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THE ACTIONS OF METABOLIC SUBSTRATES AND INHIBITORS ON THE RABBIT AURICLE*

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Knowledge of cardiac muscle metabolism has advanced during the past few years to an appreciable extent, but the correlation of metabolic processes with the various functions of the heart has been very little investigated. It is possible that some of the enzyme systems that are highly active in cardiac extracts or homogenates, or even in heart slices, are not intimately involved in the beating of the heart. It is also possible that in the intact beating heart there are enzyme systems of great importance, which are either destroyed or altered by any damage to the myocardium. Such systems may be brought to light by a more detailed analysis of the actions of metabolic substrates and inhibitors on the normally contracting cardiac tissue. For the understanding of drug actions on the heart, it is frequently necessary to correlate the metabolism with the functional aspects of impulse discharge, conduction, and contraction. The isolated rabbit auricle was used in the present work because both the amplitude of contraction and the rate of impulse discharge could be more accurately measured with it than with the entire heart where there occur problems of conduction block and complex contractile movement. It is also difficult to maintain normal conditions in the perfused heart, and the tissue soon becomes damaged, whereas auricles will beat regularly for many hours in the proper medium. This work was done in connexion with the problem of the action of acetylcholine on the heart and of how this action is influenced by the metabolic state of the myocardium. The results obtained with acetylcholine will be reported in a subsequent paper.

METHODS

The effects of metabolically important substances on the rabbit auricle were determined by addition of the substances to a bath in which the auricles were beating. Hearts from young rabbits were used, inasmuch as it was found that such preparations beat more regularly and give more reproducible results than those from older rabbits. It is possible that in older animals the auricular walls are so thick that diffusion of oxygen into the tissue, or diffusion of metabolic products from the tissue, is not adequate. It also seemed that better results were obtained when the rabbits were allowed to remain quiet for several minutes before they were killed. Auricles obtained from animals that had been killed immediately after being caught and handled often gave poor results

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and would seldom achieve a regular beat. The rabbits were killed by a blow on the head and the hearts were rapidly removed. The hearts were allowed to beat for about half a minute in a dish of physiological medium in order to allow as much blood as possible to be pumped out of the tissues. The ventricles were often massaged in order to rinse the blood from the auricles, care being taken not to touch the auricles. The auricles were then cut from the heart and carefully trimmed so that no ventricular tissue remained. Caution was exercised in the trimming of the arteries and veins that no damage be done to the adjacent regions of the auricle, for it is in these regions that the pacemaker tissue usually lies. The auricles were suspended in 70 ml. of Tyrode solution (0.137 M-NaCl, 0.0027 M-KCl, 0.0018 M-$CaCl_2$, 0.00011 M-$MgCl_2$, 0.012 M-$NaHCO_3$, 0.00042 M-NaH_2PO4, and 0.0055 M-glucose) which, after saturation with the gas mixture, had a *p*H of 8.1. Substances to be tested were added in 0.7 ml. volumes at a concentration 100 times that required in the final medium. Although this medium is a good buffer, all solutions of these substances were brought to a *p*H close to 8.1 before their addition to the bath. All measurements were made at 30° C. It was found that better results could be obtained at this temperature than at 38° C. At the higher temperature it is likely that the rapid rate often limits the recording of the full amplitude and this would introduce serious errors into the determinations of amplitude effects. The auricles also beat regularly over a much longer period of time at the lower temperature. The medium was continually saturated with a mixture of 5 per cent CO_2 and 95 per cent O_2. The substances used were obtained in as pure a form as possible. The sodium fluoroacetate was kindly supplied by Dr. Saunders, of Cambridge University.

In order to achieve quantitative results the auricular tracings were carefully measured, the amplitude being recorded in millimetres and the rate of beating in contractions per ten seconds. It was necessary to use this small time unit for measuring the rates, inasmuch as rapid rate changes could not have been accurately determined if longer intervals had been chosen. The effects produced by the addition of any substance were expressed in terms of the percentage change in the amplitude and rate. Such results might conceivably be expressed as absolute changes, percentage changes, or as final levels of activity to which the auricles were brought by the substances. For most purposes the percentage change is the most significant. In the first place, owing to the recording arrangement, the true amplitude of contraction is not measured; hence, if absolute changes were given, it would not be possible to compare one experiment with another. Furthermore, in considering the effects of substances on the auricular frequency we are usually interested in the changes in the rates of processes occurring within the pacemaker cells. It is likely that the rates of such processes are roughly proportional to the frequencies of beating. In metabolic studies the effect of an inhibitor on an enzymatic system is usually expressed in terms of percentage inhibition, and so here we might expect a more direct relationship to the underlying processes if we also express the frequency changes in similar terms. Justification for this method of expression, with regard to both rate and amplitude, was found in the quantitatively comparable results obtained from auricles beating at greatly different rates and amplitudes.

The auricles usually beat somewhat irregularly for 5–30 minutes after placing them in the bath, but eventually became quite constant with respect to both rate and amplitude. Experimental work was always initiated after the auricles had reached this steady state. Such auricles would beat for two to three days, the amplitude gradually decreasing during the second and third days. No auricles were considered to be normal after the first day and all results reported were obtained on fresh auricles. The recorded amplitude was usually adjusted to between 40–60 mm. Normal auricles beat spontaneously at an average rate of 102 contractions per minute, although there was quite large variation between the auricles from different animals in this respect, the extremes recorded being 72 and 144. At 38° C. the frequency is approximately 80 per cent

greater than at 30° C., and thus the rate at body temperature would have been approximately 184 beats per minute, a figure not far distant from the normal *in situ* rate of the rabbit heart.

RESULTS

The effects of metabolic substrates on normal auricles

It is obvious that if a substance is to alter the beating of the auricles by acting through metabolic mechanisms, its quantitative effect will depend on the metabolic state of the myocardial cells. If a particular substrate or intermediate, which is of importance in the functional metabolism of the heart, is deficient, we might expect its addition to produce an effect. Whether this would be stimulation or depression cannot be predicted until the correlation between the various metabolic reactions and the functioning of the heart cells is better understood. On the other hand, if an enzyme system is saturated with respect to its substrate (that is, if the substrate is not limiting the rate of the reaction), there may be no response to the addition of the substrate, or there may perhaps occur a retardation of the enzyme systems that operate before the particular step in which the substrate is involved. Furthermore, the observed actions of substrates on the auricular tissue may be independent of enzymic mechanisms, the primary effect being upon the cellular membrane potential or the contractile substance itself. Lastly, the possible actions of these substances on the acetylcholine equilibrium in the cardiac tissue must be borne in mind. Since there is a slow formation of acetylcholine in the auricles (either within the heart cells themselves or released from the post-ganglionic nerve endings), it is possible that a substance may stimulate or depress this formation, or alter the rate at which this endogenous acetylcholine is destroyed by modifying the activity of the cholinesterase, or antagonize the action of the acetylcholine on the auricular cells.

The results obtained on the actions of substrates on normal auricles are summarized in Table I. Changes of less than 5 per cent are probably not significant unless derived from experiments upon several auricles. An inspection of this Table will show that stimulation of either rate or amplitude is very seldom seen with any of the substrates investigated. It is likely that the only really significant stimulation is that produced by citrate on the rate. These substances, in general, have no effect on the normal auricle until a certain concentration is reached and then they begin progressively to depress it. This is seen in the concentration-action curves in Fig. 1. On the whole, it is probable that the enzymatic systems involved in providing energy for the beating of the auricles are not limited by any of the substrates tested.

The amplitude is more readily depressed by these substances than the rate. On the average, for any specific concentration of substrate, the depression of the amplitude is two to three times as great as the depression of the rate. However, substrates vary in this respect and it will be seen that acetate at lower concentrations depresses the rate as strongly as it does the amplitude. The maximum effect, in most instances, is reached from one to two minutes after the addition of the substrate to the bath. There is then, with the majority of substrates used, a partial or complete spontaneous recovery from this depression. This was observed with pyruvate, lactate, fumarate, malate, acetate, oxalacetate, propionate, and butyrate; it was not seen with citrate or caprylate, the depression produced by these being

TABLE I

THE EFFECTS OF METABOLIC SUBSTRATES ON NORMAL AURICLES

Substrate	Molar concentration	Time (min.)	% Change in rate	% Change in ampl.	Hearts	Tests
Succinate	0.0001	1	0	0	1	1
		5	0	+2	1	1
	0.001	1	0	0	1	1
		5	0	+3	1	1
	0.005	0.5	0	−7	1	1
		1	0	−9	2	5
		2	0	−12	2	4
	0.01	0.5	−18	−22	3	3
		1	−13	−26	4	4
		2	−10	−27	3	3
Fumarate	0.001	0.5	−6	−4	1	1
		1	−6	−5	1	1
		2	−3	0	1	1
	0.005	0.5	−3	−5	2	2
		1	−3	−7	3	3
		2	0	−6	2	2
	0.01	0.5	−9	−8	2	3
		1	−5	−14	3	4
		2	−2	−10	3	3
Malate	0.001	0.5	−3	0	2	2
		1	−3	0	2	2
		2	−6	−1	1	1
	0.005	0.5	−2	−10	3	6
		1	−4	−14		5
		2	0	−15	3	3
	0.01	0.5	−4	−9	2	3
		1	−4	−18	2	3
		2	−4	−20	2	2
		5	0	−15	2	2
		10	0	−8	2	2
		15	0	−5	1	1
	0.02	0.5	−7	−23	1	1
		1	−14	−32	1	1
		2	−7	−27	1	1
		5	0	−22	1	1
		8	0	−12	1	1
		15	0	−8	1	1
Oxalacetate ..	0.002	0.5	0	−2	1	1
		1	0	−2	2	2
		2	0	0	1	1
	0.01	0.5	0	−9	1	1
		1	0	−14	1	1
		2	0	−13	1	1
		3	0	−11	1	1

TABLE I—*contd.*

Substrate	Molar concentration	Time (min.)	% Change in rate	% Change in ampl.	Hearts	Tests
Pyruvate	0.0001	1	0	0	2	2
		2	0	−2	2	2
	0.001	0.5	0	−2	2	2
		1	0	−5	2	2
		2	0	−6	2	2
		5	0	0	2	2
		10	0	+3	2	2
	0.005	0.5	−1	−3	5	10
		1	−1	−6	7	13
		2	−1	−11	5	10
		3	−2	−9	3	5
		5	−2	−8	4	6
		10	−4	−5	3	3
		15	0	−3	2	2
	0.01	0.5	0	−9	2	2
		1	0	−12	4	4
		2	0	−16	5	5
		3	0	−7	2	2
		15	+5	−6	1	1
	0.02	0.5	0	−8	1	1
		1	−8	− 3	1	1
		2	0	−23	1	1
Acetate	0.00002	1	0	0	1	1
		5	0	0	1	1
	0.0001	1	−6	−8	1	1
		2	0	−4	1	1
	0.0005	0.5	−9	−4	2	3
		1	−22	−15	2	3
		2	−17	−10	2	3
	0.001	0.5	−5	−9	4	5
		1	−19	−21	3	4
		2	−11	−9	3	3
		5	−3	−2	2	3
	0.01	0.5	−4	−8	2	2
		1	−6	−23	3	4
		2	−4	−16	2	3
		5	0	−7	2	3
		10	+6	−3	2	2
		15	+6	−2	2	2
	0.03	1	−6	−27	2	2
		2	−4	−22	2	2
		5	0	−18	1	1
		10	+6	−11	1	1
		15	+6	−15	1	1

TABLE I—*contd.*

Substrate	Molar concentration	Time (min.)	% Change in rate	% Change in ampl.	Hearts	Tests
Propionate ..	0.0005	0.5	0	−1	2	2
		1	0	−4	2	2
		2	0	−3	2	2
		5	+3	−8	2	4
		10	+7	−3	2	3
	0.005	0.5	−7	−10	1	2
		1	−5	−7	1	2
		2	0	−3	1	2
		5	+5	−1	1	2
	0.01	0.5	−7	−4	1	1
		1	−7	−6	1	1
		3	0	−3	1	1
Butyrate	0.0005	1	0	−1	1	1
		5	0	0	1	1
	0.001	1	0	−10	1	2
		2	−3	−8	1	2
	0.005	1	0	−16	1	2
		2	−6	−11	1	2
		5	−3	−8	1	2
	0.01	0.5	0	−7	1	1
		2	0	−6	1	1
β-Hydroxy-butyrate	0.001	0.5	0	−4	1	1
		1	0	−6	1	1
		3	0	−10	1	1
	0.005	1	−7	−11	1	1
		3	0	−13	1	1
Caprylate	0.00002	0.5	0	0	1	2
		1	0	0	1	2
		3	0	−4	1	2
		5	0	−9	1	1
	0.0001	0.5	0	0	1	1
		1	0	−5	1	1
		3	−6	−16	1	1
		5	−6	−26	1	1
	0.0005	0.5	−6	0	1	1
		1	−17	−11	1	1
		5	−6	−24	1	1
	0.001	1	−7	−15	1	1
		2	−14	−25	1	1
	0.01	1	−22	−33	1	1
		2	−22	−42	1	1

TABLE I—*contd.*

Substrate	Molar concentration	Time (min.)	% Change in rate	% Change in ampl.	Hearts	Tests
Glutamate ..	0.001	1	0	0	1	1
		2	0	0	1	1
	0.005	1	0	0	1	1
		2	0	0	1	1
	0.01	1	0	−3	2	3
		2	0	−1	2	3
	0.03	1	0	−7	1	1
		2	0	−5	1	1
Lactate	0.001	1	0	−2	1	1
		2	0	−1	1	1
	0.005	1	0	−1	2	2
		2	0	−1	2	2
	0.01	1	0	−6	2	2
		2	0	−2	2	2
		5	0	0	2	2
	0.03	1	0	−9	1	1
		2	0	−3	1	1
		5	0	−2	1	1
Citrate	0.0001	1	0	0	1	1
		2	0	0	1	1
	0.001	0.5	0	−5	2	3
		1	−2	−11	2	3
		2	−2	−12	2	3
	0.005	0.5	0	−28	2	3
		1	+3	−47	2	3
		2	+6	−60	2	3
		4	+2	−62	2	3
	0.01	0.5	0	−35	2	2
		1	+16	−63	2	2
		2	+25	−74	2	2
		5	+28	−75	2	2
		10	+25	−74	2	2
		15	+20	−72	2	2

irreversible until the substance was washed from the bath. Typical curves illustrating this spontaneous recovery are given in Fig. 2. This recovery is not due to destruction of the substrate, for in most instances the total amount of substrate present in the bath is sufficient to prevent any appreciable decrease in the concentration resulting from amounts destroyed by the tissue in the short time intervals involved. This type of spontaneous recovery is seen in cardiac tissue under the influence of a variety of substances, and is probably to be interpreted in the light of the ability of the cardiac functional systems to adjust themselves to abnormal conditions.

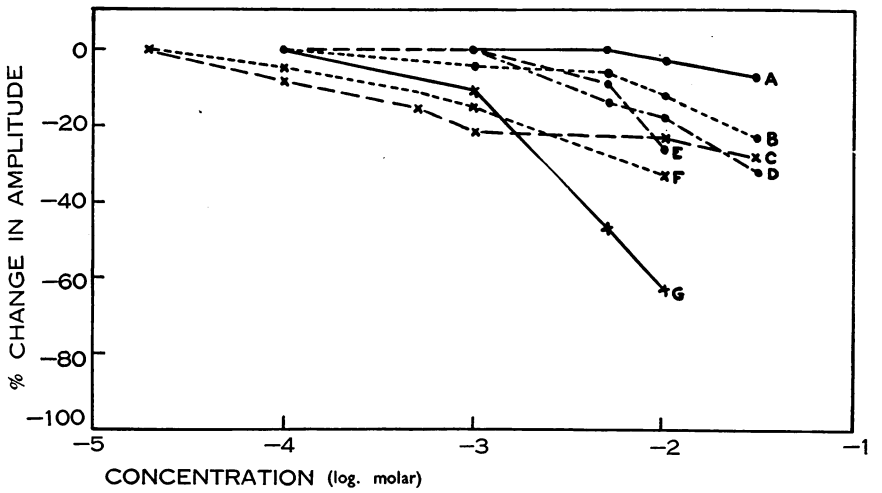

FIG. 1.—The action of substrates on the normal rabbit auricle. (A) glutamate, (B) pyruvate, (C) acetate, (D) malate, (E) succinate, (F) caprylate, (G) citrate. Time of action: 1 min.

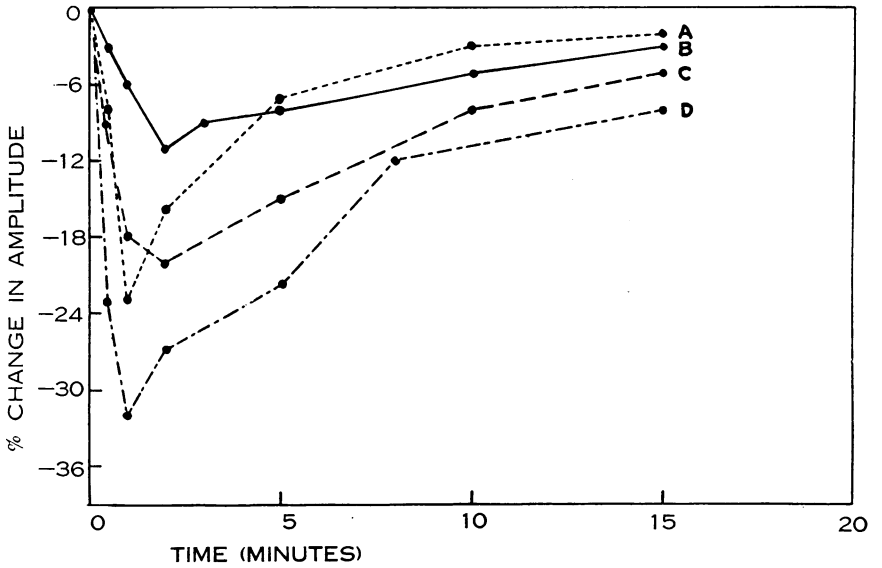

FIG. 2.—Recovery of auricles from substrate depression. (A) acetate 0.01 M, (B) pyruvate 0.005 M, (C) malate 0.01 M, (D) malate 0.02 M.

Consideration of the penetration of these substances into the cardiac cells is of the greatest importance, for, unless the heart cells, like some bacteria, possess enzymatic systems upon their surfaces, it is necessary for the substances to enter the cells in order to produce a direct effect upon the metabolism. Very little work has been done on the permeability of cell membranes to such substances. Further-

more, no analogies between the penetration of these substances and other weak acids can be drawn with confidence, inasmuch as cell membranes seem frequently to possess selective permeabilities for substances of functional importance. At the slightly alkaline *p*H of this medium, these substrates exist in solution preponderantly as anions; this may interfere with rapid penetration, especially when the substrates possess two or three such ionized groups. However, it must be borne in mind that the permeability to charged molecules may markedly increase in the heart cells during the passage of an impulse over the tissue. There is some evidence for the penetration of such substrates. It will be shown later that in substrate-depleted auricles there is an almost immediate effect of added pyruvate. Furthermore, the actions of pyruvate, lactate, β-hydroxybutyrate and other substrates are quite rapid on exhausted perfused hearts. It will also be shown that iodoacetate and fluoroacetate penetrate the auricular cells quite readily. With regard to dicarboxylic and tricarboxylic acids, I know of no evidence that is applicable to the present situation, although it seems certain that succinate and malonate are able to penetrate into heart slices. Fig. 1 shows no correlation between effect and the number of carboxyl groups. I believe that the evidence available points to a definite penetration by these substances. However, equilibrium may not be reached for one to three minutes, the times of maximum substrate action thus being expressive of the relatively slow rate of penetration compared to uncharged molecules.

It is possible that the effects observed with the substrates may have been partly or wholly non-metabolic effects. The relationship between cellular membrane potentials and the rate of discharge of the pacemaker cells or the degree of contraction is not understood, but it is conceivable that alterations brought about in the cell membrane by these substrates might influence the auricular functioning. Such organic acids are known to be capable of producing temporary electrical potential changes in nerve, muscle and plant cells. If such a mechanism of action is involved, it is likely that we must interpret the spontaneous recoveries from substrate depression as originating in the gradual restoration of the normal potential by the establishing of a concentration equilibrium between the medium and the interior of the cells. That these substrates depress the auricles by increasing in some manner the effective acetylcholine concentration in the tissue is unlikely, since it was clearly shown that atropine, at a concentration capable of blocking all acetylcholine action (1×10^{-5}), failed to alter the actions of pyruvate, citrate, acetate, malate, and fumarate. It is improbable that the depressant action originates in a simple increase in osmotic pressure of the medium, for glucose may be added up to a concentration of 0.03 M without alteration of the beat. Furthermore, if an osmotic effect were involved, we should expect the various substrates to produce similar degrees of depression at equivalent concentrations; this, however, is not the case. Since the concentration-action curves for the various substrates, as shown in Fig. 1, are not only at different positions along the concentration axis, but also possess markedly different curvatures, it would seem unlikely that the depressant effects were due to any general, non-specific type of action.

Some of the substrates, particularly acetate, malate, and propionate, showed a tendency to induce arrhythmias in the auricles. These arrhythmias were occasionally completely irregular, but usually they were of the "pulsus alternans" type, the amplitude of contraction regularly alternating each beat between two different

values. Sometimes such a rhythm was caused by the regular beating of one auricle of a pair while the other auricle beat only every other contraction, but more frequently this was not so. Rapid recoveries from such arrhythmias were observed when the substrate was removed from the bath.

The action of succinate was only depressant in these experiments. Depression of the amplitude was observed by Salant, Livingston, and Connet (1917) on the perfused frog heart (succinate 0.01–0.02 M) and by Forssmann and Lindstén (1946) on the isolated and perfused rabbit heart (succinate 0.01–0.04 M). They did not find in these preparations such marked depression of the rate as was here observed on the rabbit auricles. Forssmann and Lindstén (1946) found a mild stimulation (+15 per cent) of the amplitude by succinate in lower concentrations (0.0005–0.005 M). In the auricles no such stimulation was observed within a similar concentration range; this possibly indicates a difference in behaviour between the auricles and ventricles of the rabbit, inasmuch as they measured the contractions of the left ventricle. No stimulation was found in the isolated and perfused rat heart (Nakamura *et al.*, 1949). Despite the rapid oxidation of succinate by cardiac tissue, it would appear that the energy derived from this is, at least in the normal auricle, of no consequence in the functioning of the auricles. It will be shown later that this energy cannot be utilized to revive substrate-depleted auricles.

The relatively marked depression produced by malate on the auricles is in accord with the results of Salant, Livingston, and Connet (1917) on the frog heart; the frog heart would seem to be more sensitive to the action of this substrate than the rabbit auricle. A definite difference in the behaviour of the two tissues is seen, however, in the recovery of the rabbit auricles from the malate depression, whereas the frog heart was reported to remain in a constant state of depression as long as the malate was present (in one case for 62 minutes).

The action of citrate on the rabbit auricle is very interesting, there being a marked stimulation of the rate while the amplitude is simultaneously reduced. This phenomenon occurs only at higher concentrations of citrate (above 0.005 M); below this there is little effect on the rate, although the amplitude is depressed. The acceleration of the rate occurs quite suddenly. For example, in one experiment using 0.01 M-citrate, the rate was unchanged 30 seconds after the addition of the citrate, but it suddenly increased by 22 per cent at 40 seconds, and was up by 39 per cent at 60 seconds, after which it remained relatively constant. The depression of the amplitude observed here is very similar to that reported by Forssmann and Lindstén (1946) on the entire heart, but, unfortunately, they did not include their results on the rate changes. How much of the action of citrate was due to a decrease in the concentration of ionized calcium in solution is difficult to say. Added calcium will counteract the depression produced by citrate, but this does not prove that citrate acts by calcium depletion. When the concentration of citrate is 0.001 M or above, there must be a decrease in the ionized calcium, the original concentration of the calcium ion in the medium being 0.0018 M. If one considers the effect on the rabbit auricle of decreasing the calcium concentration (Webb, 1950), and compares this with the concentration-action curve for citrate depression of the amplitude, one must admit that the results coincide with what one might expect if citrate were acting by calcium depletion. On the other hand, the stimulation of the rate cannot be due to a decrease of ionized calcium in the medium, for low calcium concentra-

tions have very little, if any, effect on the rate of the rabbit auricle. There still remains the possibility that the citrate penetrated the pacemaker cells and within them produced a disturbance in the calcium equilibrium, thereby producing changes in discharge rate that would not have occurred as the result of a mere decrease in external calcium.

The effect of glucose deficiency on rabbit auricles

If rabbit auricles were left for 24–48 hours at 30° C. in a glucose-containing medium a marked reduction in the amplitude was usually observed, especially if the medium had been continually replaced, although the rate often remained the same as it was originally. Under such circumstances the auricles stop in the relaxed condition of diastole. These auricles could usually be restarted with acetylcholine or adrenaline (Vane, 1948; Burn and Vane, 1949) but not by any of the substrates that were tried (pyruvate, acetate, succinate, oxalacetate, and malate). This type of exhaustion occurs in the presence of glucose and is probably not related in any way to depletion of substrate. It is likely that some alterations of the cellular enzyme systems have taken place, such as the depression of the formation of acetylcholine (Bülbring and Burn, 1949) or a dissociation of the reactions of oxidation and phosphorylation.

If glucose was removed from the medium in which the auricles were beating, there was no immediate effect. It is to be expected that the auricles would have sufficient endogenous substrate—glycogen and fat—on which to operate for a period of time. The amount of work performed by beating auricles under these conditions was not great, for they were not working against the usual internal pressure of the blood. Seven experiments were performed in which the behaviour of the auricles in the absence of glucose was observed for five to seven hours. In three of these experiments there was no change of rate, and in four there was some decrease (–10 to –30 per cent). In three of these experiments there was no change in amplitude, and in four there was some decrease (–30 to –50 per cent). The auricles thus show variation in their behaviour in the absence of glucose, some apparently having a greater supply of endogenous substrate than others. Since there was essentially no change in rate or amplitude over a period of five to seven hours in the presence of glucose, these decreases in rate and amplitude, when they occurred, must be attributed to the lack of glucose. In longer experiments, when the auricles were left without glucose overnight, the exhaustion produced by substrate depletion is complicated by the simultaneous development of the type of exhaustion seen when glucose is present. Furthermore, it seems evident that lack of glucose in the medium produced some irreversible change or damage in the cardiac cells, for although stimulation was observed when glucose was added to such auricles, there was never anything like complete recovery. This type of damage may be related to that occurring when cardiac tissue is subjected to oxygen deficiency (Webb, Saunders, and Thienes, 1949a).

In order to produce rapid substrate depletion, with as little complication from other changes as possible, the auricles were driven with adrenaline (1×10^{-6}) in several experiments for varying periods of time in the absence of glucose. Such a procedure exhausted the auricles to a low level of activity in 15 to 60 minutes. If adrenaline were allowed to act for one to three hours, the auricles, after the

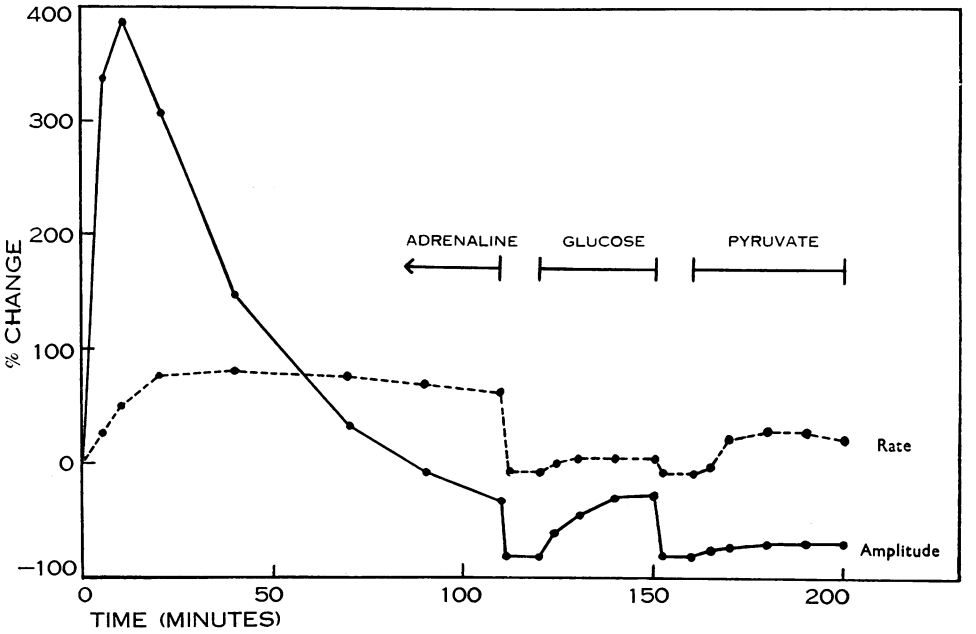

FIG. 3.—Substrate depletion of the auricles and the actions of glucose and pyruvate. Average of four experiments. Adrenaline, 10^{-6}; glucose, 0.0055 M; pyruvate, 0.005 M.

adrenaline had been washed out, beat with a very small amplitude, although the rate was usually relatively unaffected. The averaged results of four experiments are shown in Fig. 3. The rate in the presence of adrenaline remained at a high level, while the amplitude rapidly decreased to below the normal level. After the adrenaline had been removed, the rate returned to nearly its original value and the amplitude was very depressed. Exhaustion with adrenaline in the absence of glucose seems to affect the amplitude much more than the rate, the latter being quite stable. In spontaneous exhaustion in the absence of glucose the amplitude was also more easily depressed. The question whether adrenaline produces cardiac failure by depletion of substrate under such circumstances has never been settled. Some investigators have found that adrenaline-driven hearts lose their glycogen and attribute the failure to this, while others have shown that the heart may fail when there is sufficient glycogen present (Chang, 1936; Fieschi, 1933; Bogue, Evans, and Gregory, 1937; and others). This problem might be solved by determining the extent to which the cardiac tissue will recover when glucose is given. If there is complete, or nearly complete, recovery the failure may be attributed to substrate depletion, while if no recovery is seen it is likely that damage and disorganization have occurred to the functional systems. Any intermediate situation would indicate the extent of substrate depletion or cardiac damage. In the present investigation we shall see that the situation is intermediate and we must conclude that changes have taken place in the auricular tissue in addition to substrate depletion. However, for convenience we shall speak of such auricles as depleted.

When the auricles were driven with adrenaline there was invariably a gradual development of contracture as the amplitude decreased. This may also be expressed as an increase in tone or a decreased ability of the auricular muscle to relax. Auricles brought to almost complete exhaustion with adrenaline in the absence of glucose usually beat at a normal rate with a greatly diminished amplitude and were in a state of contracture. This inability to relax was also seen in the longer experiments when glucose was absent and no adrenaline was used, but it was never observed in exhaustion occurring when glucose was present. The problem of relaxation will be discussed after the results obtained with various metabolic inhibitors have been presented.

Effects of metabolic substrates on substrate depleted auricles

Auricles that had been spontaneously depleted or driven with adrenaline in the absence of glucose were invariably stimulated by the addition of 0.0055 M glucose, the concentration normally present in Tyrode solution. Furthermore, the degree of contracture of the auricles was lessened; that is, the auricles showed an increased ability to relax. Sometimes the degree of relaxation could be alternately increased and decreased by additions and removals of glucose. Complete recovery to the initial amplitude was never seen, but the rate, if it was decreased, was frequently restored to its normal value. The rate, however, in the depleted auricles was never far below its original value, while the amplitude was markedly depressed to only 10–20 per cent of the initial height. Usually there was a fairly rapid rise in amplitude over the first two minutes after glucose addition, and this was followed by a more gradual increase to reach a maximum value after 10–40 minutes. In terms of percentage, glucose increased the amplitude by 100–300 per cent over the depressed value. This is illustrated in Fig. 3, where the action of glucose may be compared with that of pyruvate.

Pyruvate likewise stimulated the substrate depleted auricles but not so well as glucose. However, the effect upon the rate was more marked with pyruvate, the rate being occasionally increased over the normal original value, while a good effect was sometimes obtained on the rate with little or no stimulation of the amplitude. The stimulation of the amplitude by pyruvate was usually between 10–60 per cent, and when compared with glucose on the same auricles this amounted to about one-fifth of the glucose effect. Pyruvate also induced relaxation of the auricles but was not so effective as glucose in this respect. The optimal concentration of pyruvate for such stimulation was found to lie between 0.001–0.005 M.

Only acetate and β-hydroxybutyrate of all other substrates tested were capable of producing any recovery in the substrate depleted auricles, and these effects were small and variable. Neither of these substances was found to induce relaxation. With acetate, the concentration had to be low (0.001 M) for any stimulation to be observed, for if higher concentrations were used only further depression resulted. In six experiments with 0.001 M-acetate, the average increase in amplitude was 18 per cent whereas the rate was not stimulated. Substrates with no ability to revive depleted auricles were fumarate, succinate, citrate, malate, oxalacetate, propionate, butyrate, and glutamate.

It is interesting to note that glucose and pyruvate would occasionally facilitate relaxation of the auricular muscle although the auricles were not beating. Here we may confidently say that depletion of substrate increases the tone of the cardiac muscle and that the presence of glucose or pyruvate decreases the tone. When the auricles were beating, the amplitude of contraction usually decreased while the tone was increasing, and vice versa, but this was not always true, and we must conclude that there is no necessary relationship between the degree of contraction, and the ability of the muscle to relax. Failure of the muscle to relax sufficiently naturally inhibited the development of a full contraction, but it was observed occasionally, when glucose or pyruvate were added while the contracture was proceeding, that the amplitude may increase without simultaneous relaxation.

The results presented here on the rabbit auricle agree well with those obtained in the heart-lung preparation and in the isolated and perfused mammalian heart, not only with respect to the substrates that are utilizable by the cardiac tissue, but also with the comparative abilities of these substrates to revive the depleted heart muscle. However, on no other tissue are the differential actions of glucose and pyruvate on the rate and amplitude so easily observed. On may conclude that auricular and ventricular functional metabolism is qualitatively similar. It is likely, however, that somewhat different systems are involved in the discharge of impulses by the pacemaker cells and in the processes of contraction.

Effects of metabolic inhibitors on normal auricles

Curves showing the relationship between the concentration of inhibitor and the action produced are presented in Figs. 4 and 5. The time course of inhibitor

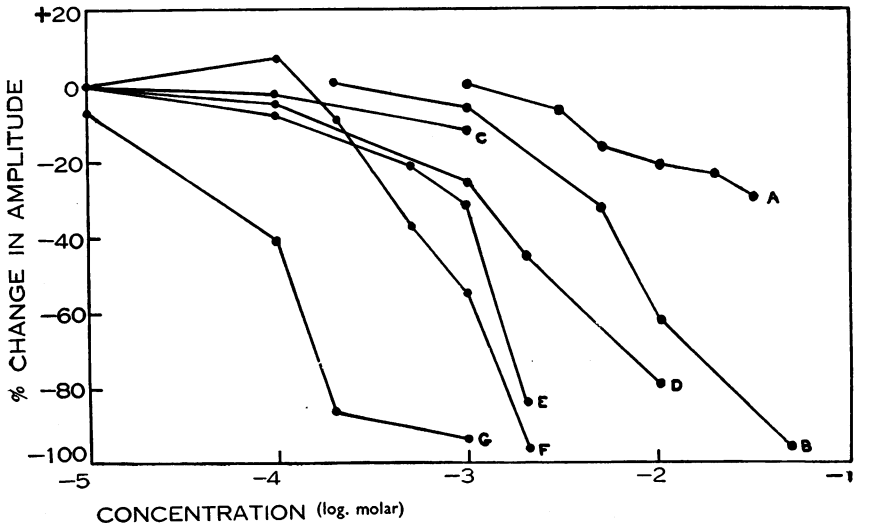

FIG. 4.—The action of inhibitors on the amplitude of normal rabbit auricles. (A) malonate—2 min., (B) fluoride—4 min., (C) phlorizin—5 min., (D) fluoroacetate—25 min., (E) azide—4 min., (F) cyanide—2 min., (G) iodoacetate—25 min. The times refer to the duration of inhibitor action.

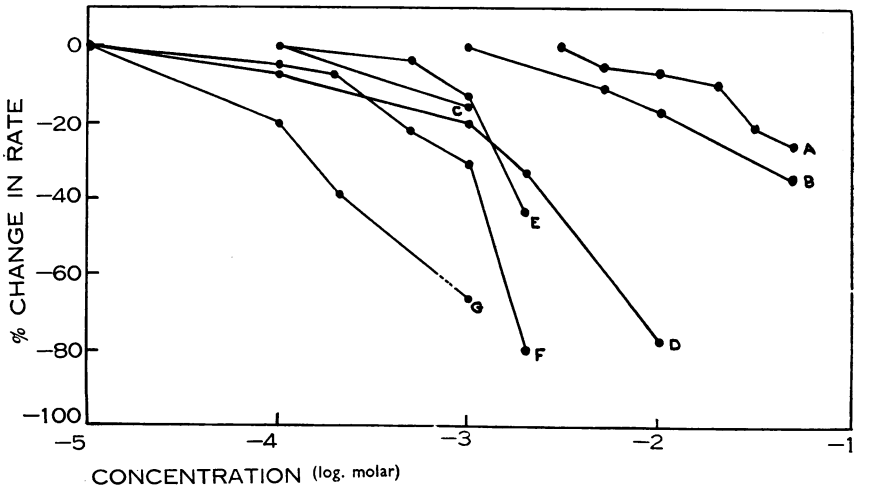

FIG. 5.—The action of inhibitors on the rate of normal rabbit auricles. Same designations as in Fig. 4.

action is illustrated in Figs. 6 to 12, where the actions upon rate and amplitude may be compared. These are all average curves obtained from three to six experiments.

Cyanide

Cyanide invariably produced a temporary stimulation before it began to depress the auricles, this stimulation being more marked in the amplitude than in the rate. The degree and duration of stimulation varied with the concentration of cyanide: at low concentrations (0.00001–0.0001 M) the stimulation was small but persisted, often not being followed by a depression, but as the concentration was increased the period of stimulation became shorter and shorter, so that at concentrations around 0.001 M the maximum stimulation was reached 20–40 seconds after addition and was followed immediately by a rapidly developing depression. Such a temporary stimulation was also seen when the auricles were made anoxic.

The rapid development of the depression would indicate that the available supply of high-energy phosphate bonds in the auricle, at least with respect to some process, is not great, unless cyanide, through some unrecognized action, prevented the direct utilization of this energy. A concentration of cyanide of 0.001 M produces a final depression of about 90 per cent in the amplitude and 50 per cent in the rate; this concentration was reported to inhibit the respiration of minced rabbit heart 45 per cent (Banga, Schneider, and Szent-Györgyi, 1931) and the respiration of rat heart slices 74 per cent (Webb, Saunders, and Thienes, 1949b). The rabbit auricle will usually stop in a concentration of 0.002 M-cyanide. It is probable that, when the auricles stop, there is still a definite fraction of the respiration proceeding; the level of activity to which the auricles are brought by any concentration of cyanide is not a quantitative measure of the cyanide-resistant respiration. Impulse discharge from the pacemaker cells was not inhibited so readily as the amplitude of contraction; this has been observed with other cardiac

preparations (Loewi, 1897; Beresin, 1913; and others). Most workers have reported that hearts poisoned by cyanide stop in diastole (Loewi, 1897; and others) but some have observed systolic standstill (Fujimaki, 1924). In the rabbit auricle both may be observed according to the conditions of the inhibition. When the auricles were stopped rapidly by higher concentrations of cyanide, they stopped in the relaxed condition of diastole; however, if the action of cyanide at concentrations just below those required to stop the auricles was prolonged, the auricles began to go into contracture and eventually stopped in systole. Occasionally both phenomena were observed in the same experiment. In one instance, the auricles were stopped in 80 seconds by 0.002 M-cyanide, but they slowly and partially recovered in the presence of cyanide, so that at 30 minutes the rate was depressed to only 40 per cent of its original value and the amplitude 92 per cent of its original value. At this stage the auricles were in a state of partial contracture, an inability to relax having been developed during this time. Eventually they stopped in systole. However, contracture is less commonly observed with cyanide than with inhibitors like iodoacetate, fluoroacetate, and fluoride.

Spontaneous recovery from depression in the presence of cyanide was very seldom observed, the example cited above being the only definite instance. Normally the auricles would reach a certain level of depression and remain there, or, if the depression were sufficient, they would slowly pass into a state of contracture. The auricular tissue seems to have very little ability to adjust itself to conditions in which the oxygen utilization is impaired; no ability to adjust to anoxic conditions was observed. During very slight cyanide depressions, there was occasionally some recovery, but this may be explained by loss of cyanide from the medium, some being carried off by the vigorous gassing. When the cyanide was removed from the medium, the auricles recovered completely, the rapidity of recovery depending on the concentration of cyanide and the duration of exposure. After marked depression following exposures of three to six minutes to 0.001 M-cyanide, the auricles would recover completely within ten minutes, but if the exposure time was only one minute the recovery took place within three to five minutes. No irreversible damage to the cells was observed at these concentrations, unless full contracture was allowed to occur. After the cyanide had been washed out a stimulation of the rate during the recovery phase was frequently observed, amounting to a 20–30 per cent increase over the normal rate before the cyanide had been added. Various arrhythmias commonly occurred during this recovery phase. It is possible that during the depression some energy-containing or energy-supplying substance had accumulated in the cells and that when oxygen was again available this resulted in a temporary stimulation of activity; this stimulation, however, was observed only with regard to the rate.

When excess calcium was added to the medium there was a marked stimulation of the amplitude, the rate remaining unchanged (Webb, 1950). Cyanide (0.001 M) antagonized this action, so that lower concentrations of calcium (0.0001–0.0005 M) were almost without effect, while the action of higher concentrations was markedly diminished. Cyanide also diminished the stimulating action of adrenaline on the auricles. In one experiment, adrenaline (10^{-6}) normally increased the rate by 69 per cent and the amplitude by 390 per cent, but in the presence of cyanide (0.001 M) the observed effects of adrenaline were zero and 105 per cent respectively.

Azide

It is interesting to compare the actions of cyanide and azide because of the usually accepted similarity of their enzymatic inhibitions. Azide was not as potent an inhibitor of the auricles as cyanide. In equal concentrations, azide acted more slowly than cyanide and at any particular time produced only 1/2 to 1/3 the depression that would have been produced by cyanide. In Figs. 6 and 7 comparison may be made of their actions at 0.0005 M. Azide did not produce the temporary stimulation observed with cyanide, nor did low concentrations ever stimulate. As with cyanide, azide depressed the amplitude more than the rate. This was also observed on the isolated hearts of cats and rabbits by Graham (1949).

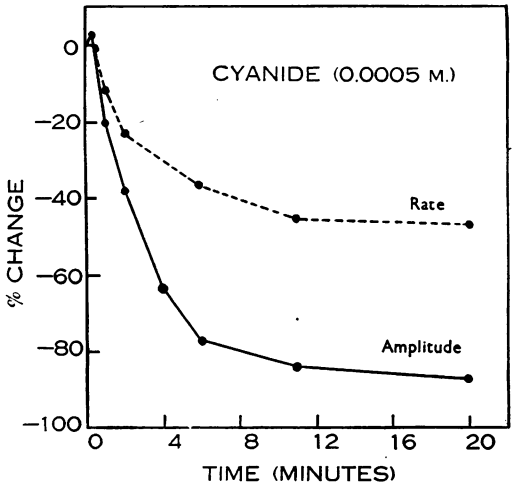

FIG. 6.—The action of cyanide on the rabbit auricle.

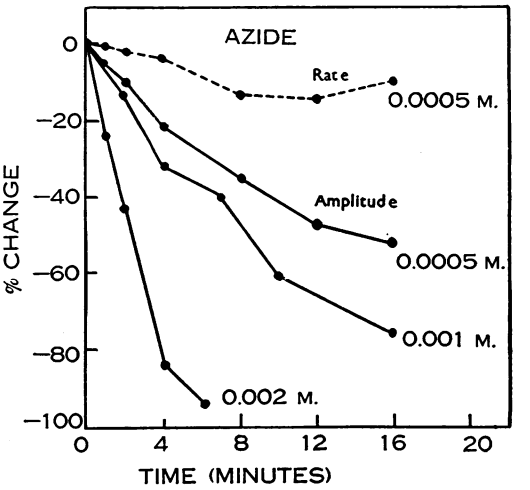

FIG. 7.—The action of azide on the rabbit auricle.

Recovery of the auricles after the azide had been removed was slower than with cyanide; 10 minutes were required after an exposure of 12 minutes to a concentration of 0.0005 M, and 20 minutes after a four-minute exposure to a concentration of 0.002 M, indicating that the azide is more tightly bound than cyanide.

Armstrong and Fisher (1940) concluded that cyanide and azide acted differently on fish hearts because the cyanide-stable respiration was different from the azide-stable respiration. However, this may be merely an expression of the wider range of action of cyanide at higher concentrations, for it is known that cyanide above a concentration of 0.001 M begins to inhibit dehydrogenases in addition to its action on metal-containing enzymes. However, it is clear that even in lower concentrations there was some difference in behaviour between the two inhibitors in their actions on the rabbit auricle.

Fluoride

Fluoride at concentrations between 0.005–0.01 M first depressed both the rate and amplitude of the auricles, but after approximately five minutes the amplitude began to increase and eventually reached values above the normal original level

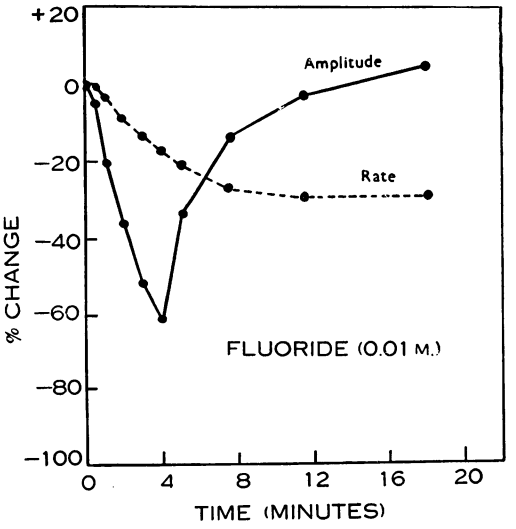

FIG. 8.—The action of fluoride on the rabbit auricle.

(see Fig. 8). Meanwhile, the rate remained at its depressed level or continued to decrease slightly. After 20–40 minutes the auricles were beating slowly but with a large amplitude. However, occasionally contracture began and the amplitude again decreased owing to the increasing inability of the auricles to relax. Previous reports on the action of fluoride on cardiac tissue have been equivocal. Several investigators have found only depression: Vernon (1910a, b) with 0.24–1.2 M-fluoride on the perfused tortoise heart, De Nitto (1928) with 0.00012–0.0012 M-fluoride on isolated mammalian hearts, and Chang (1938) with 0.0078 M-fluoride on rabbit auricles. Gottdenker and Rothberger (1935a, b) found depression of the perfused frog heart and the dog heart-lung by fluoride, but on guinea-pig and rabbit auricular strips they usually observed an increase in the amplitude when the fluoride concentration was 0.003 M. Ferrannini (1936) also found fluoride (0.0024–0.0072 M) to increase the force of contraction of rabbit auricular strips. In most work it has been observed that cardiac tissue is able to function surprisingly well in the presence of fluoride at concentrations which should inhibit glycolysis completely. Chang (1938) stated that fluoride, except for a slight depression, had little effect on the mechanic ability of rabbit auricles over a period of two to three hours as long as oxygen was present. Furthermore, he apparently observed no increases in amplitude. In the present work with the same tissue such a concentration of fluoride produced a marked effect. No explanation can be offered for these discrepancies.

With regard to the mechanism of fluoride action, most workers have concluded that depletion of calcium from the medium is not important. However, at least in the present experiments, it is difficult to see how it could fail to be of some importance. The calcium concentration in Tyrode solution is 0.0018 M; if sodium fluoride is added to the medium in a concentration of 0.005 M, there are approximately equivalent amounts of calcium and fluoride to form CaF_2. Since CaF_2 is soluble to the extent of 0.00022 M and since it is very little ionized (solubility product is 3.4×10^{-11}), it would seem likely that a definite fraction of the calcium would be withdrawn from the medium. When more fluoride is added the calcium should be reduced even more. No visible precipitation of CaF_2 was observed, but the walls of the tissue bath slowly became covered with a white layer. Reduction of calcium in the medium leads to a marked depression of the auricles with respect to amplitude, but has no effect on the rate (Webb, 1950). Thus it would seem that the observed depression of rate and the subsequent increase in

amplitude, in the presence of fluoride, must have been due to an action of fluoride other than that of altering the calcium concentration in the medium. As to how much the initial depression of amplitude is due to decreased calcium it is impossible to say.

Since fluoride inhibits cholinesterase, the possibility exists that at least part of the depressant action was due to prevention of destruction of endogenous acetylcholine. However, fluoride was found to give a typical depression of the auricles in the presence of physostigmine (10^{-6}), when the cholinesterase was already inhibited, and also in the presence of atropine (10^{-5}), when the action of acetylcholine was completely prevented. It is thus unlikely that the action of fluoride is related to the acetylcholine equilibrium in the auricles.

Such concentrations of fluoride as were found to have definite actions on the auricles (0.005–0.05 M) should inhibit glycolysis completely. Muscle enolase is usually inhibited 50 per cent by 0.001 M-fluoride and completely by 0.005 M-fluoride. A certain decrease in fluoride concentration would result from combination with calcium, but in the higher concentrations this could not have been very great. We must, therefore, conclude that the auricles can function quite well in the absence of the formation of phosphopyruvate from 2-phosphoglycerate. One possibility is that the auricles used fat for the necessary energy. Another possibility is that the 2-phosphoglycerate was utilized through some other system without the formation of pyruvate. It is interesting to speculate that the secondary increase in amplitude is due to this new source of energy, brought into play because of the enolase block. In rat heart slices the respiration was never depressed more than 42 per cent by fluoride at concentrations as high as 0.1 M (Webb, Saunders, and Thienes, 1949b), so it is evident that in cardiac tissue there is an appreciable fraction of the metabolism insensitive to fluoride.

Fluoride inhibition is reversible, and after the fluoride had been washed out recovery was usually complete within 5–10 minutes. After long exposures there was occasionally some stimulation of the rate above the normal figure during the post-wash recovery stage. It was also noticed that the auricles during this period frequently had difficulty in relaxing and the tone varied correspondingly. In passing, it may be remarked that fluoride (0.03 M) quite effectively diminished the action of adrenaline (10^{-7}–10^{-6}), the stimulation of amplitude being almost abolished while the rate effect remained but was made weaker.

Iodoacetate

Iodoacetate in concentrations between 0.0001–0.001 M is a slowly acting depressant of the rabbit auricle, requiring from three to five minutes for appreciable reduction of the amplitude and from 10 to 20 minutes for a slowing of the rate. At certain stages of the depression, the amplitude was markedly reduced while the rate remained unaffected, but eventually both were depressed almost equally before the auricles stopped. During the later stages of this depression the auricles began to go into contracture and they stopped in systole, a phenomenon that has been invariably observed in work with iodoacetate (Weicker, 1934 ; Chang, 1938 ; and others). Although slight depressions produced by low concentrations of iodoacetate acting for short periods of time could be partially reversed by removing the iodoacetate, the usual result was that after the iodoacetate had been washed out the inhibition

proceeded, exactly as if the inhibitor were still present in the medium. This is, of course, what one would expect from considerations of the irreversible cellular reactions of iodoacetate, and it confirms previous results on other tissues and preparations.

Iodoacetate is not a specific inhibitor of glycolysis in mammalian cardiac tissue (Webb, Saunders, and Thienes, 1949b), and even in low concentrations it is likely that other enzyme systems are involved in its action. Not only is the synthesis of ATP diminished, by inhibition of the utilization of pyruvate and fatty acid breakdown products as well as by prevention of glycolysis, but it is likely that iodoacetate interferes with the utilization of ATP, for Buchtal, Deutsch, and Knappeis (1944) have reported that iodoacetate blocked the stimulating effect of ATP on skeletal muscle. It would not be surprising if iodoacetate reacted directly with myosin, since this protein complex possesses a number of sulphhydryl groups important to its functioning. This may explain the observation (Chang, 1938) that there is some ATP present in rabbit hearts that have been stopped by iodoacetate.

Adrenaline produced very little stimulation of the iodoacetate depressed auricle, and occasionally a reversal of the rate effect was observed when the depression was well advanced. In one typical experiment the concentration of iodoacetate was 0.001 M, the exposure time was 35 minutes, and the auricles were markedly depressed (rate –42 per cent, amplitude –96 per cent); adrenaline (10^{-6}) stimulated the amplitude slightly (28 per cent), but the rate was decreased to –75 per cent of the original value. It is not surprising that such auricles would fail to be stimulated by adrenaline, but the reversal of the rate effect was not expected. It is possible that iodo-acetate blocks some step in the series of reactions through which adrenaline normally produces a stimulation of the rate, leaving the systems by which it may depress the rate at higher concentrations relatively unaffected.

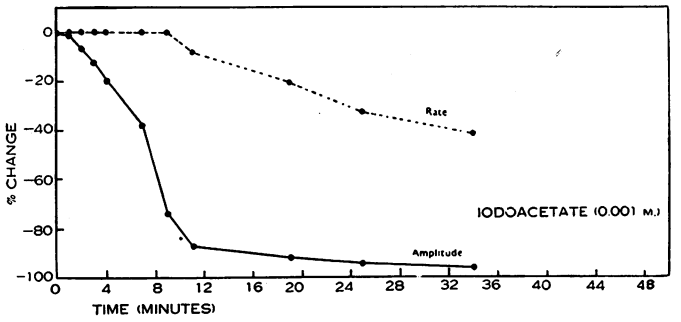

FIG. 9.—The action of iodoacetate on the rabbit auricle.

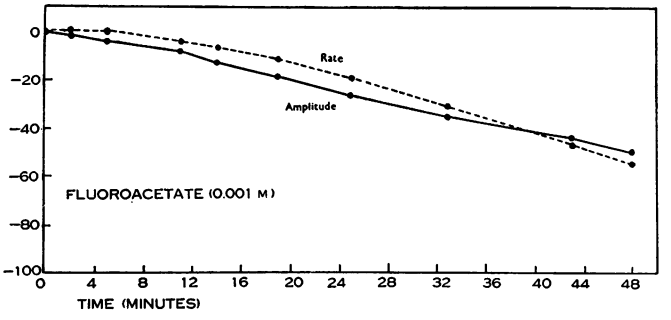

FIG. 10.—The action of fluoroacetate on the rabbit auricle.

Fluoroacetate

Fluoroacetate is the most slowly acting inhibitor of those investigated, depressing the auricles gradually and steadily for a period of two to three hours at a concentration of 0.001 M. It characteristically depressed both rate and amplitude equally, in this respect differing from any other inhibitor used in this study. The metabolic systems involved in impulse discharge and contraction may at some point possess a common path which is attacked by fluoroacetate. At the present time the exact mechanism of action of fluoroacetate is unknown, but it is likely that it inhibits some reaction, or reactions, in the tricarboxylic acid cycle. Accumulation of citrate and acetate during fluoroacetate action has been reported, but the depression of the auricles was not due to these intermediates, as may be seen by comparing the action of fluoroacetate with the actions of these substances. Furthermore, if such were the explanation, we should expect some degree of recovery when fluoroacetate was removed from the bath, but this did not occur. The action of fluoroacetate was quite irreversible. Occasionally the inhibition could be arrested by washing out the inhibitor, but the auricles never showed any tendency to return to their normal rate and amplitude.

Phlorizin

A nearly saturated solution of phlorizin in the medium (0.001M) produced a slight depression of the auricles, the effect on the rate being somewhat greater than on the amplitude, the latter showing some ability to recover spontaneously in the presence of the inhibitor. The mechanism of action of phlorizin at these higher concentrations is not well understood, and therefore it is difficult at the present time to interpret these results or correlate the actions with any particular inhibition of enzymatic systems.

Pyrophosphate

Pyrophosphate at concentrations between 0.002–0.005 M produced a marked depression in the amplitude of the auricles but had little effect on the rate, slight

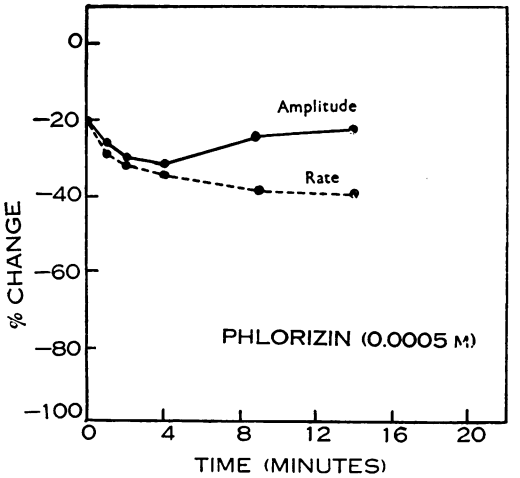

FIG. 11.—The action of phlorizin on the rabbit auricle.

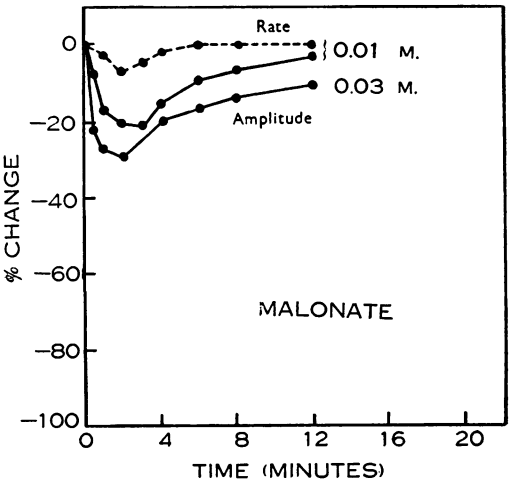

FIG. 12.—The action of malonate on the rabbit auricle.

stimulation occasionally being the only effect observed. Since pyrophosphate combines with calcium and the medium became slightly turbid after its addition, the possibility was considered that the action upon the auricle was due to a decrease of ionized calcium in the medium. If calcium was added to the medium during pyrophosphate depression the amplitude returned to normal, but the increased rate was unaffected. Lack of calcium in the medium leads to depression of the amplitude, but the rate is never stimulated and is occasionally slightly reduced. Recovery of the auricles after low calcium was more rapid than after pyrophosphate depression. We may conclude, therefore, that the effects produced by pyrophosphate were partially due to a lack of ionized calcium, but that the pyrophosphate also had a definite action of its own. Since these actions could not be separated conveniently, the action of pyrophosphate was not studied farther and the curves of its action are not included in the Figures.

Malonate

The rabbit auricle was not strongly depressed by malonate; at high concentrations (0.02–0.03 M) a maximum inhibition of only 30 per cent in rate and amplitude was reached. Furthermore, the auricles spontaneously recovered in the presence of malonate at these high concentrations and returned to normal, or nearly normal, rates and amplitudes after 8–10 minutes. When the malonate was washed from the bath during the period of greatest depression, the recovery was very rapid, requiring only two to three minutes. Forssmann and Lindstén (1946) found that malonate in concentrations between 0.01–0.04 M depressed the ventricular contractions of the perfused rabbit heart but had little effect on the rate. It is remarkable that malonate at these concentrations should have so little depressant action on the auricles, for it is generally believed that malonate at a concentration of 0.005 M completely inhibits succinic dehydrogenase and therefore blocks the tricarboxylic acid cycle, a system upon which the cardiac muscle is thought to depend. Recently it has been reported that somewhat higher malonate concentrations, such as those used here, will prevent the oxidation of oxalacetate in homogenates, this action producing an additional derangement of the tricarboxylic acid cycle (Pardee and Potter, 1949). It seems unlikely that failure to penetrate the muscle cells could have been responsible for this lack of action, inasmuch as exposures of 20–30 minutes to malonate did not lead to any marked alteration of auricular function. The strange behaviour of cardiac cells under the influence of malonate was noted previously on rat heart slices (Webb, Saunders, and Thienes, 1949b). In this preparation malonate produced an initial depression of the respiration, but this was followed by a recovery to values near the normal rate. Furthermore, in the rat heart slices it was known that the succinic dehydrogenase was inhibited, for no rapid increase in oxygen uptake was observed when succinate was added as invariably happened when malonate was absent. Rat heart slices can respire adequately in the presence of high concentrations of malonate, and auricles can function quite normally under these conditions. We must conclude that cardiac tissue can respire and function when this particular step in the tricarboxylic acid cycle is blocked. It may well be that when this block is imposed in an organized cellular system, the cycle can be short-circuited; perhaps under such conditions oxalacetate may be formed directly from α-ketoglutarate, or pyruvate or its activated fragment be taken into the

cycle by combination with something other than oxalacetate. It will be shown later that the small amount of depression observed with malonate is probably not due to the inhibition of succinic dehydrogenase.

Interactions of substrates and inhibitors on the auricles

Experiments were performed to investigate the abilities of various substrates to counteract or antagonize the depressions produced by metabolic inhibitors. Theoretically, if an inhibitor acts specifically on one enzyme in a series of enzymic reactions, the addition of the intermediate substrate that is normally formed by the action of this enzyme should allow at least partial recovery of the functional activity of the tissue. If the inhibitor acts not only on this one enzyme, but upon others as well, the amount of recovery produced by the added intermediate will be roughly proportional to the specificity of the inhibitor effect. The possibility must always be considered that an added substrate, in the presence of an inhibitor, may initiate energy-forming reactions that are not normally important; thus, a stimulation of the auricles by a substrate during an inhibitor depression does not necessarily mean that there is a direct relationship between the inhibitor and the substrate in the cellular metabolism.

The relatively weak effect of malonate on the auricles was puzzling, and attempts were therefore made to determine whether the depression that was produced was related to the commonly accepted action of malonate on succinic dehydrogenase. Malonate prevents the formation of fumarate from succinate, and recovery from malonate depression should, therefore, be observed after the addition of fumarate. If malonate acted simultaneously elsewhere in the tricarboxylic acid cycle this would not necessarily be true. Three types of experiment were performed.

(1) In two experiments the effects of malonate (0.01–0.03 M) were first determined, and these were compared with the effects of adding malonate and fumarate (0.001–0.01 M) together. No modification of the action by fumarate was observed. In a typical experiment the average maximum depression of amplitude produced by malonate alone was 25.7 per cent; when both malonate and fumarate were added together the depression was 27.7 per cent. The changes in rate were likewise comparable.

(2) In the second type of experiment fumarate (0.001–0.01 M) was added to auricles already depressed by malonate (0.02 M). No stimulating effect was observed; in fact, an additional depression was usually seen.

(3) In the third type of experiment the auricles were allowed to remain in contact with fumarate (0.005 M) for five minutes before the addition of the malonate (0.01 M) in order to ensure penetration of the fumarate. Again no change in the response to malonate was observed.

It therefore seems likely that the malonate depression is not due solely to inhibition of succinic dehydrogenase. It is more reasonable, I believe, to assume that malonate at such concentrations exerts a toxic action on the cell surface, a depression such as is seen with any of such substances under similar conditions. Acetate (0.001 M) and pyruvate (0.005 M) were also unable to counteract the depression produced by malonate (0.02 M), only additional depression being observed.

The depression produced by fluoride (0.005–0.02 M) was not counteracted by succinate (0.001 M), acetate (0.001 M), or citrate (0.001 M). However, pyruvate (0.005 M) showed some ability to stimulate the rate of the auricle in the presence of fluoride, but the amplitude was usually unaffected. This is in harmony with most of the results obtained with pyruvate, namely that it acts more particularly upon the mechanisms involved in rate than upon those involved in the amplitude of contraction. Complete antagonism of fluoride with pyruvate was never observed.

Iodoacetate, like fluoride, is generally supposed to act predominantly on the glycolytic stages of metabolism, inhibiting the formation of pyruvate from glycogen or glucose. However, there are reasons for supposing that in cardiac tissue the action is not specific. The heart muscle presumably obtains energy from both glycolytic and post-glycolytic phases of metabolism, although a relatively small amount from the former (approximately 15–20 per cent of the total). If iodoacetate acted only on glycolysis, the addition of pyruvate should restore the activity of the heart muscle to about 80 per cent of its normal value, only the energy from the glycolysis now being lost to the tissues. Three experiments were performed to test this.

(1) In the first experiment the concentration of iodoacetate was quite high (0.001 M), the auricles had been in contact with the inhibitor for 15 minutes, and the resulting depression was severe (rate −40 per cent, amplitude −95 per cent). Addition of pyruvate (0.01 M) gave no stimulation, the rate and amplitude continuing to decrease and contracture to develop. After the auricles were completely stopped at 30 minutes the addition of pyruvate was without effect.

(2) In the second experiment the concentration of iodoacetate was lower (0.0002 M), the time of exposure to the inhibitor was 25 minutes, and the auricles were very depressed (rate −83 per cent, amplitude −87 per cent). The addition of pyruvate (0.005 M) slightly stimulated the rate but had no effect on the amplitude. The normal rate before iodoacetate was added was 72, the iodoacetate had reduced it to 12; pyruvate increased the rate to 18 in two minutes and to 24 in four minutes. Later in the depression pyruvate had no effect at all.

(3) In the third experiment the iodoacetate (0.0002 M) was allowed to act for 15 minutes, the auricles being moderately depressed (rate −19 per cent, amplitude −53 per cent). Pyruvate (0.005 M) slightly stimulated both rate and amplitude, the rate increasing by 15 per cent in two minutes and the amplitude increasing by 32 per cent within 30 seconds; these were the maximum effects and were only temporary, the depression of the auricles proceeding as if the pyruvate had not been present. Later in the depression pyruvate was added at both 0.001 M and 0.01 M without any effect.

One may conclude that pyruvate is only partially able to counteract the depression produced by iodoacetate, and that the higher the concentration of iodoacetate and the longer its action, the less recovery may be observed. These experiments confirm previous conclusions that iodoacetate is not a specific poison of the glycolytic phase of metabolism in mammalian cardiac muscle. Acetate (0.001–0.01 M) showed no ability to counteract the iodoacetate depression.

Depression of the auricles produced by fluoroacetate was partially antagonized by pyruvate. This was demonstrated in two experiments.

(1) In the first experiment the concentration of fluoroacetate was 0.001 M, the time of exposure was two hours, and the depression was very marked (rate −79 per cent, amplitude −83 per cent). Pyruvate (0.01 M) increased the rate by 133 per cent and the amplitude by 18 per cent within one minute, the rate returning to half its normal level and the amplitude to only 20 per cent of its normal value. The addition of pyruvate at the same concentration 35 minutes later produced even greater stimulation, the rate increasing by 200 per cent and the amplitude by 126 per cent in two minutes.

(2) In the second experiment the fluoroacetate (0.001 M) was allowed to act for only 25 minutes and the auricles were only moderately depressed (rate −24 per cent, amplitude −25 per cent). Pyruvate (0.005 M) gave some stimulation, increasing the rate by 8 per cent and the amplitude by 4 per cent within one minute. After an exposure of 50 minutes, when the auricles were more depressed (rate −41 per cent, amplitude −62 per cent), pyruvate at the same concentration stimulated more successfully, the rate increasing by 40 per cent and the amplitude by 21 per cent. The effect on the amplitude was quite temporary and after three or four minutes the auricles were again being progressively inhibited, but the rate effect remained for a much longer time.

Discussion

Each metabolic inhibitor used in the present investigation has been found to exert a unique and characteristic action upon the auricle, the effects differing from one another with respect to (*a*) the concentration-action curves, (*b*) the time course of action, (*c*) the relative actions on rate, amplitude of contraction, and muscular relaxation, (*d*) the ability of the cardiac muscle to recover spontaneously in the presence of the inhibitor, and (*e*) the ability to recover after the inhibitor has been removed. Each inhibitor can be distinguished from the rest by its action on the rabbit auricle. The problem, however, is to determine whether any useful information on the mechanisms of cardiac function may be derived from such data in conjunction with the results on substrates, substrate depletion, and anoxia.

The auricles may be stopped in either diastole (state of relaxation) or systole (state of contraction). Substances which arrest the auricles rapidly always stop them in a state of relaxation. The process of contracture, by which the auricles stop in systole, requires time for its development and is not observed early in the action of any substance. There are substances which will rapidly arrest the auricles in diastole and, with continuing action, will induce contracture, the end result being systolic arrest. In the present experiments with rabbit auricles, the following have been found to lead to contracture: iodoacetate, fluoroacetate, fluoride, cyanide, anoxia, and substrate depletion. All of these would be expected to produce a state of reduced metabolic activity with a decrease in the amount of energy available to the muscle. This inhibition of the flow of energy would result in a gradual loss of ATP (and other substances containing high-energy phosphate bonds), such having been observed in the past under similar conditions. Simultaneously with the depletion of ATP the cardiac muscle becomes less and less able to relax, and when the ATP level becomes sufficiently low the muscle goes into complete contracture and

will beat no longer. The ATP concentration need not drop to zero for this to occur. This conception is in harmony with the views of Szent-Györgyi (1947) on skeletal muscle, ATP being required for the relaxation of the muscle fibrils as well as for their contractile activity. The auricular muscle is in a low-energy state when it is contracted and in a high-energy state when it is relaxed. Adding glucose or pyruvate to a muscle in contracture would provide some ATP and the muscle would be able to relax and beat again.

Let us consider auricles that have been stopped in the relaxed state of diastole by some metabolic inhibitor. Why does the muscle not contract? There is certainly present a sufficient concentration of ATP. There is nothing fundamentally wrong with the contractile mechanisms, for the muscle can be made to contract by electrical stimulation, and it will recover from such contractions for a period of time. The initiating reaction for contraction must be depressed. The functioning of cardiac muscle involves not only the mechanisms of contraction and relaxation, but also processes that are rhythmic in nature and serve to stimulate these contractions. Normal contraction is induced by a wave of depolarization (accompanied simultaneously by other changes) which sweeps over the muscle and in some manner causes the actomyosin to be transformed into its low-energy state. This stimulating process can be roughly divided into three phases: the discharge of the impulse from the pacemaker cells, the conduction of this impulse, and the excitatory reaction by which the actomyosin is affected by the impulse. These process are all dependent on metabolic reactions for their energy. Maintenance and restoration of the cellular membrane potentials require energy, and further energy may be required for the synthesis and distribution of substances involved in these processes. It is likely that these excitatory mechanisms are inhibited in auricles that have been arrested in diastole. Occasionally a substance will stop the heart by depressing the discharge of impulses from the pacemaker cells, but usually it would seem to be a matter of depression of conduction or of the excitatory reaction (the process by which the actomyosin is stimulated by the conducted impulse will be here termed the "excitatory reaction"). In many cases the amplitude of the auricles was gradually depressed until arrest occurred; immediately before arrest the rate was depressed relatively little. Observation of such auricles leads one to the conclusion that the pacemaker cells are still discharging after the auricles have ceased to beat, the failure being due to disturbances in conduction or the excitatory reaction. If such is the case, it lends support to the view that partial inhibition of the amplitude is due to the failure of a certain fraction of the contractile units to react, the contraction of each unit being all-or-none in nature. Progressive inhibition of the excitatory reaction would lead to a gradual decrease in the number of units responding to the conducted impulse, and this in turn would lead to a progressive inhibition of the amplitude of contraction. Conduction and the excitatory reaction may be more sensitive than the processes of contraction and relaxation to metabolic inhibitors and anoxia because they do not possess a store of ATP, most of this substance being found in the actomyosin or in the enzymes involved in phosphate transfer.

When the amplitude of contraction of cardiac tissue is reduced to some fraction of its normal value (no change in the degree of relaxation having taken place), we may interpret this in two general ways. Either there is a smaller fraction of

all-or-none units contracting, or each unit in the muscle is contracting proportionately less. The solution to this problem is fundamental to cardiac physiology and pharmacology. Does cyanide, for example, when it inhibits the auricular amplitude by 20 per cent, prevent completely the contractions of 20 per cent of the contractile units, the remaining units contracting normally, or does it reduce the contraction of each unit an average of 20 per cent? This is not merely the old question of whether the concentration-action curve is an expression of the action of a substance on cellular processes or whether it is derived from the distribution of susceptibilities of the individual cells. A depressant may inhibit the excitatory reaction either by raising the threshold of response of the contractile units or by reducing the excitatory state that is initiated by the passage of the impulse. In the former case we should be measuring a distribution of susceptibilities, but in the latter case it would be a matter of a true action on a cellular process. There is no *a priori* reason why both actions may not be of importance, depending on the substance that is producing the depression. It should also be pointed out that, although the contractile units may normally behave in an all-or-none manner, it is entirely possible that various conditions may modify the degree of contraction.

How must we interpret the changes in amplitude brought about by the action of these metabolically important substances on the rabbit auricle? The results of the present investigation can be best explained by assuming that these substances modify the excitatory reaction and not the contractile mechanisms directly. Only when the cardiac muscle begins to go into contracture is the contractile mechanism itself altered. If this is the correct interpretation, it would seem that our conceptions of the actions of substances on cardiac muscle must be modified. Such an interpretation would also apply to the actions of drugs on the heart.

Throughout this investigation it was found that the changes in rate and amplitude of the auricles were relatively independent of each other. An increase or decrease in the amplitude had no particular effect on the rate, nor did changes in the rate, within limits, affect the amplitude. Quite frequently one would remain constant during a marked change in the other, and occasionally they would change in the opposite directions. The fundamental energy-forming enzymatic reactions may be quite similar in the processes that control rate and amplitude, but the paths of energy flow and utilization are different. It is true that most substances change the rate and amplitude in the same direction. This is perhaps more readily understandable when we consider changes in amplitude as arising, not from alterations in the contractile mechanisms, but from modifications of some cellular surface phenomenon that is involved in the excitatory reaction, such a surface state being related to the cellular membrane changes occurring in the pacemaker cells. In whatever way we consider the cyclical discharge of impulses, we must assume that there is the development of a depolarized state and that this is propagated over the cardiac muscle, this being responsible, directly or indirectly, for the excitatory reaction and the contraction of the myofibrils that lie in the region over which the impulse is passing. We can thus see a possible connexion between rate and amplitude, a relationship that implies no necessary simultaneous behaviour, but which, in the majority of cases, will lead to parallel

behaviour with respect to direction of change. However, the magnitudes of the changes in rate and amplitude need bear no relationship to one another.

Development of contracture was always a late process, following earlier alterations in the auricular rate and amplitude. We have supposed that this was due to a greater reserve of ATP or other substances containing high-energy bonds. It is indeed possible that the membrane phenomena of impulse discharge, conduction, and the excitatory reaction do not utilize ATP or similar substances, but that the cyclical processes involved are more directly dependent on the oxidative sequence of enzymatic reactions. Most of the metabolic inhibitors investigated, as well as substrate depletion and anoxia, depressed the amplitude more than the rate. This might be explained by assuming that the rate processes require less energy than those involved in the excitatory reaction, but this can hardly be the entire answer since there are instances in which the rate is depressed as much as the amplitude. The metabolic systems involved in the rate and amplitude may also be assumed to be somewhat different, and this is quite likely from a consideration of the characteristic actions of the various inhibitors, but it would explain only the differences in behaviour and not the basic fact that the rate is more resistant to change than the amplitude. Another explanation might be that the rate and amplitude mechanisms alter their functional level to different degrees in response to an equivalent change in some common factor. For example, if we assume that both the rate of discharge and the effectiveness of the impulse on the contractile units depend on changes in the degree of electrical polarization of the cell membrane, it is conceivable that a depression of the resting potential or a depression of the rate of repolarization would inhibit both processes. However, owing to the particular circumstances, the number of units reacting would be depressed more readily than the discharge rate. The excitatory reaction is probably quite delicately adjusted to the changes occurring at or near the cellular surface. This delicate balance assures a ready adaptability of the cardiac muscle.

We might expect, for the same reasons, that glucose and pyruvate would stimulate the amplitude more than the rate in substrate depleted auricles. This was found to be true for glucose, but pyruvate acted more particularly on the rate. Quantitative comparisons are complicated by the third action of these substrates, the stimulation of relaxation, an action somewhat more readily produced by glucose than by pyruvate. However, it was clear that pyruvate, both in substrate depleted auricles and in those poisoned by inhibitors, stimulated the rate more effectively than the amplitude and more effectively than it produced relaxation of the muscle. From this it might be concluded that the oxidations in the tricarboxylic acid cycle are the main source of energy for the rate processes. Supplying glucose does not lead to a sufficiently rapid formation of pyruvate, and therefore giving pyruvate directly has a greater stimulating effect. Why, however, is pyruvate less effective than glucose in stimulating the amplitude? The energy derived from the breakdown of glucose to pyruvate must be of greater importance to the processes involved in the contraction and relaxation of the muscle than it is to the rate processes. It is doubtful whether we are measuring the effect of either substrate on the excitatory reaction under such circumstances, the stimulation of ATP formation being the primary action in increasing activity of contraction. According to Szent-Györgyi (1947) the actomyosin requires so much ATP to

be active at all, aside from the ATP that is utilized for relaxation. Since ATP can be formed from energy derived from the breakdown of glucose to pyruvate it is not so surprising that glucose is somewhat more effective than pyruvate in this respect. However, in the cellular membrane processes of impulse discharge and the excitatory reaction the conditions are different; as we have seen, it is possible that these mechanisms do not require ATP and may operate directly from the oxidative systems, especially the tricarboxylic acid cycle. Since the tricarboxylic acid cycle can continue to operate in the metabolism of fat, the observation that the rate is more steady during inhibition of glycolysis (iodoacetate) than it is during inhibition of the tricarboxylic acid cycle (fluoroacetate) is explained. There remain, however, a number of problems—for example, the anomalous behaviours of malonate and fluoride—and these ideas are presented only as a working hypothesis.

The metabolic activities of contracted and relaxed cardiac muscle may be different. The metabolism may occur in cycles, in phase with contraction and relaxation, so that the conception of the metabolism as a steady state would be erroneous. Deductions based on steady state conditions would not necessarily be applicable, nor would arguments based on limiting reactions always be valid. This must be kept in mind in considerations of the action of metabolic substrates and inhibitors on the heart. Determinations of the ability of heart slices to oxidize various substrates may give information on only one phase of the cycle; it is likely that such slices are in a state of contracture. The same reasoning would be applicable to the actions of inhibitors. Substrates that are well oxidized by the contracted muscle may produce no stimulation of the normally beating auricle because their oxidation occurs at phases of the cycle where their energy cannot be utilized; only when the cardiac muscle has become abnormal, when these cyclical processes have become distorted, or when the muscle has gone into contracture will these substrates be oxidized with the production of utilizable energy. Similar metabolic cycles of activity probably occur in the pacemaker cells during rhythmic discharge of impulses and near the surfaces of the muscle cells as the propagated impulses pass.

Furthermore, one is not justified in assuming that there is a general synthesis and supply of ATP upon which all the processes in the cardiac cell must draw. ATP is probably used in only certain functional mechanisms and is synthesized in these particular regions. The concentration of ATP as determined by analysis of the entire muscle can seldom be correlated with the functional activity of the tissue for this reason. When one considers the various processes which control the beat of cardiac tissue, the different metabolic requirements of these processes, the spatial relationships, and the cyclical nature of the metabolism, it is easy to understand why it is so difficult to correlate analyses for substances containing high-energy bonds and for other metabolic intermediates with the level of activity of the cardiac muscle.

SUMMARY

1. The actions of thirteen metabolic substrates and intermediates on the rabbit auricle have been investigated, and it was found that they all depress the normal auricle, with regard to both rate and amplitude, except citrate which at higher concentrations stimulated the rate.

2. The behaviour of the beating auricles during depletion of substrate was observed and the abilities of various substrates to revive the auricles have been determined. It was found that only glucose and pyruvate produced marked effects; glucose produced a more marked effect on muscular relaxation, and pyruvate acted particularly upon the rate.

3. The actions of eight metabolic inhibitors on the rabbit auricle have been investigated and each inhibitor was found to produce a characteristic effect. The actions of these inhibitors on the functioning of the auricles does not always parallel their commonly accepted actions on enzymatic systems, and it is concluded that unknown pathways of metabolism exist in cardiac muscle.

4. The abilities of substrates to revive auricles that have been depressed by inhibitors was studied, and the bearing of these results on the specificity of inhibitor action and the nature of myocardial enzyme systems are discussed.

5. None of the observed actions of metabolic substrates and inhibitors are due to an action on the acetylcholine equilibrium in the auricular muscle, because the presence of atropine did not modify them.

6. The mechanisms by which the auricles can be stopped in diastole or systole are discussed in the light of the information obtained on the actions of substrates and inhibitors.

7. It is questioned whether changes in amplitude are directly related to contractile mechanisms. The results obtained could be best explained by assuming that the actions of the substances investigated were upon the mechanisms by which the propagated impulse stimulates the actomyosin to contract into its low-energy state. This important phase of the cardiac functioning is termed the "excitatory reaction." Depression of this excitatory reaction, either by reducing the excitatory state at the cellular membrane or by increasing the stimulation threshold of the contractile units, leads to a reduced number of units contracting and consequently to a reduced amplitude.

8. Changes in rate, amplitude, and degree of relaxation were shown to be relatively independent of each other. From the actions of substrates and inhibitors, as well as substrate depletion and anoxia, on the auricles, an attempt was made to correlate certain phases of metabolism with the mechanisms involved in rate, amplitude, and relaxation. It was concluded that contraction and relaxation are dependent on a certain supply of ATP but that the rhythmic discharge of impulses and the excitatory reaction are more directly dependent on the oxidative reactions of metabolism, particularly the tricarboxylic acid cycle.

9. Some general problems in the correlation of cardiac metabolism with cardiac function are discussed.

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