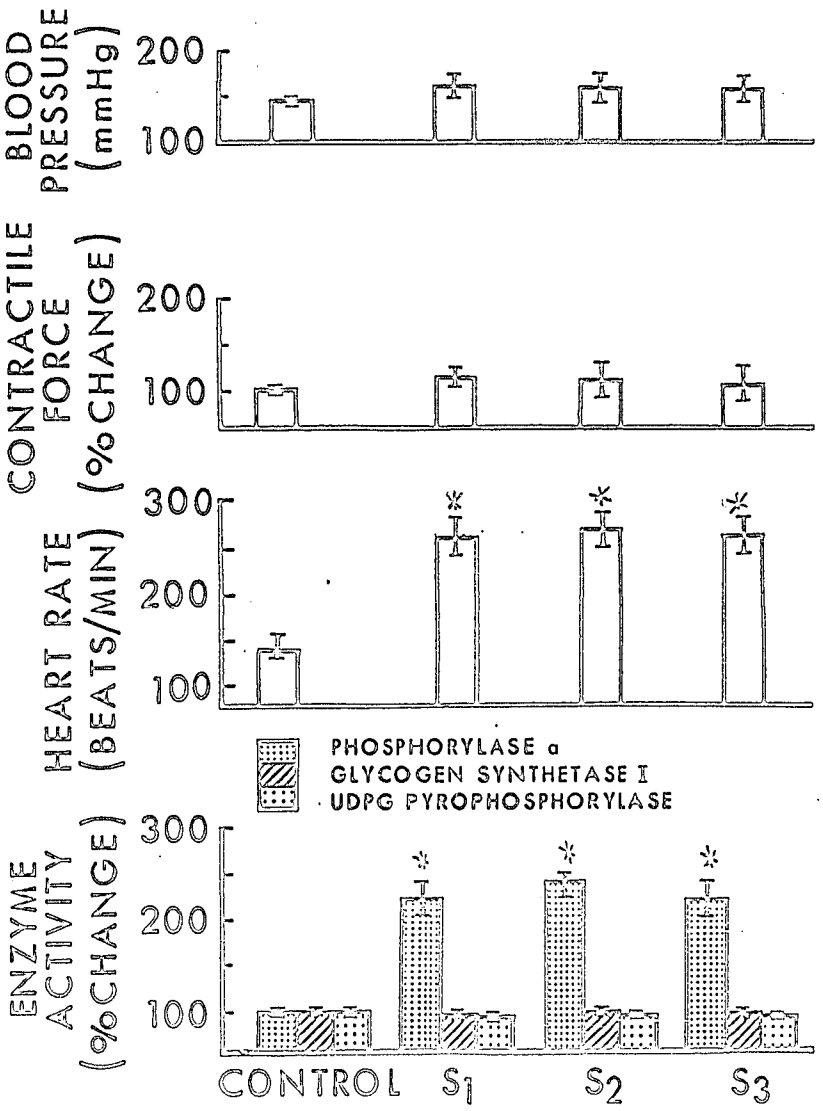

Fig. 5 Concurrent changes in phosphorylase a, glycogen synthetase I, UDPG pyrophosphorylase, heart rate, contractile force and blood pressure induced by cranial cardiosympathetic nerve stimulation at 20 min intervals. S_1 denotes the first stimulus. Control values of enzyme activities and contractile force were taken as 100%.
Values are mean ± S.E. of 3 experiments and * denotes significance at the 95% confidence level.

2. Influence of Cranial Cardiosympathetic Nerve Excitation

The cranial cardiosympathetic nerve predominantly innervates the sino-atrial node (see Methods). Preliminary experiments indicated that the time course of the enzymatic changes induced by electrical stimulation of this nerve were similar to those described for the medial cardiosympathetic nerve (see Figure 2). Hence, biopsies were taken in each experiment at 10 sec after the onset of the stimulus. Figure 5 summarizes the results obtained during repeated stimulation at 20 min intervals. With the onset of stimulation, phosphorylase a activity increased to a maximum of 224 $\pm$ 21% from the control value of 100%. However, neither glycogen synthetase I nor UDPG pyrophosphorylase activity was affected. Associated changes in heart rate, contractile force and blood pressure are also shown. While there were no significant alterations in blood pressure and contractile force, heart rate increased significantly ($P < 0.01$) to 263 $\pm$ 4 from the control rate of 163 $\pm$ 4 beats/minute. These values were readily repeatable as shown in the Figure. In similar experiments it was observed that the total cardiac glycogen concentration was decreased to a value of 85 $\pm$ 7% below control.

The influence of cranial cardiosympathetic nerve stimulation upon the ECG, heart rate, contractile force and blood pressure is illustrated in Figure 6. A significant increase in heart rate

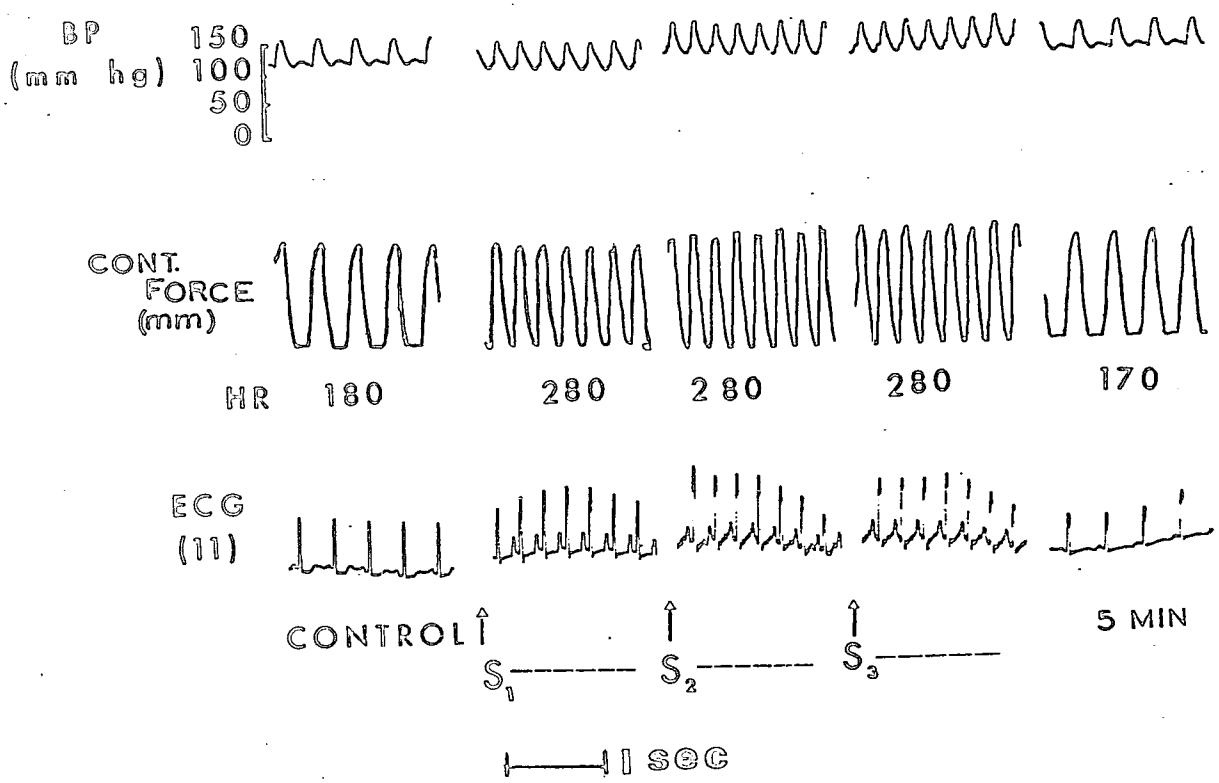

Fig. 6 Illustrating the influence of cranial cardiosympathetic nerve stimulation upon ECG, heart rate, contractile force and blood pressure. S_1 - S_3 denote consecutive stimuli at 20 min intervals.

associated with an increase in blood pressure but no concurrent alteration in contractile force is readily observed. The flattened P waves observed during the control period became quite prominent during stimulation. The record also illustrates that the control pattern was restored within 5 min after cessation of the stimulus.

When compared to the enzymatic changes induced by stimulation of the medial cardiosympathetic nerve, it was observed that a significantly greater ($P < 0.01$) increase in phosphorylase a activity was produced by medial cardiosympathetic nerve stimulation than by cranial cardiosympathetic nerve excitation. Whereas glycogen synthetase I activity was greatly increased by medial cardiosympathetic nerve stimulation ($200 \pm 4\%$), the activity of this enzyme remained unchanged upon cranial cardiosympathetic nerve excitation.

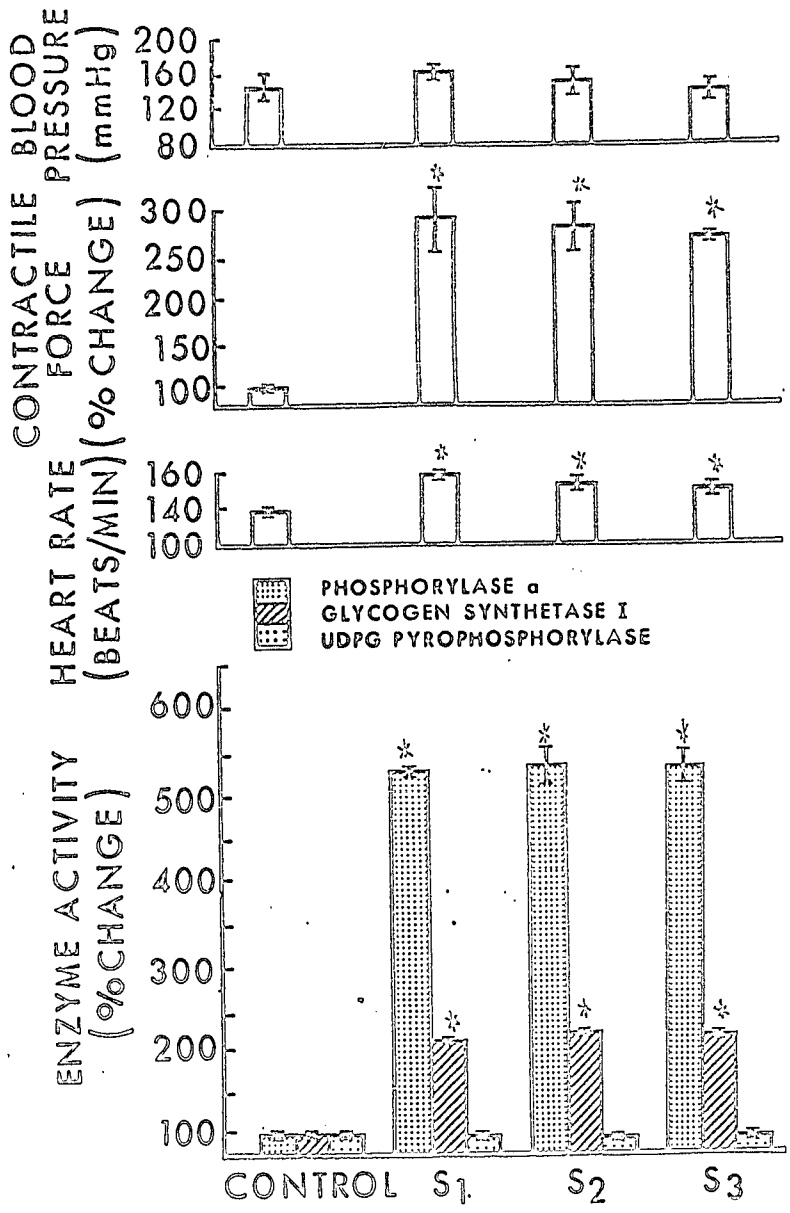

Fig. 7 Concurrent changes induced by combined stimulation of the medial cardiosympathetic and cranial cardiosympathetic nerves upon phosphorylase a, glycogen synthetase I, UDPG pyrophosphorylase, heart rate, contractile force and blood pressure at 20 min intervals. S_1 denotes the first stimulus. Control enzyme activities and contractile force were taken as 100%. Values are mean ± S.E. of 3 experiments and * denotes significance at the 95% confidence level.

3. Influence of Combined Cardiac Sympathetic Nerve Stimulation

It was of interest to determine to what extent the parameters previously described could be influenced by simultaneous excitation of both cardiac sympathetic nerves. Figure 7 is a histogram of the results obtained when both the medial cardiosympathetic and cranial cardiosympathetic nerves were simultaneously excited. There was a marked increase in phosphorylase a activity during stimulation (517 $\pm$ 6%). This high level of activity was significantly greater ($P < 0.01$) than values obtained when either nerve was stimulated separately. The increase in glycogen synthetase I activity (207 $\pm$ 0%) was almost identical to values obtained when the medial cardiosympathetic nerve was stimulated (200 $\pm$ 4%). UDPG pyrophosphorylase activity was not affected. These values could also be repeated at 20 min intervals as the figure shows. No determinations of total cardiac glycogen were made in these experiments.

Although the blood pressure was slightly increased both heart rate and contractile force were significantly enhanced by each stimulus. In Figure 8 concurrent changes in ECG, heart rate, contractile force and blood pressure are illustrated. The marked increases in contractile force and heart rate are quite apparent.

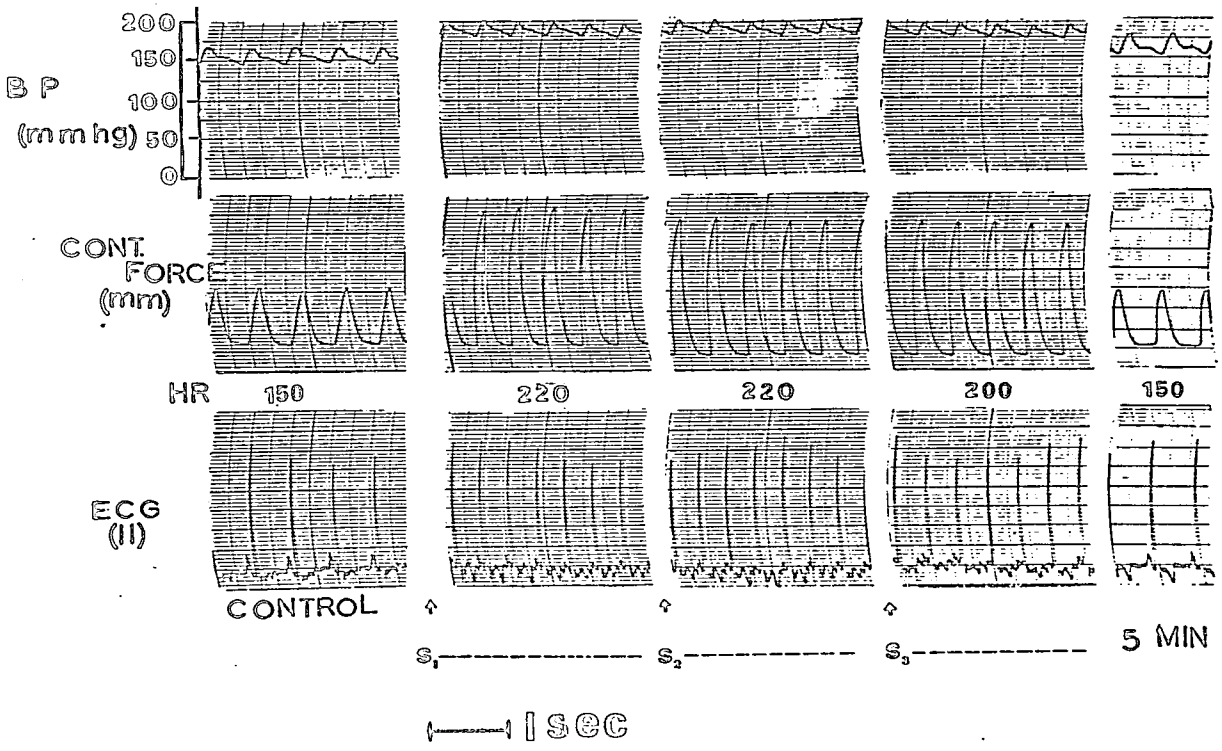

Fig. 8 Illustrating the influence of combined medial cardiosympathetic and cranial cardiosympathetic nerve excitation upon ECG, heart rate, contractile force and blood pressure. $S_1 - S_3$ represents stimuli at 20 min intervals.

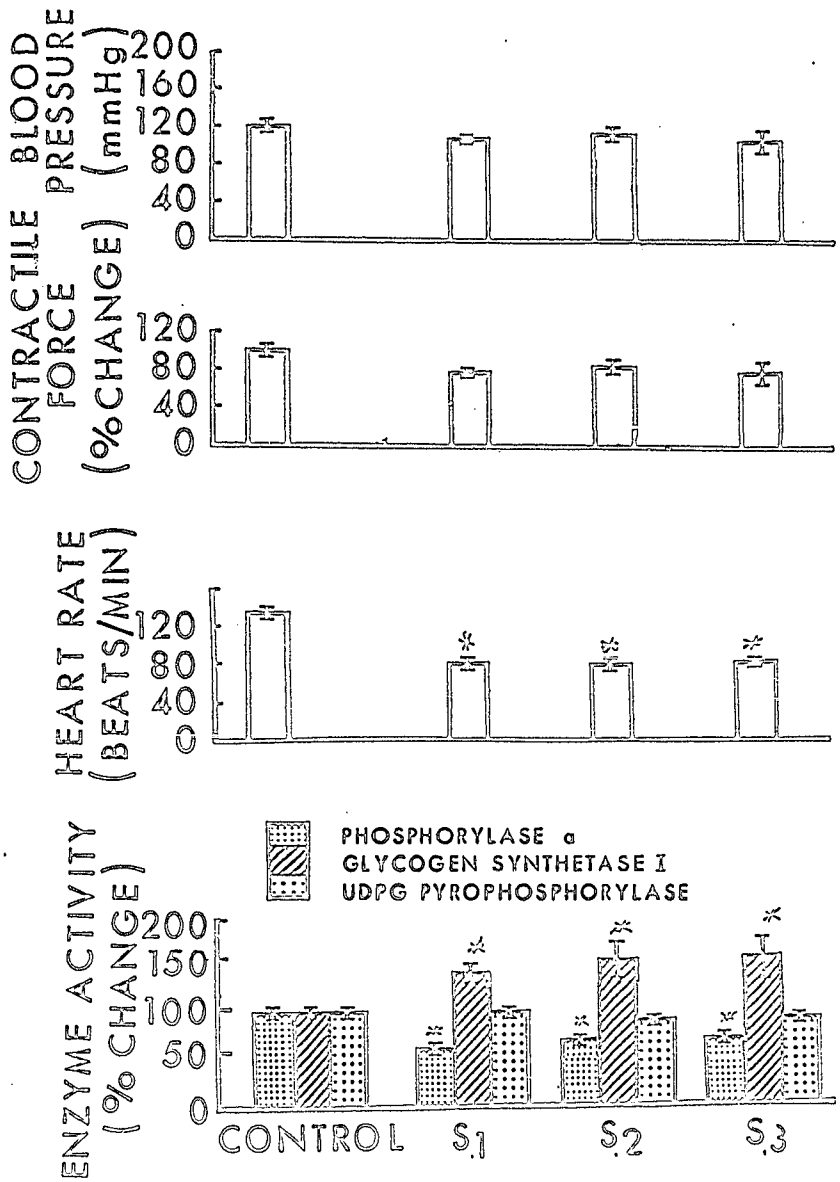

Fig. 9 The influence of cardiac slowing upon phosphorylase a, glycogen synthetase I, UDPG pyrophosphorylase, heart rate, contractile force and blood pressure is shown. S_1 - S_3 represent 3 consecutive stimuli at 20 min intervals. Control enzymatic activities and contractile force values were taken as 100%. Values are mean ± S.E. of 3 experiments and * denotes significance at the 95% confidence level.

B. Influence of Cardiovagal Nerve Stimulation Upon the Cardiac Glycogen Metabolizing Enzymes and Associated Parameters

Having investigated the influence of the sympathetic division of the cardiac autonomic nervous system upon the cardiac glycogen metabolizing enzymes, total cardiac glycogen content and concurrent alterations in blood pressure, heart rate and cardiac contractility, it was of interest to further extend these investigations to a study of the influence of the parasympathetic division upon these parameters. Cardiac slowing induced by cardiovagal nerve excitation was first studied. This was followed by a study of the effect of temporary cardiac arrest upon changes in cardiac glycogen metabolism.

1. Cardiac Slowing

A terminal branch of the right cardiovagal nerve was isolated and stimulated at an intensity which induced a decrease of 40-50 beats/minute. This ranged from 1-2 volts in each experiment (see Methods). Biopsies were taken within 10 sec after the onset of stimulation. Figure 9 summarizes the results of three consecutive stimuli at 20 min intervals.

It was observed that cardiac slowing resulted in a significant decrease in phosphorylase *a* activity (51 $\pm$ 8%). A concurrent increase in the activity of glycogen synthetase I (141 $\pm$ 5%) was also observed. There was no associated alteration in the activity

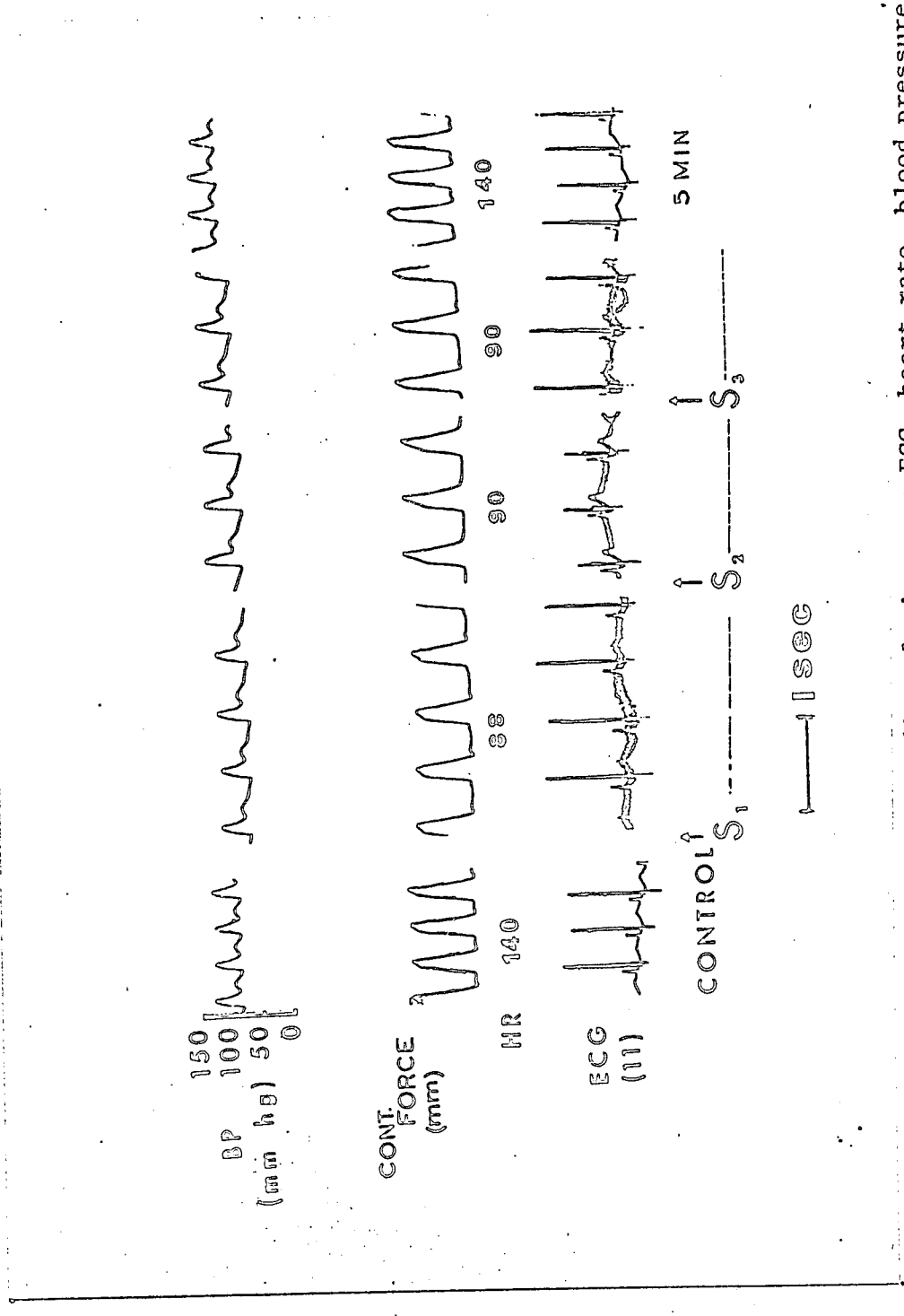

Fig. 10 Illustrating the effects of cardiac slowing upon ECG, heart rate, blood pressure and contractile force. S_1 - S_3 denote 3 consecutive stimuli at 20 min intervals.

of UDPG pyrophosphorylase. In similar experiments it was observed that the total cardiac glycogen concentration increased to a maximum value of 202 $\pm$ 13% from the control value of 100%. While only slight insignificant decreases in blood pressure and contractile force were observed, heart rate decreased significantly to a rate of 85 $\pm$ 5 from the control rate of 140 $\pm$ 7 beats/minute.

Figure 10 illustrates a pronounced decrease in heart rate associated with a slight decrease in blood pressure and contractile force but no alteration in the form of the ECG during nerve stimulation.

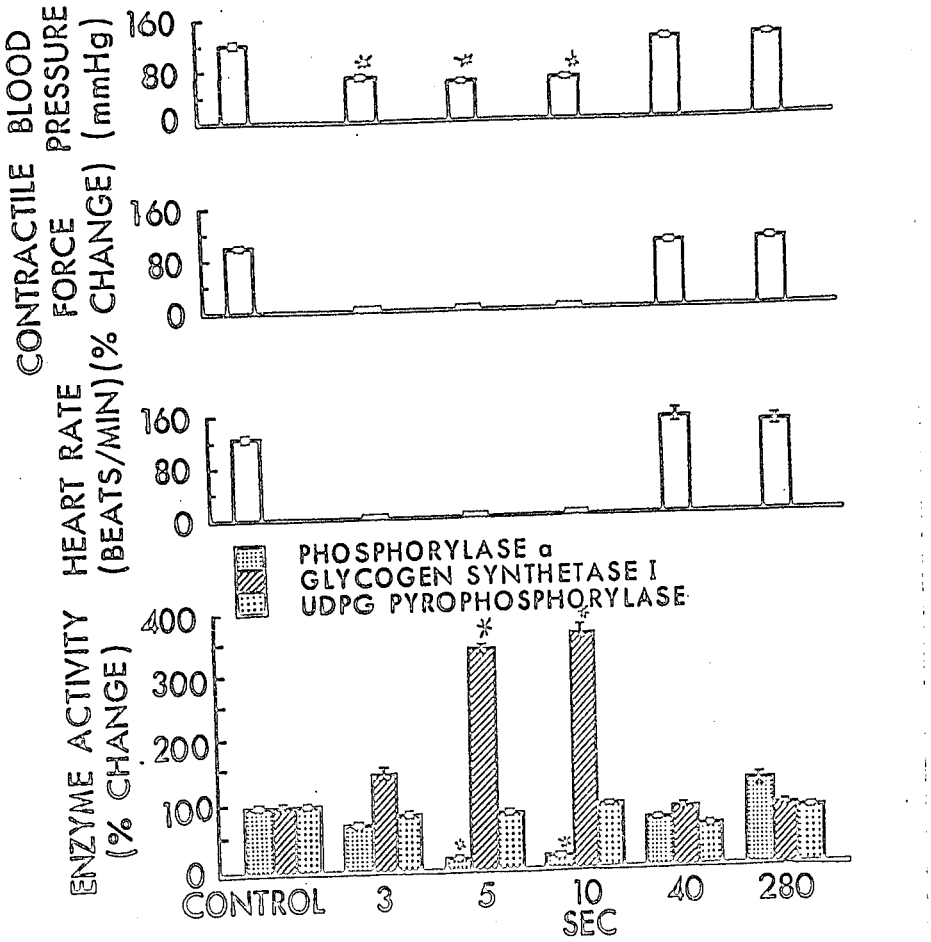

Fig. 11 Concurrent alterations in phosphorylase a, glycogen synthetase I, UDPG pyrophosphorylase, heart rate, contractile force and blood pressure induced by temporary cardiac arrest. Values at 3, 5 and 10 sec were recorded from biopsies taken during stimulation while values at 40 and 280 sec were recorded from post-stimulation biopsies. Control enzyme activities and contractile force were taken as 100%. Values are mean ± S.E. of 6 experiments and * denotes significance at the 95% confidence level.

2. Temporary Cardiac Arrest

Figure 11 shows that with the onset of cardiac arrest, induced by intense cardiovagal excitation, phosphorylase a activity decreased from the control of 100% to 69 ± 4% within 3 seconds. A further decrease to a minimum value of 18 ± 1% ensued within 10 seconds. After cessation of the stimulus, phosphorylase a activity increased to 75 ± 5% within 40 sec and a restoration to values above the control level (128 ± 12%) was achieved within 280 seconds. Changes in glycogen synthetase I activity were opposite to those observed for phosphorylase a activity. Within 3 sec an increase to a value of 148 ± 10% was observed, and the maximum increase (364 ± 11%) occurred within 10 seconds. Cessation of the stimulus was followed by a decline to 114 ± 8% within 40 sec, the control level being restored within 280 seconds. As previously observed following sympathetic nerve stimulation and cardiac slowing, UDPG pyrophosphorylase activity was not significantly affected by temporary cardiac arrest. In similar experiments, the level of total cardiac glycogen was increased (306 ± 20%) within 10 sec following the onset of cardiac arrest.

As Figure 11 also shows, there was a cessation of cardiac contraction with a resultant dramatic fall in blood pressure from a control value of 121 ± 5 to a minimum of 60 ± 5 mm Hg within 10 seconds. Cessation of the stimulus was followed by subsequent restoration of control heart rate, contractile force and blood

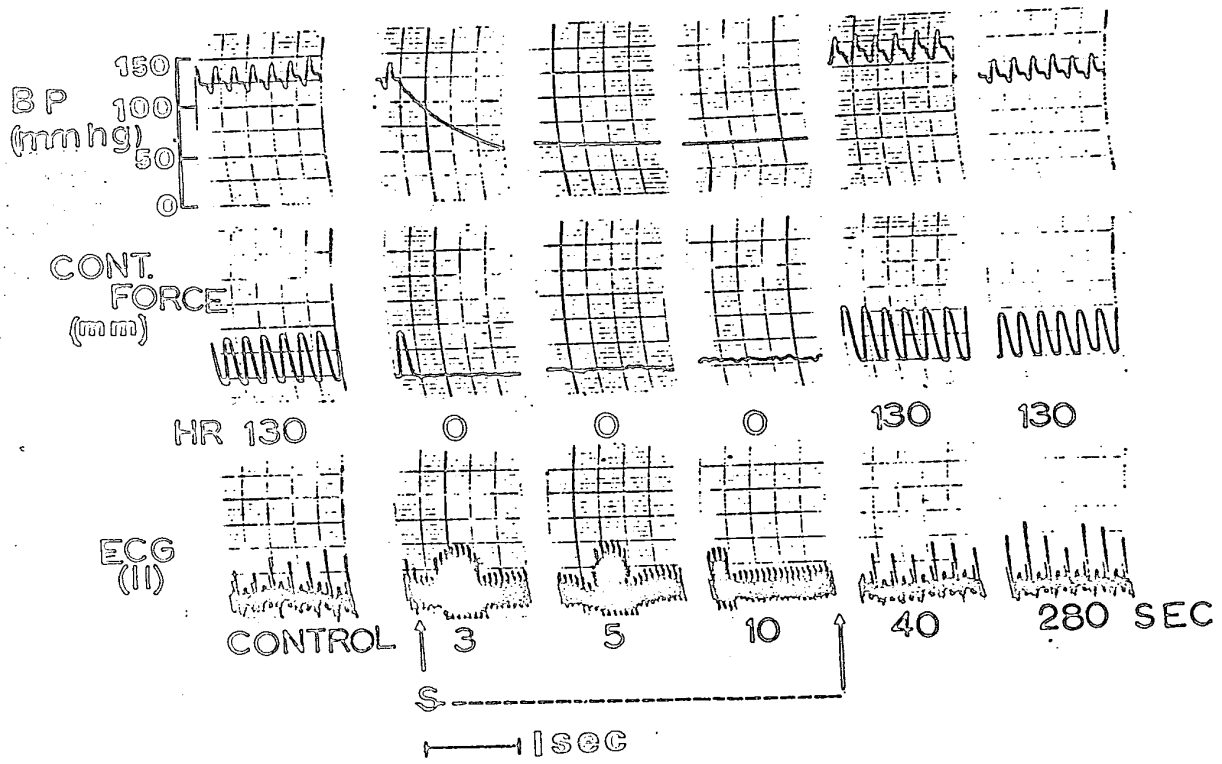

Fig. 12 Illustrating the effects of intense cardiovagal nerve excitation upon ECG, heart rate, contractile force and blood pressure over a 280 sec period. S represents the onset of stimulation.

pressure. These changes are clearly illustrated in Figure 12. It can be observed that within 3 sec after the onset of stimulation, there was a cessation of contraction and a precipitous fall in blood pressure followed by subsequent recovery of all parameters within 280 seconds.

Figure 13 is a summary of the results obtained following 3 consecutive stimuli at 20 min intervals. It is seen that identical decreases in phosphorylase a activity (19 $\pm$ 0%) were induced by successive stimuli (S1, S2, S3). Glycogen synthetase I activity was increased (330 $\pm$ 11%) by all 3 stimuli while UDPG pyrophosphorylase activity remained unchanged.

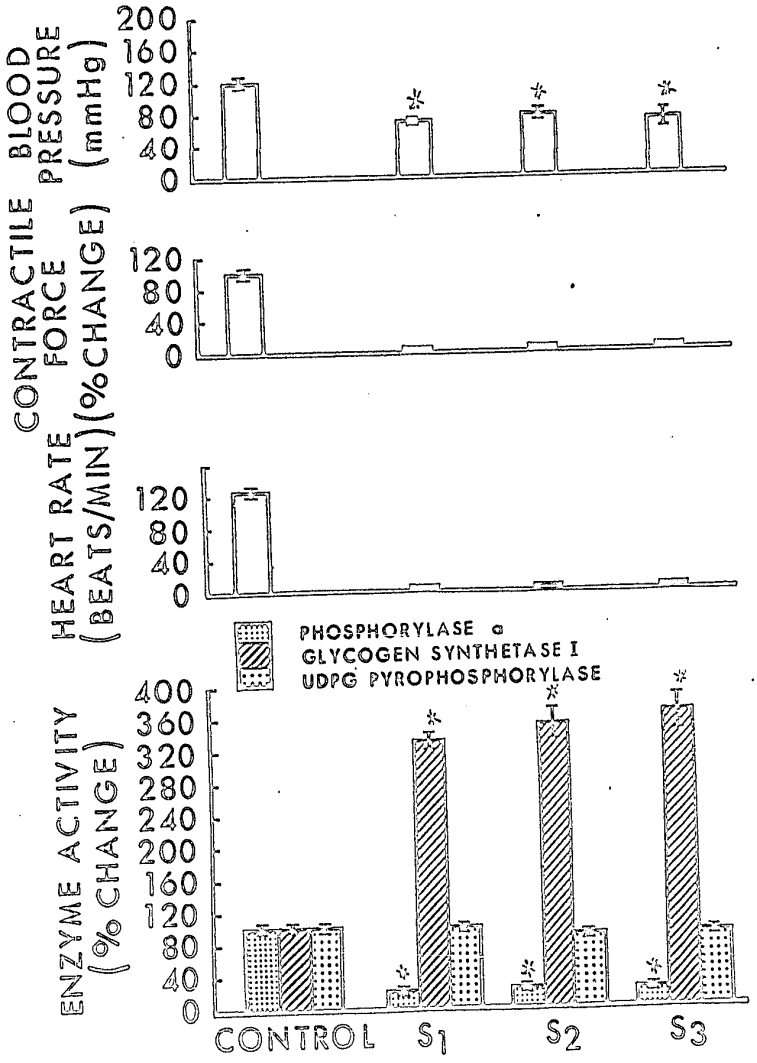

Fig. 13 Concurrent alterations induced by intense cardiovagal nerve stimulation upon phosphorylase a, glycogen synthetase I, UDPG pyrophosphorylase, heart rate, contractile force and blood pressure. $S_1 - S_3$ represent 3 consecutive stimuli at 20 min intervals. Control values for the enzymatic activities and contractile force were taken as 100%. Values are mean $\pm$ S.E. of 6 experiments and * denotes significance at the 95% confidence level.

C. The Effects of Cardiac Drugs Upon Modifications in Cardiac Glycogen Metabolizing Enzyme Activity

Cardiac glycosides exert profound effects upon myocardial contractility. It is not known whether this effect is mediated through a direct action upon cardiac glycogen stores, or through a modification of the autonomic nervous system activity (Gillis, 1969; McLain, 1969; Garvey, 1970). A local anaesthetic agent lidocaine has shown considerable promise as a potential cardiac drug in antagonizing digitalis intoxication. Its influence upon the cardiac glycogen metabolizing enzymes has not been previously investigated. In this section, the influence of ouabain and lidocaine upon these enzymes was investigated separately. This was followed by an examination of the influence of lidocaine upon ouabain-induced changes in cardiac glycogen metabolism.

1. Effects of Ouabain

Maximal changes in heart rate, contractile force and blood pressure were observed within 15 min after ouabain administration, and hence, biopsies were taken at this point. Changes in cardiac enzyme activity as well as concurrent alterations in heart rate, blood pressure and contractile force induced by ouabain (20 μg/kg) are summarized in Figure 14. There was a marked increase in phosphorylase *a* activity from the control level of 100% to a maximum of 969 ± 109%. This was associated with a decrease in

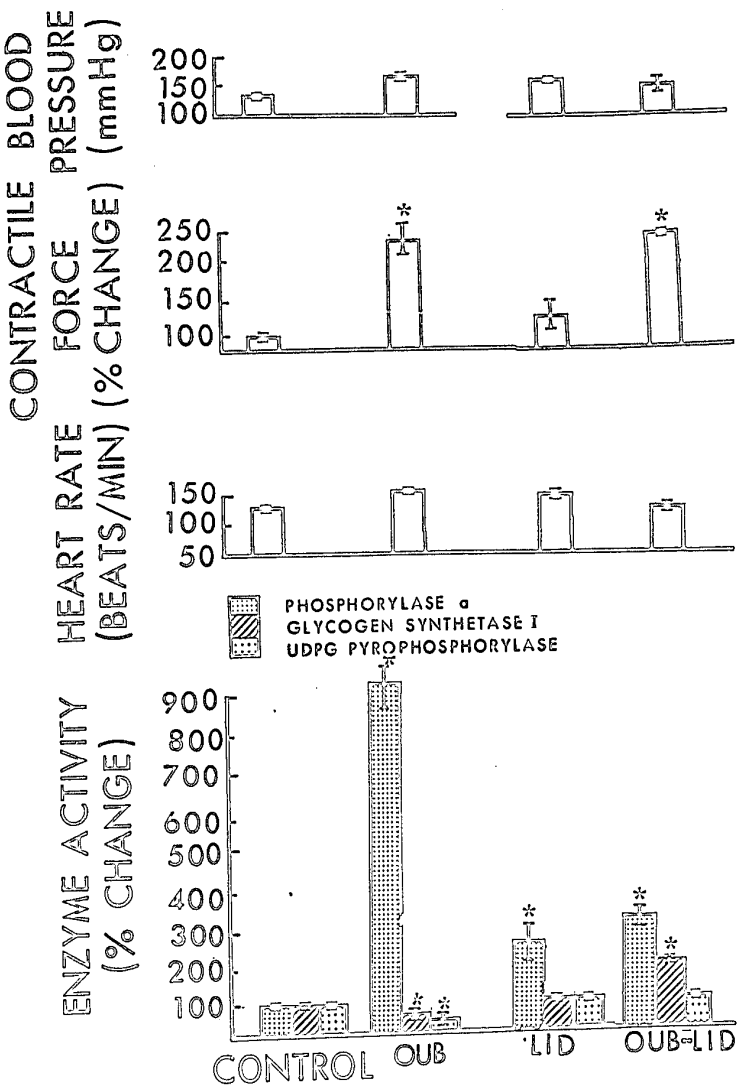

Fig. 14 The influence of ouabain (20 µg/kg), lidocaine (10 µg/kg) and a combination of ouabain and lidocaine upon phosphorylase a, glycogen synthetaseI, UDPG pyrophosphorylase, heart rate, contractile force and blood pressure. Control enzymatic activity and contractile force were taken as 100%. Values are mean ± S.E. of 6 experiments and * denotes significance at the 95% confidence level.

glycogen synthetase I activity (54 ± 6%). Surprisingly, UDPG pyrophosphorylase activity was also markedly reduced to a value of 12 ± 1% from the control value of 100%. These changes in enzyme activity were associated with a striking increase in cardiac contractile force (262 ± 46%) but no pronounced alterations in blood pressure and heart rate. An example of a typical experiment is illustrated in Figure 15.

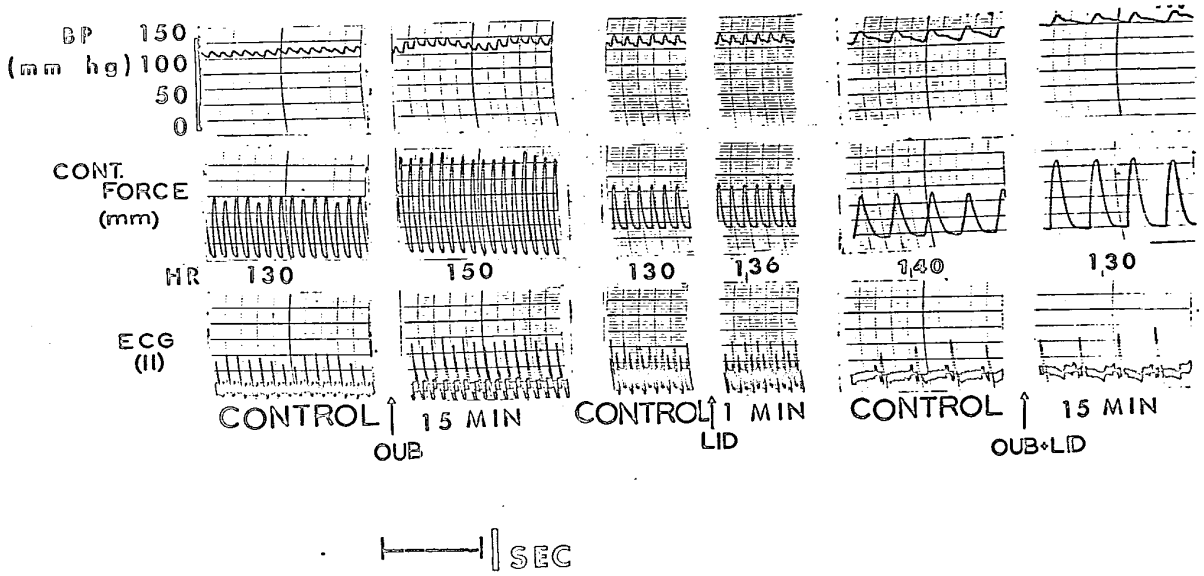

Fig. 15 Illustrates the effects of ouabain (20 µg/kg), lidocaine (10 µg/kg) and a combination of both drugs upon ECG, heart rate, contractile force and blood pressure.

2. Effects of Lidocaine

The alterations in cardiac enzyme activity as well as in blood pressure, heart rate and contractile force induced by lidocaine (10 μg/kg) are also summarized in Figure 14. Biopsies were taken within 1 min after lidocaine administration, since maximal changes in heart rate, blood pressure and contractile force were observed within this interval. There was a significant increase in phosphorylase *a* activity from 100% to 230 ± 10%. However, glycogen synthetase I as well as UDPG pyrophosphorylase activity remained unchanged. There were no significant changes in heart rate, blood pressure and contractile force.

3. Influence of Lidocaine Upon the Responses to Ouabain

The influence of lidocaine upon changes in the cardiac glycogen metabolizing enzymes induced by ouabain administration was next assessed. The results obtained are also summarized in Figure 14. As illustrated, lidocaine (10 μg/kg) pretreatment induced no antagonism of the ouabain-induced changes in blood pressure, contractile force and heart rate. However, phosphorylase a activity was now significantly ($P < 0.01$) reduced to a value of 352 ± 10% from a value of 969 ± 109%, observed during ouabain administration. In addition, glycogen synthetase I activity was now increased to a value of 223 ± 13% in contrast to a decrease of 54 ± 6% induced by ouabain. The control level of UDPG pyrophosphorylase activity (decreased to 12 ± 1% by ouabain) was now restored (101 ± 1%).

As illustrated in Figure 15 maximal increases in contractile force associated with ectopic beats were observed within 15 minutes after ouabain administration. These ectopic beats were readily antagonized by lidocaine with the maintenance of a normal sinus rhythm. Ouabain-induced increases in contractile force were not affected by lidocaine pretreatment.

V GENERAL DISCUSSIONS

A. The Role of the Cardiac Sympathetic Nervous System in Glycogen Metabolism

A comprehensive investigation of the influence of the autonomic nervous system upon cardiac glycogen metabolism should involve a study of the activity of the key rate-limiting enzymes responsible for glycogen breakdown (phosphorylase a) and synthesis (glycogen synthetase I). While Mayer and Moran (1960) have reported enhanced glycogenolysis (increased phosphorylase a activity) following electrical excitation of the cardiac sympathetic nerves, there are no previous studies on concurrent changes in the activities of the enzymes glycogen synthetase I and UDPG pyrophosphorylase, which are involved in glycogenesis.

Excitation of the cardiac sympathetic nerves increases the force of cardiac contraction and the heart rate. It is now known that these two effects are mediated by separate nerves (Miller, 1962; Kaye et al, 1970). The medial cardiosympathetic nerve, otherwise known as the cardioaugmentor nerve, innervates mainly the ventricles, and on excitation augments contractile force without concurrent alterations in heart rate. On the other hand, the cranial cardiosympathetic nerve, otherwise known as the cardioaccelerator nerve, innervates predominately the SA and AV nodes. Its excitation increases heart rate without associated changes in contractile force. The separation of these nerves

permitted an evaluation of the relative importance of augmented contractile force and increased heart rate upon cardiac glycogen metabolism. This has not been previously reported in the literature.

In the present study, it was observed that electrical excitation of the medial cardiosympathetic nerve resulted in enhanced glycogenolysis as evidenced by an increased activity of the enzyme phosphorylase a. This was also associated with increased glycogenesis as indicated by the augmented glycogen synthetase I activity. Cessation of the stimulus was associated with enhanced synthesis, which was significantly greater than the levels observed during stimulation. In skeletal muscle a different response has been reported during excitation. Whereas there is an augmented glycogen breakdown, no concurrent synthesis was observed (Williams and Mayer, 1966). Glycogen is an important source of energy in myocardial tissue (Stetten and Stetten, 1960). It would appear that, unlike skeletal muscle, the heart is continually active, and must adapt its performances to meet increased demands without signs of fatigue. It is therefore necessary that concurrent glycogenesis occur during enhanced cardiac contractility, as demonstrated in the present investigation. Similar findings have been previously reported by Neely and Morgan (1966). In their investigation, augmented glycogen synthesis was observed, when the external work of the perfused rat heart was increased.

In contrast to the results obtained following medial cardiosympathetic nerve stimulation, when the cranial cardiosympathetic or cardioaccelerator nerve was excited, a marked increase in heart rate was observed. This was associated with elevated phosphorylase a activity but no alteration in the activity of glycogen synthetase I. The observed increase in phosphorylase a activity (224 $\pm$ 21%) was significantly less ($P < 0.01$) than values obtained (451 $\pm$ 44%) when myocardial contractility was augmented by medial cardiosympathetic nerve excitation. It would appear that an enhanced cardiac contractile force imposes a much greater demand upon cardiac glycogen stores than an increase in the frequency of cardiac contraction. Similarly, a comparison of the total cardiac glycogen concentration, following excitation of these two nerves, indicates a greater decrease during increased contractility than during elevated heart rate. It would also appear from these observations that there might be a definite level (70 $\pm$ 5%) above which concurrent glycogen synthesis does not occur. This aspect, however, requires further investigation before any definite conclusion can be drawn.

Simultaneous excitation of both nerves enhanced heart rate and contractile force. Whereas the elevation in rate was lower than during cranial cardiosympathetic nerve excitation, the augmentation in myocardial contractile force was greater than values observed during stimulation of the medial cardiosympathetic nerve.

Concurrent alterations in cardiac glycogen metabolism indicate a greatly enhanced phosphorylase a activity (517 ± 16%) as well as increased synthesis. Mayer and Moran (1960) reported an increase of 300% in phosphorylase a activity following post-ganglionic cardiac sympathetic nerve stimulation. This value was recorded 45 sec after the onset of stimulation. In the present investigation, maximal changes were observed within 10 sec after the onset of the stimulus. Maximal increases in contractile force were also observed during this interval.

It might be assumed that combined sympathetic nerve stimulation predominantly influences cardiac contractile force, which is reflected as an augmentation in glycogen breakdown and concurrent synthesis. It is interesting to note, that although glycogenolysis had increased over the level observed following medial cardiosympathetic nerve excitation, the level of glycogenesis was not additionally increased by combined stimulation.

The activity of UDPG pyrophosphorylase was not significantly altered by cardiac sympathetic nerve stimulation. In glycogen metabolism this enzyme is not rate-limiting, since its activity is much greater than that of glycogen synthetase. Williams and Mayer (1966) had suggested that the activity of UDPG pyrophosphorylase might become rate-limiting when glycogen synthetase I attained exceedingly high levels. It would appear that under the conditions of this investigation the level of glycogen synthesis required to

induce significant changes in the activity of UDPG pyrophosphorylase was not achieved. The possibility therefore exists that all of the substrate UDPG, that is synthesized, is not utilized under conditions of enhanced cardiac activity, which is induced by augmented sympathetic nerve activity.

B. The Role of the Parasympathetic Nervous System in Cardiac Glycogen Metabolism

The cardiac parasympathetic nervous system is generally thought to oppose the action of the sympathetic nervous system. Consequently, an enhancement of glycogenesis associated with reduced glycogenolysis might be anticipated when the cardiovagal nerves are excited, or following acetylcholine administration. This has been previously demonstrated by Hess et al (1961, 1962) as well as by Ellis and Vincent (1961, 1963). These investigators observed a reduction in phosphorylase a activity (25% below control values) following vagal stimulation as well as administration of acetylcholine. It is well known that the vagus is a mixed nerve and contains cardiovagal as well as sympathetic fibres (Randall et al, 1969); and hence, the possibility exists that sympathetic fibres might have been simultaneously excited in these investigations. Moreover, no estimations of glycogen synthetase I or UDPG pyrophosphorylase activity were made. The influence of parasympathetic nerve excitation upon the activity of the cardiac glycogen synthesizing enzymes has not been previously reported in the literature.

In the present study the vagus nerve was not used. Instead, postganglionic cardiovagal nerves beyond the level of the stellate ganglion were isolated and stimulated. It was observed that when slowing, instead of cardiac arrest, was induced, cardiac contractile

force was not significantly affected but heart rate decreased significantly (reduction of 40-50 beats/min below control levels). Under these conditions an increase in glycogen synthetase I activity occurred associated with a corresponding reduction in the activity of phosphorylase a. Moreover, the level of total cardiac glycogen was significantly ($P < 0.01$) increased ($202 \pm 13\%$).

On the other hand, when temporary cardiac arrest was induced by increasing the voltage of stimulation, a pronounced elevation in glycogen synthetase I activity associated with a dramatic reduction in the activity of phosphorylase a resulted. Cessation of the stimulus induced an enhancement of phosphorylase a activity above the control level. The total cardiac glycogen concentration was also markedly increased ($306 \pm 20\%$) during temporary cardiac arrest.

Under the conditions of these experiments, increased parasympathetic activity was observed to be succeeded by a reflex sympathetic predominance (increases in heart rate, force of contraction and blood pressure above the control levels). It might be assumed that during increased parasympathetic activity, a state of readiness, as evidenced by augmented glycogenesis, is induced in order to cope with the enhanced glycogenolysis, which occurred during this reflex enhancement of sympathetic activity.

As observed following excitation of the cardiac sympathetic nerves, UDPG pyrophosphorylase activity was unchanged by cardiovagal

nerve excitation. According to the postulation of Williams and Mayer (1966), the level of UDPG pyrophosphorylase activity might be expected to be increased at high levels of glycogen synthetase I activity. This was not observed in the present investigation. It is possible that even though the rate of synthesis was extremely high during cardiac arrest, the level of glycogen synthetase I activity was still lower than that of UDPG pyrophosphorylase.

C. The Influence of Cardiac Drugs Upon Glycogen Metabolism

A clear understanding of the mechanism by which cardiac glycosides exert their positive inotropic as well as toxic action(s) remains to be demonstrated. The results obtained in this study indicate that ouabain induced a pronounced increase in glycogenolysis and an inhibitory effect upon glycogenesis, as indicated by the dramatic enhancement in phosphorylase a activity and the significant reduction in the activities of glycogen synthetase I and UDPG pyrophosphorylase. These results are not in agreement with those of Mayer and Moran (1960), who demonstrated no change in phosphorylase a activity following ouabain administration. It must be noted that in their investigation cardiac biopsies were taken within 25 sec after ouabain administration. The time of onset of ouabain's action ranges from 3 to 10 min, with maximum effects being observed within 30 min to 2 hours (Goodman and Gilman, 1966). In the present investigation, a maximum effect upon myocardial contractility was observed within 15 min and ectopic beats appeared within 15-25 minutes. Biopsies were therefore taken during this period. It is not surprising that these authors failed to demonstrate any alterations in the cardiac glycogen metabolizing enzyme, phosphorylase a. Belford and Feinlieb (1959) observed that K-strophanthin could induce a 200-400% increase in phosphorylase a activity in the perfused guinea pig auricle. This observation is in agreement with the findings in the present study; however,

these investigators failed to measure concurrent changes in glycogen synthesis. Further evidence supporting ouabain-induced glycogenolysis is provided by the study of Arese et al (1967). These investigators demonstrated an elevated glucose-6-phosphate level accompanied by a concurrent decrease in glucose-1-phosphate concentration, following ouabain administration to frog ventricular slices.

It is the concensus of opinion that cardiac glycosides induce a more efficient utilization of energy in the myocardium. It is therefore, conceivable that by causing an increased glycogenolysis, more substrate is made available in the form of glucose-1-phosphate, which can be readily utilized. Unlike sympathetic nerve stimulation, ouabain's glycogenolytic action was not associated with concurrent glycogen synthesis, since the levels of glycogen synthetase I and UDPG pyrophosphorylase activities were significantly decreased below control values.

Glycogen is a major source of energy in cardiac tissue (Stetten and Stetten, 1960). It is conceivable that with the augmented level of glycogen breakdown, observed following ouabain administration, eventual glycogen depletion might occur. This might very well be involved in the manifestations of ouabain's toxic action (ectopic beats observed in the present study). Recent investigations by Garvey and Kacew (1970) have indicated that following ouabain administration to open-chest dogs, the onset

of toxic effects were associated with a marked depletion of cardiac glycogen stores. Moreover, ventricular fibrillation occurred uniformly at the point of maximum glycogen depletion. This is further supported by the recent investigations of Lavigne (1970), who observed a correlative decrease in the total cardiac glycogen content of cat hearts during progressive ouabain intoxication. It would appear from the present investigation that the arrhythmias observed might be related to a depletion of cardiac glycogen stores. It might, therefore, be postulated that at therapeutic dose levels, the cardiac glycosides exert their beneficial effects by increasing the availability of glucose-1-phosphate, which can readily be utilized to yield energy. However, at toxic dose levels excess glycogen breakdown might occur, leading to eventual glycogen depletion.

It was observed that UDPG pyrophosphorylase, the non rate-limiting enzyme, was depressed by ouabain administration. This is in direct contradiction to the suggestion that this enzyme might become rate-limiting when the level of glycogen synthetase I activity is exceedingly high (Williams and Mayer, 1966), since the glycogen synthetase I activity was also decreased. It is possible that ouabain inhibits glycogen synthesis by a direct depression of glycogen synthetase I and UDPG pyrophosphorylase activities. Measurements of total cardiac glycogen content are in agreement with these findings.

When the results obtained following ouabain administration are compared with those obtained during sympathetic nerve stimulation, it is seen that although both procedures induced almost identical responses in cardiac contractility as well as enhanced phosphorylase a activity, the influence upon glycogen synthesis differed; i.e., cardiac sympathetic nerve stimulation was associated with a concurrent increase in glycogen synthetase I activity, while ouabain depressed the activity of this enzyme. Whereas the level of cardiac contractility returned to control levels upon termination of sympathetic nerve excitation, the increased level of myocardial contractility was maintained following ouabain administration. It was not determined whether, or not, the activities of glycogen synthetase I or UDPG pyrophosphorylase were augmented during the initial stages of ouabain's positive ionotropic action, nor were investigations made to determine the influence of sustained cardiac sympathetic nerve activity upon the level of activities of these enzymes.

Lidocaine is a local anaesthetic which has been recently introduced into the therapy of cardiac arrhythmias. Its greatest use is the correction of arrhythmias of recent origin, as well as the antagonism of digitalis intoxication (Katz and Zitnik, 1966; Morrow and Bosomworth, 1967; Grossman et al, 1968; Mendez and Kabela, 1970). Unlike most antiarrhythmic agents which depress myocardial contractility, lidocaine increases cardiac contractility

(Mendez and Kabela, 1970). In the present investigation, only slight increases in phosphorylase a activity and myocardial contractility were observed following lidocaine administration. Similar observations were made by Belliveau and Covino (1968), who demonstrated that the administration of lidocaine to rat ventricular slices resulted in a reduction of the glycogen concentration.

When lidocaine was administered to ouabain pretreated animals, the augmentation in contractile force induced by ouabain was not prevented. However, the arrhythmias were abolished resulting in the maintenance of a stable sinus rhythm. The marked elevation in phosphorylase a activity was significantly reduced, while the ouabain induced depression of synthesis was reversed, and a concurrent glycogen synthesis was induced. From these observations, it would appear that lidocaine permitted a more efficient utilization of cardiac glycogen and stimulated glycogen synthesis at high levels of glycogenolysis; i.e., lidocaine might antagonize the "glycogen wasting" action of ouabain; and thus, prevent glycogen depletion. In a recent study (Garvey and Kacew, 1970) it was demonstrated that whereas lidocaine pretreatment did not antagonize the influence of ouabain upon myocardial contractility, the onset of ouabain's toxic action was significantly delayed. This was associated with a significantly decreased level of glycogen breakdown as well as a concurrently enhanced glycogen synthesis. Thus, it would appear that the antiarrhythmic action of lidocaine,

during digitalis arrhythmias, might not be related to the myocardial depressant or "quinidine-like" action of other antiarrhythmic drugs, but might be associated with an antagonism of both "wasteful" glycogen breakdown and the depression of glycogen synthesis induced by cardiac glycosides.

VI SUMMARY AND CONCLUSIONS

Experiments were conducted to determine the influence of the cardiac autonomic nervous system, as well as cardiac drugs, upon the cardiac glycogen metabolizing enzymes and the total cardiac glycogen concentration.

The results obtained may be summarized as follows:

(1) Stimulation of the medial cardiosympathetic nerve (predominantly innervating the ventricles) induced a significant elevation in contractile force associated with concurrently enhanced phosphorylase *a* and glycogen synthetase I activity, but no marked changes in UDPG pyrophosphorylase activity, heart rate or blood pressure.

(2) Cranial cardiosympathetic nerve stimulation (mainly innervating the SA node) induced a significant increase in heart rate and phosphorylase *a* activity without concurrent marked changes in glycogen synthetase I, UDPG pyrophosphorylase, blood pressure or contractile force.

(3) Combined sympathetic nerve excitation greatly increased phosphorylase *a* and glycogen synthetase I activities without concurrent UDPG pyrophosphorylase alterations.

(4) Cardiac slowing induced an increase in glycogen synthetase I, a reduction in phosphorylase *a*, but no alteration in UDPG pyrophosphorylase activity.

(5) Temporary cardiac arrest resulted in more pronounced alterations, as compared to cardiac slowing, in the activities of glycogen synthetase I and phosphorylase a without affecting UDPG pyrophosphorylase activity.

(6) Ouabain administration augmented contractile force and phosphorylase a activity, and significantly reduced glycogen synthetase I and UDPG pyrophosphorylase activities.

(7) Ouabain-induced glycogenolysis was much greater than that observed following sympathetic nerve excitation, and was not accompanied by a concurrent enhanced glycogenesis.

(8) Lidocaine administration induced insignificant changes in all parameters except phosphorylase a activity.

(9) Lidocaine pretreatment did not influence ouabain-induced changes in heart rate, blood pressure or contractile force, but decreased phosphorylase a activity, as compared to ouabain alone, and reversed ouabain's effect upon glycogen synthetase I and UDPG pyrophosphorylase activities. Ouabain-induced ectopic beats were also antagonized.

From the foregoing observations, the following conclusions are drawn:

(1) The role of the sympathetic nervous system is primarily concerned with glycogenolysis, whereas the parasympathetic

is concerned with glycogenesis.

(2) Ouabain administration induces a dramatic glycogenolysis.

(3) The mechanism of lidocaine's action in antagonizing ouabain-induced arrhythmias may be related to a prevention of cardiac glycogen depletion.

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TABLE OF RESULTS

CONTROL

PROCEDURE	PHOS. a		GLY. SYN. I		UDPG Pyro.		GLY.		HR.	BP	CONT. FORCE	
	%1	%2	%1	%2	µmoles UDPG/ g/min	%2	µg/g	%2	BEATS/MIN	mm Hg	mm	%2
Medial Cardiosympathetic Nerve Stimulation	5.35±0.72	100±0	9.67±0.35	100±0	1.91±0.02	100±0	6142±17	100±0	125±14	116±4	6.25±0.20	100±0
Cranial Cardiosympathetic Nerve Stimulation	5.35±0.72	100±0	9.67±0.35	100±0	1.91±0.02	100±0	6142±17	100±0	163±4	141±1	18.70±3.0	100±0
Combined Cardiac Sympathetic Nerve Stimulation	5.35±0.72	100±0	9.67±0.35	100±0	1.91±0.02	100±0	6142±17	100±0	137±7	146±12	8.57±1.0	100±0
Cardiac Slowing	5.35±0.72	100±0	9.67±0.35	100±0	1.91±0.02	100±0	6142±17	100±0	173±18	114±13	26.30±1.0	100±0
Temporary Cardiac Arrest	5.35±0.72	100±0	9.67±0.35	100±0	1.91±0.02	100±0	6142±17	100±0	126±8	121±5	6.67±1.0	100±0
Ouabain	4.67±0.64	100±0	9.67±0.35	100±0	1.91±0.02	100±0	6142±17	100±0	138±9	146±5	11.25±3.0	100±0
Lidocaine	4.67±0.64	100±0	9.67±0.35	100±0	1.91±0.02	100±0	6142±17	100±0	146±8	140±5	12.5±2.0	100±0
Ouabain and Lidocaine	5.35±0.72	100±	9.67±0.35	100±0	1.91±0.02	100±0	6142±17	100±0	150±20	145±26	12.0±1.0	100±0

Legend: PHOS. = PHOSPHORYLASE
GLY.SYN = GLYCOGEN SYNTHETASE
UDPG Pyro. = UDPG PYROPHOSPHORYLASE
GLY. = GLYCOGEN
HR = HEART RATE
BP = BLOOD PRESSURE
Cont. Force = CONTRACTILE FORCE

%1 = ACTUAL %
%2 = CONTROL VALUE TAKEN AS 100%
NS = NO SAMPLES TAKEN
* = DENOTES SIGNIFICANCE AT 95% CONFIDENCE LEVEL

MAXIMUM EFFECT

ORCE	PHOS. a		GLY. SYN. I		UDPG Pyro.	GLY.			HR	BP	CONT. FORCE	
%2	%1	%2	%1	%2	µmoles UDPG/g/min	%2	µg/g	%2	BEATS/MIN	mm Hg	mm	%2
100±0	24.17±2.40	452±44*	22.50±0.41	233±12*	1.71±0.01	90±3	4,399±56	70±5*	123±5	131±3	13.75±2.30	220±20*
100±0	12.0 ±1.0	224±21*	9.33±0.25	96±3	1.78±0.07	93±1	5,167±34	85±7	263±4*	141±1	17.80±2.0	95±7
100±0	27. 67±4.82	517±6*	20.0±0	207±0*	1.83±0.04	96±2	NS	NS	178±7	164±9	25.0±4.0	293±40*
100±0	2.67±0.31	50±8*	13.73±0.15	141±5*	2.45±0.47	128±3	12,400±159	202±13*	110±12*	103±10	23.41±2.32	88±4
100±0	0.95±0.01	18±1*	35.17±0.71	364±11*	1.82±0.24	95±3	18,795±207	306±20*	0*	66±5*	0*	0*
100±0	45.25±7.24	969±109*	5.0±0.86	52±6*	0.22±0.01	12±1*	840±18	14±2*	140±10	154±11	29.75±3.27	264±46*
100±0	10.75±3.90	230±10*	10.0±0	104±0	1.78±0.80	93±3	NS	NS	146±15	144±7	14.14±0	112±11
100±0	20.64±7.24	385±10*	22.74±0.53	235±13*	1.92±0.91	101±1	NS	NS	156±25	140±36	25.46±2.29	212±8*