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Metabolism of the failing heart

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TABLE OF CONTENTS

I INTRODUCTION

The problem of heart failure is one of the largest today's physician has to face. There are some three million so called "cardiacs" in this country today and the number is continually growing. To meet all those patients with shortness of breath and swollen ankles the physician is equipped with a few well proven agents but the large part of therapy is empirical and often quite resembles the days of foxglove and blood letting. The reason for this is obvious and lies with the fact that the basic mechanisms of failure are still largely unknown.

The use of digitalis is an illustration of this point. It is not enough to state that in failure the heart muscle has weakened and lost its reserve capacity and digitalis aids by somehow slowing and strengthening the beat. The question still remains as to what phase of the metabolic cycle producing contraction it benefits. If digitalis is to be assigned a role in the correction of metabolic errors, these errors must be sought for and understood before the mode of action of the drug, and others like it, can be appreciated. The volumnious and contradictory literature appearing on cardiac stroke volumes, circulation times, intra-cardiac pressures and other highly involved procedures is another indication of the confusion of thought in this field. These data may be

quite valuable in explaining certain clinical situations but are, in all probability, manifestations of an underlying difficulty on which they shed little light. The problem thus again resolves itself into a better understanding of the metabolism of failing hearts. It is only in this way that a more logical and precise mode of therapy may be found.

The purpose of this thesis is to review the work of qualified observers in this field. The author is both unwilling and unable to draw any dogmatic conclusions or fanciful theories from the results presented. What can be done, however, is to examine closely and attempt to correllate the various experimental results, and to see if a patteren forms which might be useful in explaining the basic mechanisms of failure.

II NORMAL CARDIAC METABOLISM

Before a discussion of abnormal cardiac metabolism is attempted, a brief review of the normal state is necessary in order to evaluate any abnormal occurrences. Since cardiac metabolism follows in general outline the better known metabolism of skeletal muscle, stress will be laid on the differences between the two. The discussion will be separated into two main phases; A - the steps required for production of high energy phosphates and B - the utilization of that energy for the contraction of muscle.

A Production of Energy

The heart can use as fuel any normally oxidizable substrate. As in skeletal muscle, these substrates may be either brought to the heart via the circulation or derived from the cardiac muscle cell itself. Conditions such as physical activity, endocrine balance, state of nutrition, and extreme limits of age may of course influence the extent to which the varicus substrates may be used.

Of the carbohydrate fuels, significant is the importance assumed in the heart by the oxidation of lactic acid. This has been emphasized by the finding that whereas in skeletal muscles venous blood has a higher lactic acid concentration than arterial blood, coronary sinus blood has a lower lactic acid content than arterial blood. (22) This indicates that while skeletal muscle liberates lactic acid cardiac muscle, when well oxygenated, removes lactic acid from the blood perfusing it. It has also been shown by studies with the venous catheter that above certain critical levels lactate, as well as glucose and pyruvate, are removed by the myocardium in proportion to their espective arterial concentrations. In normal postpriandial subjects Goodale and others (23) showed that the arterio-venous differences of glucose, lactate, and

pyruvate would, if complete combustion were assumed, account for 90-100 per cent of the simultaneous oxygen consumption. This indicates, then, that these three carbohydrates serve as the important oxidizable substrates in cardiac metabolism.

While it has been shown that glucose can be easily metabolized in the heart, cardiac muscle glycogen is maintained at very stable levels under normal conditions of cardiac work.(64) In anoxemia, especially when the oxygen saturation falls below 25 per cent, lactic acid utilization fails and it is produced instead of absorbed by the heart. (26) Production is associated with the disappearance of myocardial glycogen and ceases when the glycogen stores have been exhausted. The process is assumed to be the chief source of energy available to the anaerobic heart. However this may be, the anaerobic energy supplies of the heart are extremely limited and the heart is capable of accumulating an oxygen debt of but small proportions and little significance.(52)

Fats: That the heart is able to utilize fats is indicated by; 1- Its ability to survive for considerable periods of time without glucose, lactate, or glycogen(16), 2- In heart-lung preparations the total lipid of the heart decreases during cardiac work (64), 3- The low

respiratory quotient at times observed, and 4- The direct observation of utilization of beta-hydroxy butyric acid by tissue homogenates. (17) The extent to which fat utilization contributes toward energy production in the normal heart, however, is not known but is in all probability quite small.

Proteins: Amino acids derived from proteins may also participate in the energy production of the heart, but apparently only after transfer of their amino groups to a suitable receptor. Studies in the heart-lung preparation suggest, however, that amino acid production is relatively unimportant in the total energy production of the heart.(52) The importance of amino acids as a source of keto-acids such as pyruvate and alpha-keto glutarate following deamination in other tissues and transfer to the heart has not been evaluated.

Enzymes: The relative concentration of various enzymes furnishes another basis for a marked difference between skeletal and cardiac muscle. While in skeletal muscle the anaerobic (glycolytic) system of enzymes is most prominent, cardiac muscle is quite richly endowed with oxidative enzymes.(45) The concentrations of cytochrome C, cytochrome oxidase, and succinic acid dehydrogenase in the heart are the highest found in any tissue

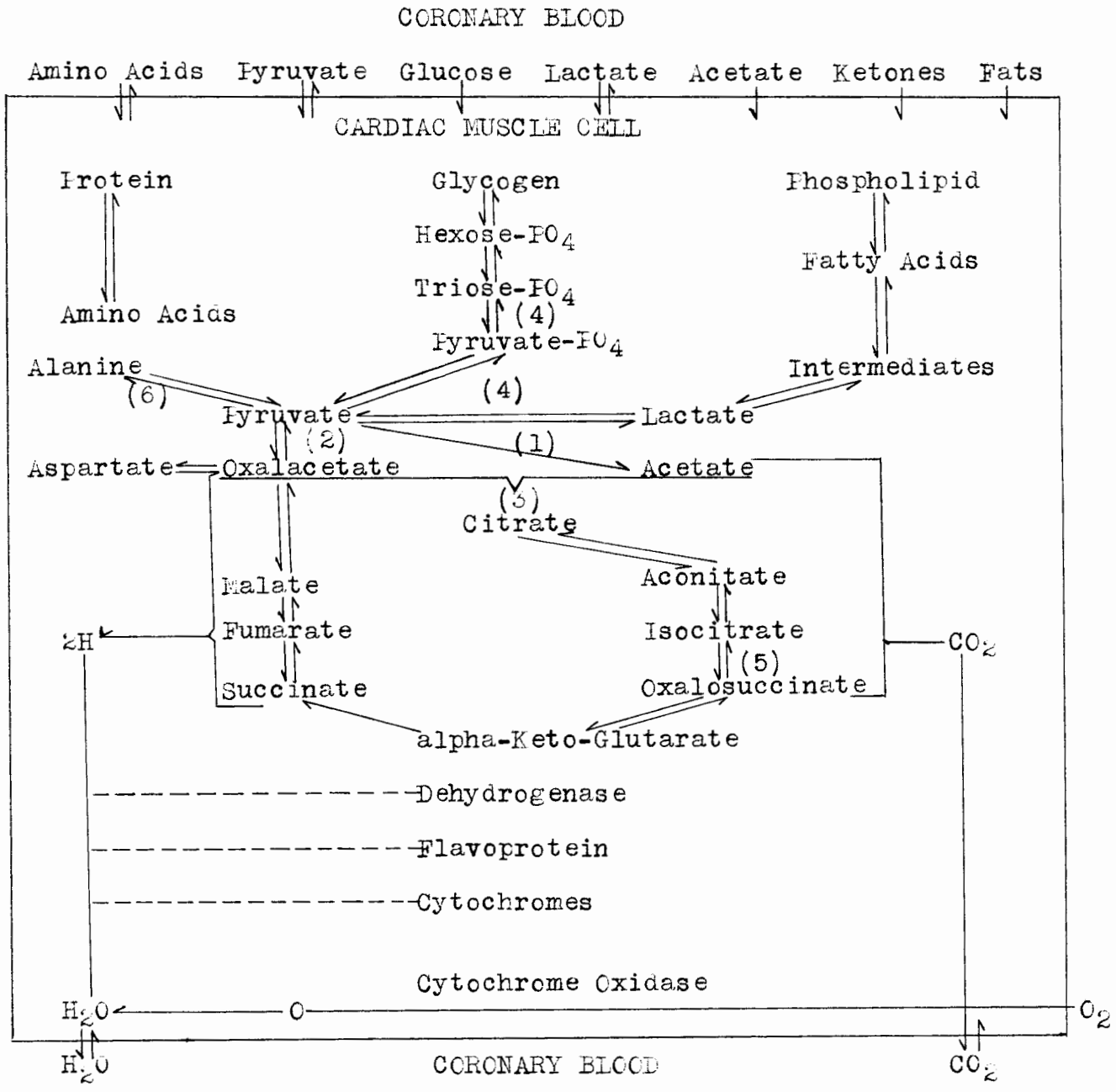
in the body. This, together with the profuse blood supply and absence of a myolemma, illustrate that the heart is adapted to function on an aerobic basis, and in anatomical arrangement and physical properties it is designed to receive and make use of a large, uninterrupted oxygen supply.

Course of Events:

The chain of reactions leading up to the production of energy for contraction is summarized in figure 1. This figure, as well as much of the discussion, is taken from the excellent review by Olson and Schwartz (52).

When glucose is extracted from the coronary blood, the initial steps involve its fission into two molecules of pyruvic acid through a series of phosphorylated intermediates. Under normal conditions this pyruvate is then oxidized to CO_2 and H_2O via a sequence of additional reactions known commonly as the Krebs-Johnson tricarboxylic acid cycle. This cycle, however, cannot function without the presence of oxygen, and in any condition producing anoxemia the pyruvate formed from glucose is reduced to lactate which then accumualtes as an end product. When this occurs only about 10 per cent of the inherent energy content of the glucose is realized. This again serves to illustrate the dependence of the myocardium on a plentiful

Intermediary Metabolism of Cardiac Muscle



1. Requires Thiamine as Cocarboxylase
2. Requires Biotin
3. Requires Pantothenic Acid as Co A
4. Requires Niacin as DPN
5. Requires Niacin as TPN
6. Requires Pyridoxine as Pyridaoxyl Phosphate

Figure 1

supply of oxygen.

As has been demonstrated by the previously mentioned experiments on coronary sinus catheterization(23), lactate and pyruvate may be absorbed directly from the coronary blood. When this occurs, these substances are oxidized to a common two-carbon fragment, acetate. The oxidation of lactate to pyruvate requires DPN (diphosphopyridine nucleotide), a derivative of nicotinic acid, as a conenzyme, while the oxidation of pyruvate to acetate requires cocarboxlase (thiamine pyrophosphate). The acetate also serves (see fig. 1) as a common denominator for the entrance of fatty acids, which yield acetate in heart muscle by the process of multiple beta oxidations.

The final combustion of the acetate and pyruvate is believed to occur through the tricarboxlyic acid cycle previously mentioned. In this the acetate is combined enzymatically with oxalacetic acid to yield citric acid, from which two carbon atoms and their associated hydrogen atoms are then removed in oxidative reactions. As shown in figure 1, these steps require the presence of varied enzyme systems. The overall effect of this limited citrate oxidation in the cardiac muscle cell is to liberate free energy from two-carbon fragments with a regeneration of oxalacetate. It appears to provide a nice solution to the problem of oxidizing a small molecule to CO_2 and H_2O and

at the same time developing and conserving the energy of that oxidation. The importance of the Krebs cycle in human cardiac muscle has been conclusively shown by Burdette (9) who obtained muscle from auricular appendage at the time of thoracotomy in twelve patients and studied its respiration by means of the Warburg apparatus. He demonstrated that the addition of pyruvate, acetate, citrate, beta-hydroxy butyrate, succinate, malate, and fumarate all resulted in an increased oxygen consumption. The addition of inhibitors of the cycle such as malonate (see fig. 1) conversely limited or stopped respiration.

Since these oxidative processes provide a source of potential energy, the next problem is its transfer and the generation of high energy bonds. The oxidations which occur in the Krebs cycle are enzymatic dehydrogenations. The hydrogen released does not immediately combine with oxygen but passes along a chain of enzymatic hydrogen carriers before combining with oxygen to form water. These enzymes include the flavoproteins, which contain riboflavin, and the cytochromes, which contain iron porpyrin moieties. Most of the bond energy accompanies the hydrogen atoms and is released stepwise during its passage from carrier to carrier until it finally combines with oxygen at a low energy level. This stepwise release of the energy

of hydrogen atoms derived from the substrate and its conversion into the energy of bonds of certain organic phosphates is the key to the slow utilization of energy by the cell. Should the combination of hydrogen and oxygen occur spontaneously, the instantaneous liberation of energy would be so great that it could not be harnessed for cell work.

In order to make phosphate bond energy more readily in the cell, certain compounds serve as acceptors and donors of phosphate bonds. Two such carriers are creatine and adenylic acid which accept, without loss of energy, phosphate moieties formed during the oxidation of substrate and donate them where needed for cell work. Adenylic acid, which is a nucleotide composed of adenine, ribose, and phosphoric acid, may accept one high energy phosphate group to become adenosine diphosphate (ADP) or two such groups to become adenosine triphosphate(ATP). Creatine is able to accept only one high energy phosphate group and becomes phosphocreatine. Thus ATP becomes the primary high energy phosphate donor in the cell, being the immediate source of energy for muscular contraction, while phosphocreatine provides a reserve supply of high energy phosphate bonds.

In brief, then, the steps toward production of energy

in the cardiac muscle cell can be described as a chain of events, each event dependent for continuation upon the step before it. It is, like a chain, only as strong as its weakest link, and a biochemical defect anywhere along this chain will result in a decrease of the production of energy required for contraction.

B Utilization of Energy

While the reactions leading to the production of energy have been more completely studied, the next phase, that is the utilization of that energy, is certainly as important to the total picture of cardiac muscle cell contraction. Knowledge along this important line was given impetus by the excellent work of Szent-Gyorgi (60, 61). According to him, the main contractile unit of muscle is composed of a long, thin protein thread called myosin. These. like any other long, thin protein strands, tend to fold up or shorten and are kept in the extended state by a cloud of potassium ions surronding each myosin particle. If myosin or another charged particle approaches it, it will be these positively charged atmospheres which meet first and repel one another, and thus keep the myosin in solution. This atmosphere will also have the tendency to expand because the positive ions in it repel one another. The end result will be that not only are other particles

kept away, but that the particles will be kept straight and extended.

While this may explain how the particle is kept straight, the next question is how these particles can be made to contract not at a moment's notice but at a thousandth second's notice. Since this dicharge or shortening may be termed precipitation, nature accomplishes this shortening by adding another specifically built protein, a precipitin called actin. If an actin and a myosin particle meet in the presence of a physiological salt concentration they unite to form a complex, actomyosin, which becomes partially discharged and shortened. The method, then, by which nature manages to produce the sudden folding of the myosin particle at a sigma's notice is to keep another protein, actin, at its side. The two proteins in resting muscle are separated by the repulsive forces discussed before. The repulsive and attractive forces are carefully balanced and this balance is disturbed by the wave of depolarization which travels along the membrane and is called by physiologists "excitation". As a consequence of this disturbance actin and myosin get together, interact, and contract.

Although this is a rather ingenious scheme for outlining contraction of muscle cells, the very important

problem of energy relationships must still be answered. While contraction has been described as the natural tendency for the protein to return to its low energy compact form, the contraction liberates energy which must be replaced. This is done with ATP which is present in relatively high concentrations in heart muscle. The ATP, by virtue of its high energy bonds, thus provides the energy for relaxation or lengthening of the muscle fiber. This concept, then, may be briefly and rather sketchily summarized as follows: During rest, the muscle fibers are kept extended by repulsion of like ionic charges surronding the fiber. When a wave of depolarization passes across the membrane, these repulsive charges are disturbed and the fiber can then return to its natural contracted state. When this occurs, energy is lost and work is performed. In order to re-extend the fiber, energy is needed and is provided by introducing high energy bonds by means of ATP into the molecule. This energy is used to extend the fiber and is again released during contraction.

II METABOLISM DURING FAILURE

From the previous discussion it can be seen that there are basically two processes leading to the heart's pumping action. The first is the development of high energy via oxido-reduction of substrates, and the second is the transfer or utilization of that energy by the cardiac muscle cell. The question of what produces failure can

then be restated in a simpler fashion; Is heart failure due to a defect in generation of energy or due to a defect in the utilization of that energy for useful work? This is a basic question which must be answered. If the answer lies in the first possibility, then the processes of oxidative metabolism must be better understood. If, on the other hand, failure is due to a defective utilization of energy, then the mysterious half-world of actin and myosin must be more thoroughly investigated.

In order to determine which of these factors is at work, several types of investigations have been undertaken. Throughout these experiments the investigators are primarily concerned with failure of the classic human congestive type as exemplified by valvular, hypertensive, or arteriosclerotic disease. It is realized, of course, that there may be a variety of causes of heart failure such as acute deficiency states such as beri-beri, acute anoxia, thyrotoxicosis, and so forth. In these cases, however, the metabolic defect is obvious and is thus not the type of failure that will be studied primarily in this thesis.

A MYOCARDIAL EFFICIENCY DURING FAILURE

The total potential energy let loose by the heart

can either be measured directly by physical measurements of heat and work or by chemical measurements of the underlying metabolism. The physical methods have not been extensively applied to the case of cardiac contraction mainly because of technical difficulties and as a result indirect calorimetry, that is energy measurements by analysis of oxygen uptake, has been the method mainly used.

The basic work in this field has been done by Starling and his collaborators using the dog heart-lung preparation.(59,18,63) They demonstrated initially what has come to be regarded as "Starling's law of the heart" which states that the mechanical energy set free in the contraction of the heart depends on its diastolic volume, They also demonstrated that oxygen consumption was a function of diastolic fiber length and thus oxygen consumption could be used as a guide or index of the mechanical energy set free during a contraction. Later Starling and Visscher (59) in applying these principles to the failing heart, showed that regardless of whether the heart failed under conditions of constant work or of constant diastolic volume, failure was always associated with a decreased efficiency. A typical result from one experiment is shown in Table I. In this experiment, the work was held constant

over four hours during which the efficiency declined from 9.5 to 5.8%, associated with an increase in diastolic ventricular volume of 42 ccs. over the value at the beginning of the period of observation.

TABLE I

Oxygen consumption of a 100 gm. dog heart in the heart-lung preparation in which work was held constant over 4 hours at 450 cc. total ventricular output per minute and 100 mm. Hg aortic pressure

Time	O_2 consumption	Diastolic Ventricular Volume above initial	Efficiency
0 min.	360 cc/hr	0 cc	9.5%
60 "	455 " "	15 "	7.5%
120"	515 " "	18 "	6.6%
180"	565 " "	32 "	6.1%
240"	590 " "	42 "	5.8%

Similar observations of a decreased efficiency during experimental failure in the heart-lung preparation have been confirmed by Gremels(30), Gollwitzer-Meiir and Kruger(25), and Peters and Visscher(54). Since the

interpretaion of data and validity of conclusions rest largely on the usefulness of the experimental apparatus, an example of the type of preparation used is shown in Figure 2. (63)

In drawing significance from these results, it has been shown that the failing heart suffers first simply from a decrease in mechanical efficiency. The energy liberated at a given external diastolic volume remains the same, only the proportion which can be put to work falls off. In other words, since oxygen is being taken up in normal or even high proportions, it is assumed that the oxidative processes leading to the production of high-energy substances are intact, but the utilization and transference of that energy is faulty as demonstrated by a decrease in work.

Although these observations have been confirmed by others and appear to be rather widely accepted, they have been challenged by Katz and his collaborators. (67, 42,43) Also using the heart-lung preparation, Katz found a simultaneous decrease in oxygen consumption together with a decrease in work and therefore concluded that myocardial efficiency did not decrease during failure. In attempting to reconcile these results, Moe pointed out that Katz estimated oxygen consumption by measurement

of coronary arterio-venous differences and assumed the Thebesian vein oxygen content to be identical. Moe and Visscher(63) collected coronary sinus and Thebesian blood simultaneously and found that the arterio-venous differences for these two fractions varied widely and would thus lead to error in oxygen consumption data. Katz, however, repeated the experiments and measured oxygen consumption by sampling aortic and pulmonary arterial blood, thus obviating the error of coronary sinus sampling, and obtained approximately the same results as before. Furthermore Katz criticized the work of Moe and all in that they did not take spontaneous dilatation of the coronary arteries into account when estimating the total cardiac output. He pointed out that an increase in coronary flow would reduce the amount of blood passing through the aorta and thus the amount of work done by the heart would appear to be lowered and a decrease in efficiency could be erroneously found.

These difficulties have not as yet been surmonted. It is quite obvious that the heart-lung preparation such as is shown in figure 2 is subject to many inherent technical errors. Also, the advisability of trying to correllate the findings obtained from a dog heart-lung preparation to humans in congestive failure may be open to

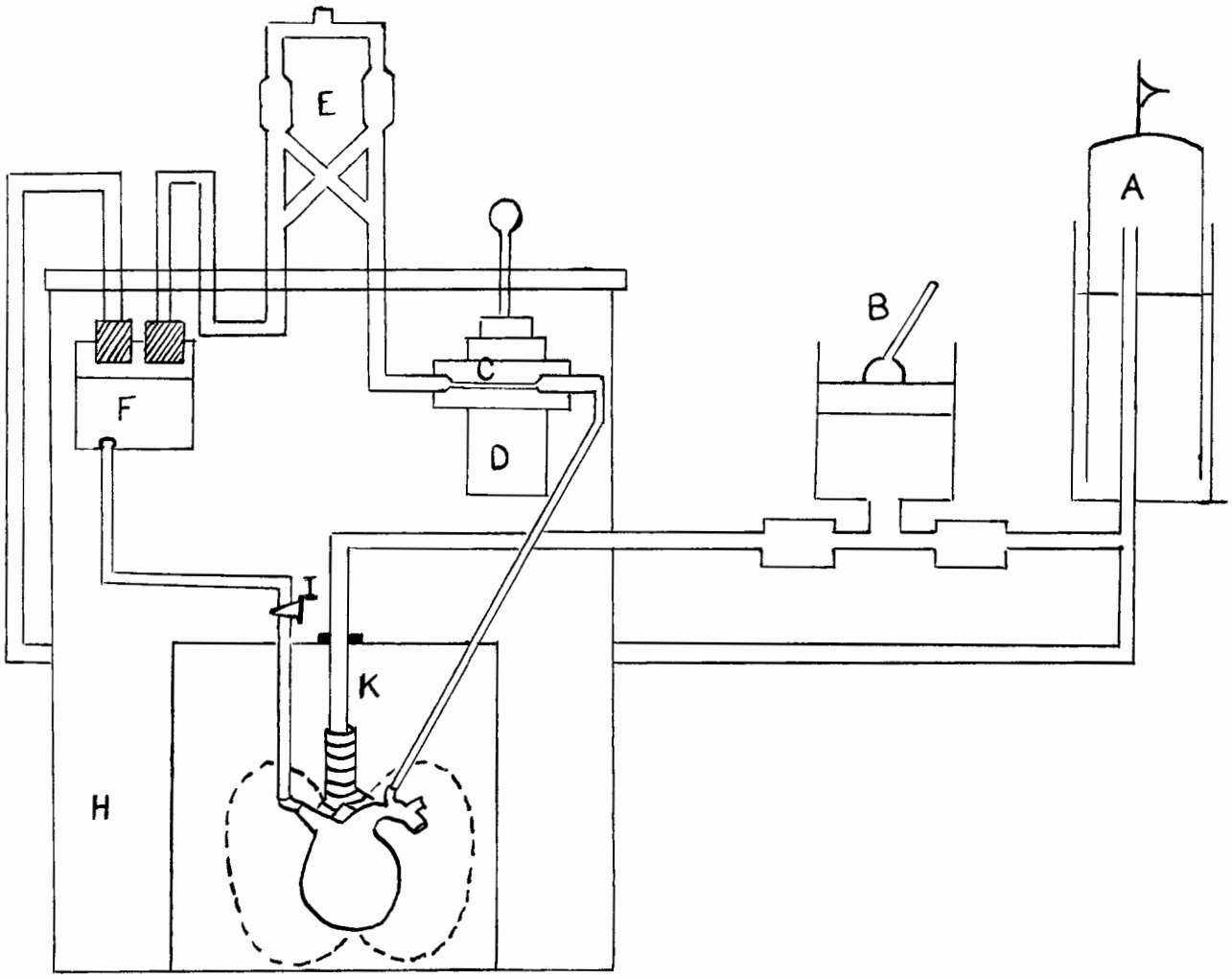


Figure 2

A Oxygen Spirometer
B Respiration Pump
C Artificial Resistance
D Pressure Bottle
E Stromuhr
F Venous Resevoir
H Constant Temperature Bath
I Stopcock for Adjustment of Venous Return
K Tracheal Cannula

considerable question.

In light of these points, Bing et all (3,4,5,24) have reported efficiency determinations in human failing and non-failing hearts by means of cardiac catheterizations. Their work is especially enlightening in view of the fact that they managed, by dint of meticulous care and the aid of flouroscopy, to place their catheter within the coronary sinus. They were therefore able to make distinct advances in the measurement of coronary blood flow and oxygen consumption in the intact human heart. In their data they present results obtained from twenty normal hearts together with fourteen cases of obvious clinical decompensation, the causes being valvular, hypertensive, or arteriosclerotic in nature. They determined coronary flow by the nitrous oxide method, total cardiac output by the Fick principle, and then calculated efficiency as the ratio of the work of the left ventricle to the oxygen consumption of the left ventricle. In their results they found that the output of the heart in failure was markedly reduced but that the oxygen consumption remained normal or became slightly elevated, these factors resulting in a definite decrease in efficiency. They also found, however, that there was some degree of cardiac dilatation in almost all of their fourteen cases of failure, thus

throwing some doubt on Starling's original hypothesis that oxygen consumption is a function of fiber length. Despite this discrepancy, in their conclusions they echoed and substantiated the findings of Starling et all that a failing heart is inefficient and the failure is in all probability due to decreased utilization of energy rather than to a decrease in its production.

Aside from the conclusions drawn, this work is also important in that it correllates well with the findings obtained from heart-lung preparations, thereby demonstrating the latter's usefulness toward investigating human heart disease. It also directly opposes the view, long held by Harrison(36) and others, that myocardial anoxia is the underlying factor in human congestive failure. Conversely, these findings support Dock's(21) anatomical studies showing that an enlarged, hypertrophied heart has a normal capacity for blood flow.

In summary of the work in this aspect, it can be said that the majority of workers feel that a failing heart is inefficient, and thus able to produce but not utilize energy. The work of Katz, however, can hardly be ignored and a definite answer is not yet present.

B BALANCE OF ENERGY RICH PHOSPHATES IN FAILURE

Another, and quite logical method of investigating

the basic disturbance in heart failure is to determine the amount of high energy present in the heart by a measurement of those compounds containing high energy phosphate bonds. In other words, if in a failing heart chemical analyses reveal absent or low amounts of phosphocreatine, ATP, or ADP, it can be assumed that their oxidative production has been interfered with. Conversely if these substances are found in normal or high amounts, then failure has not been due to any defect in their development.

The initial work along this line was done by Cowan.(14) Since phosphocreatine and ATP are very labile substances, their determination is difficult in fresh tissue and almost impossible in autopsy material. For this reason Cowan merely determined total creatine values and assumed that the changes in creatine and phosphocreatine would parallel each other. Obtaining left ventricular musculature from normal and decompensated hearts he found an average level of 194 mgm per 100 gm of tissue and in failing hearts demonstrated an average of some 50 mgm lower. He concluded that a deficiency of oxidative metabolism was present.

Much the same type of work was done later by Herrman and Dechard(37) who also showed a decrease of

creatine values in cases of congestive failure at autopsy. They reasoned that the basic disturbance in cases of this sort was anoxia of the myocardium, which in turn leads to diminished production of high energy bonds and subsequent loss of creatine. Still later Myers (50) again analysed hearts for creatine and again obtained results quite similar to those previously mentioned. At the same time he also analysed the hearts for their potassium content and found concomitant decreases in the potassium levels. Postulating that ATP and phosphocreatine probably exist in the cell as potassium salts, Myers cites the well known potassium depletions often observed in failing hearts (1,12,41) as further evidence that ATP and phosphocreatine decrease during decompensation.

A number of other workers have investigated the phosphocreatine and ATP concentrations within isolated hearts or under various other conditions. Chang(10), for example, subjected rabbits to anoxia for varying periods of time and examined their hearts for content of phosphocreatine, glycogen, and ATP immediately afterwards. He found a decline of these substances in all cases and also found that they declined in the order given, that is phosphocreatine suffered the sharpest and earliest of the falls. He interpreted this last result, curiosly enough,

as indicating that phosphocreatine was the important high energy phosphate donor to the cell rather than ATP. Also studied have been the effects of iodoacetate(8), cyanide(16), thiamine deficiency, hypothyroidism(13), and hyperthyroidism(57). All of these conditions exhibited some degree of loss of their high energy phosphates.

While the results presented so far seem to indicate that failing hearts are deficient in ATP and phosphocreatine, the results and conclusions are open to a great deal of question. The inferences drawn from the autopsy material, for example, proceed under the assumption that the concentrations of creatine and phosphocreatine parallel each other, an idea to which Wollenberger(69) presents two serious objections; First, it has been shown by several workers that the amount of creatine bound to phosphate in the myocardium is merely a minor fraction of the total creatine, in one series(19) the amount bound to phosphate amounted to only one per cent of the total. Under these conditions it is difficult to see that phosphocreatine levels must necessarily parallel total creatine values. Secondly Wollenberger(69) presents data obtained by analysis of muscle of dogs in which the ratio of phosphocreatine to creatine varies widely not only from heart to heart but also in different portions of the same heart. This indicates that while total creatine

values may indeed decrease in failure, its significance may be entirely unrelated to changes in the level of phosphocreatine.

The demonstration of decreased high-energy phosphates in conditions such as hypo or hyperthyroidism, thiamine deficiency, or poisonings are illuminating, but in no way lend support to any theory attempting to explain all human congestive failure on the basis of decreased energy production. In these cases the defect in oxidative metabolism is readily apparent and has been previously well defined. The mechanisms of failure, then, are in all probability quite different than in heart-lung or human congestive failures.

As an illustration of this point, Wollenberger(69,70) carried out extensive analyses of energy rich phosphate concentrations in the spontaneously failing heart-lung preparation of the dog. In this important experiment he found that at no point during failure was there any diminution of these phosphate compounds, finding either normal or elevated concentrations throughout. Since this represented direct measurement in a well nourished heart free from any metabolic inhibitors, Wollenberger concluded that this was an example of failure because of inadequate utilization of energy, rather than a def-

iciency of supply. An example of his experimental data is hown in Table II. These findings would then be consistent with the theories of Bing, Starling et all as outlined in the previous section.

That these results are not peculiar to the animal used or the type of preparation employed has been ably demonstrated by Burns and Cruikshank(8). Using the hearts of cats, rabbits, or dogs either as isolated hearts or in the heart-lung preparation, they also found normal levels of ATP and phosphocreatine, provided the preparation remained well oxygenated. With any appreciable degree of anoxia either from decreased tension or iodoacetate poisoning the levels were shown to decrease, again illustrating the basic difference between these two types of failure. It is also worthwhile to mention the work of Clark(15) in this regard, who found no phosphagen (phosphocreatine) depletion in fatigued hearts beating over considerable periods of time against heavy resistance.

The significance of the results from this form of investigation is hard to evaluate. It seems definite that the failing heart of the heart-lung preparation has no decrease in energy rich stores which may, in the light of the resemblance of this type of failure to that seen in human congestive failure, indicate that human

TABLE II

Condition of heart	Mean Systemic Arterial Pressure mm Hg	Total Output cc/min	Work Kg-M/min	P, mgm per 100 gm apex		
				Inorganic Phosphate	Phospho Creatine	Labile P of ATP
Normal	111	515	0.870	28.9	14.1	37.4
Normal	109	491	0.814	25.7	14.7	33.4
Normal	107	498	0.794	22.7	15.7	33.7
Normal	100	566	0.940	29.9	13.6	35.4
Normal	114	560	0.834	27.6	17.4	36.6
Mean	108	536	0.840	26.6	14.9	35.2
Failing	101	247	0.439	27.4	18.4	31.2
Failing	62	120	0.137	29.1	22.7	34.7
Failing	79	310	0.399	24.0	15.3	28.1
Failing	102	388	0.647	28.2	20.4	41.1
Failing	88	275	0.604	20.5	22.5	31.2
Mean	87	260	0.450	25.7	19.9	34.2

hearts alsc fail with their energy stores intact. Why creatine is diminished in autopsied decompensated hearts then becomes hard to explain. It has been suggested (70) that creatine plays a role in contraction which is separte from its association with phosphate bonds, but no experimental evidence has been put forward in support of this. The crucial experiment of determining directly increased energy stores in failing human hearts would clear much of the argument and confusion but is a difficult clinical accomplishment and will probably be a long time in appearing.

C CARBOHYDRATE METABOLISM DURING FAILURE

Yet another method of attack on the basic process of failure is a study of the reactions comprising the oxidative cycle. If a substrate is not utilized, or an enzyme can be shown to be inactive during failure, the whole question could be neatly resolved.

One of the first attempts along this line was the work of Visscher and Mulder (64). Using the dog heart-lung preparation they were able to demonstrate that glycogen levels remained fairly constant even after six hours at which time failure usually became quite pronounced. Gremels(31), however, reported that spontaneous failure of the heart in this type of a preparation was preceeded

and accompanied by a reduction in glucose uptake and showed, along with others(53), that the utilization was improved by the addition of insulin. This seems difficult to follow in view of the normal glycogen level during failure and the normal oxygen uptake in failure(4). Gremels postulated that the heart, because of decreased glucose utilization, was in a state of "energetic insufficiency" which was a prerequisite to failure. This work has not as yet been repeated and has in fact been strongly repudiated by in vivo studies as will be mentioned shortly.

Since lactic acid uptake was initially thought to be one of the heart's main sources of energy, the changes during failure have been studied, with a striking disparity of results among different investigators. The uptake has been described as increased(55), others have shown the uptake to be normal(23), and still others have shown a progressive decrease until the process is reversed and lactic acid is given off instead of being metabolized (26). It is hard to reconcile these results but perhaps part of the answer lies in the fact that during normal metabolism the uptake of glucose,lactate, and pyruvate varies directly with their arterial concentrations. It has been pointed out in the section on normal metabolism that the lactate blood level also varies with the type

of preparation used. Since a variation in levels may have been present at the time of the experimental procedure, this could account for the discrepancy with regard to the uptake of lactate.

There have been numerous reports on substrate and enzyme concentrations during anoxia. It has been demonstarted, for example, that anoxia causes a decrease in levels of cytochrome c(45), coenzyme I(45), glycogen (10), and that the effects of decreased coenzyme I can be relieved by nicotinamide(11). As has been pointed out before, these results are interesting but reflect a specific metabolic defect which has not been shown to be present in classical human congestive failure.

Perhaps the most significant results have been presented by Goodale and his co-workers(23). In their methods they made use of the coronary sinus catheter as devised by Bing(24) and directly measured glucose, lactate, and pyruvate uptake in normal and failing hearts. After analysing their results they could find no appreciable difference between the uptake of these substrates in normal and failing hearts. They the re-echoed the conclusion that there is no defect in oxidative metabolism in failing hearts.

In summary of the effects of failure on carbohydrate

metabolism, it can be said that no conclusive evidence has as yet been presented to show any defect in this respect. Although negative in nature, this tends to support the concept of failure as being due to defective utilization rather than defective generation of energy.

D MEMBRANE PERMEABILITY AND METABOLISM OF ACETYLCHOLINE

The possibility that failure might not be related to oxidative metabolism led some workers to investigate other possible methods whereby a heart loses its contractile force. One of the possibilities that had suggested itself was a defect in cardiac muscle cell permeability. Clark (16) early put forth the hypothesis that the hypodynamic condition of the frog's heart when beating in saline was due to the loss of phospho-lipids from the surface of the cell with resultant increased permeability and swelling. Other indications of a decreased membrane integrity during failure came from the oft repeated experiments showing a decrease in potassium concentration in autopsied human hearts from cases of congestive failure. That this was not due to digitalis administration was shown by Clarke (12) who included in his series many cases of untreated cardiac decompensation.

Although it seemed on this basis that membrane permeability might play a part in failure, methods of invest-

igation were slow in forthcoming because of the rather striking lack of knowledge about the maintenance of normal membrane integrity. While considerable time has been spent on nerve-membrane permeability, considerably less has been done in relation to the cardiac muscle cell. Welsh(66), admittedly on little actual experimental basis, presented theories on the mode of action of acetylcholine and its relationship to membrane permeability in the heart. In his concept, acetylcholine may be acting as a coenzyme to regulate the activity of a "receptor substance" located in or near the cell membrane. The role of this enzyme is to alter the excitability of the cell through processes leading to changes in membrane polarity and permeability. In support of this hypothesis he cites the infinitesmally small amounts of acetylcholine needed to affect the heart of the Venus mercenaria (clam heart), pointing out that this would be more consistent with the action of a coenzyme rather than with a substance taking direct part in the reactions of the cell.

Despite these theories, the relationship between acetylcholine and cellular permeability remained relatively obscure until the work of Greig and Holland appeared. They first of all demonstrated that permeability of red blood cells to potassium seems to depend on the integrity of the metabolic cycle of acetylcholine. (29)

They showed that when the cholinesterase is inhibited, or the substrates necessary for acetylcholine synthesis are limited, hemolysis of the red blood cell and loss of potassium would occur. They extended their work to include brain(29a), cornea(29b), and heart muscle(39), in each case showing that when either the synthesis or the breakdown of acetylcholine is blocked, the respective membranes become more permeable to potassium. A simplified diagram illustrating these features is shown in figure 3.

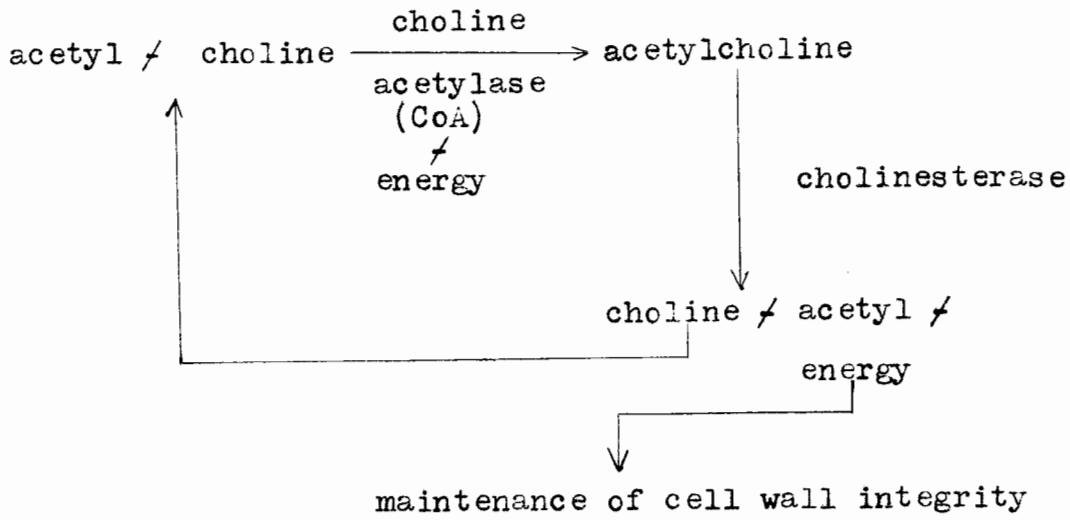


Figure 3

It can be seen from this diagram that subsrates in themselves are not responsible for membrane integrity, but it is rather the energy releasing metabolism of these

substances that provides for normal membrane integrity.

With this work in mind, Govier et all became interested in applying it to the problem of congestive failure and have presented a very stimulating paper in the subject(28). In their methods they used heart-lung preparations of dogs set up in the classical manner and then proceeded to test the effects of various known inhibitors of the choline (acetylcholine) cycle on the hearts. They found the following: 1- Inhibitors of cholinesterase such as physostigmine and DFP (diisoflourophosphate) procuce cardiac decompensation with a measureable loss of non-specific cholinesterase activity of the heart muscle, 2- Penicillin G, a choline acetylase inhibitor, produces cardiac decompensation, 3- Pentobarbital, also a choline acetylase inhibitor, produces cardiac decompensation often accompanied by a decrease in cholinesterase activity, 4- Benzoylcholine, an esterase substrate, is positively inotropic in failure produced by acetylase inhibition and in addition impedes decompensation by cholinesterase inhibitors, 5- Choline is positively inotropic in atropinized hearts failed by any of the above compounds. Its administration hindered subsequent failure by physostigmine or pentobarbital.

With these results, the authors then concluded that

failure in their heart preparations was due to increased membrane permeability as a result of depression of the metabolic cycle of acetylcholine. Their results are especially interesting with respect to the effects shown by the administration of choline. They demonstrated that in non-atropinized heart-lung preparations choline was negatively inotropic, but that in the atropinized preparations choline exhibited strong and rapid inotropic action and in certain cases managed to prevent failure. This may serve to illustrate the relative independence of the effects of choline on effector celld and its role in maintaining membrane integrity.

Although all cases of failure were associated with known metabolic inhibition of the acetylcholine cycle, the authors postulated that perhaps other types of decompensation may have a similar etiology. In tenuous support of this theory, many previously reported but poorly understood actions of acetylcholine may be cited. Burn(71), for example, was able to restart exhausted rabbit auricles with very minute additions of acetylcholine to the perfusing medium. Fatigue in his cases was not due to oxidative deficiency, for the auricles were placed in well-oxygenated, buffered media normally supplied with substrates. Other workers have also shown this

effect, not only in fatigued muscle but also in some instances of relatively normal hearts (38,47,48,62). The stimulation of cardiac activity can also be seen with relatively large doses of acetylcholine in atropinized hearts. This unusual result naturally led to speculation as to the possible mechanism. Hoffman and all (38) demonstrated that the perfusate from isolated, atropinized hearts stimulated with acetylcholine was inhibitory to isolated rabbit intestine and was also capable of pupillary dilatation. They concluded that acetylcholine caused liberation of epinepherine or an epinepherine-like from sympathetic ganglia which in turn stimulated the heart. In support of this hypothesis they were able to show that ganglionic blocking agents such as nicotine and TEM prevented this stimulating action. In view of the fact that their hearts were isolated, these workers were also forced to the conclusion that sympathetic ganglion cells exist in the heart, an assumption that has been in no way borne out by anatomic studies. Also in argument with this hypothesis is McDowall (47) who was able to stimulate hearts with acetylcholine but could not demonstrate blockage of this effect by known ganglionic blocking agents. While the workers mentioned have been able to report stimulation by acetylcholine with regularity,

it is apparently a phenomenon not easily observed by all investigators. Numerous studies, either attempting specifically to show stimulation by acetylcholine (72) or in observing the relationship of acetylcholine to the heart (20,65), have not shown any observable increase in rate or amplitude either with small doses of acetylcholines on non-atropinized hearts or larger doses on atropinized hearts.

Granted that acetylcholine may at times stimulate the heart, a satisfactory explanation must still be sought. In light of the work of Greig and Holland and on his own experiments as previously outlined, Govier (28) attributes the beneficial action of acetylcholine to strengthening of its metabolic cycle, either through by-passing a block or overcoming it with an excess of substrate. Thus the disparity of results obtained by different workers might be explained on the basis that the status of the metabolic cycle of acetylcholine varied in the different cases. In other words the most beneficial effects of acetylcholine would be logically expected in cases where its metabolic cycle had deteriorated, and in comparatively normal hearts with no disturbance in this cycle its administration could not be expected to aid the heart.

The attempt to attribute other types of heart failure

to this mechanism, however, reaches an impasse at this point. Although Wollenberger (69) has shown that failure in the heart-lung preparation induced by pentobarbital resembles the failure spontaneously occurring, there is still no direct evidence of a disturbance in the acetylcholine cycle of experimental hearts in failure. While the work of Govier and all is intriguing, the authors themselves freely admit that the conclusions drawn are largely conjecture and will need much more experimental attention before any of their theories can be verified.

Of considerable interest with regard to the status of the membrane during failure is the work of Szent-Gyorgi and S. Hajdu (33,34). These workers were particularly interested in the phenomenon of staircase, or "treppe", in the isolated heart. Bauditch, and early American physiologist, had illustrated the principle that if an isolated, electrically driven heart is stopped for a few seconds and then driven electrically again, the contractions would be small at first and then gradually increase in amplitude until they had reached their previous level. This effect when recorded on a smoked drum somewhat resembles a staircase. (fig. 3)

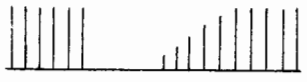


figure 3

Using an isolated frog heart perfused through the aorta with Ringer's solution, Szent-Gyorgi demonstrated first that the staircase could be abolished by a low potassium concentration or by low temperature. After a period of rest, in other words, the contractions would begin again at a normal level without first showing the gradual rise. He then tested the effects of various substances on this staircase and found that serum, desoxy-corticosterone, and digitalis were able to abolish it while substances such as epinepherine had no effect. (fig. 4)

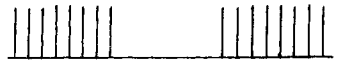


Figure 4
After addition of serum, desoxy-corticosterone, or digitalis

From these relatively simple experiments, Szent-Gyorgi draws some extensive ideas. In his concept, when the cardiac muscle cell contracts, potassium is extruded and during the process of relaxation potassium re-enters the cell. He postulates that the contractile elements within the cell are geared so as to produce maximal contractions at a certain level of potassium concentration. If too much time elapses between contractions the potassium concentration within the cell is abnormally elevated and the contractions become depressed. This feature then

accounts for the small initial contraction after the extended period of rest. If the concentration of potassium in the surronding media is artificially lowered, abnormal potassium re-entry into the cell should be prevented and the staircase abolished. He further theorizes that this may be applicable to the case of the failing human heart. In this situation he envisions a heart unable to prevent excessive re-entry of potassium. In an attempt to contract in a more favorable ionic milieu the heart begins to beat more rapidly. This in turn leads to a further decrease in amplitude of contraction when diastole becomes too short for re-extension of the muscle fibers. A type of vicious circle is then established, cumulating eventually in failure.

This theoretical interpretation meets many obstacles. First, it is directly opposed to the assumed fact that myocardial potassium is lowered during failure. Secondly, the phenomenon of staircase is one seen only in isolated hearts perfused with artificial media and it is therefore difficult to apply what may be a laboratory artefact to considerations of human congestive failure.

Significance:

Since the work on acetylcholine and membrane permeability is recent and still highly theoretical, it is not easy to fit into a patteren of either defective

utilization or defective generation of energy. Since the synthesis of acetylcholine involves the transfer of high energy bonds from ATP via coenzyme A, it might be assumed that a defect in oxidative metabolism could lead to abnormal membrane permeability. That this is probably not the usual case can be illustrated by the finding that choline can stimulate atropinized hearts, while if decreased energy store were present the heart should theoretically not be able to use choline in the synthesis of acetylcholine. Wollenberger's findings (69) of normal or high energy rich phosphate concentrations in hearts failing through barbiturate administration also indicates that failure of the acetylcholine cycle need not be associated with a defect in oxidative metabolism. Along this same line, there are many possibilities for a disturbance in the choline-acetylcholine cycle other than a defect in energy production. Augustinsson (2), for example, has shown that the cholinesterases are subject to many and varied metabolic influences, and choline itself undergoes numerous cyclic changes before even entering into the reaction with acetyl groups. It is quite conceivable to assume that a metabolic disturbance, if present, may have nothing to do with the generation of high energy bonds.

E CONTRACTILE PROTEINS DURING FAILURE

Again assuming that oxidative metabolism is not at fault, another distinct possibility of the mechanism of failure lies in the transference of energy to the contractile proteins within the cardiac muscle cell. Perhaps because this is a comparatively new field of research, very little has been demonstrated with respect to the heart in failure and most of the evidence is of an indirect nature.

Several workers have investigated the effects of the digitalis glycosides on cardiac muscle protein strands. Of special interest are the results of Horvath(40) who observed the effects of digitalis on increasing the polymerization of actin. Actin may exist in two forms, globular or fibrous, and the transformation of globular to fibrous actin, the so called polymerization, is necessary before union with myosin can occur. (see part II) Horvath tenatively postulates that failing hearts may have abnormal degrees of depolymerization and that digitalis tends to reverse this process, thus enabling stronger union of actin and myosin.

In a similar vein, Mallov and Robb (46) have shown that after actomyosin is formed if it is first mixed with a cardiac glycoside and allowed to stand for one to two hours, considerable relaxation ensues and the

contraction when it occurs is stronger. Following the concept that ATP is necessary for proper relaxation of the actomyosin, this experiment might conceivably illustrate a potentiating effect of digitalis on the reaction between ATP and the cardiac muscle cell. Bowen (7) has also noted a strengthening effect of digitalis on myosin B threads. He points out that no known oxidative metabolism occurs within myosin B fibers and the effect of digitalis is therefore not in that direction.

A discussion of the detailed action of digitalis is beyond the scope of this paper. Assuming, however, that digitalis aids in correcting some metabolic defect, these experiments lend some indirect evidence to the concept of failure occurring because of an inability of the contractile protein of muscle to use ATP or other high energy donors. Before too many conclusions are drawn, it should be pointed out that the heart muscle used in these experiments was from normal hearts, a fact which considerably lessens their value. The field is broad, however, and further studies will most surely be undertaken.

III DISCUSSION AND CONCLUSIONS

In looking back over all the various data and conclusions presented, several features become quite striking. First, it appears that cardiac failure can be definitely divided into two main groups. The first group includes those due to substrate deficiencies, anoxia, or poisonings and which appear to fail because of a decrease in production of energy. The second group, which includes spontaneously failing heart-lung preparations and human congestive failure and with which this thesis is largely concerned, seem to fail with their energy stores intact and without any demonstrable change in oxidative metabolism. At present, these are the only statements that can be made with any degree of assurance. The investigations into possible disturbances of membrane permeability or protein contractility are still in a nebulous stage. Indications for further investigation may be drawn from them, but conclusions are hardly justified.

A separation of heart failure into two basic groups in this manner gives rise to some fascinating and rewarding clinical correllations. It has been well known, for example, that the classical indications for digitalis therapy are those cases of failure due to valvular disease, hypertension, and to a lesser degree arteriosclerotic heart disease. These are the types of failure that seem

to respond best to digitalis therapy and it will be noted that they fall into the second group, that is those hearts that fail with their energy stores intact. It might be then tenatively assumed that digitalis aids either by helping the muscle fibril assimilate phosphate bond energy or perhaps by its effect in strengthening the acetylcholine cycle.

If this hypothesis is valid, digitalis would conversely be ineffective in cases of group I, where the defect lies in the production of energy. A review of the literature shows this to be true. Digitalis is of little benefit in cardiac failure resulting from thiamine deficiency(ber-beri), thyrotoxicosis, acute anoxia, or anemia(35). Thus it seems likely that a prerequisite for the action of digitalis is the presence within the heart of a plentiful supply of high energy phosphates.

As in almost every other field of abnormal physiology, this topic is still subject to argument from many sources. To defer any conclusions until all workers are in agreement would, however, be deferring them indefinitely. If the conclusion can be drawn that the failing heart suffers from an inability to use energy, many new avenues for research will be opened which may lead eventually to a definitive answer.

IV SUMMARY

1. Normal cardiac metabolism has been discussed.
2. Possible mechanisms of failure were presented and the failing heart was discussed with respect to myocardial efficiency, balance of energy rich phosphates, carbohydrate metabolism, acetylcholine metabolism, and contraction of myocardial protein strands.
3. It was concluded that the balance of evidence favored an interpretation of heart failure of the classic human congestive type as being due to an inability to use available energy and not to a decreased production of energy.

BIBLIOGRAPHY

1. Alexander, L.C., Boyle, A.J., Iseri, L.T., McCaughey, R.S., and Myers, G.B. Electrolyte and Water Content of Cardiac and Skeletal Muscle in Normals, Ventricular Hypertrophy, and Infarction Jour. Lab.&Clin. Med. 36;796, 1950

2. Augustinsson, K.B. Cholinesterases Acta Physiol. Scandinavia Suppl. 52, 1948

3. Bing, R.J., Hammond, M.M., Handelsman, J.C., Powers, S.R., Goodale, W.T., and Kety, S.S. The Measurement of Coronary Blood Flow, Oxygen Consumption, and Efficiency of the Left Ventricle in Man. Am. Ht. Jour. 38:1, 1949

4. Bing, R.J., Falholt, W., Heimbecker, R. and Carrol, D. Myocardial Oxidative Metabolism and Initial Fiber Length of Failing Human Hearts. Jour. Clin. Invest. 30:630, 1951

5. Bing, R.J. and Daley, R. Behavior of the Myocardium in Health and Disease as Studied by Coronary Sinus Catheterization. Am. Jour. Med. 16:711, 1951

6. Bouge, Y.J., Chang, I. and Gregory, R.A. Metabolism of Isolated Mammalian Heart Under Partial Anoxia. Quart. Jour. Exp. Physiol. 27:319, 1938

7. Bowen, W.J. Effect of Digitoxin on Rate of Shortening of Myosin B Threads. Fed. Proc. 11:16, 1952

8. Burns, W., and Cruikshank, E.W.H. Changes in Creatine, Phosphagen, and Adenylpyrophosphate in Relation to the Gaseous Metabolism of the Heart. Jour. Physiol. 91:314, 1937

9. Burdette, W.J. The Krebs Cycle in Human Cardiac Muscle. Am. Ht. Jour. 44:823, 1952

10. Chang, I. Effect of Asphyxia on the ATP Content of the Rabbit's Heart. Quart. Jour. Exp. Physiol. 28:3, 1938

11. Calder, R.M. Effect of Nicotinic Acid on Myocardial Systole, Coronary Flow, and Arrhythmias of Isolated Heart. Proc. Soc. Exper. Biol. & Med. 65:76, 1947

12. Clarke, N.E. and Mosher, R. Electrolyte Changes in the

Myocardium, Reported at the Regional Meeting of the American Federation for Clinical Research, Michigan, 1951. Abstract Jour. Michigan Med. Soc. 50:622, 1951

13. Cheng, G., and Geiling, E.M.K. The Effect of Thiamine Deficiency, Quinidine, Hyperthyroidism, and Hypothyroidism on the ATP and ATP-ase Activity of Heart Muscle of Rats. Fed Proc. 5:169, 1946

14. Cowan, D.W. The Creatine Content of the Myocardium of Normal and Abnormal Human Hearts. Am. Ht. Jour. 9: 378, 1933

15. Clark, A.J., Eggleton, and Eggleton, P. Phosphagen in the Perfused Heart of the Frog. Jour. Physiol. 75: 332, 1932

16. Clark, A.J. The Metabolism of the Frog's Heart. Edinburg and London 1938

17. Cruikshank, E.W.H. Metabolism of the Heart Physiol. Rev. 16:597, 1936

18. Dechard, G.M. and Visscher M.B. Energy Metabolism of the Failing Heart. Jour. Exper. Med. 59:195, 1934

19. Davies, F., Francis, E.T.B., and Stoner, H.B. The Distribution of Nucleotide, Phosphocreatine, and Glycogen in the Heart. Jour. Physiol. 106:154,1947

20. De Elio, F.J. Acetylcholine Antagonists; A Comparison of their Action in Different Tissues. Brit. Jour. Pharmacol. 3:108, 1948

21. Dock, W. Capacity of the Coronary Bed in Cardiac Hypertrophy. Jour. Exp. Med. 74:177, 1941

22. Evans, C.L.A. The Metabolism of Cardiac Muscle. Recent Advances in Physiology Blakiston, Philadelphia, 1936

23. Goodale, T., Olson, R.E. and Hackel, D.B. Myocardial Glucose, Lactate, and Pyruvate Metabolism of Normal and Failing Hearts Studied by Coronary Venous Catheterization. Fed. Proc. 9:49, 1950

24. Goodale, W.T., Lubin, M., Eckenhoff, J.E., Hafkenschiel,J and Banfield, W.G. Coronary Sinus Catheterization for

Studying Coronary Blood Flow and Myocardial Metabolism. Am. Jour. Physiol. 152:340, 1948

25. Gollwitzer-Meiir Quoted in Visscher, Blood, Heart, and circulation. AAAS Pub. 13:176, 1940

26. Gottdenker, F. and Rothberger, C.J. Quoted in Wollenberger, Pharm. Rev. 1:311, 1949

27. Govier, W.M. The Effect of Experimental Coronary Artery Occlusion on the Coenzyme I and Cocarboxylase Content of the Myocardium of the Dog. Am. Ht. Jour. 29:384, 1945

28. Govier, W.M., Freyburger, W.A., Gibbons, A.J., Howes, B.G and Smits, E. The Relation of the Choline Cycle to Cardiac Decompensation: Acetylcholine Metabolism in the Dog Heart-Lung Preparation. Am. Ht. Jour. 45: 122, 1953

29. Greig, M.E. and Holland, W.C. The Relationship between Cholinesterase activity and Permeability of Dog Erythrocytes. Arch. Biochem. 23: 370, 1949

29a Greig, M.E. and Holland, W.C. Increased Permeability of the Hemoencephalic Barrier Produced by Physostigmine and Acetylcholine. Science 110:237, 1949

29b Greig, M.E. and Holland, W.C. The Anesthetization of the Rabbit's Cornea by Non-Surface Anesthetics. Brit. Jour. Pharmacol. and Chemotherapy 5:461, 1950

30. Gremels, H. Quoted in Visscher, Blood, Heart, and Circulation. AAAS Pub. 13: 176,1940

31. Gremels, H. Quoted in Wollenberger, Pharm. Rev. 1:311, 1949

32. Hackel, D.B., Goodale, W.T., and Johnson, R.P. Myocardial Metabolism in Thiamine Deficient Dogs as studied by IV Catheterization of the Coronary Sinus. Fed. Proc. 8:65, 1949

33. Hajdu, S. and Szent-Gyorgi, A. Action of Digitalis Glucosides on Isolated Frog Heart. Am. Jour. Physiol. 168: 171, 1952

34. Hajdu, S. and Szent-Gyorgi, A. Action of DOC and Serum on the Frog Heart. Am. Jour. of Physiol. 168: 159, 1952

35. Harrison, T.R. In Principles of Internal Medicine Blakiston Co. 1951

36. Harrison, T.R. Failure of the Circulation. Baltimore, 1939 Williams and Wilkins

37. Herrman, G. and Dechard, G.M. The Chemical Nature of Heart Failure. Ann. Int. Med. 12: 1234, 1939

38. Hoffman, E.J., Middleton, S. and Talesnik, J. The Stimulating Effect of Acetylcholine on the Mammalian Heart and the Liberation of an Epinepherine-Like Substance by the Isolated Heart. Am. Jour. Physiol. 144: 189, 1945

39. Holland, W.C., Dunn, C.E., and Greig, M.E. Effect of Acetylcholine on the Permeability to Na and K of Isolated Guinea Pig Hearts. Fed. Proc. 11: 357, 1952

40. Horvath, I., Kiraly, C., and Szerb, J. Action of Cardiac Glycosides on the Polymerization of Actin. Nature 164: 792, 1949

41. Iseri, L.T., Alexander, L.C., McCaughey, R.S., Boyle, A.J., and Myers, G.B. Water and Electrolyte Content of Cardiac and Skeletal Muscle in Heart Failure and Myocardial Infarction. Am. Ht. Jour. 43: 215, 1952

42. Katz, L.N., Wise, W., and Jochim, K. The Dynamic Alterations in Heart Failure in the Isolated Heart and Heart-lung Preparation. Am. Jour. Physiol. 143: 507

43. Katz, L.N., Wise, W., Meyer, J., Lendrum, B., and Jochim, K. Mechanical Efficiency of the Heart in Experimental Heart Failure. Fed. Proc. 5:52, 1946

44. Kruger, E. Quoted in Visscher, Blood, Heart, and Circulation. AAAS Pub. 13:176, 1940

45. Lemley, J.M., and Meneely, G.R. Effects of Anoxia on Metabolism of Myocardial Tissue Am. Jour. Physiol. 169: 66, 1952

46. Mallov, S. and Robb, J.S. Behavior of Actomyosin Threads. Fed. Proc. 8:104, 1949

47. McDowal, R.J.S. The Stimulating Action of Acetyl-Choline on the Heart. Jour. of Physiol. 104:392, 1945

48. McNamara, B., Kropp, S., and McKay, E.A. The Stimulating Action of Acetylcholine on the Isolated Mammalian Heart. Jour. Pharmacol. 92: 153, 1948

49. Mosonyi, L. and Porzasz, J. Digitalis Sensitivity of Persons Treated with Penicillin. Orvosi Hetil. 89: 149, 1948 Chem. Abstracts 43: 8547h

50. Myers, V.C. Some Chemical Changes in the Myocardium Accompanying Heart Failure. Bull. N.Y. Acad. Med. 18: 303, 1942

51. Nakamura, K., Sauders, P.R., Webb, J.L., Lawson, H.C., and Thienes C.H. Metabolism of the Hear in Relation to Drug Action. Am. Jour. Physiol. 158: 269, 1949

52. Olson, R. E. and Schwartz, W.B. Myocardial Metabolism in Congestive Failure. Medicine 30: 21, 1951

53. Pearson, C.H., Hsieh, C.K., Dutoit, C.H., and Hastings, A.B. Metabolism of Cardiac Muscle. Utilization of C14 Labeled Pyruvate and Acetate in Diabetic Rat Heart and Diaphragm. Am. Jour. Physiol. 158: 261, 1949

54. Peters, H.C. and Visscher, M.B. Energy Metabolism of the Heart in Failure and the Influence of Drugs upon it. Am. Ht. Jour. 11:273, 1936

55. Rolshoven, H. Quoted in Wollenberger, Pharm. Rev. 1:311, 1949

56. Sherrod, T. R. Effects of Digitalis on Electrolytes of Heart Muscle. Proc. Soc. Exp. Biol. & Med. 65:89,1947

57. Shelby, W.B., Code, C.E., and Visscher, M.B. Influence of Thyroid, Dinitrophenol, and Swimming on Glycogen and Phosphocreatine Levels of Rat Heart in Relation to Cardiac Hypertrophy. Am. Jour. Physiol. 138: 652, 1942

58. Stadie, W.C., Haugaard, N., and Perlmutter, M. Synthesis of Glycogen by Heart Slices. Jour. Biol. Chem. 171:419, 1947

59. Starling, E.H. and Visscher, M.B. The Regulation of the Energy Output of the Heart. Jour. Physiol. 62: 243, 1927

60. Szent-Gyorgi, A. Contraction in the Heart Muscle Fiber. Bull. N. Y. Acad. Med. 28: 1, 1952

61. Szent-Gyorgi, A. Chemistry of Muscular Contraction. New York, Academic Press, Inc., 1951

62. Vane, J. R. The action of Choline Derivatives on Isolated Rabbit Auricles when Arrested by Paludrine. Brit. Jour. Pharm. 3: 341, 1948

63. Visscher, M.B. Energy Transformations by the Heart and the Mechanism of Experimental Cardiac Failure. Blood, Heart, and Circulation AAAS Pub. 13: 176, 1940

64. Visscher, M. B. and Mulder, A.G. The Carbohydrate Metabolism of the Heart. Am. Jour. Physiol. 94: 630,1930

65. Webb, J. L. The Action of Acetylcholine on Isolated Rabbit Auricles. Brit. Jour. Pharmacol. 5: 87, 1950

66. Welsh, J.H. Concerning the Mode of Action of Acetylcholine. Bull. John's Hop. Hosp. 83: 568, 1948

67. Wise, W., Walter, J., Katz, L.N., and Jochim, K. The Oxygen Consumption and Mechanical Efficiency of the Heart Before and During Heart Failure. Am. Jour. Physiol. 147: 28, 1946

68. Wollenberger, A. Utilization of C14-labeled Glucose by Cardiac Muscle Traeted with a Cardiac Glycoside. Science 113: 64, 1951

69. Wollenberger, A. On the Energy Rich Phosphate Supply of the Failing Heart. Am. Jour. Physiol. 150: 733, 1947

70. Wollenberger, A. The Energy Metabolism of the Failing Heart and the Metabolic Action of the Cardiac Glycosides. Pharm. Rev. 1:311, 1949

71. Burn, J.H. Relation of Motor and Inhibitor Effects of Local Hormones. Physiol. Rev. 30: 177, 1950

72. Isacson, E. P. Unpublished Observations, Dept. of Physiol. and Pharmacol. University of Nebraska School of Medicine, 1951