Cardiac Active Principles in Blood Plasma

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A study of isolated frog hearts suggests that the amphibian heart is provided normally with a substance that maintains contractility and that disappears slowly from the heart during perfusion with saline solution. A search for substances that might fulfill this role in the frog heart has led to the isolation of many materials that have some degree of cardiotoxic activity, but no conclusion can yet be reached about which, if any, of these substances is present in the intact frog heart. The search has led, however, to the discovery of 2 substances of mammalian origin that are as potent as the cardiac glycosides with respect to their inotropic action on frog heart. One of these is a phospholipid called lyssolecithin, the other is a system of 3 plasma globulins called ‘‘cardioglobulin A, B, and C.’’ The concentration of cardioglobulin C in man is increased in essential hypertension and aortic stenosis, 2 unrelated conditions that have in common the development of increased left ventricular isometric tension in systole. Conversely, cardioglobulin C is decreased in a group of patients with idiopathic congestive heart failure. The discovery of these substances is relevant to the question whether isolated mammalian cardiac tissue becomes hypodynamic in physiologic saline because of the loss of a system that helps maintain normal myocardial contractility.

We have noted that most of the studies on isolated strips of mammalian cardiac tissue fail to answer this question, since the strips were probably hypodynamic because of impaired oxygenation or nonphysiologic saline media. Studies from our laboratory indicate that a slow decline in contractility on prolonged washing does occur in isolated mammalian heart tissue, despite good oxygenation and a normal environment with respect to inorganic ions. This can be prevented or reversed by perfusion with mammalian plasma. Along similar lines, it is of interest that, although the decline of performance characteristics of in situ mammalian hearts may be due to many factors, the decline can be prevented by perfusing the coronary system of the in situ heart with blood from a healthy donor animal. The problem, then, has 2 aspects. On the one hand, we must discover the physiologic significance of the potent glycoside-like substances already isolated from mammalian tissue; on the other, we must investigate the beneficial effects of plasma on heart strips or of donor dog blood on in situ hearts. Do these actions occur because of an effect on myocardial metabolism or do they come about because of a plasma substance that enhances myocardial contractility directly?

A WIDE VARIETY of experience has led to the conclusion that blood plasma has a beneficial effect on cardiac contractility. Thus, cardiac function declines when hearts are bathed in artificial saline media and improves when fresh whole blood or plasma is added. This effect was first noted by Ringer in 1885.1 Recent interest in this phenomenon stems from two different lines of interest. On the one hand, now that much information has been obtained about the contractile protein of muscle and about the metabolic events that yield energy for contraction, it is appropriate to consider the problem of how the force of muscle contraction is regulated. The same question about the regulation of muscular contraction presses to the fore in the field of cardiovascular research. We may mention as examples the abnormal increase in arteriolar tone in essential hypertension, the congestive heart failure that occurs in a number of patients in the absence of any known structural or inflammatory disease of the myocardium, and the cardiovascular collapse that often supervenes in patients subjected to a prolonged period of extracorporeal circulation. All these instances are characterized by abnormalities of involuntary muscle function, despite the absence of any gross anatomic or metabolic defects that might be invoked as causes of the disturbance. In this lecture we will review some experimental situations in which alterations in cardiac function might be due to the addition or depletion of cardiac active substances of biologic origin.

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We shall begin with the isolated amphibian heart. The decline of myocardial contractility that occurs when hearts are bathed in artificial media was studied in detail by A. J. Clark in 1913; he said that "excised frog hearts after perfusion for a few hours pass into a hypodynamic state in which both the force of contraction and the rate of conduction are markedly impaired." He concluded that the development of a hypodynamic state was associated with the loss of some essential substance from the frog heart, which was washed away slowly by prolonged contact with large volumes of saline solution.

There are several features about the hypodynamic heart that we would now like to enumerate. First, we may note that, immediately following excision and immersion in saline, a slow decline in contractility begins that continues steadily for a number of hours. The rate of decline depends to some extent on the thoroughness of the washing, that is to say, on the volume of perfusing solution and frequency of exchange. The first signs of the decline cannot be detected at high rates of stimulation but can be observed in the form of decreased twitch tension at lower frequencies. It should be emphasized that this decline occurs despite an environment that is ideal with respect to inorganic ions and oxygenation. Unlike mammalian heart muscle, the frog heart has no coronary system and depends for oxygenation on the movement of blood through the sponge-like network of muscle cells that constitute the ventricular wall. It is therefore no problem for oxygenated Ringer solution to perfuse the various parts of the frog ventricle adequately. Not only is there no impairment in oxygenation, but it is likely that the development of the hypodynamic state occurs in the absence of any decrease of high energy phosphate. Although this has not been studied in frog ventricle, Furchgott and de Gubareff have shown that the development of the hypodynamic state in guinea-pig atria is not associated with any change in the concentration of high energy phosphate compounds in the atrial muscle. It would appear therefore that the hypodynamic state in frog heart muscle is not due to a defect in the supply of phosphate energy to the contractile protein. For a more detailed discussion of the question of whether the defect in experimental hypodynamic states is due to a defect in energy supply or energy utilization see reviews by Hajdu and Leonard and by Wollenberger.

We may now consider the substances that can restore frog-heart contractility to the original level. Actually, a very great number and variety of substances of plasma origin can improve the hypodynamic heart. Ringer concluded that the plasma activity resided in a nondialyzable fraction. Clark found that a positive inotropic effect could be obtained not only with soaps, such as sodium oleate, but also with various phospholipids and even amino acids. Other surface-active substances, such as bile acids, also improved contractility. In addition, beneficial effects have been obtained with adrenalin, adenosine triphosphate (ATP), and with pharmacologic concentrations of certain steroids, such as deoxycorticosterone and progesterone. A great many investigators have worked in this field, and the interested reader can find more references in the review by Amberson. Most of the substances we have mentioned are capable of bringing the contractility of the hypodynamic frog heart back to normal, if they are added to the bathing solution in sufficient concentration. Their effect can be appreciated by reference to figure 1, in which twitch tension is plotted on the ordinate and the time interval between stimuli is plotted on the abscissa. The twitch tension of both the fresh heart and the washed hypodynamic heart varies with the frequency of stimulation. Comparison of these curves shows that, whereas the twitch tension of the hypodynamic heart approaches that of the fresh heart at high frequencies, at intermediate frequencies fresh heart is capable of developing much greater twitch tension than is the hypodynamic heart. The beneficial substances we have discussed above cause the hypodynamic curve to shift back toward the fresh heart curve.
In one respect, however, the hypodynamic frog heart, which is improved by the addition of these materials, is still different from a normal heart: on subsequent washing, the hypodynamic state develops very rapidly. This is in marked contrast to the slow and gradual decline in contractility that occurs with a fresh heart. It seems reasonable to postulate that the development of the hypodynamic state in the frog heart is due to the slow loss of some material from the muscle. One would suspect that it is present in low concentrations in frog blood, and that an appropriate cardiac content is maintained because of the constant perfusion and the high affinity of the material for the heart. If so, perfusion of a hypodynamic frog heart with a large volume of normal frog plasma should result in the slow accumulation of the material, so that once again the heart would become hypodynamic only after prolonged washing.

Recently, a material that may fulfill these conditions has been isolated. It is a phospholipid called "β-palmitoyl-lysolecithin," which can be found in small concentrations in mammalian plasma. It has been isolated from adrenal medulla and may be rather widely distributed in the chromaffin system. Although this substance is present in mammalian plasma in concentrations too low to affect the hypodynamic frog heart immediately, it becomes bound to frog cardiac muscle so that, if a frog heart is perfused with successive changes of serum, a significant amount of lysolecithin gradually accumulates. And, in contrast to the other substances discussed, after exposure to this material the restored heart becomes hypodynamic only gradually in the course of prolonged washing. Attempts have not yet been made to isolate lysolecithin from frog tissues. If it is found in frog plasma, it would be reasonable to postulate that lysolecithin is the naturally occurring substance that is slowly washed away from the frog heart during the development of the hypodynamic state.

Lysolecithin differs in another way from substances previously shown to restore the contractility of the hypodynamic frog heart. This can be appreciated by referring once again to figure 1, which shows how twitch tension varies with the interval between stimuli. Lysolecithin, like the cardiac glycosides, can cause maximal twitch tension even at very low rates of stimulation, which is represented in the figure by the nearly horizontal line at the top of the graph. In higher or toxic concentrations, the glycosides or lysolecithin induce systolic arrest or contracture, a phenomenon that is not seen with the other substances we have been discussing. Their positive inotropic effect is therefore profoundly greater than that of any of the other substances. Recently we have isolated from mammalian plasma a cardiotonic protein system of great potency that is comprised of three globulins that have been called cardioglobulin A, B, and C. The effect of cardioglobulin on the frog heart in a concentration comparable to that found in normal human plasma is similar to that of a nontoxic concentration of cardiac glycosides. (This protein system also causes constriction of the peripheral vasculature of the frog in a Trendelenburg preparation, and therefore the vasoconstrictor globulin studied by Sakai and Hiramatsu many years ago may have been cardioglobulin.)

A new question of great interest now arises as a result of these studies of the hypodynamic...
amphibian heart. The discovery of 2 inotropic systems, lysolceithin and cardioglobulin, with cardiotonic potency comparable to that of the cardiae glycosides, indicates that we must determine whether either of these systems is important in the maintenance of normal myocardial contractility in the mammal. In this regard, the plasma concentration of cardioglobulin in various clinical states is of interest. We have compared the plasma concentration of cardioglobulin C in normals, in patients with aortic stenosis, and in patients with essential hypertension. In both aortic stenosis and essential hypertension, 2 conditions characterized by increased left ventricular isometric tension in systole, the concentration of cardioglobulin C is significantly increased above normal.12,13 This is consistent with the idea that cardioglobulin could be a naturally occurring cardiotonic system that is increased in the hyperdynamic states noted. The increased cardioglobulin seems to be related to the increased pressure developed by the left ventricle and not simply to increased work, since in aortic insufficiency (characterized by increased stroke work without an increase in pressure), cardioglobulin is normal. The concentration of cardioglobulin has also been measured in various types of cardiac failure, and it has been found that values for patients with failure secondary to valvular disease (aortic or mitral insufficiency) are normal. In contrast, about half of the patients with cardiac failure secondary to idiopathic myocardial disease appeared to fall into a separate population with extremely low values of cardioglobulin.12,13 The question whether the myocardial failure of this group may be caused by the observed cardioglobulin deficiency cannot be answered at this time.

The questions about cardioglobulin raised by these clinical correlations lead us to consider studies on experimentally induced hypodynamic states in mammalian heart muscle. Investigations on surviving strips of mammalian heart received considerable impetus when Cattell and Gold introduced the cat papillary-muscle preparation for the study of the action of cardiac glycosides.14 Since that time a great variety of substances of mammalian origin have been found to increase the contractility of isolated cat papillary muscle: including phospholipids, amines, amino acids, serum albumin, a dialysate of serum albumin, and various adrenal steroids.18 The substances studied have been isolated not only from serum but also from fractions of dried spleen and from liver.15

In attempting to evaluate these various results, it is pertinent to recall that the contractility of the isolated frog heart in saline declines slowly and steadily over a period of hours, despite normal energy metabolism and ionic environment, suggesting that a substance which maintains normal contractility is gradually washed away from the heart. Does a comparable situation exist in the case of mammalian hearts? Unfortunately the studies under consideration cast no light on this question because the comparable experimental conditions do not exist in the mammalian heart preparations. The mammalian tissues studied were hypodynamic because of abnormally low calcium or bicarbonate concentration in the extracellular medium in some cases, and in others it is probable that impaired oxygenation caused the hypodynamic state. In contrast to the frog heart,
which has no coronary system, oxygenation of dense mammalian ventricular tissue is difficult to accomplish once the coronary circulation is interrupted. Because of the difficulty in oxygenation, we would not expect cat papillary muscle stimulated at rapid rates to survive without some metabolic abnormality. Tanz, for example, has recently published photomicrographs showing severe histologic damage in cat papillary muscle stimulated at 1 per second at 37 C. for 6 hours.\(^1\) Therefore, although a great variety of substances of biologic origin have been found to improve the performance of mammalian heart preparations, it is difficult to judge the physiologic significance of these findings, and none of the studies provides an answer to our question about whether there is a cardiotonic material bound to mammalian cardiac muscle that is lost when the tissue is removed from its normal environment.

Some recent results of our own, which are addressed to this problem, can be seen in figure 2. The studies were made on thin strips of right ventricle from very young rats, and the graph shows twitch tension plotted as a function of the interval between stimuli. The upper curve represents the normal pattern found in freshly prepared rat ventricle. After 3 hours of washing in Krebs bicarbonate solution, the lower curve is obtained, with considerably lower twitch tension over a wide frequency range. This can be reversed with digitalis or mammalian plasma, and the original decline can be prevented if the strip is maintained from the beginning in mammalian plasma. In general, it appears that mammalian cardiac muscle strips immersed in an environment which is ideal with respect to inorganic ions and oxygenation becomes hypodynamic on prolonged perfusion, and it is probable that this decline in contractility can be prevented if heart strips are maintained in mammalian plasma.

We will now turn to a brief consideration of studies on mammalian hearts in situ in which the vascular connections between the heart and other organs are partly or completely severed so that it is possible both to achieve some control over factors that affect myocardial function and to measure the rate of utilization of oxygen and other metabolic substrates. The prototype preparation is, of course, the heart-lung preparation, but there are also various interesting modifications in which 1 or more organs of the body are excluded from the general circulation. It was clearly stated by Starling and Visscher\(^2\) that the classical heart-lung preparation deteriorates over a period of several hours, a decline which is reflected in both a decrease in contractility and in efficiency. It is apparent that many factors may contribute to the decline of myocardial performance in the heart-lung preparation. For example, a period of impaired coronary blood flow is almost inevitable and, if prolonged, will produce irreversible myocardial damage. The heart-lung preparation is deprived of sympathetic tone and probably undergoes progressive depletion of the cardiac sympathomimetic amines, which are an important determinant of myocardial contractility. Various substances produced by other organs of the body will not be available to the heart-lung preparation and these may conceivably be of importance at the level of either energy production or of energy utilization. Finally, disturbances may be produced by the tubing of the extracorporeal part of the system, and emboli may arise either from small clots or from bits of dried blood that form on the walls of the venous reservoir. In fact, one cannot overemphasize what a poor performance the heart-lung preparation renders compared to the intact heart. (For interesting recent data see references 21, 22, and 23.)

Although it is therefore practically impossible to find one's way among the various difficulties inherent in these preparations, 1 or 2 facts of interest stand out. In the first place, it becomes apparent from the results of several groups of investigators that myocardial performance is better when the liver and spleen are included in the circulation.\(^2\)\(^2\)\(^-\)\(^2\)\(^6\) In some studies the effect of liver and spleen appear to be primarily on the
metabolism of the heart,²²,²⁴ whereas in others some factor from the liver and spleen seems to have a primary effect on myocardial contractility.²⁵,²⁶ The nature of such a substance isolated from hepatic venous blood after stimulation of the splenic nerve has been said by Schmier to be a polypeptide.²⁶ Recently Sayers' group has suggested that hormones from the adrenal cortex may delay the decline of performance seen in a heart-lung preparation of the rat.²⁷ These findings, like those reviewed for isolated cardiac muscle, must be evaluated by asking why the heart-lung preparation is depressed in the first place, and whether the beneficial substances reported are important only in these experimental protocols or whether they have a general physiologic significance.

A new and encouraging note in the field of in situ heart preparations has been introduced by perfusing the coronary system of the experimental heart with blood from a healthy donor dog.²³,²⁸ This preparation, which has been studied by Sarnoff and co-workers, sometimes maintains a steady level of contractility for several hours. Therefore, despite all the difficulties that we have enumerated, it is possible for a denervated heart to maintain a fair level of functional capacity over a period of time, provided that vascular contact with a normal donor dog is maintained. One cannot say whether one of the beneficial effects of the donor animal is the maintenance at normal levels of some hypothetic cardiac active substance, but at least this preparation might provide a basis for experimental investigation of the point.

It would be of great interest, for example, to determine cardioglobulin concentrations in such a preparation. We know in the case of the rat that an extracorporeal circuit that includes a filter with a large air-blood interface is associated with rapid destruction of cardioglobulin C. A comparable destruction occurs in patients subjected to a period on a heart-lung machine. Whereas there is a comparatively small change in cardioglobulin C concentration during the pumping period of 30 to 45 minutes, we have found that there is a marked drop in cardioglobulin concentration during the first few hours after surgery. During the ensuing 24 hours considerable recovery occurs, with cardioglobulin concentrations again approaching normal.

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