THE INNERVATION OF THE CORONARY VESSELS.

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Introductory and method.

IN the course of our experiments upon the isolated mammalian heart we had occasion to study the action of various substances upon the heart beat and upon the rate of flow of the perfusion fluid through the coronary vessels. In the latter connection we gained evidence that in many instances adrenalin caused what appeared to be a preliminary constriction of the coronary vessels, the dilatation described by other observers being apparently a secondary effect. This led us to carry out a number of experiments more particularly directed to the determination of the action of adrenalin upon the coronary vessels, because of the important bearing this point has upon the question of a sympathetic vaso-constrictor nerve supply to these vessels. Work upon this question has already appeared. Schäfer[1], employing the isolated hearts of rabbits and cats, injected as much as 0·00015 to 0·0006 grm. of adrenalin. He observed no effect upon the rate of flow of the perfusion fluid although the heart was greatly affected. Nor did he succeed in effecting any change by stimulating the extracardiac nerves. He therefore concluded that the coronary vessels were not supplied with vaso-constrictor nerve fibres. The problem was next investigated by Langendorff[2] who in the first instance measured the outflow from the coronary vessels during perfusion. Using this method

[1] Schäfer. Quoted from *Zntrlb. f. Physiol.* XIX. p. 218. 1905.

[2] Langendorff. *Zntrlbl. Physiol.* XXI. p. 551. 1907.

he came to no conclusion on account of the difficulty of determining whether a change of flow was due to vaso-motor effects or was of mechanical origin produced by variations in force of the heart beat. Experiments upon the fibrillating heart, upon hearts arrested by the administration of a dose of potassium chloride as also upon dead hearts in *rigor mortis* all gave unsatisfactory results. In the last series of experiments he adopted the method of O. B. Meyer[1] of recording changes in length of rings of arteries immersed in saline solution. For this purpose the coronary vessels of the ox heart were chosen. Under these conditions adrenalin produced lengthening and Langendorff therefore concluded that the coronary vessels receive vaso-dilator fibres from the sympathetic. This last result has been confirmed by Cow[2] upon the coronary vessels of the sheep's heart.

In our first set of experiments we recorded changes in the rate at which the perfused fluid dropped from the heart, the heart hanging vertically from a cannula tied in the aorta. In all instances the heart was perfused with oxygenated saline solution. The method of recording the rate of outflow of fluid was as follows. The fluid dropping from the heart was caught in a large funnel and carried into a vertical tube 53 cms. long and 6 mm. in diameter. This was one of three tubes passing through a cork into the interior of a small bottle. The second tube, also vertical, was connected at the top to a sensitive volume recorder. The third was a thick-walled capillary tube, turned horizontally immediately above the cork of the bottle. This tube was 60 cms. long and of such bore that it took a pressure head of from 10 to 30 cms. of water to force fluid through it at the rate at which the saline passes through the heart during perfusion. The principle of the method then was to collect all the fluid from the heart in the one vertical tube and allow it to escape through the horizontal capillary tube. The fluid at once begins to rise in the two vertical tubes and continues to do so until a height is reached at which the fluid is driven through the horizontal capillary at the same pace as it is entering the apparatus. This height is then registered by the volume recorder attached to the second vertical tube. Hence if the volume recorder registers a horizontal line it indicates that the rate of flow of fluid remains unaltered. A rise of the record indicates an increase in rate of flow and a fall a decrease. The apparatus was calibrated at the outset so that a definite height in the recording tube was known to indicate a definite flow. All that was

[1] Meyer. *Ztschr. Biol.* xxx. p. 352. 1906.
[2] Cow. This *Journal*, xlii. p. 132. 1911.

necessary in an experiment then was to take readings occasionally of the height of the fluid in the vertical tubes. In addition to this, frequent estimations of the rate at which fluid was dropping from the horizontal tube were also taken. This method proved very convenient for recording changes in rate of flow through an organ which is being perfused, the main disadvantage being that a fresh equilibrium for a new rate of flow is not attained instantaneously.

In Fig. 1 we produce a tracing obtained in this way illustrating the action of adrenalin. The first effect upon the rate of flow is seen to be a diminution commencing synchronously with the cardiac acceleration and augmentation. Later this passes off and is followed by an increase in flow. In the application of this method to the particular case of the heart there is however a disadvantage which made us in the end have recourse to a different procedure. This is that the fluid issuing from the coronary vessels is in the first instance collected in the heart

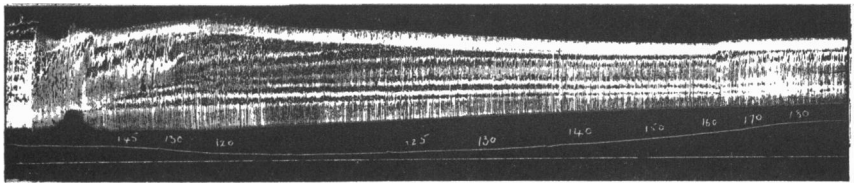

Fig. 1. Rabbit's heart. Injection of 0·000002 grm. adrenalin hydrochloride at point indicated on abscissa line. The middle tracing indicates change in rate of flow of the perfusion fluid. The figures above this line give the heights of the liquid in the recording vertical tube.

cavities both right and left and in nearly all cases not completely emptied with each systole. If then the heart begins to empty itself more vigorously the extra amount discharged from the right ventricle appears on the tracing as an increased flow through the heart and conversely retention of fluid in the ventricle may be interpreted as a diminished flow. We therefore adopted another and more sensitive method. This consists in placing the heart in a closed chamber (Fig. 2) in the form of a wide horizontal tube tapering at one end. The wide end is closed with a rubber cork through which the perfusion cannula passes and the heart lies in the first portion of the tube. The opposite end of the tube has a wide receptacle blown on its under surface, and this terminates in a narrow tube with a glass tap. Lastly the free end of the horizontal tube is drawn out to a capillary orifice wide

enough to allow a thin silk thread to pass through freely. The fluid after passing through the coronary vessels collects within the receptacle and is allowed to drop away through the tap. To record the heart beat a fine hook is attached to the ventricle of the heart, and by means of a thread passing through the capillary orifice the contractions are recorded by a crank lever. The capillary orifice is rendered air-tight by running a drop of oil up the capillary, thus converting the chamber into a plethysmograph. Lastly, by a short lateral tube the heart chamber is connected by rubber tubing to a sensitive volume recorder. If the tap be closed the recorder registers the rate at which the perfusion fluid is

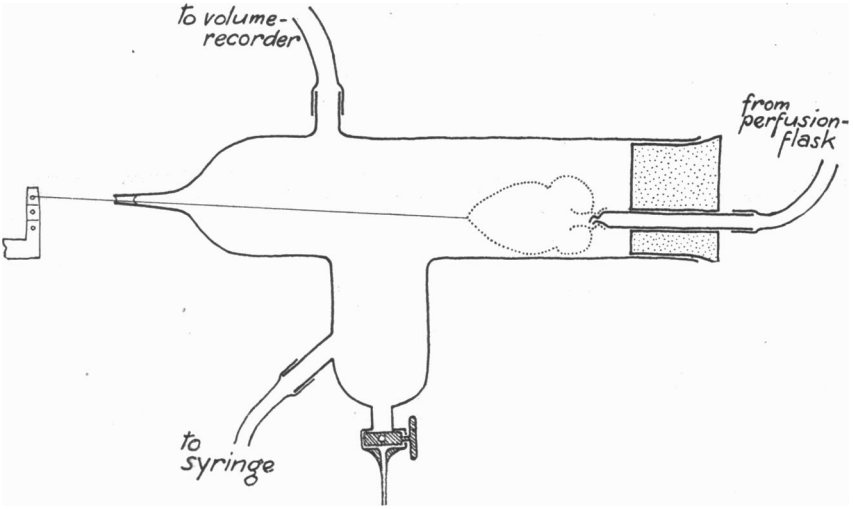

Fig. 2. Heart chamber.

passing through the coronary vessels. The tap is now opened and adjusted until the recorder writes a straight line. The rate at which the fluid is dropping from the heart chamber is the rate of flow through the heart and any change in that rate of flow is at once indicated by a rise or fall of the tracing. In order to keep the record within the best range for the volume recorder, and to keep the tracing within convenient limits when any change in rate of flow of some duration has been produced, we either inject or withdraw small volumes of solution directly into the heart chamber by means of a graduated syringe. In the tracings reproduced in this paper the amount injected or withdrawn each time was 1 c.c.

The effect of a small dose of adrenalin as recorded by this method is given in Fig. 3. The tracing shows the onset of a marked fall in rate of flow commencing a few heart beats before the cardiac augmentation and acceleration and at once attaining its maximum intensity. This persisted for 80 secs., during which time it was necessary to inject 1 c.c. on five successive occasions. Between the 80th and 100th seconds the diminution in flow was converted into an increase of a much more prolonged character (450 secs.). This tracing can be further analysed in the following way. The rate at which fluid was dropping away from the heart chamber was 8·7 c.c. per minute. During the fall in rate of flow it was necessary to inject 4 c.c. during the minute in order to maintain the tracing at the original level. Hence the flow had fallen to 4·7 c.c. per minute, *i.e.* it was nearly reduced to one half. At the height of the subsequent increase in flow 2·6 c.c. of fluid were withdrawn during a minute so that the flow had risen to 11·3 c.c. per

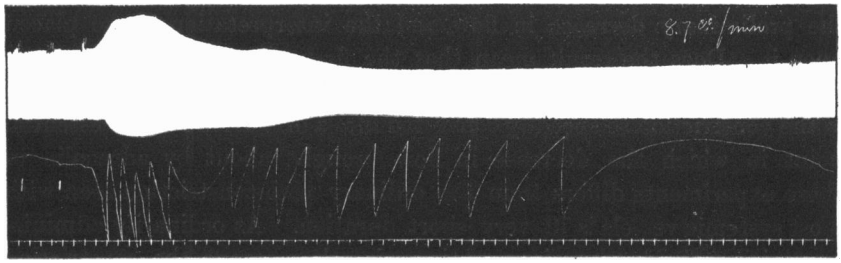

Fig. 3. Rabbit's heart. Injection of 0·00001 grm. adrenalin hydrochloride in 1 c.c. saline solution. Upper record, ventricular contractions. The separate contractions are indistinguishable in the Figure. Lower record, rate of flow. A horizontal line in this tracing indicates a rate of flow of 8·7 c.c. per minute. The verticals indicate the rapid withdrawal or injection of 1 c.c. into the heart chamber. Time tracing records intervals of 10 secs.

minute. We would also call attention to the rapidity with which the increase in flow disappears at the end of the tracing. This method has proved very convenient and simple. It is also, as the tracings show, extremely sensitive. There is only one disadvantage which, however, does not occur frequently. This is that the left ventricle may now and again discharge some of its contents into the aorta and cannula which causes a sudden fall in the volume tracing. On account of its suddenness this can always be detected at once and so never leads to any doubt as to the interpretation to be placed on the tracing. In all

perfusions of the heart, fluid invariably collects in the left ventricle; this fluid comes either from a slight leakage through the aortic valves or as usually from the coronary vessels through the veins of Thebesius. If the mitral valve be intact the fluid commonly collects within the ventricle, for in a perfused heart the force of the contraction is but rarely forcible enough to empty the ventricle at each beat into the distended aorta. The difficulty may be obviated entirely either by destroying the mitral valve, which is the best plan, or by inserting a small rubber tube through the auricle into the interior of the ventricle. In the latter plan there is some difficulty in maintaining the tube in its place without interfering with the freedom of the ventricular movements.

The action of adrenalin on the coronary vessels.

We have already stated the general result we have obtained and a typical tracing has been given. The most important point is, of course, the preliminary decrease in flow and the interpretation to be placed upon it. Does the result mean that the first effect of adrenalin upon the coronary vessels is to cause a constriction? If this is the case, why is the constriction so speedily converted into a dilatation? The essential point in which the decrease in flow brought about by adrenalin in these experiments differs from the ordinary vaso-constriction seen with most systemic vessels is its very short duration. As ordinarily administered in perfusion experiments through other organs the constriction produced by adrenalin is extremely persistent. In the instance of the heart we have found that the period of decreased flow is frequently even shorter than that seen in Fig. 3. At times indeed it may be almost absent. On the other hand, the extent of the decrease in flow is often so marked as to be quite comparable to the similar action of adrenalin on other vessels. Further, the change exactly reproduces in the suddenness of its onset the typical reaction of adrenalin upon innervated vessels. The chief difficulty then is to explain how this big diminution in flow is so rapidly converted into an increase.

Before we proceed further there is one possibility other than vaso-constriction to be considered. This is that the diminution in flow may not be due to contraction of the coronary vessels at all but may owe its production to a mechanical cause. As the adrenalin produces its augmentor and accelerator effect upon the cardiac muscle, so in proportion a mechanical hindrance to the flow through the coronary

capillaries may be produced. It is not known whether the flow through the coronary vessels is impeded with each cardiac contraction. Perhaps it is even arrested at each beat, so that blood flow through the heart only occurs during the diastolic period. If then the heart be made to beat more rapidly, a change brought about at the expense of the diastolic pause, the result must be a decrease in the total volume of fluid passing through the heart muscle per minute. There is, however, one point which clearly proves that this cannot explain the diminution in rate of flow in our experiments. This is that the decrease in rate usually precedes the cardiac muscle effect. Also it attains its maximum intensity at once. The augmentation and acceleration is much more gradual in its onset and often does not attain its maximum until the diminution in flow has been converted into an increase. Hence we conclude that the decrease in flow after administering adrenalin is in reality due to contraction of the coronary vessels. The corollary to this is that these vessels receive a set of vaso-constrictor nerve fibres from the sympathetic system.

Let us next turn attention to the second phase, the increase in rate of flow. From reasoning similar to that given above this again is a vascular response, a dilatation of coronary vessels. There are two possible explanations to be considered. Either the reaction is due to excitation of vaso-dilator nerve endings, or to the direct action upon the vessels of some substance produced within the heart as a result of the excitatory effect of the adrenalin upon the heart muscle. Let us consider the latter alternative first. The marked increase in cardiac activity due to the adrenalin is of course accompanied by an increased output of metabolites, foremost among which we may think of carbonic acid. We have on many occasions tested directly the action of carbonic acid upon the coronary and other vessels. In all instances in confirmation of the results of several workers upon systemic vessels we found carbonic acid or any other dilute acid to produce very decided dilatation. In the case of the coronary vessels it is very marked, often doubling the flow. One other point of interest is that its onset is gradual, much more so than is usual with most drugs. The method used in such experiments was to take a sample of the saline in use for the perfusion and saturate it with carbonic acid. This was then mixed in varying proportions with fresh saline and injected. A quantity as small as 1 c.c. of a 30% saturated solution will produce considerable dilatation without exerting any effect upon the cardiac contractions. Stronger doses produce greater dilatation and at the same time depress

or even abolish the heart beat. We have also proved in experiments to be described in a later paper that adrenalin causes a considerable increase in the carbonic acid output of the isolated heart perfused with saline. The effect then must come into play after the increased activity of the heart has been effected. The question then is: Is it the sole cause?

We attempted to decide this in a variety of ways in the majority of which the aim was to exclude the cardiac activity. In the first place we studied the action of adrenalin upon a heart perfused with a calcium-free saline. This plan was not successful as after perfusing for some hours with this saline the heart was still beating, though only feebly, and the flow of fluid had fallen to such an extent on changing from the ordinary saline to that from which the calcium had been excluded that we could not expect to obtain much further change. Even in such a heart adrenalin still produced a decided effect upon the cardiac muscle. Its effect upon the rate of flow was of the same character as before, first constriction then dilatation, but it was very insignificant in amount.

Secondly, as has been previously attempted by Langendorff, we examined the effect upon a heart whose activity had been brought to a standstill by the injection of a fairly strong dose of potassium chloride. Here again the results were quite unsatisfactory, for the effect of the potassium by itself was first constriction followed by dilatation and it was impossible, on injecting the adrenalin, to detect any difference which we could with certainty ascribe to the adrenalin rather than to the potassium; certainly there was no further constriction. Again, in these hearts the adrenalin markedly accelerated the recovery of the heart from the potassium standstill.

In the third place we performed some experiments upon hearts which had been excised and kept at laboratory temperature for some hours in the hope that we could obtain one in which the cardiac muscle was inexcitable while the coronary vessels still retained their irritability. In this again we were unsuccessful, no matter whether we kept the heart for 24 hours or for as short a period as 5 hours. Such change as was produced was in the direction of constriction, but the effect was so slight that we do not feel warranted in laying any emphasis upon the result.

Failing in these directions we next experimented to see whether carbonic acid could abolish the constrictor effect of adrenalin. The plan of the experiments was to inject a small dose of saline containing carbonic acid in varying proportions and to follow this by an injection

of adrenalin. After testing the two separately the adrenalin was added directly to the carbonic acid saline so that the two influences were acting simultaneously. In one experiment the constrictor response was abolished in a heart that had been previously treated with carbonic acid saline in three injections. In another experiment in which a high percentage of carbonic acid was present in the adrenalin solution the constrictor effect was much weakened in the first injection and abolished in the second. In other instances, however, the reaction was quite inconclusive. At first sight it seems very improbable that so powerful an action as that of adrenalin upon innervated vessels could be abolished by carbonic acid, but in this connection we must remember that possibly other metabolites exerting a vaso-dilator effect are also produced. Possibly, however, it is not necessary to hypothecate any other for carbonic acid is certainly produced in considerable quantity and with a slow rate of perfusion the rise of carbonic acid tension locally must be considerable, even higher than that of the fluids we injected.

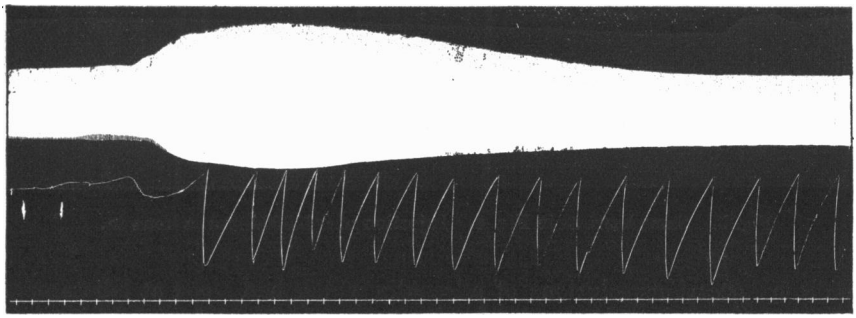

Fig. 4. Rabbit's heart. Injection of 0·000005 grm. adrenalin hydrochloride. 1 c.c. withdrawals. The separate contractions of the ventricle are indistinguishable in the Figure. Time tracing 10 secs.

Although unsuccessful in these directions we finally obtained valuable evidence by studying what happened when the adrenalin injections were repeated. As is well known, the stimulatory effect of adrenalin on cardiac muscle rapidly becomes weakened by repetition. This is especially the case for a heart perfused with saline solution. All our tracings show that as the effect upon the cardiac muscle diminishes so the constrictor effect becomes more pronounced and especially more prolonged. In illustration of this we reproduce two further tracings in Figs. 4 and 5. These are two successive injections of adrenalin with an interval of about 30 minutes. In the first the constriction is seen to

be of very short duration. It is indeed the shortest we have ever recorded. The dilatation it will be noticed only attains its maximum between the 50th and 60th second after its commencement and then persists several minutes. In the tracing of Fig. 5 the reaction is very different. The constriction lasts 110 seconds only, becoming converted into a dilatation after the maximum effect upon the cardiac muscle has been passed.

In yet another direction we also obtained very conclusive evidence and at the same time an explanation of the divergence of our results from those of previous workers. This arose from the study of the effects of excessively minute doses of adrenalin, such for instance as 0·001 to 0·0001 of a milligram. We were led to this through having employed

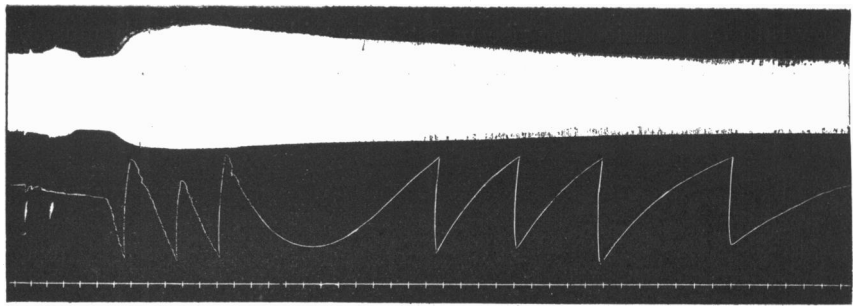

Fig. 5. Same heart as in Fig. 4. Injection of 0·000004 grm. adrenalin.

on a few occasions solutions of adrenalin which had stood at laboratory temperature for a few hours. It had been our practice to inject 1 c.c. of a 1 in 100,000 solution, and at this strength the adrenalin is of course oxidised fairly rapidly at room temperature. We found that with these old solutions we obtained a much better constrictor phase and that the dilatation when produced was less marked. At first we thought that this was due to the action of the oxidation products, but subsequently found that it was simply the result of the weakening of the adrenalin, for extremely minute doses of freshly prepared adrenalin produce exactly the same result. Thus in one experiment 0·0000001 grm. of adrenalin (Fig. 6) produced constriction only. This came on quite gradually but was very persistent. This amount of adrenalin produces no effect upon the cardiac muscle. In the next place the dose was increased, with the result that the constriction becomes less marked and is followed by a dilatation, as is seen in Fig. 7. In this tracing

moreover it is seen that this amount of adrenalin causes definite augmentation and acceleration of the heart. With a still further increase of dose both constrictor and dilator effects are more pronounced and sharper in onset. Finally, with very large doses the constrictor effect is very quickly over, and the dilator most marked. The main number of these results are quite well explained on the view that the dilatation is caused by the direct action upon the vessel walls of metabolites produced within the heart muscle by the adrenalin stimulus.

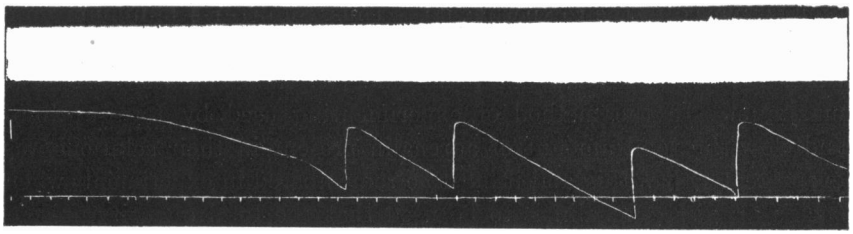

Fig. 6. Rabbit's heart. Injection of 0·0000001 grm. adrenalin between points marked at commencement of tracing. Production of constriction only. The separate contractions of the ventricle are indistinguishable in the Figure. Time tracing gives ten second intervals.

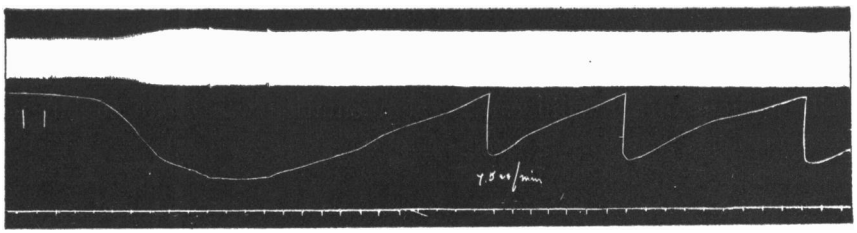

Fig. 7. Same experiment as in Fig. 6. Injection of 0·000001 grm. adrenalin. Constriction followed by dilatation.

But there is one observation which indicates that in addition to this there is yet another influence at work. This is that with large doses of adrenalin the dilatation may come on as soon as the cardiac muscle effect is produced, that is before sufficient time has elapsed for the accumulation of any quantity of metabolites. Moreover both Langendorff and Cow found that adrenalin acting on excised rings of coronary arteries produced relaxation. Hence we conclude that the dilatation produced by adrenalin is of double origin. The main cause

is the excitation of vaso-dilator "nerve-endings," the secondary cause the action of metabolites, particularly of carbonic acid.

The full results of our experiments then show that the coronary vessels are supplied with both vaso-constrictors and vaso-dilators from the sympathetic system and that the former are more excitable by adrenalin than the latter. Under the influence of a large dose of adrenalin the constrictor effect is rapidly overpowered by the dilator.

The different results we have obtained as compared with previous workers are explained we think by the much larger dose of adrenalin they employed in their experiments. Thus, Schäfer's minimum dose was 15 times greater than our maximum, and his maximum dose 6000 times greater than our minimum and 60 times greater than our maximum. In the method of experimenting used by Langendorff and Cow the total amount of adrenalin present in their solutions was even greater than this, and it must be remembered in experiments when such a drug as adrenalin is to be used that the total quantity present rather than the concentration is the most important point.

In connection with the action of adrenalin upon the heart muscle there are two points we would make. The first is that with very minute doses no effect is produced upon the heart muscle although constriction is effected. Probably this means that all the adrenalin has been removed from the saline solution during its passage through the coronary arterioles. The second is that when in some experiments we perfused for two or three minutes with a solution containing 1 in 100 millions of adrenalin cardiac augmentation was produced but no acceleration.

(A part of the expenses of this research has been defrayed from a grant from the Government Grant Committee of the Royal Society.)