THE ENERGY METABOLISM OF THE FAILING HEART AND THE METABOLIC ACTION OF THE CARDIAC GLYCOSIDES[1]

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I. INTRODUCTION

Few classes of drugs have been the subject of such extensive and thorough pharmacological investigation as the cardioactive glycosides of digitalis, strophanthus, and other plants. From the vast literature representing more than a century and a half of research (cf. the comprehensive monographs of Straub (227), Lendle (141), and Weese (241)) emerges a fairly complete and accurate picture of the circulatory effects of these drugs. Above all, the specific action on the heart stands out with great clarity. But to the present day only the gross manifestations of altered organ and tissue function are well understood. The underlying mechanism of the cardiac action is still more or less obscure.

The elucidation of this mechanism, *i.e.*, the analysis of the cardiac action in

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terms of changes on the cellular, subcellular and molecular level, is a research problem whose solution demands much wider use of biochemical methods of approach than has hitherto been the case. Past attacks on the problem along this line have centered mainly around three aspects of the action of the cardiac glycosides: (a) the relation of this action to the physiological role and the metabolism of certain inorganic cations, particularly calcium and potassium (for reviews or summaries of the literature, see 10, 141, 189, 241); (b) effects on the physicochemical state of the protoplasm (141, 241); and (c) effects on the energy transformations in the heart, notably the catabolic processes furnishing the energy for the recovery phase of the cardiac cycle. The present review is concerned chiefly with the last-named effects; the cardiac glycoside-ion relation and the physicochemical changes are discussed only in so far as they supplement information gained from studies of the changes in energy metabolism. Certain aspects of the subject have been reviewed by Lendle (141), Weese (241) and Mardones (159).

Perhaps the most striking of all the effects of the cardiac glycosides on the heart is the strengthening of its contractile power, the positive inotropic action. This effect is most convincingly demonstrated in the failing heart or the hypodynamic heart muscle preparation, in which the impaired contractility can be restored to normal. It may be assumed that the cardiac glycosides reverse whatever chemical or physicochemical change is responsible for the impairment of contractility. Hence, information concerning the mechanism of myocardial failure responding to cardiac glycosides, as well as that of heart failure known to be refractory to these drugs, would also provide clues to the mechanism of the positive inotropic action. With this in mind a discussion of the pertinent literature on the metabolism of the failing heart has been included in the review.

In discussing the relation between the metabolic and contractility changes, the heart will be regarded as a machine which converts chemical into mechanical energy. Studies chiefly on skeletal muscle (67, 211, 228) indicate that the contractile protein complex actomyosin is the structural basis of the muscular machine and adenosine triphosphate (ATP) its immediate source of energy. On stimulation the muscle is somehow activated and contracts, releasing energy during these processes which appears as heat and as tension or work. Enzymatic hydrolysis of ATP releases the free energy which, according to one viewpoint (228), recharges the contractile system, enabling it to return to the relaxed state. Resynthesis of ATP, and of phosphocreatine which is believed to function as a reservoir of energy-rich phosphate bonds (150) (for a contrary view, see (58)), is accomplished during recovery, the energy required for these reactions being produced by the degradation of foodstuff through the processes of glycolysis and respiration.

The indications are that these reactions are also the main chemical events in cardiac muscle. Actomyosin obtained from heart muscle is, according to Szent-Györgyi (228), indistinguishable from that extracted from skeletal muscle, though certain quantitative differences in physical properties have been reported (156); even if it should be true that the energy source for the cardiac systole is not

ATP but a closely related dinucleotide (8), which is doubtful (151), this would not constitute a fundamental difference. The recovery metabolism in the heart follows essentially the same pattern as in skeletal muscle. Differences are chiefly quantitative and reflect adaptation to specific energy requirements. As a muscle which is uninterruptedly working throughout its lifetime, nearly always at a steady state, not subject to energy demands imposed by sudden great outbursts of activity such as occur in skeletal muscle, and richly supplied with oxygen and nutrients, the heart, particularly in the warm-blooded animal, is a predominantly aerobic organ, powerfully equipped with respiratory enzymes, but possessing a relatively low ability for anaerobic recovery, as shown by its small phosphocreatine reserve and its low glycolytic power. Detailed information on these and other features of cardiac muscle metabolism is contained in several excellent reviews (38, 44, 63, 152, 181, 217a).

II. THE ENERGY METABOLISM OF THE FAILING HEART

A. Total Energy Liberation and Utilization

If intermediary metabolic steps and energy transferring mechanisms are left out of consideration, the problem of where the defect underlying a given type of heart failure is located in the above outlined energy cycle in the heart, reduces itself to the following question: Does the defect concern metabolic energy liberation or does it concern the utilization of the liberated energy for work?

1. *Spontaneous Failure of the Isolated Heart.* A systematic study of the changes in the energy transformations of the heart occurring in failure is feasible only in the isolated organ where the variables of circulatory dynamics cannot only be measured, but where they can be controlled.

The question has been examined most carefully in the mammalian heart, usually the dog heart, isolated in the form of the heart-lung or similar preparation. In this arrangement, the heart is well supplied with oxygenated blood and performs work in a physiological manner, *i.e.*, by pumping blood into the periphery. At first the heart works very competently, but then less and less so, as its contractile power inevitably weakens and it gradually goes into a failure which in many respects is comparable to chronic congestive heart failure and which lends itself easily to experimental analysis. The external work done by this preparation can be accurately measured and, knowing the caloric equivalent of the fuel burned, the total energy set free can be estimated from the oxygen consumption. This is a valid procedure since the heart in the warm-blooded animal contracts an oxygen debt only for brief periods, if at all (63, 84).

The usefulness of the heart-lung preparation in the study of cardiac energetics has been questioned on account of its low mechanical efficiency (195). Indeed, values of the order of 3 per cent are quite common. The low efficiency, interpreted by some authors as indicating failure from the outset, has variously been attributed to unfavorable mechanical conditions inherent in the preparation (79), to denervation of the heart (87, 96), and to the absence of metabolic control through known hormones (98) and unknown substances released by the liver (186, 196). In part, however, it is undoubtedly the consequence of low work

levels, at which the resting oxygen consumption constitutes a relatively large fraction of the total metabolism, and is not necessarily a reflection of a poor physiological state (84).

The theoretical and experimental basis for the study of the energetics of the failing heart was laid by the work of Starling and collaborators (187, 225). They demonstrated that the mechanical energy set free in the contraction of the heart depends on its diastolic volume, *i.e.*, the initial length of its fibers. At a given diastolic volume, the failing heart has a smaller capacity for doing work than the "physiological" heart; in order to maintain a constant level of work, it has to increase its diastolic volume. Later, Starling and Visscher (226) presented data showing that when rate, temperature and chemical conditions are held constant, the oxygen consumed by the mammalian heart is determined by its diastolic volume. According to these authors this is also the case in spontaneous failure of the heart-lung heart. As the heart goes into failure and dilates, the same amount of work can be performed only at the cost of greater oxygen consumption; or when the diastolic volume is kept constant, the oxygen consumption remains the same but the work declines. In either case the mechanical efficiency is decreased. The failing heart is still able to liberate energy from foodstuff at a normal rate, but its ability to convert this energy into work has suffered.

These findings and conclusions concerning the mechanism of the spontaneous failure in the isolated mammalian heart have been confirmed in Visscher's laboratory (169, 185, 235) as well as by other workers (45, 86, 95). They have been challenged by Rühl (203, 206) and by Katz and collaborators, (18, 121, 250), who failed to note a loss of efficiency during failure. Since Rühl did not measure or control diastolic volume and work, his results are not as relevant in this connection as those of Katz *et al.* (121) who maintain that when the heart is permitted to fail at approximately constant diastolic volume, there is a coincident decrease in work and oxygen consumption, whereas at a constant work level the diastolic volume increases during development of failure without significant changes in oxygen consumption and mechanical efficiency. In these experiments, apparently, the loss of contractility was associated with a reduction in total energy release and not with a reduced ability to utilize the liberated energy for work.

Katz (118) suggests that diastolic volume may not be the sole factor governing the release of energy by the heart. As other authors (38, 84, 123) have pointed out, if the amount of energy released by the heart were independent of the mechanical conditions prevailing during contraction, cardiac muscle would be in a class apart from skeletal muscle, which liberates extra energy for work (Fenn effect) (cf. 67). The crucial experiment to decide this question, namely, the measurement of the heat and work output of cardiac muscle under conditions also permitting isometric contraction, such as in the papillary muscle preparation, has not yet been performed.

Since impairment of energy utilization was always observed to be the cause of spontaneous heart failure in those studies in which oxygen uptake was determined with a spirometer, whereas impairment of energy liberation was found

as the causative factor when the oxygen uptake was estimated from the arteriovenous oxygen difference and the coronary flow, the discrepancy of the results was thought at first to be attributable to the difference in analytical methods. The drawbacks of each method in the determination of the oxygen uptake of the heart have been amply stressed by its opponents (86, 98, 121, 234). To obviate criticism and counter-criticism both methods were improved and refined (118, 169, 185, 250), but without yielding results differing from those obtained previously by the respective investigators. Moe and Visscher (169), using the heart-oxygenator preparation, made simultaneous determinations of the oxygen uptake by the spirometer and blood analysis methods. The methods checked satisfactorily and gave values showing failure in this preparation to be associated with the sharp decline in mechanical efficiency. The explanation for the divergent results of Katz *et al.*, using the same type of preparation, must probably be sought in peculiarities of experimental technic other than the analytical method.

Spontaneous failure of the isolated frog and tortoise heart perfused with Ringer fluid containing blood or serum is entirely due to decreased energy utilization, the oxygen consumption remaining relatively high (39, 40, 53, 61). When some of the same preparations are perfused with pure Ringer fluid, the resulting hypodynamic condition is characterized by a parallel decrease in mechanical activity and total oxygen consumption (39, 40). The resting oxygen consumption is not decreased in this condition. Prolonged perfusion of the mammalian heart with a balanced ion solution likewise leads to a simultaneous decline in work and oxygen consumption (197, 198). In this instance the oxygen supply may be a limiting factor.

The chief cause of the hypodynamic condition of the frog heart after long perfusion with Ringer solution is, according to Clark (35), a loss of lipids from the surface of the cell. The claim that loss of myocardial lipids, in the form of phospholipids, is a cause of human congestive heart failure (138) has not been substantiated (51), and neither has a noticeable decrease in the lipid content of the heart been found in experimental myocardial damage (237).

As Clark (35) has shown, the weakened contractility of the frog heart depleted of lipids can be restored to normal by addition of serum or alcoholic extracts of serum, the active principle being probably a lipid, as well as by low concentrations of sodium oleate, the activity of which is believed to be due to the formation of an insoluble calcium soap at the cellular membrane. The restoration of the contractility of the heart by these substances is accompanied by an increase in oxygen consumption to the normal level (39). Clark (38) draws attention to the observation of Meyerhof (165) that the oxygen consumption of minced muscle is greatly increased on addition of the phospholipid, lecithin, or of one of its components, linolenic acid. Lecithin has been shown by Clark (35) and others (38) to strengthen the beat of the frog heart under the same conditions in which oleate and serum are effective. That phospholipids play a role in oxidative processes has long been suspected (221). A recent study (6) suggests that lipids and phospholipids may serve as "cement substances" to hold in close association groups of enzymes that perform together in metabolic cycles.

2. *Experimental Heart Failure Induced by Pharmacological Means.* There is no need here for a discussion of the general nature of heart failure caused by acute anoxia of the myocardium, whether produced by deprivation of oxygen or by specific respiratory inhibitors such as cyanide. The evidence that these types of failure are due to interference with the supply of energy is clear-cut, even in the case of the frog heart which has some ability for anaerobic survival (38). It is noteworthy that both in oxygen lack as well as in cyanide poisoning, the cardiac glycosides are incapable of restoring the contractility of the myocardium (p. 328).

On the other hand, there are a number of agents with a negative inotropic action on the heart which are known to be antagonized by the cardiac glycosides. The study of the changes in energy metabolism which accompany this action have been confined mainly to heart failure caused by narcotics (general anesthetics, hypnotics) and by alterations in the inorganic ionic environment.

The literature on the action of the narcotics on the cold-blooded heart has been reviewed in the monograph of Clark *et al.* (38). It appears that these compounds depress contractility more severely than oxidative metabolism. The relation was studied in detail by Clark and White (39). They found that with increasing concentrations of such narcotics as ethyl urethane and ethyl alcohol the mechanical response declines at a faster rate than the metabolism, *i.e.*, the mechanical efficiency decreases progressively until complete arrest. At this point, the rate of metabolism is about 75 per cent below the value at full activity, a level comparable to that of the normal resting rate. Deducting this residual from the total respiration, the authors found the proportion between the mechanical and the metabolic depression to be constant over the whole concentration range. They concluded, as they did in a subsequent study (40), that the resting respiration of the frog heart differs from the activity respiration in being more resistant to depressant drugs. As Clark and his collaborators (38) emphasize, these results can be explained without the need of postulating two separate respiratory systems by the hypothesis that inhibition of respiration in the heart by narcotics is secondary to inhibition of the contractile process. In other words, in concentrations which depress the contractile power of the frog heart, the narcotics do not interfere specifically with energy liberation.

The effect of narcotics (chloral hydrate, ethyl alcohol) on the metabolism and the function of the isolated mammalian heart perfused with Locke's solution is the same as in the corresponding cold-blooded preparation, namely, a comparatively more pronounced decline in mechanical performance than in oxygen consumption (70, 199). Whether this relation can be explained by the above hypothesis of Clark is not certain, since values for the resting respiration are not given.

A series of studies on heart failure caused by narcotics in the heart-lung preparation of the dog has yielded conflicting results. According to Gremels (95) the rate of oxygen consumption rises markedly in failure caused by barbiturates, although the work simultaneously declines. An increased oxygen consumption was also observed by Fahr and Buehler (65) in hearts poisoned with chloral hydrate.

The results of other investigators are in sharp disagreement with these reports. According to Rühl (204, 206) and Kiese and Garan (123), the oxygen uptake declines regularly in barbiturate-induced failure, and this decline may even be relatively greater than that of the work. Rühl finds this also to be the case in heart failure caused by avertin (204) and histamine (203). However, he is reluctant to regard this as an indication of improved mechanical efficiency since the carbon dioxide output greatly exceeds the oxygen uptake, which suggests to him that lactic acid is being produced. However, according to Visscher's (234) recalculation and reevaluation of the data, Rühl's oxygen consumption figures are too low and his deductions are consequently misleading. Kiese and Garan (123) leave it undecided whether they were dealing with anaerobic energy production or enhanced conversion of chemical energy into mechanical work. Rein (194) and Gollwitzer-Meier and Krüger (86) found that both the oxygen uptake and the work were reduced in moderate and severe barbiturate-induced failure, and similar observations have been made in the intact dog poisoned with chloral hydrate (108).

According to Gollwitzer-Meier and Krüger (86), the decrease in the oxygen uptake of the heart in barbiturate failure occurs in spite of an increase in diastolic volume. With their sensitive recording technic these authors were also able to observe a decrease in oxygen uptake and consequently an improvement of efficiency after small doses of barbiturate which left the circulation unaffected. The efficiency remained improved when mild failure occurred. These phenomena are taken as an indication of a specific inhibitory effect of barbiturates on the oxidative metabolism of the myocardium. In agreement with this conclusion is the observation (256) that low concentrations of pentobarbital and chlorobutanol reduce the work done by the dog heart more than they reduce what is presumed to be its resting respiration. As was pointed out above, a primary effect of narcotics on the oxidative metabolism of the cold-blooded heart has been negated. These substances are depressants of various cellular functions; depending on the state of the tissue and on external conditions such as, for example, temperature, one or the other function may be the first to be affected. In the cold-blooded heart the primary effect of narcotics over a wide range of concentration appears to be an inhibition of the energy utilizing processes in contraction. In the warm-blooded mammalian heart the energy liberating metabolism may be the first function to suffer; but, as a serious stage of myocardial depression is approached, the conversion of chemical into mechanical energy may become impaired to a greater extent.

Reduction of the Ca^{++}/K^{+} ratio in the extracellular fluid has long been known to diminish the contractile power of the heart. Clark and White (39, 40) have shown that lowering the Ca^{++} and increasing the K^{+} concentration in the perfusion fluid of the isolated frog heart produce a relatively greater depression of the amplitude of contraction than of oxygen uptake. The mechanical efficiency of the heart declines steadily until the contractions cease. At this time the heart respires at its normal resting rate, and further changes in ion concentration have little or no effect on this residual fraction of the respiration. On the other hand,

there is a linear relation between the activity (total minus resting) respiration and the mechanical response at all concentrations of ions. Like the analogous effects of narcotics, these phenomena were later interpreted by Clark *et al.* (38) to mean that the reduction of the respiratory metabolism is merely the consequence of a depression of the contractile process. Eismayer and Quincke's data (62), on the other hand, suggest that lack of calcium may lower the oxygen uptake of the frog heart more than its mechanical response. The implication that the oxidative metabolism is specifically depressed in the absence of calcium is hard to accept not only in view of the contrary results of Clark and White (39, 40), but also because the depression of the contractility of the frog heart due to calcium lack occurs with a speed much faster than the depression caused by simultaneous complete inhibition of respiration and glycolysis by means of asphyxia and iodoacetate (49). Furthermore, the rapidity of the calcium effect is independent of the frequency of beat, *i.e.*, one of the principal factors determining the rate at which chemical energy is utilized, whereas the rapidity of the effects of asphyxia and iodoacetate is proportional to the frequency of beat (38, 49). It has also been shown that reduction and even complete omission of calcium or excess of potassium in the bathing fluid of non-beating mammalian heart muscle has no immediate effect on the rate of respiration (11, 145, 184).

In heart failure produced in the intact dog by intravenous infusion of potassium chloride, the oxygen consumption of the heart is unchanged or slightly increased; the mechanical efficiency is markedly lowered (108).

These facts speak strongly against a direct effect of extracellular calcium lack or potassium excess on the energy yielding metabolism of the heart. The interpretation of the potassium effect is complicated by the fact that excess of this ion impairs the conducting mechanism in the heart (38, 265). This does not seem to be the case in calcium lack. There is ample evidence (38) that the electrocardiogram is unaffected even when the heart is almost completely paralyzed due to the absence of calcium. It is of particular significance that the height of the monophasic electrogram is unaltered (17). This has also been shown to be the case in heart failure caused by barbiturates (133). It can be inferred that barbiturates and lack of calcium weaken the heart beat either by direct impairment of the contractile process or by impairment of the processes intervening between the passage of the action potential and the onset of the contractile response. The latter alternative is the more likely one in the case of calcium lack, since the action of calcium on the heart is believed to be located at the surface of the cell (134, 247).

3. *Chronic Congestive Heart Failure.* Nothing definite is known about the mechanism underlying this syndrome and information concerning the point at issue is scant. Harrison (107) has postulated—and this is widely accepted—that a decisive factor in the development of the condition is anoxia of the myocardium due to compensatory cardiac hypertrophy. According to his calculations, the thickness of the hypertrophic fibers does not permit adequate diffusion of oxygen into the internal portions. It has also been estimated (238) that the total capillary surface per unit volume of hypertrophic heart muscle is only one

half as large as in normal heart muscle. However, since the measurements of Dock (55) indicate that in cardiac hypertrophy without coronary disease the coronary flow is more than adequate to provide oxygen in excess of the needs of even the thickest fibers, the anoxia theory of congestive heart failure must be regarded with skepticism. Bing and coworkers (12) report that in patients with congestive heart failure due to mitral stenosis or insufficiency and to arteriosclerotic heart disease the rate of oxygen consumption of the left ventricle per unit weight of muscle is slightly elevated and the mechanical efficiency of work is greatly reduced. The increase in the oxygen consumption, however, is negligible compared to the marked left ventricular enlargement. As the authors point out, this finding raises renewed doubts concerning the validity of Starling and Visscher's (226) postulate that when temperature and the chemical environment are held constant the total energy set free in the heart beat is solely a function of the diastolic volume.

B. Carbohydrate Metabolism

Spontaneous failure of the heart in the heart-lung preparation has been reported by Gremels (98) to be preceded and accompanied by a reduction in glucose uptake. As is to be expected from the work of Evans, Grande and Hsu (64) the utilization of glucose is improved by administration of insulin. The heart failure is stated to be correlated with a (hypothetical) depletion of glycogen, and insulin is said to prevent this depletion. But actual determinations of the glycogen content of the heart in the heart-lung preparation show that it remains unchanged even after six hours of work (236), by which time failure is usually pronounced. Confirming earlier results of Bayliss, Müller and Starling (7) Gremels (98) finds that insulin lowers the oxygen consumption of the heart. This effect is attributed to potentiation of a metabolic sparing action believed to be exerted by the peripheral vagus trunk, and the finding is stressed that oxygen consumption is lowered also following administration of acetylcholine (96), a change said to be independent of a simultaneous decrease of the heart rate. According to Bayliss *et al.* the decrease in cardiac oxygen consumption produced by (impure ?) insulin is secondary to a decrease in cardiac volume and is not related to metabolic changes. Gremels theorizes that the lower rate of glucose utilization before the administration of insulin and the higher rate of oxygen utilization before the administration of acetylcholine constitute a so-called "energetic insufficiency" of the heart, which he thinks is a necessary condition for the development of dynamic failure. This terminology, which gives hypothetical mechanisms the air of having an experimental foundation, has been extended by Hegglin (110, 111) to clinical heart failure characterized by lengthened electrical systole and simultaneously shortened ejection time. Hegglin's original assumption that this "energetic-dynamic" heart failure is associated with a decreased myocardial glycogen content is not supported by his own experimental studies (94, 111, 112); moreover, there does not seem to be a first order correlation between the amount of glycogen in the heart and the electrocardiogram (93).

Because the heart avidly takes up lactic acid from the blood as a source of energy (see 63) it has been suspected that this process may be disturbed in heart failure. The problem has been studied in the mammalian heart-lung preparation where glycolysis in the lungs and the blood provides the heart with a continuous supply of lactate. The uptake (or output) of lactic acid by the heart has been measured from the coronary arterio-venous lactic acid difference and the coronary flow rate as estimated from the coronary sinus outflow. Since it is not known whether the lactic acid content of the coronary sinus blood and of the Thebesian blood are the same under varying conditions—in view of marked differences in oxygen content (169) this may be doubted—and since the partition of the coronary flow between the two venous channels may change during the development of failure, the results obtained must be accepted with reservations.

An increase in the uptake of lactic acid by the heart is stated by Rühl and Rolshoven (202, 205) to occur during spontaneous failure. According to other investigators (89, 162) the lactic acid uptake in this condition decreases progressively until the balance is finally reversed and the metabolite is given off. An increase in the acidity of the coronary sinus blood during the development of the failure has been interpreted in support of this finding (85). Decreased utilization of lactic acid rather than accelerated breakdown of precursors is held to be the reason for the shift in the balance (85, 89). Since the glycogen content remains unchanged (236) this seems to be a plausible explanation. However, the appearance of excess lactic acid in the coronary sinus blood has to be accounted for. Stimulation of glycolysis from glucose is a possibility which cannot be entirely dismissed.

Granted that the spontaneously failing heart does not retain lactic acid because it is unable to oxidize it, the question arises whether it is only the step to pyruvic acid which is blocked. A study of the fate of added pyruvate, which in the normal heart is rapidly removed (20) and oxidized, presumably by way of the tricarboxylic acid cycle (222), would furnish the answer. That the lactic dehydrogenase system in heart muscle may be particularly sensitive to damage is suggested by the finding (176) that in failure of the Langendorff heart caused by depletion of substrates, partial recovery can be effected by administration of pyruvate, but not of lactate. Information on the metabolism of pyruvate in spontaneous heart failure in the heart-lung preparation would be highly desirable also for the reason that the energy sources of the spontaneously failing heart need to be identified. This is a question of particular concern to those who maintain that total energy liberation in this type of failure is undiminished.

Rühl and Rolshoven (202, 205) also claim to have found an increased lactic acid utilization by the heart in barbiturate- and avertin-induced failure, occurring concomitantly with a reduced oxygen consumption and in spite of a postulated stimulation of anaerobic glycolysis (204). The authors had no way of telling what proportion of lactic acid taken up was synthesized to carbohydrate and what was oxidized. Rühl (205) merely states that if all the lactic acid taken up during failure is completely oxidized, 65 per cent of the total oxygen consumption can be accounted for by this reaction. Since the total oxygen consumption is markedly decreased, this would mean that the oxidation of lactic acid and

also of pyruvic acid in the heart is not inhibited by doses of barbiturates and avertin which inhibit the oxidation of other substrates. In support of this contention one could cite the observation (11) which, however, has been questioned (256) that the oxidation of lactate and pyruvate by heart muscle tissue *in vitro* is highly resistant to narcotics such as avertin. Rühl (205) does not specifically attribute the increased uptake of lactic acid to a suppression of the oxidation of other substrates, but ascribes it to a facilitation of its absorption from the capillaries due to dilatation of the coronary vessels.

In mild anoxemia of the mammalian heart-lung or heart-oxygenator preparation lasting even for considerable periods, the function and metabolism of the organ do not seem to become impaired to an appreciably greater extent than occurs with time at a normal oxygen tension. The usage of oxygen (123) and of lactate (16, 207) is maintained at or near the normal levels, and there is no marked depletion of the glycogen stores, provided glucose or lactate are added (16, 207). Sudden failure of the heart occurs when the oxygen tension in the blood is lowered to below 30 to 40 per cent saturation (16, 99, 123), and this failure is associated with exhaustion of the reserves of glycogen (16, 207) and of energy-rich phosphates (p. 322). It is not surprising that in severe myocardial anoxia brought about by occlusion of the coronaries the uptake of lactic acid is arrested (115). This is accompanied and perhaps preceded by destruction of diphosphopyridinenucleotide (DPN), the coenzyme of lactic dehydrogenase (91). It remains to be seen whether this also occurs in less severe myocardial anoxia. The possibility has been suggested (24).

C. Balance of Energy-Rich Phosphate

Knowing that the free energy made available in oxido-reductions is used in muscle for mechanical work through the intercession of ATP and, indirectly, phosphocreatine, with which ATP is in rapid equilibrium, the question of what constitutes, from the point of view of energetics, the cause of failure of the heart can be reformulated somewhat differently: Is the heart failure due to impairment of the generation or of the utilization of phosphate bond energy?

The ATP and phosphocreatine content of the heart at any time reflects the balance between the rates of synthesis and breakdown. The interpretation of changes in content has therefore to take into account the likelihood of changes in the rate of both processes. Unfortunately this is made difficult by the paucity of information concerning the effect of variations in frequency of beat, work, diastolic volume, and other cardiodynamic factors on the ATP and phosphocreatine content of the heart. However, there are indications that normally, within fairly wide limits, it is independent of the activity of the heart (245a, 259). Ordinarily, therefore, a decrease in the ATP and phosphocreatine content of a failing heart may be interpreted as an indication of deficient synthesis, although the possibility of wasteful ATP hydrolysis must be taken into consideration, whereas a constancy or an increase in the content of ATP and phosphocreatine can be accepted as evidence of deficient utilization.

In the rapidly beating warm-blooded heart the continued resynthesis of phos-

phocreatine and ATP is highly dependent upon an efficient aerobic metabolism. Under anaerobic conditions phosphocreatine disappears rapidly, followed by depletion of ATP, and the heart fails rapidly and may go into contracture (23, 28, 243). In the frog heart deprived of oxygen these changes are much slower and just enough energy-rich phosphate can be formed by glycolysis to maintain the beat at only moderately reduced strength for as long as an hour, provided the perfusion fluid is alkaline enough to neutralize the lactic acid formed (37).

A decrease in the ATP content of the warm-blooded heart has also been noted in thiamine deficiency (30). In this condition, which eventually leads to disturbances of cardiac function, one of the main pathways of metabolic energy production is blocked due to inactivation of pyruvic oxidase through lack of its coenzyme, thiamine diphosphate. The metabolic defect has been demonstrated in heart muscle of thiamine deficient animals (180, 193). Another important metabolic disturbance affecting cardiac function is caused by excess of thyroid hormone. The disturbance is characterized by exhaustion of the cardiac stores of ATP (9, 161) and phosphocreatine (68, 161, 217, 219). Concomitant losses in glycogen content have been attributed to increases in the rate of beat. However, heart rate increases of the magnitude produced by excess thyroid hormone have no effect *per se* on the phosphocreatine and ATP content of the muscle (259). The mechanism of the metabolic derangement in the thyrotoxic heart is not known. In view of the fact that oxygen consumption is elevated and energy utilization certainly not improved, the depletion of the energy reserves would be consistent with the assumption that either the oxidative synthesis of ATP and phosphocreatine is inefficient or that the energy set free on dephosphorylation of ATP is wasted. Both thiamine deficiency and thyrotoxicosis lead to heart failure which, like cardiac failure produced by acute anoxia, is resistant to the action of cardiac glycosides (p. 328).

In the asphyxiated heart, cold- or warm-blooded, the decline in work capacity is roughly proportional to the decline of the phosphocreatine concentration (27, 28, 37, 243), and on admission of oxygen both return to normal (37). The loss of ATP does not ordinarily become serious under these conditions until the phosphocreatine stores are exhausted, for the reason that ATP is resynthesized at the expense of phosphocreatine, and not necessarily because phosphocreatine might be the primary energy donor for the cardiac systole, as some authors (28, 29) contend. On the basis of experiments such as these, the so-called "phosphagen index"—the ratio of the phosphate of phosphocreatine to the inorganic orthophosphate—has been introduced as a chemical measure of the physiological state of the heart (44). Apart from the fact that this index is objectionable on chemical grounds because in muscle phosphocreatine is not in direct equilibrium with inorganic phosphate, the interpretation given has validity only when the work capacity of the muscle is strictly a function of the rate of formation of energy-rich phosphate.

There are situations where this is not the case. Clark, Eggleton and Eggleton (37) found that when the isolated frog heart is arrested or its beat weakened by lack of calcium, excess of potassium, or by a narcotic such as ethyl urethane,

its phosphocreatine content remains undiminished. Hence, these forms of failure are due to inactivation of the energy utilizing mechanisms and not to metabolic exhaustion. It will be recalled that a similar conclusion was reached by Clark (38) on the basis of respiratory data.

In later experiments Clark and Eggleton (36) observed that frog hearts poisoned with iodoacetic acid in the presence of oxygen eventually reached the stage of contracture or rigor with their phosphocreatine stores intact. Arrest could be hastened by reducing the oxygen pressure and yet, in contrast to arrest in complete anaerobiosis, there was no loss of phosphocreatine. These results are doubly interesting because the occurrence of contracture or rigor without depletion of phosphocreatine is unusual and because the depression of cellular function on reducing the oxygen pressure was not due to deficient synthesis of phosphocreatine. Failure of mammalian heart muscle treated aerobically with iodoacetate is disassociated from phosphocreatine depletion insofar as it occurs considerably ahead of the depletion (29). This is believed to indicate that the contractile mechanism is depressed to a greater extent than the metabolism. This hypothesis, however, could not explain the results of Clark and Eggleton (36), since in their experiments the same concentration of iodoacetate which caused contracture within fifteen minutes at low oxygen pressure had no effect for hours in air. Hence, contractility could not have been directly impaired.

Spontaneous failure of the isolated mammalian heart may or may not be associated with "chemical" failure, depending upon experimental conditions. When the heart is perfused with a salt solution the phosphocreatine content is decreased at the onset of failure and continues to decrease as failure progresses (Mügge, 172; Weicker, 243, 244). According to Mügge the ATP concentration remains unchanged throughout; according to Weicker it is diminished, and, as reported earlier by Parnas and Ostern (182), there is also a loss of adenylic acid, probably due to deamination and possibly further breakdown, as is to be expected when synthesis of ATP is deficient. This failure is also accompanied by a decrease in oxygen uptake (197, 198). The danger of anoxia looms large in the mammalian heart perfused with Ringer, particularly during the setting up of the preparation, and other essential cell constituents besides adenylic acid may be destroyed or washed out (24). These dangers are minimized in the heart supplied with blood. Indeed, analyses performed by the present author (254) reveal that the spontaneously failing heart in the heart-lung preparation retains its normal complement of ATP and is even richer in phosphocreatine than the nonfailing heart. In this form of heart failure, therefore, it is the utilization and not the generation of phosphate bond energy which is at fault. In the same publication, unchanged ATP and elevated phosphocreatine values are also reported to have been found in heart failure resulting from the administration of various anesthetics and other toxic drugs, in spite of the fact that some of these compounds have a direct depressant action on the oxidative metabolism of heart muscle (86, 256). It has been suggested (119) that the high phosphocreatine and ATP content of the majority of the failing hearts in these experiments may have been the result of a decrease in the work load. However, the data provide a few

examples showing that a spontaneously failing heart may perform a normal amount of work without differing appreciably, with respect to the distribution of the energy-rich phosphates, from a heart doing little work. Furthermore, it has been found (259) that even very wide variations in "volume" work have little effect on the ATP and phosphocreatine levels in the heart of the heart-lung preparation. Variations in "pressure" work, on the other hand, produce changes in the phosphocreatine level in a direction opposite to the changes in arterial pressure. But in order to produce significant effects the pressure changes have to be pronounced, more pronounced, in fact, than those which occurred in spontaneous failure and in the majority of the drug-induced failures in my above mentioned experiments. Therefore, the adequacy of the energy-rich phosphate supply of the heart in spontaneous experimental failure can hardly be attributed to a sparing action of decreased work. The same can probably be said of failure induced by drugs which do not specifically inhibit the oxidative metabolism.

The instability of phosphocreatine and ATP precludes their determination in human autopsy material. Reasoning that the estimation of creatine after death might be a substitute for the estimation of phosphocreatine in life, Herrman and Decherd (113) and Myers and Mangun (173, 175) have amassed an impressive amount of clinical data, showing, in confirmation of earlier reports (15, 41, 42), the creatine content of the heart to be abnormally low in patients who had died of congestive heart failure. The creatine content in the myocardium of animals with experimentally damaged hearts was likewise found to be lower (113). The authors believe that in all probability the heart in congestive failure has an inadequate supply of phosphocreatine.

Little factual evidence exists as yet to back up this contention. The amount of creatine bound to phosphate in the myocardium is merely a minor fraction of the total creatine (22, 48, 190, 254), and the ratio between the two appears to vary widely from heart to heart (254). Hence caution is required in interpreting differences in myocardial creatine in terms of changes in phosphocreatine. The reported decreases in the creatine content of the heart in congestive failure may turn out to have a significance unrelated to hypothetical decreases in phosphocreatine. It is not known at present what other vital role creatine plays in muscle in addition to serving in phosphate transfer. There are indications (48) that the inherent strength of cardiac muscle varies with its creatine and not with its phosphocreatine content.

The studies of the human heart were extended by Mangun and Myers (157) to include determinations of total acid-soluble purines, the larger part of which was presumably present before death in the form of adenosine compounds. The results show a deficiency of the acid-soluble purines in the cardiac ventricles of patients who had died of myocardial failure. Whether this actually means that the ATP content was low before death remains to be verified.

The same authors (158, 173) suggest that, since phosphocreatine and ATP probably exist in the cell largely as potassium salts (174), loss of these compounds might account for the low potassium content found in the myocardium of patients who had died of congestive heart failure (107, 158, 248). However, the low

potassium content of the heart in congestive failure may also have other causes. There are reasons to believe that the amount of potassium inside the muscle fiber is closely correlated to the physical state of myosin (cf. 67, 228). In view of evidence indicating that the solubility (60, 171) and electrophoretic pattern (59, 117) of myosin of fatigued muscles differ from those of rested muscles, the possibility should not be overlooked that the state of myosin in the myocardium might be altered in heart failure.

In a review of their work, Myers and Mangun (175) refer briefly to unpublished observations on dogs with aortic insufficiency. No losses in acid-soluble phosphates were seen in the left ventricles during the early stages of cardiac failure, but in the late stages a decrease in the ATP and phosphocreatine content was noted in two dogs. More determinations of this sort are needed before the significance of the clinical data can be properly assessed. Until such time any pronouncement as to the status of ATP and phosphocreatine in the heart in chronic congestive failure will be more or less arbitrary.

III. THE METABOLIC ACTION OF THE CARDIAC GLYCOSIDES

A. Total Energy Liberation and Utilization by the Heart

1. *Therapeutic Concentrations.* a. *Absence of Heart Failure.* The cardiac glycosides do not appreciably increase the work of the heart in a good physiological condition. It is important to know whether they may, nevertheless, influence its metabolism. Using the isolated frog heart perfused with Ringer's solution, Eismayer and Quincke (62) obtained considerable increases in oxygen uptake and carbon dioxide output with a very low concentration of strophanthin ($1:10^7$) which had no influence on the work output. On addition of small amounts of strophanthin to the isolated frog auricle, David (47) noted a similar increase in respiration, which was later followed by a decline to below the normal level. Since the frequency of beat was lowered and the amplitude of contraction not augmented, the stimulation of metabolism can be regarded, as in Eismayer and Quincke's experiments, to represent a primary effect. In earlier experiments on the isolated frog heart, Gottschalk (90) had failed to note any increase in oxygen consumption by strophanthin $1:10^6$ or by higher concentrations which eventually produced toxic effects. Because an increase in oxygen consumption may be obtainable only under favorable experimental conditions, the findings of Eismayer and Quincke and of David probably carry greater weight than the negative results of Gottschalk.

The fact that in the experiments of Eismayer and Quincke and of David the mechanical efficiency declined, due to the rise in oxygen uptake, is perhaps indicative of a dislocation in the normal interplay of the various cellular functions and may therefore be regarded as a toxic effect. There is no way of telling from the data whether the excess energy liberated is completely wasted as heat or whether some energy requiring activity other than contraction–relaxation is stimulated. Since under different circumstances the heart is made to work more economically by the cardiac glycosides, the latter alternative seems more likely.

The argument that the liberation of the extra energy is itself a phenomenon characteristic of the "toxic" as contrasted to the "therapeutic" phase of action of cardiac glycosides on the heart (84, 97) is not very helpful because the processes underlying both phases may be the same.

The gaseous metabolism of the non-failing heart-lung preparation of the dog as well as its work and diastolic volume has been found by Gollwitzer-Meier and Krüger (86) to be practically unchanged after administration of therapeutic doses of strophanthin. Gremels (97) has presented data showing the oxygen consumption and the frequency of beat of the freshly prepared heart-lung preparation to be markedly reduced following the administration of small amounts (5 μgm.) of strophanthin or digitoxin. These effects seem to be due to sensitization of the denervated heart to residual vagal activity because they can be reproduced by infusion of minute amounts of acetylcholine which in themselves are not effective (cf. also 46). Gremels believes that the decrease in oxygen consumption is a phenomenon independent of the decrease in heart rate.

Cattell (26) has found that the tension and the total heat produced in the twitch of the frog sartorius suspended in a gaseous environment are both increased after previous exposure to ouabain, the mechanical efficiency remaining unchanged. This is soon followed by a decrease in tension, heat output, and efficiency, and finally by loss of excitability. The initial changes and the inexcitability, but not the decrease in efficiency, are probably related to the escape of potassium from the muscle fiber and its accumulation at the membrane (103). Increased movement of potassium is a prominent chemical feature of the toxic and perhaps also of the positive inotropic action of the cardiac glycosides on cardiac muscle (see p. 343).

b. *Spontaneous Failure of the Isolated Heart.* In the isolated mammalian heart perfused with Locke solution in which spontaneous failure is characterized by a proportionally equal decline in work and total energy liberation (197, 198), the positive inotropic action of strophanthin is associated with an increase in oxygen uptake, without appreciable changes in mechanical efficiency (199). This is also true in hearts beating isometrically at approximately constant diastolic volume.

The changes in cardiac energetics characterizing the spontaneous failure of the heart in the mammalian heart-lung preparation are likewise reversed by the cardiac glycosides. Most authors agree that the heart is enabled to perform more work with a relatively or even absolutely smaller expenditure of energy (86, 95, 168, 185). Claims that the oxygen consumption increases in proportion to the work done and that consequently the improvement in work performance is due to increased liberation of energy (206, 208) have been dismissed as based on questionable methods of estimating work (84) and oxygen consumption (234). When the venous blood supply to the heart is kept constant during recovery from spontaneous failure the increase in work is accompanied by an actual decrease in oxygen consumption (86, 95). The decreased oxygen consumption is said to be proportional to a decrease in external diastolic volume (86). While there cannot be the slightest doubt concerning the improvement of energy utiliza-

tion, the complexity of the changes renders their interpretation difficult. The analysis of the relation between the cardiodynamic and energetic changes becomes easier if either work or diastolic volume is kept constant. The latter is the procedure followed by Peters and Visscher (185). At constant external diastolic volume the oxygen consumption of the spontaneously failing heart is found to increase following the administration of various cardiac glycosides (scillaren, ouabain, digilanid). The utilization of liberated chemical energy for work is improved even more. At the peak of the therapeutic effect, just before irregularities occurred, the increases in oxygen consumption amounted to 16 to 52 per cent; the increases in mechanical efficiency, to 60 to 153 per cent. Peters and Visscher conclude that this represents an improvement in "true" myocardial efficiency. The increase in metabolism is probably not merely a borderline manifestation of poisoning, as Gollwitzer-Meier (84) suggests, since Peters and Visscher's curves show it to occur before the maximum therapeutic effect is reached. However, these authors think that it may be due to a change in the hydration of the myocardium which, judging from their discussion of hydration changes in failure, presumably results in increases in the internal and mean diastolic volumes. Moe and Visscher (168) state, though this is by no means apparent from their published data, that there is no change in total energy output following small doses of digilanid (0.04 to 0.1 mg.) which increase the efficiency of the spontaneously failing heart in the heart-lung preparation. In view of these interpretations and findings and the apparent absence of a metabolic effect in the nonfailing mammalian heart (86), it is not possible at present to attach decisive significance to the reported increases in energy liberation, although there is some justification for doing so in the case of the frog heart (p. 325).

c. *Chemically Induced Heart Failure.* In the isolated frog heart weakened by ethyl alcohol or lack of calcium, low concentrations of strophanthin ($1:2 \times 10^7$) produce an increase in respiratory activity which appears to be secondary to the increase in mechanical activity (62). In the mammalian heart-lung preparation a variety of substances with a negative inotropic action have been shown to be antagonized by the cardiac glycosides. Where the energetics of the heart have been studied a reversal of the changes produced by the depressants has usually been seen. Conflicting statements concerning the direction of the reversal stem from disagreement as to the nature of the changes (see pp. 316–317). According to Fahr and Buehler (65) therapeutic doses of digilanid lower the supposedly elevated oxygen uptake of the heart in failure induced by chloral hydrate apparently in proportion to a reduction in diastolic volume. At approximately constant diastolic volume the oxygen uptake remains unaltered. But Peters and Visscher (185) present an example of a dramatic relief by scillaren of heart failure due to ethyl alcohol where at constant diastolic volume the oxygen consumption rose 77 per cent and the mechanical efficiency 204 per cent. Gremels' assertion (95) that the respiration of the heart weakened by barbituric acid derivatives is lowered by cardiac glycosides has been repeatedly denied (86, 206, 208). Although the diastolic volume of the barbiturate-poisoned heart is decreased by strophanthin (86), the oxygen uptake may actually increase (208).

This probably depends on the changes in work performance. There may also be an increase in mechanical efficiency (86); however, as in the reversal of heart failure produced by camphor (95), this increase can be accounted for by the resumption of the normal relation between work and oxygen consumption with respect to output, *i.e.*, by the well-known improvement of mechanical efficiency accompanying the rise in volume work of the non-failing heart (63).

General agreement exists (86, 185, 206, 208) that in failure due to histamine, strophanthin increases the oxygen consumption of the heart, a change which occurs in spite of a reduction in external diastolic volume (86, 185). Since at the same time the work increases to an even greater extent, the mechanical efficiency is increased (86, 185). According to a hypothesis advanced by Rühl (206), the therapeutic action is a consequence of improved supply of oxygen to the myocardium due to facilitation of the diffusion of the gas across the walls of the capillary vessels. The capillary vessel action is held to be responsible also for the improvement of cardiac function in other types of heart failure. Observations on human subjects showing that injection of strophanthin into the cubital artery increases the oxygen uptake in the cubital region are likewise interpreted as indicative of changes in the capillaries favoring the diffusion of oxygen into the tissue (128).

d. *Heart Failure Refractory to Cardiac Glycosides.* It is not known what influence, if any, the cardiac glycosides may have on cardiac metabolism in heart failure in which they afford no relief, but it is worth recording that they have been found to be of little or no help in disturbances of cardiac metabolism such as myocardial anoxia (127, 129, 154, 223, 245) (but see also 232, 244), thiamine deficiency (71), and thyrotoxicosis (71), in which the contractile power of the myocardium is known or presumed to be weakened by interference with its energy supply. The ineffectiveness of the cardiac glycosides in myocardial anoxia has been interpreted as indicating that they must act during the aerobic phase of contraction (223); but the alternative interpretation, namely, that these drugs restore some non-oxidative process and hence are powerless when the heart fails due to interference with the energy supply for recovery, is just as plausible and is more consistent with what is known about the changes in the energy transformations in the heart during relief of types of failures which respond to cardiac glycosides.

e. *Conclusion.* The results of the studies examined above do not permit an unequivocal decision as to whether the mechanism of cardiac glycoside action is bound up with energy liberation or with energy utilization. The evidence in favor of improved utilization of chemical energy is strong. At the same time it is clear that energy liberation by the heart may also be increased, apparently independently of other changes, and that this increase is particularly pronounced when the metabolism is depressed in failure. In view of the high specificity of action of the cardiac glycosides and of their effectiveness in trace amounts, it seems unlikely that these drugs relieve different forms of myocardial failure by more than one basic mechanism and that they act directly upon more than one cellular system in the myocardium. That this must be a system of general

importance in the activity of the tissue is also suggested by the great variety of chemical changes and pharmacological agents which are antagonized by the cardiac glycosides, comprising, in addition to alterations of the extra-cellular ionic environment and the changes responsible for the development of spontaneous heart failure, not only hypnotics, histamine, and camphor, but a host of compounds not used in the above studies, such as volatile anesthetics, cocaine, heavy metals and quinoline derivatives.

2. *Toxic Concentrations.* Doses of strophanthin leading eventually to ventricular contracture[2] have been reported to reduce the respiration of the isolated ventricle of the frog heart (62, 90). The same effect has been observed in the strophanthin-poisoned frog auricle (47). In all these experiments the decline in respiration paralleled the decline in work or amplitude of contraction and was interpreted as a secondary effect. Near the point of contracture at minimal amplitudes of beat, the oxygen uptake, according to Gottschalk (90), is only about 30 per cent of the initial rate, which is a value only slightly higher than the normal resting respiration of the frog ventricle (38). This observation has not been confirmed. On the contrary, frog ventricular muscle treated with toxic doses of ouabain and described to be in a state of contracture has repeatedly been found to respire at a rate exceeding by far the resting respiration (233, 262). That Niemeyer and Lira, in Mardones' laboratory (159), could not find a change in the oxygen uptake of the isolated frog heart treated with high concentrations of digilanid is perhaps not surprising since their measurements extended over a period of only ten minutes. Salomon and Riesser's (210) data which show no change in the oxygen uptake of isolated frog and mouse hearts in highly toxic solutions of digitoxin and strophanthin are not pertinent to this discussion, because no information on the mechanical activity or the physical condition of the muscle is given.

Ventricular contracture due to poisoning with cardiac glycosides rarely occurs in the mammalian heart supplied with blood. Instead, foci of discharge are set up in the ventricular musculature which give rise to tachycardia and, in combination with a slowing or block of conduction, to severe irregularities of rhythm and eventually to fibrillation. The oxygen uptake of the heart in tachycardia can be expected to be high, but it seems to be elevated even in bradycardia, if there are extrasystoles (168). In the isolated warm-blooded heart perfused with Locke's solution, contracture is the usual endpoint of cardiac glycoside poisoning, and in this state oxygen consumption is markedly augmented (199). This has also been observed in an instance of contracture produced by strophanthin in a mammalian heart-lung preparation (86). No information is available on the metabolism of skeletal muscle in contracture produced by high concen-

[2] This effect of cardiac glycosides, incorrectly (227) called "systolic" standstill, belongs to the group of phenomena covered by Gasser's (78) definition of "contracture" as a sustained, but non-propagated and reversible muscular shortening or tension development. The reversibility of the cardiac glycoside contracture has been demonstrated in the frog heart by means of washing with heart muscle extracts (109) and with thiamine triphosphate (188).

trations of cardiac glycosides, but skeletal muscle contracture due to various other agents is characterized by an increased oxygen consumption (66).

Recent evidence indicates that depolarization of the muscle fiber membrane is the process which initiates contracture, and propagated contraction as well, and that the depolarized state is required for the maintenance of the contracture (136, 137). From studies on nerve (13, 153) it has become apparent that the energy supplied by oxidative metabolism is the essential factor determining the resting membrane potential and that membrane depolarization will bring about an increase in oxygen consumption. In muscle, such a relation could account for at least a fraction of the increased oxygen uptake in contracture, the shortened state of the fibers being possibly another factor favoring increased metabolism (228).

If, for convenience, one considers the membrane changes apart from the energy requirements of the contractile system, the contracture which the cardiac glycosides produce in the heart of cold-blooded animals and in the warm-blooded heart perfused with a salt solution may be assumed to be due to a depolarizing action on the membrane of the myocardial fiber. Such a mechanism seems to be responsible for the increased ventricular automaticity (82). The absence of contracture in the warm-blooded heart supplied with blood might then be attributed to the circumstance that under this condition the tissue is able to release energy at a rate sufficient to restore the decreased membrane potential to a level permitting relaxation, hence making possible renewed initiation of contraction. The ventricular contracture could thus be regarded as the sequence or the counterpart, respectively, of the increased ventricular automaticity.

B. Total Energy Yielding Metabolism of Non-Beating Heart Muscle and Other Tissues

If the cardiac glycosides have an influence on the metabolic activity of the myocardium which is independent of changes produced in mechanical activity—and studies with non-failing frog hearts (p. 325) give us reason to believe that this is the case—this influence may be expected to be visible in the non-beating muscle. For this reason and in order to study single reaction steps, investigators have turned to experiments with cardiac muscle tissue *in vitro*. Other tissues have been used, by some to test for selectivity of action, by others in the hope of demonstrating absence of selectivity since the main pathways of substrate catabolism are largely the same in a great many living cells. In evaluating the significance of the results of these studies with regard to the action on the beating heart, two criteria above all have to be applied, namely, the specificity and the order of potency of the agent. At high enough concentrations any substance can be expected to affect in some way or other various enzyme proteins and their prosthetic groups. The cardiac glycosides, while combining with proteins in the blood plasma (83) and being bound to organs other than the heart (241), have a particularly high affinity for the myocardium, but the effective concentrations in this tissue are extremely low. Furthermore, the cardiac activity depends on a number of characteristic structural features of the active molecule and may be

abolished or greatly weakened by relatively minor structural alterations. One would expect the relation between structure and cardiac activity to hold for any effect *in vitro* having a bearing on the action *in vivo*.

1. *Fermentation.* Incidental observations made during studies on yeast reveal that high concentrations of cardiac glycosides can both stimulate (177) and depress (216) alcoholic fermentation.

Of a different sort are investigations (75, 100, 142, 192, 213, 214, 231) reported on by Freund (72, 73). These investigations, as well as other studies from Freund's laboratory to be discussed below, constitute the first systematic attempt to attack the problem of the mode of action of the cardiac glycosides by the methods of enzyme biochemistry, and herein lies their merit. Data are published according to which "therapeutic" concentrations of strophanthin and digitoxin and their genins ($1:5 \times 10^9$(!)–$1:5 \times 10^7$) increase the anaerobic glycolysis of minced mammalian heart and skeletal muscle, whereas higher, "toxic" concentrations have as a rule a depressant effect. The anaerobic glycolysis of brain is said to be increased at all concentrations. A particularly pronounced loss of glycolytic power is reported to occur in the heart and skeletal muscle of strophanthin-poisoned animals (231). Freund (73) classifies the cardiac glycosides as agents which modify the carbohydrate metabolism not only of cardiac muscle but of living tissue in general and implies that this constitutes the mechanism of their therapeutic and toxic action on the heart. The fact that the experimental work on which these claims are based was performed by dental and medical students explains perhaps why the results were not confirmed by more responsible investigators.

An inhibition of glycolysis in fresh unwashed horse erythrocytes by strophanthin in concentrations as low as $1:5 \times 10^5$ has been reported by Segre (218). The inhibition is ascribed to a shift in the lactic acid-pyruvic acid equilibrium in favor of the latter compound, the accumulation of which is stated to cause inactivation of glycolytic enzymes. When the experiment is repeated in the presence of cyanide, strophanthin no longer inhibits but stimulates glycolysis, due to the removal of pyruvate by the keto reagent. These and other effects, to be discussed below, can be obtained in hemolysates, *i.e.*, in the absence of protoplasmic structure.

Profound changes in glycolytic metabolism are produced by the cardiac glycosides in the brain. Weese and Wiegand (242), using slices of guinea pig brain, noted that strophanthin not only strongly inhibits anaerobic glycolysis and moderately depresses respiration, but brings about a qualitative change in the aerobic metabolism which, depending on whether the tabular or the textual presentation of their results is to be believed, constitutes either a pronounced rise of the respiratory quotient (R. Q.) or a stimulation of glycolysis. Detailed reinvestigation of this matter in the same tissue and with additional glycosides (ouabain, digitoxin, scillaren, and others) showed that the R. Q. remains unchanged and that aerobic glycolysis is set in motion (258). At the same time respiration is temporarily increased and anaerobic glycolysis, as in Weese and Wiegand's experiments, permanently depressed. This inhibition of the Pasteur

effect is complete in phosphate-buffered salt solution in which the aerobic glycolysis quickly reaches the normal anaerobic level. None of these changes is seen in cell-free brain preparations or, like the effect of high concentrations of potassium (3, 54) to which they have a striking resemblance, in tissues other than the brain, except that the respiratory action also occurs in heart muscle (cf. below). An inhibition of anaerobic glycolysis has been noticed in heart muscle slices of the rat (147), but not in feebly glycolyzing guinea pig heart slices (258). That stimulation of glycolysis is not noticeable in heart muscle is probably a consequence of the low glycolytic capacity of this tissue (22, 120, 178). The brain effect is also specific in that cardio-inactive derivatives of the cardiac glycosides as well as a number of structurally unrelated cardiac agents are unable to produce it at a concentration (2×10^{-5} M) which is 5 to 20 times the concentration at which the various cardiac glycosides are highly effective. On the other hand, it can be duplicated with very low concentrations of the structurally related steroid alkaloids protoveratrine and veratridine and the triterpenoid alkaloid coumingine, all of which have an action on the heart similar to that of the cardiac glycosides (131, 155, 212). Veratrine, the most active component of which is veratridine, has been shown to have a marked stimulating effect on the metabolism of peripheral nerve (215). It is quite possible that the metabolic brain effect is related to the disturbance in the function of the central nervous system seen in digitalis and veratrine poisoning, and the cellular changes in the poisoned brain may be comparable to those in the poisoned heart. Nevertheless, the deduction (242) that the inhibition of anaerobic glycolysis and of respiration in the brain explains the depression of impulse conduction in the heart seems farfetched.

2. *Respiration.* A bewildering mass of data on the influence of cardiac glycosides upon tissue respiration is contained in a series of dissertations from the laboratory of Freund (21, 74, 76, 77, 135, 191, 214). Concentrations of various cardiac glycosides and aglycones of the order of $1:10^9$ to $1:10^7$ are credited with possessing a stimulating and in other cases an inhibiting action on the oxygen uptake of mammalian and frog heart muscle suspended in phosphate buffer. At "toxic" concentrations ($1:10^6$) inhibition of the respiratory metabolism is said by some authors (21, 74, 76, 191) to be the predominant effect in these tissues, whereas others (77, 135) insist on observing a stimulant action; in brain and liver an increase in respiration is generally noted at all concentrations. Unless one accepts the existence of radical differences between the individual drugs, there is no way of predicting, from the kind of substrate added or otherwise, the quality or intensity of the action. Of all these findings only the inhibition of respiration in mammalian cardiac muscle, emphasized by Frühauf (76) could be interpreted as being in agreement with the work of later authors (69, 259), though not in the extreme dilution range. But even this action, or any other, is denied by Salomon and Riesser (210) who repeated Frühauf's experiments. Neither do these authors admit any effect of low or of high concentrations of strophanthin when the plain phosphate buffer is replaced by phosphate-Ringer. Genuit and Haarmann (80), too, obtained only insignificant changes in the

metabolism of minced rat and guinea pig hearts after the addition of strophanthin to give final concentrations ranging from $1:5 \times 10^7$ to $1:3 \times 10^6$. They conclude, and this may be accepted as a fair appraisal of the significance of the preceding work as well, that experiments with minced tissue are not very useful in bringing to light possible metabolic effects of the cardiac glycosides on the beating heart. Attention must be called to the fact that in all the above studies the cardiac tissue preparations were respiring at abnormally low rates.

When, instead of the ill-defined mixture of whole and fragmented cells and free enzymes which goes to make up a fine tissue mince, relatively intact and more steadily respiring pieces of cardiac muscle in the form of slices are used, profound effects of the cardiac glycosides on the respiration become apparent. This has been, with a single exception (101), the common experience in several laboratories as attested by the recent contributions of Lévy and Libert (143, 144, 146, 147), Wollenberger (251, 252, 253, 257, 258), Finkelstein and Bodansky (69) and DuBois *et al.* (57). In general the respiratory action can be obtained at low, pharmacologically relevant concentrations of the cardiac glycosides. Its quality and intensity depend, among other factors, on the concentration of the drug, the composition of the medium, and the species of animal. Increases in the rate of oxygen uptake of as much as 200 per cent (146, 147) as well as total suppression of activity (259) have been observed. Significant changes in the R. Q. have not been noticed (253). In buffered Ringer or similar solution containing glucose or lactate, the oxygen consumption is, as a rule, accelerated at all glycoside concentrations, and magnitude and rapidity of this effect are, up to a maximum, proportional to the concentration (69, 251, 253). An analogous proportionality characterizes the decline in the rate of oxygen uptake which at higher than minimal effective concentrations follows the acceleration in cardiac slices of guinea pigs (251, 253) and rats (144). High concentrations of ouabain have been reported to depress the oxygen uptake of rat heart slices in normal medium from the outset (144), but in these experiments readings were taken only once an hour, and it is therefore not unlikely that an initial rise in activity might have been missed. In ventricular muscle slices of the heart of the cat (69) and the dog (251), the sole action of glycosides even at maximal effective concentrations is to produce a rise in respiration lasting for as long as four to five hours. The reason for this species difference is obscure, but in experiments in which only small amounts of dog heart tissues were used (20 mg. wet weight or less per 3 cc.) the duration of the rise in respiration was reduced and the rate fell below the control level (259). The dependence of all these effects on environmental factors is illustrated by observations that respiration may not be increased and may instead be depressed at once if the incubation medium is altered by acidification (144), omission of substrate (147, 253), or omission of calcium ions (69), and that it has been possible to prevent the inhibition, but not the stimulation, of respiration in guinea pig heart slices by incubating them in boiled heart muscle extracts (252).

So far only one other tissue besides cardiac muscle has been found which responds to cardiac glycosides with an increase in respiration, namely, brain

cortex (253). Its sensitivity, however, is considerably lower than that of heart muscle. Inhibitory effects on cellular respiration have been observed in a variety of other tissues (57, 253), but these effects are not comparable in intensity to the pronounced inhibition of metabolism which can be produced in slices of brain cortex and cardiac muscle. The absence of a specific response in skeletal (146, 253) and smooth (259) muscle is worth noting.

Intactness of the structure of the cardiac muscle fiber appears to be a prerequisite for the action of the cardiac glycosides on the respiration of the tissue. Ouabain in 100 times the concentration exerting a maximal effect on sliced heart muscle has not the slightest effect on the respiration of the homogenized tissue. This has been demonstrated both in concentrated unfortified (253) and in dilute fortified (259) preparations. A marked inhibition by ouabain of the respiration of isotonic homogenates of brain has been attributed to the presence of intact cells (253), and this is conceivably also the explanation for a similar inhibitory action of bufagin (57). As a matter of fact, no significant effect of ouabain is seen in hypotonic (253) or in cell-free isotonic (258) brain homogenates. It has been concluded (253) that the cardiac glycosides have no *direct* effect on the catalytic activity of oxidative enzymes.

The question naturally arises whether the action of the cardiac glycosides on the respiration of heart muscle tissue *in vitro* is of relevance with regard to the action on the beating heart. In the light of available evidence, both direct and indirect, this question can without hesitation be answered in the affirmative. First of all there is the convincing observation (143, 144, 146, 257) that slices prepared from hearts of animals which have been given injections of cardiac glycosides, in therapeutic to lethal doses, can be distinguished from cardiac muscle slices of control animals by virtue of differences in the rate of oxygen consumption, the first hour values following cardiac glycoside administration being, as a rule, markedly elevated above the control level.

Secondly, from what has become known so far about the effect of cardiac glycosides on cardiac tissue respiration, this effect appears comparable in a number of ways to the effect on cardiac function, particularly with respect to its intensity and specificity. This parallelism is brought out by the following features of the respiratory response: (a) The response can be elicited by concentrations and doses of cardiac glycosides which lie within the therapeutic or at least the non-lethal range for the beating heart (69, 251, 253). (b) The order of potency among individual glycosides and aglycones corresponds to that of their positive inotropic and toxic action on the isolated heart (252, 259). (c) The sensitivity of the respiratory response to a given cardiac glycoside varies according to the species of the animal in much the same way as the sensitivity to the inotropic and toxic action of the compound (146). (d) Of all animal tissues tested the myocardium is by far the most sensitive to the respiratory action of the cardiac glycosides and is, besides brain cortex (253), the only one which responds with an increase in respiratory activity (146, 253, 259). (e) The respiratory effect is highly dependent upon certain structural characteristics of the cardiac glycoside molecule which are also required for its typical cardiac action. As was found in

brain (258), changes in the molecule which abolish cardiac activity, such as hydrogenation of the lactone ring or allomerization at carbon 17, also abolish the respiratory response (259). Among the metabolically inactive compounds are also simpler unsaturated lactones which produce contracture in the perfused frog heart and several structurally unrelated cardiac agents, among them epinephrine. Again, like in brain (258), the metabolic effect is elicited by low, physiologically relevant concentrations of structurally related alkaloids which have a digitalis-like action on the heart, *viz.*, the steroid veratrum alkaloids veratridine and protoveratrine and the triterpenoid erythrophleum alkaloid coumingine (259).

3. *Mechanisms.* No satisfactory explanation has been given for the increase or the subsequent decrease in the rate of respiration of cardiac and brain cortex slices by the cardiac glycosides. I suggested at one time (253) that at least a fraction of the increase in respiration is due to increased permeability to exogenous oxidizable substrates and attributed the inhibition of respiration and also of anaerobic glycolysis to a loss of diffusable oxidative catalysts.

Changes in cell permeability have been invoked on a number of occasions, usually on purely speculative grounds, in order to account for unexplained effects of cardiac glycosides. Some evidence of both increases and decreases in membrane permeability to various substances has been obtained in experiments with erythrocytes and model membranes (141, 241). The finding that the fixation of Brilliant Congo Red by the isolated frog heart is slowed in the presence of small amounts of ouabain has been taken as an indication of a decreased permeability of the cardiac muscle fiber (209). On the other hand, abnormal deposition of vital dyes in the myocardium of animals poisoned with digoxin is suggestive of an increased permeability (249), but this change may be entirely incidental to the development of anatomical lesions.

The permeability hypothesis of the stimulant action of the cardiac glycosides on heart and brain tissue respiration is mainly based on experiments (147, 253) showing that in guinea pig and rat heart slices the increase in respiration is proportional, within limits, to the concentration of glucose or lactate in the surrounding medium and does not occur in the absence of these substrates. In support of this hypothesis could also be cited the findings that there is no increase but only a decline of the rate of metabolism when the permeability of the cell is abnormally high to start with, such as in the absence of calcium (69) and in anaerobiosis (147, 252).

That not more than a fraction of the increase in the oxygen uptake caused by cardiac glycosides may be due to accelerated oxidation of exogenous substrate is emphasized by the findings that in cat heart slices this increase is not entirely dependent on the presence of added substrate (69) and that in dog heart slices incubated in glucose-containing solution it is greater than can be accounted for by an increase in glucose uptake, even assuming complete combustion of the extra sugar taken up (259). In the resting ventricular muscle of the frog the stimulating effect of cardiac glycosides on the respiration is entirely independent of the presence or concentration of exogenous glucose (262). These findings, particu-

larly the latter, indicate that an increase in permeability to substrates is not the cause, certainly not the most important cause, of the enhanced respiratory activity of cardiac muscle exposed to cardiac glycosides. The increased rate of permeation of glucose into dog and probably into other mammalian heart muscle, in which the cardiac glycoside effect is partly or wholly dependent upon extracellular substrate, may be merely a consequence and not a cause of the accelerated oxidation of this substrate inside the cell.

The hypothesis that increased permeability, by allowing oxidative catalysts to diffuse out of the cell, is responsible for the inhibitory action of the cardiac glycosides on the metabolism of heart and brain slices fits a number of experimental facts very well, such as the intensification of the inhibitory effect by washing (253); its prevention by contact with boiled heart muscle extract (252), a medium rich in various metabolites and inorganic and organic cofactors; the unimpaired activity of the succinic oxidase system, which functions without pyridine nucleotide, in otherwise severely poisoned cardiac slices (253); and, finally, the inverse relationship between tissue concentration and the rapidity and intensity of respiratory inhibition in heart slices from ouabain-poisoned dogs (257). Interestingly enough, intensification of certain toxic effects of cardiac glycosides by means of washing with Ringer's solution occurs also in contracting skeletal muscle (200), and reversal of the toxic action on the frog heart can be effected by means of washing with extracts of heart muscle (109).

The permeability hypothesis, however, is not helpful in explaining the lack of correlation between tissue concentration and intensity of the inhibitory effect on the metabolism of brain cortex (253), nor does it explain the effectiveness of nicotinamide (0.04 M) and the ineffectiveness of DPN (0.001 M) in preventing the inhibition of anaerobic glycolysis in ouabain-treated brain (258). The latter phenomenon is taken to mean that not outward diffusion, but outright destruction of the coenzyme by its nucleosidase is a cause of the inhibition of glycolysis. Nicotinamide, however, does not protect against inhibition of the aerobic metabolism. Increased DPN-ase activity, therefore, could not be the sole effect of the cardiac glycosides. Other hydrolytic enzymes might conceivably be released from an inactive state. The stimulation of aerobic glycolysis in brain, not explainable on the basis of interference with oxidative phosphorylation (258), likewise points to the removal of a barrier normally restricting cellular enzymatic activity. Changes in the colloidal state of protoplasm, reported to occur in the presence of cardiac glycosides (141, 241), might conceivably be responsible for these effects by making ordinarily inaccessible enzyme surfaces available to their substrates.

Finkelstein and Bodansky (69) have called attention to the possibility that the increased oxygen consumption of cardiac slices incubated in the presence of cardiac glycosides may be associated with contracture of the muscle. Indeed, Victor (233), who reported that ouabain in rather high concentrations greatly increases the oxygen consumption of the resting frog ventricle, found the muscle in a firmly contracted state at the end of his experiments. The contracture hypothesis, however, could not explain the increased oxygen uptake in brain tissue. Furthermore, Yaffe (262) has found that the rise in the oxygen uptake of resting

frog ventricular muscle by high concentrations of ouabain precedes the onset of contracture and occurs also in the presence of low concentrations of the glycoside not producing contracture.

The increase in the respiration of cardiac muscle tissue even by "non-toxic" amounts of cardiac glycosides has been suspected to be an early sign of tissue damage (253), and at any rate perhaps indicates that some energy requiring process, for example, membrane polarization, is placed under heavy stress. It has not been my belief (253) that this increase in respiration might be responsible for the increase in myocardial contractility in heart failure in the sense that it might furnish extra energy needed for restoring to normal the contractile response. Such a contention would be difficult to reconcile with the finding (see 254) that the cardiac glycosides are fully effective in cases of heart failure in which metabolic energy production is perfectly adequate for keeping the muscle plentifully supplied with ATP and phosphocreatine.

On the other hand, I was tempted to postulate a causal relationship between the inhibition of respiration by the cardiac glycosides in cardiac muscle and their toxic action on the function of the organ (253), having been impressed by the finding that the minimal lethal doses of various cardiac glycosides for the isolated guinea pig heart produce a 50 per cent inhibition of what is probably the basal oxygen consumption of the muscle, and also for the supplementary reason that the anatomical lesions and the electrocardiographic abnormalities seen in the myocardium of animals poisoned with digitalis are indistinguishable from those produced by prolonged anoxia (50). This hypothesis is no longer tenable, at least not as far as the acute toxic effects are concerned. The rate of respiration *in vitro* of cardiac muscle of dogs following slow infusion of toxic doses of ouabain is at first much higher than that of cardiac muscle of control dogs (257). Only during the course of prolonged incubation does the rate of respiration fall below the control level. The depression of respiration may be due to additional damage caused by the artificial bathing fluid or other unphysiological conditions of experimentation. The possibility remains that some of the manifestations of chronic cardiac poisoning, including the development of anatomical lesions, may be accompanied or even preceded by a decline in respiratory power. This possibility is, in fact, suggested by the finding of Libert (146) that the oxygen uptake of slices cut from hearts of rats which have been injected with a toxic dose of digitoxin is markedly depressed twenty-four hours following the injection, while it is higher than normal if the animals are sacrificed during the first five hours.

C. Intermediary Metabolism

1. *Carbohydrate Metabolism of the Beating Heart.* Gremels (98) reports that the utilization of glucose by the heart in the freshly prepared as well as in the spontaneously deteriorating heart-lung preparation of the dog is markedly increased, at a constant work level, by cardiac glycosides. This effect which can be elicited by as little as 1 μgm., for example, of strophanthoside, is stated to precede the improvement of the efficiency of work and is arbitrarily interpreted as a potenti-

ation of the action of traces of insulin in the blood. A rise of the respiratory quotient of the isolated frog heart accompanying the positive inotropic action of strophanthin in hypodynamy due to ethyl alcohol or lack of calcium points to an increased oxidation of carbohydrate (62).

Mel'nikova (162) finds that the utilization of lactic acid by the heart-lung preparation heart in spontaneous failure is greatly improved by strophanthin concurrently with the restoration of the contractile power of the muscle. The negative lactic acid balance of the heart is reversed and the rate of uptake of the metabolite from the blood eventually exceeds the pre-failure rate. This effect is reflected also in a lowered acidity of the coronary venous blood (85). A sharp rise in the lactic acid uptake by the heart following the administration of non-toxic doses of strophanthin occurs in the heart-lung preparation also in the absence of heart failure (34). It has also been seen in other experiments (14) in which, however, the physiological state of the heart was not determined. The lactic acid utilization of the barbiturate- and avertin-poisoned heart-lung heart, claimed by Rühl (205) to be abnormally high, is according to the same source unchanged following reversal of the poisoning by strophanthin. An increased uptake of lactic acid but not of pyruvic acid has been observed by Mardones and collaborators (159) in the isolated frog heart beating in a medium containing digilanid. Since under the same conditions the oxygen uptake is unchanged, it is concluded that the extra lactate consumed is not oxidized, but is converted to glycogen. This is a debatable conclusion since addition of readily oxidizable substrate can be expected to suppress the oxidation of endogenous metabolites.

Whether or not the cardiac glycosides promote glycogenesis in the heart is not clear from the published data, at least not so far as the therapeutic dose range is concerned. Following the administration of non-toxic doses of cardiac glycosides to rats and rabbits the glycogen content of the heart has been reported to be both increased (2, 32, 33, 263), decreased (18, 148), and not significantly changed (124, 263). Data concerning the effect of non-toxic doses on skeletal muscle are just as conflicting (18, 124, 148). Kimura (124) reports that the glycogen content in the liver of rats given non-toxic doses of digitoxin is elevated, and this effect is due to increased glycogenesis and not to decreased glycogenolysis. Other authors (18, 148) report the liver glycogen to be diminished under similar circumstances. Sharp rises in the glycogen content of the heart and to a lesser extent of the liver have been noted in pigeons treated with a dose of digitoxin bordering on the toxic (105).

In animals manifesting signs of cardiac glycoside poisoning the glycogen stores in the heart (32, 33, 106), liver (124, 264) and skeletal muscle (264) become depleted. Results to the contrary presented by Lasch and Triger (139) are not convincing because excessively high analytical values show that the determinations were not specific for glycogen. Cherkes (32, 33) finds the decrease in the glycogen content of the heart of rats and dogs acutely poisoned with digitoxin and strophanthin to be associated with a correspondingly sharp rise in lactic acid. In conformity with these changes is the diminished uptake or the actual release of lactic acid by the dog heart which he (34) and v. Blumencron (14) find in the

strophanthin- and digitoxin-poisoned heart-lung preparation. According to Cherkes' interpretation (32) the decrease in lactic acid uptake is due to impairment of its oxidation and its resynthesis to glycogen resulting from myocardial anoxia and does not represent a specific toxic effect of the cardiac glycosides. However, since the oxygen utilization by the digitalis poisoned heart in the heart-lung preparation has been shown to be very high (97, 168), a more likely alternative is that the observed shift in the lactic acid balance is the result of accelerated glycogenolysis, perhaps in response to heavy demands imposed upon the energy providing mechanisms (see also 148).

2. *Isolated Tissues and Oxidative Enzymes.* The influence of various cardiac glycosides on the dehydrogenases involved in the oxidation of glucose and some of its intermediaries has been studied by students of Freund (72) by means of the Thunberg technic and using washed cardiac and skeletal muscle minces as sources of the enzymes. The results are highly conflicting. Thus strophanthin in concentrations $1:10^3$ and higher is reported to have an accelerating action on the anaerobic oxidation of glucose, hexose diphosphate, glycerophosphate and lactate (4), in contrast to inhibitory effects of corresponding concentrations of scillaren and cymarin (126) and digitoxin and lanadigin (214). Succinate oxidation is stated to be increased by strophanthin (4) and to be unaffected by scillaren and cymarin (126). Still other qualitative differences between various cardiac glycosides are claimed (149), and finally it is found that adonidine and convallamarin have no significant effect on the various dehydrogenases, even in concentrations up to $1:4 \times 10^3$ (183). Only the latter finding is in line with later reports from other laboratories, which indicate that no effect is exerted by ouabain (253) and digitoxigenin (259) on glucose dehydrogenase, and by bufagin (57) on succinic dehydrogenase. On the other hand, it is reported (230) that the anaerobic reduction of m-dinitrobenzene by minced cardiac muscle of rabbits is accelerated by previous digitalization of the animals.

The cytochrome oxidase system appears to be unaffected even by high concentrations of ouabain (253), digitoxigenin (259), and bufagin (57). Neither does the presence of a cardiac glycoside have any influence on the activity of a complete hydrogen and electron transfer system assembled from substrate and its dehydrogenase, oxygen and cytochrome oxidase, and the intervening carriers (253). Inhibition of such a system by a narcotic in a concentration corresponding to that producing severe failure of the isolated heart is not reversed by cardiac glycosides even in 1000 times the concentration in which they relieve this type of heart failure (251). These findings again illustrate, as did the experiments with unpurified enzyme systems in homogenates, that the cardiac glycosides have no direct effect on respiratory enzymes.

Govier and collaborators (92) have reported that minute amounts of digitoxin markedly stimulate the anaerobic lactic dehydrogenase system in homogenates of hearts of vitamin E deficient guinea pigs and have attributed this effect to a protection of DPN, the coenzyme of lactic dehydrogenase, from hydrolysis by its nucleosidase. The same observation was made in anaerobic preparations of rat brain, a tissue high in nucleosidase activity. Curiously enough, exactly the op-

posite effect of digitoxin and other cardiac glycosides on DPN-ase, namely, its activation, has been postulated to occur in brain slices (p. 336). But in contrast to this and other effects of the cardiac glycosides on the metabolism of relatively intact brain and heart tissue, the stimulation of lactate oxidation in the cell-free tissue preparations is also obtained with steroids devoid of cardiac activity, such as cholesterol, digitonin and certain sex hormones. Nonetheless, Govier *et al.* (92) have suggested that the therapeutic action of digitoxin on the failing heart may be due to preservation of DPN. This hypothesis no longer has a foundation since the demonstration (224) that DPN-ase activity is not inhibited by digitoxin.

Another interesting action on lactic dehydrogenase activity occurs, according to Segre (218), in suspensions of erythrocytes and hemolysates of horse blood. In these preparations, strophanthin in a concentration $1:5 \times 10^5$ or higher is reported to accelerate the oxidation of added lactate and to restore the reaction to normal when inhibited by cyanide. The latter effect is explained by attributing to the cardiac glycoside the property of being a hydrogen acceptor, replacing molecular oxygen. Strophanthin is believed to function also as a hydrogen carrier, capable, in the same manner as ascorbic acid, of being alternately reduced and oxidized by virtue of a hypothetical reactivity of its unsaturated lactone ring. In support of this hypothesis is adduced, in addition to the accelerant effect on lactate oxidation, the finding that aged and repeatedly washed red cells whose glycolytic capacity is diminished, probably largely through destruction or loss of DPN, are enabled by strophanthin to glycolyze glucose at a rate approaching that of fresh, slightly washed cells.

Significant as the actual observations may be, these far-reaching inferences are out of proportion to the weight of the evidence presented, and neither are there other data in the literature in support of this line of thought. True, ascorbic acid and certain other simple unsaturated lactones have an effect in the isolated frog heart resembling that of the cardiac glycosides; but this effect results from the formation of peroxides (132, 163), the oxidizing action of which possibly interferes with the normal function of sulfhydryl enzymes (164) or perhaps of the sulfhydryl groups of myosin (cf. 140). No peroxides have been detected in perfusates of frog hearts treated with cardiac glycosides (130). This still leaves open the possibility that they might be formed at the site of action of the drug in the cell. But speculative theories (81) of a mechanism of action of the cardiac glycosides based on the peroxide effect and other properties of unsaturated lactones must face an immediate objection against their implicit assumption of a different mechanism for the cardiac glycoside-like action of certain veratrum and erythrophleum alkaloids, compounds structurally related to the cardiac glycosides, but lacking the lactone ring. Yet these compounds share with the cardiac glycosides not only the positive inotropic and the toxic action both on the frog and the mammalian heart (131, 155, 212) but also the characteristic effects on the metabolism of heart and brain tissue *in vitro* (258, 259), and veratridine has been shown to have the same influence as the cardiac glycosides on cardiac energetics (167). It would seem that in attempting to correlate the chemical structure and cardiac activity of these drugs, the emphasis should be placed on common structural

features rather than on characteristics which distinguish one molecule from the other (cf. also 43). Of added interest in this connection is that the same basic biochemical change has been implicated, not wholly without experimental support, in both the action of the cardiac glycosides (189) and that of veratrine (88), namely, a displacement of calcium, presumably bound to phospholipids, from the surface of the cell.

D. Energy-Rich Phosphate Content and Transfer

Non-toxic doses of cardiac glycosides do not produce significant changes in the ATP and phosphocreatine content of the non-failing heart, whether in the intact circulation (124, 259), the Starling heart-lung preparation (255), or the completely isolated Langendorff preparation perfused with Ringer's solution (244). Neither are noticeable changes produced by non-toxic doses in the heart-lung preparation during or following recovery from spontaneous failure or from failure induced by anesthetics (259).

In these types of experimental heart failure, the content of energy-rich phosphates in the heart is normal or even somewhat elevated (p. 323), and this fact itself has been taken as a basis for the generalization (254) that the primary action of the cardiac glycosides on the heart must be concerned with the utilization and not with the generation of phosphate bond energy. This is also the view adopted by Mardones (159) after evaluation of the pertinent digitalis literature in the light of Szent-Györgyi's (228) theory of muscular contraction and on the basis of his own findings (160, 179) that digitalis glycosides restore the contractility of the isolated rabbit intestine and of the isolated guinea pig heart depressed by phlorhizin, an inhibitor of phosphorylation. However, this antagonism to phlorhizin is also open to exactly the opposite interpretation, namely, that phosphorylation may somehow be reactivated by the cardiac glycosides, and hence is best omitted at present as an argument in the discussion.

The constancy of the ATP and phosphocreatine levels in the heart treated with a cardiac glycoside merely signifies that the equilibrium between the rates of their breakdown and synthesis is kept at the same level, but it does not tell anything about whether and how the rates of these reactions are changed. Direct information on this question is difficult to obtain. However, the fact that administration of a cardiac glycoside to the spontaneously failing heart in the heart-lung preparation is known to produce a rise both in oxygen consumption and work performance (185) is strongly indicative of an increased ATP and phosphocreatine metabolism, and this may also be the case in the non-failing heart.

As described earlier in this review (pp. 315, 323) spontaneous failure of the mammalian heart perfused with Locke solution is associated with a decline in oxygen consumption, phosphocreatine, and possibly ATP; restoration of normal contractility by a cardiac glycoside (strophanthin) is associated with a parallel increase in oxygen consumption. Weicker (244) finds the phosphocreatine and ATP content to be likewise restored to normal and the concentration of free adenylic acid to be substantially raised from near zero levels to 50 per cent of the normal value. A simultaneous increase in coronary flow points to the possibility that the im-

provement of the metabolism may be a secondary effect of an improved oxygen supply, which in this heart preparation is at a critical level even under favorable conditions. The observation was made in this study that administration of strophanthin in heart failure due to lack of oxygen sometimes produces a temporary increase in anaerobic work performance which is accompanied by a rise of the phosphocreatine content to the normal level, while the ATP content remains low. This finding suggests that anaerobic synthesis of phosphocreatine may be stimulated by strophanthin. If credence is given to Abdon and Nielsen's (1) claim that strophanthin inhibits the enzymatic hydrolysis of phosphocreatine, Weicker's finding could be interpreted in the sense that the drug prevents uneconomical dissipation of energy-rich phosphate which, under conditions of failure, might be pronounced. Weicker, however, contents himself with regarding the anaerobic as well as the aerobic action of strophanthin on the phosphocreatine metabolism of the heart as secondary to unknown physicochemical changes favoring anabolic activity.

Ide's (116) report that ouabain restores to normal the depleted phosphocreatine content of the hypodynamic heart cannot be evaluated since no information is given as to the origin of failure and the experimental procedures.

The finding of Herrman and Decherd and collaborators (52, 114) that digitalization of rabbits with experimental cardiac hypertrophy leads to significant increases in the creatine content of the injured hearts is of interest, but whether this indicates, as the authors imply (113), a corresponding increase in phosphocreatine must be questioned for reasons given on p. 324.

The ATP and phosphocreatine content of the frog's heart is not significantly changed by poisoning the animal with cardiac glycosides (31), but the toxic action of the drugs on the mammalian heart is characterized, particularly in its advanced stages, by a depletion of the energy-rich phosphate store. In the isolated cat heart perfused with Ringer's solution, contracture-producing doses of strophanthin cause a 50 per cent loss of phosphocreatine and an even greater reduction of ATP (244). Such disappearance of energy-rich phosphate in muscle is a characteristic feature of all kinds of contractures and rigor (166, 228). In the heart-lung preparation of the dog poisoned with ouabain or digoxin, a progressive decrease of phosphocreatine sets in with the appearance of extrasystoles and ventricular tachycardia, and at the onset of ventricular fibrillation 75 per cent of the amount originally present has disappeared (255). A similar depletion is seen in the intact animal (259). The loss of phosphocreatine is not a consequence of tachycardia *per se*, since the same increase in heart rate produced by electrical stimulation or by epinephrine is ineffective; it represents, therefore, a specific effect of the cardiac glycosides. The ATP content of the poisoned dog heart, whether *in situ* or in the heart-lung preparation, is not significantly altered by poisoning with cardiac glycosides. It is not possible to say whether this has any bearing on the fact that, in contrast to the ATP-depleted Langendorff preparation, the heart under these conditions rarely goes into contracture.

In conformity with the view that phosphocreatine functions as an energy reservoir for the adenylic system (150), the observed loss of phosphocreatine in

the cardiac glycoside-poisoned heart-lung preparation has been interpreted as reflecting a lag of the oxidative synthesis of ATP relative to its breakdown, which, however, is at once compensated for by phosphate transfer from phosphocreatine. Impairment of the synthesis as well as acceleration of the breakdown of ATP have been proposed as possible causes for the decrease in phosphocreatine (255). Impairment of the synthesis of energy-rich phosphate could result from interference with either oxidation or oxidative phosphorylation. The indications are that in the heart neither of these two processes is interfered with by the cardiac glycosides. The oxygen consumption is, on the contrary, sharply elevated (97, 168, 199) and oxidative phosphorylation in respiring heart muscle homogenates has been found, as in brain (258) and kidney (56), to be unaffected even by high concentrations of cardiac glycosides (259).

There remains the second alternative, namely, that the loss of phosphocreatine in the heart poisoned by a cardiac glycoside is a consequence of an increase in the rate of breakdown of ATP. As was pointed out above, an accelerated breakdown of ATP in all likelihood also accompanies the positive inotropic action of these drugs on the heart. However, as long as there are no extrasystoles and tachycardia, this is not brought to light by chemical analysis because of complete resynthesis of ATP and phosphocreatine during the diastolic pause. Prolongation of diastole, which is usually seen following the administration of therapeutic doses of cardiac glycosides, would thus compensate for the intensification of breakdown processes in systole. Some authors (240) have even gone so far as to declare the lengthened diastole to be the basis of the positive inotropic action of the cardiac glycosides. At any rate, it appears that what at first sight seems to be a purely toxic effect of the cardiac glycosides on the heart, namely, depletion of phosphocreatine, is probably a manifestation of a change associated also with the therapeutic action. Wood and Moe (261) have emphasized that the escape of intracellular potassium, which they (260) and others (25, 104, 220, 239) have noted in hearts poisoned with cardiac glycosides and which most likely is connected to some extent with the metabolism of the organic phosphates in the cell, is likewise a phenomenon characteristic of the therapeutic effect as well. That the potassium content of the heart exposed to therapeutic doses of cardiac glycosides may be undiminished (25, 239) or even increased (19, 104) might be due, like the constancy of the ATP and phosphocreatine levels under the same or similar conditions, to intensification of restorative processes, in this instance to an increase in the rate of reabsorption of the ion.

The possibility that the cardiac glycosides might accelerate the dephosphorylation of ATP through a stimulant action on ATP-ase has been explored by Guerra and collaborators (102). Their data show, notwithstanding the contrary claim of these authors, that ouabain in concentrations of $1:10^6$ to $1:5 \times 10^5$ has no significant effect on the activity of myosin-ATP-ase preparations from cardiac muscle. It is not inconceivable, however, that the activity of this calcium-activated enzyme might be stimulated by the drug in the intact cardiac muscle, perhaps through mobilization of calcium. According to Kimura and Du Bois (125) digitoxin in a concentration 4.7×10^{-6} M and ouabain in higher concentrations

are capable of inhibiting the splitting of ATP in cardiac muscle preparations by a phosphatase not activated by calcium, possibly Kielley and Meyerhof's (122) magnesium-activated ATP-ase.

An indication that the cardiac glycosides may have an influence, at least under certain conditions, on the interaction of actomyosin and ATP is given in the announcement by Mallov and Robb (156) of their finding that actomyosin preparations treated with a cardiac glycoside in a concentration of $1:2 \times 10^{-6}$ yield threads which, in contrast to threads drawn from untreated preparations, exhibit, in the presence of ATP and magnesium, considerable "relaxation" followed by maximal shortening. It would be of interest to know whether this effect of the cardiac glycoside is restricted to substances which enhance the contractile power of cardiac and other muscle. In any case, whatever the significance of the observations of Mallov and Robb may prove to be, the statement made by one of the authors (201) in reviewing recent literature on cardiac biochemistry and metabolism[3], that "after some two hundred years, almost any day, the complete mechanism of cardiac glycoside action may become known," seems unduly optimistic. Much progress remains to be achieved in elucidating the basic molecular events in muscular activity, before there will be justification for stating that the problem of elucidating the mechanism of action of agents such as the cardiac glycosides is about to be solved.[4]

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[3] In this otherwise very informative review several of the findings of the present author are unfortunately not correctly represented.

[4] As this review goes to press, the author has learned of renewed attempts to relate the positive inotropic action of the cardiac glycosides on the failing heart to an increase in ATP-ase activity said to have been observed in muscle homogenates (R. Hegglin, H. Grauer, and R. Münchinger, Experientia, 5: 127, 1949; G. Segre, Arch. int. pharmacodyn., 80: 336–436, 1949). The suppression of aerobic glycolysis in the stomach musculature of the guinea pig by low concentrations of ouabain is claimed (G. Werner, Arch. int. pharmacodyn., 79: 323–331, 1949). K. P. DuBois (personal communication) finds that bufagin produces the same kind of stimulation of the respiration of guinea pig heart slices as do digitoxin and strophanthin. E. C. del Pozo and E. G. Pardo (J. Pharmacol. and Exper. Therap., 97: 144–149, 1949) report that the amplitude of contraction of ischemic skeletal muscle is increased following intravenous injection of k-strophanthoside.

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