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EXTRAVASCULAR FLUID LOSSES IN THE PERFUSED ISOLATED RABBIT HEART

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The isolated heart perfused via the coronary arteries (Langendorff, 1895) has been extensively studied in regard to its responses to drugs or to changes in the ionic composition of the perfusing fluid. The weight of the perfused heart has been studied in the present work to see if the permeability of the myocardium to non-electrolytes could be measured. It was hoped that the addition of non-electrolytes to the perfusing fluid would induce an osmotic loss of water, and hence a reduction in weight, and that, as the non-electrolyte penetrated the interstitial spaces and muscle cells, this initial phase would be followed by a regain in weight. The time course of the 'osmotic transient' would be an indication of the rate of penetration of the non-electrolyte. The use of osmotic transients for determining capillary penetration rates in the perfused hind limb has been described by Pappenheimer & Soto-Rivera (1948), but in that study the weight of the perfused limb was maintained constant by varying the perfusion pressures.

The early experiments showed that osmotic transients in the beating perfused heart produced effects on the force of contraction and heart rate, and supplementary experiments showed that these factors could bring about changes in weight even when the perfusion fluid was of constant osmolarity. Stimulation of the heart by adrenaline, for instance, is associated with a change in weight, and a preliminary account of such experiments has already been reported (Stubbs & Widdas, 1958).

This paper describes further investigations into loss of weight produced by adrenaline and by excess calcium ions, which are shown to be due to a loss of extravascular fluid. In a further paper are described the variations in coronary flow which accompany changes in weight (Stubbs & Widdas, 1959).

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METHODS

The hearts used weighed 5–20 g and were from rabbits of either sex. The apparatus used for weighing the perfused heart and recording the ballistic effect of its beat was essentially that already described (Widdas, 1957), but a number of minor modifications were found desirable. The most important of these was the use of a long-stemmed T-shaped cannula instead of the L-shaped cannula previously described. One end of the cross-piece was shaped to take the aorta while the other was drawn out into a fine tube and turned over as the hook to suspend the cannula from the weighing apparatus. The fine tube was continued in polythene along the stem, and 'bleeding' at a small constant rate was allowed. Thus gas bubbles forming in the cannula were able to escape without passing into the coronary arteries. Large polythene cannulae were inserted into both ventricles near the apex of the heart to drain the cavities.

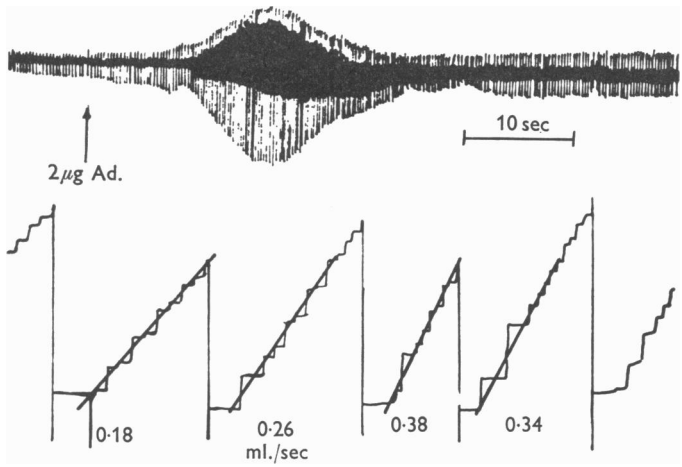

Fig. 1. Tracing from double channel recorder. Upper record shows the 'ballistic' effect which is an indication of the force of contraction. Lower record shows the rate of increase of contents of buckets used to measure the coronary outflow. The outflow is obtained from the slope of the lines. The record illustrates the effect of adrenaline on heart rate, ballistic response and coronary outflow.

In experiments in which hearts were perfused with Locke's solution containing dye, the outflow was either collected in sampling tubes and its dye concentration measured in an EEL colorimeter, or was passed through a constant-bore tube in a block mounting between a barrier-layer photocell and a light source. Another tube in front of a second barrier-layer photocell was illuminated by the same light source and served as a reference. The differential output from the photocells was amplified and recorded on a pen recorder, so that the dye concentration was being monitored continuously.

The coronary outflow was measured by weighing the outflow as it collected in one side of a double celluloid bucket supported by a spring-loaded arm. When one side bucket was full, it automatically emptied and as the spring-loaded arm returned to the original position, the other bucket came under the outflow tube. The supporting arm was linked to a linear potentiometer and the change in weight was electrically recorded by a pen-writing voltmeter. A trace of the 'ballistogram' and coronary flow recording is shown in Fig. 1. In a few experiments, coronary flow was measured by a drop recorder.

Each litre of perfusing fluid (Locke's solution) contained Na^+ 155·8, K^+ 5·7, Ca^{2+} 4·1, Cl^- 163·8,

HCO_3^- 1·8 (m-equiv) and glucose 5·6 (m-mole). The solution was aerated with oxygen. In some experiments Na lactate was used instead of glucose. The dye experiments were carried out with either Evans Blue (T1824) 25 mg/l., or washed rabbit erythrocytes (10 ml. packed cells/l.).

RESULTS

Adrenaline stimulation

When about 1–5μg adrenaline/g heart was injected into the Locke's solution near the cannula, the average weight of the heart rapidly reduced by about 5–10%. At the same time the increase in rate and force of contraction gave a ballistic response of increased magnitude which could be observed on the fast response recorder (Fig. 1). A typical result from the slow pen recorder, which gives the average change in weight, is shown in Fig. 2.

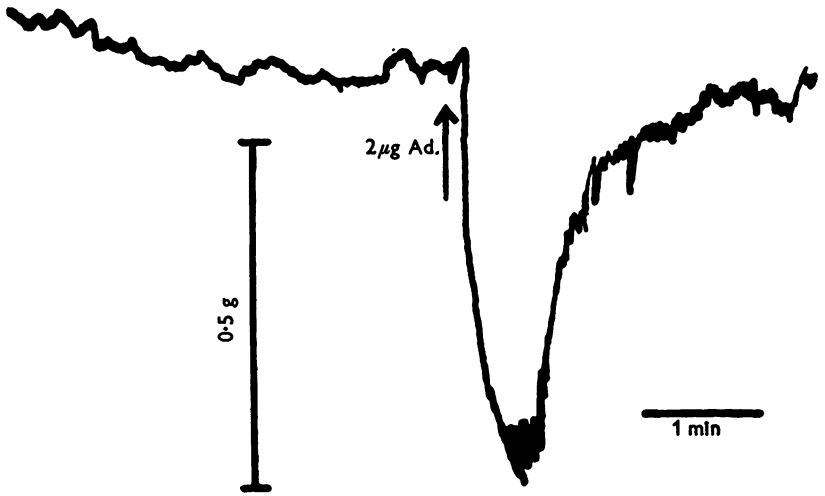

Fig. 2. Effect of adrenaline on the average weight of the heart. A typical tracing of the slow pen recorder (0·5 sec time constant) where the ballistic excursions are heavily damped but the sensitivity to change in weight is increased. Heart weight 5·5 g.

The coronary outflow, at first unchanged or reduced, showed an increase which persisted for some time after the increased ballistic effect had subsided and the heart had returned to its previous average weight. The coronary flow results agreed with those observed by Hammouda & Kinosita (1926).

The increased coronary outflow is not consistent with a reduction in the coronary vascular bed, and the most likely cause of the loss in weight (a reduction of the end-systolic volume of fluid in the ventricles) had been eliminated by draining the cavities with polythene cannulae which remained *in situ* during the experiment. As a heart stimulated by adrenaline still lost weight when the apex had been removed to expose the ventricular cavities directly, it was deduced that extravascular fluid must be involved in the loss (Stubbs *et al.* 1958).

As the physiological interpretation of the loss in weight would be fundamentally influenced by the source (intravascular or extravascular) of the fluid lost, it seemed desirable to investigate the loss by an independent method. Perfusion with Locke's solution containing Evans Blue dye was therefore carried out and the outflow from the heart monitored colorimetrically.

Stimulation by adrenaline, which produced a loss of weight, caused a drop in dye concentration quantitatively in keeping with its dilution by extravascular fluid of comparable mass to the loss of weight. Essentially similar results were obtained when washed rabbit red cells were added to the Locke's solution and used as a colorimetric label.

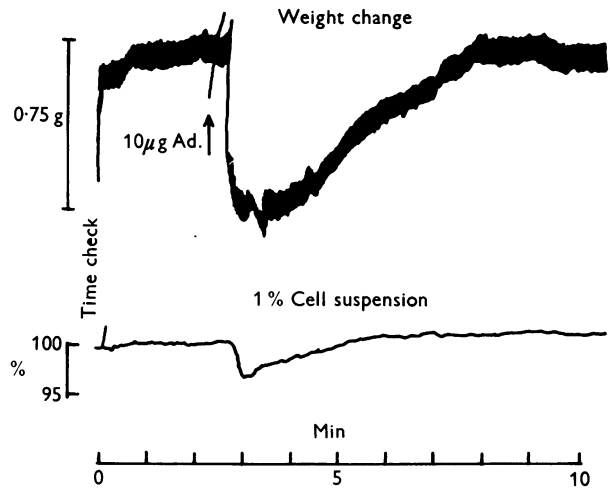

Fig. 3. Simultaneous tracings of the average weight change of the heart and colorimetric monitoring of the coronary effluent, showing loss in weight and corresponding dilution of effluent after injection of 10μg adrenaline. Mean coronary flow 0·56 ml./sec; calculated extravascular fluid loss 0·67 ml. over 2 min; heart weight 20 g.

The result of a typical experiment with washed red cells is shown in Fig. 3. Adrenaline stimulation classically affects the rate (chronotropic effect) and the force of contraction (inotropic effect). Either could conceivably play a part in producing the weight loss. To investigate the effect of rate on the weight of the heart, experiments were carried out in which the heart was stimulated electrically at speeds up to and beyond those attained with adrenaline stimulation. No significant loss in weight was produced, but there were modest changes in coronary flow. The results are summarized in Fig. 4.

Stimulation by excess Ca ions

Perfusion with Locke's solution containing excess Ca^{2+} ions sufficient to cause a rigor caused a loss of the order of 10–25% of the weight of the heart. The result of an experiment in which the outflow was collected and the dye

concentration determined with the EEL colorimeter is shown in Fig. 5. Calculated either on the basis of weight loss or on the dilution of the effluent, the maximum rate of loss of extravascular fluid attained during such experiments was 0·01 g/sec/g heart. This is a greater rate of loss of extravascular fluid than is seen under any other experimental conditions, and suggests that the operating factor is the great and prolonged force of contraction of the heart in rigor (see also, Stubbs & Widdas, 1959, Fig. 5).

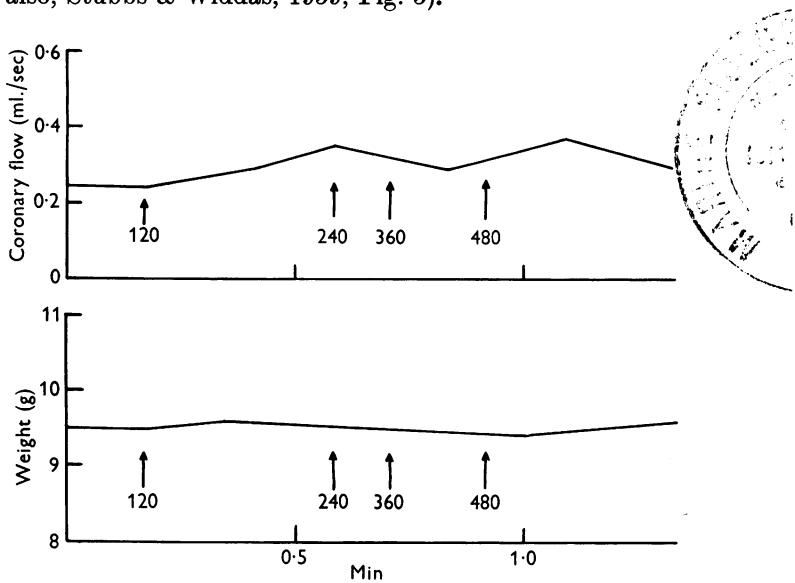

Fig. 4. Effect of electrical stimulation on coronary flow and weight of the heart. Between the arrows the rate per minute was increased slowly over the range shown.

To make a comparison of effects due to adrenaline and to excess Ca^{2+} ions the e.c.g. was recorded during experiments similar to those described above. Adrenaline in the early part of its effect appeared to prolong the period of systole as measured by the Q–T interval relative to a complete cycle. The results could be interpreted as an encroachment of systole on diastole. In the calcium experiment, however, the Q–T interval shortened in the early stages, although a pronounced loss in weight occurred. Just before the regular phasic activity was lost (during the rigor) there were typically a few bursts of repetitive discharges associated with a faster rate of weight loss. The measurements of the Q–T intervals as a fraction of each cycle are illustrated in Fig. 6, but it is doubtful if any firm conclusions can be drawn from these results, for it is known that the duration of contraction is not necessarily defined by the electrical changes (Wiggers & Banus, 1926; see also, the review by Katz, 1947).

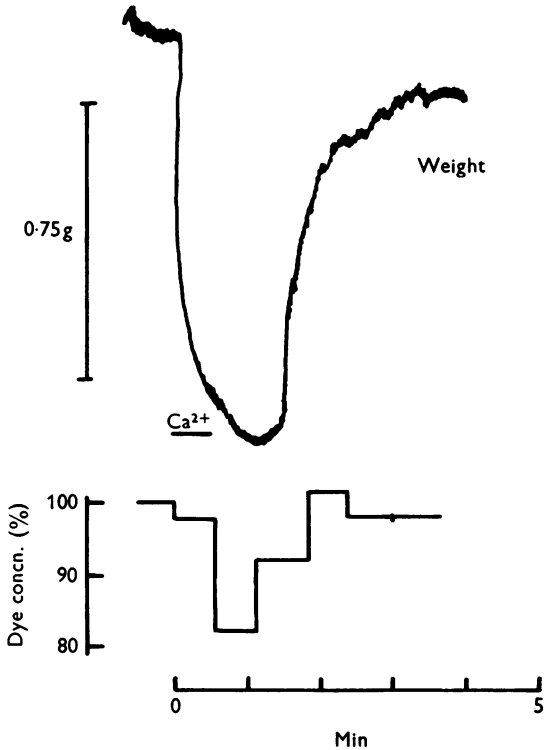

Fig. 5. Effect of calcium rigor on weight and concentration of Evans Blue dye in effluent from the heart. In this experiment samples were collected and estimated in the EEL colorimeter.

Extra-systoles

Sudden losses in weight of a smaller magnitude were occasionally observed in a perfused heart not undergoing any experimental procedure. These were associated with a double beat or extra-systole which could be identified on the ballistic record. In one heart, perfused with Locke's solution containing washed rabbit red cells, such extra-systoles occurred at fairly regular intervals. Each was associated with a small but just significant reduction in colour of the effluent, showing the extra-vascular nature of the fluid lost.

Part of the record from this experiment is shown in Fig. 7. The observation is of interest because it suggests that extra-systoles could be beneficial in reducing the interstitial fluid in a heart tending toward oedema. Since the extra-systole is presumably no more powerful than the preceding normal beat this also indicates that a contraction taking place when the fibres of the cardiac muscle are already shortened is more likely to expel interstitial fluid from the extravascular space than is a normal beat. The beat next after an extra-systole

is known to be stronger (Abbott & Mommaerts, 1959), but an inspection of the fast pen record has shown that the weight is lost before the compensatory pause and not immediately after it.

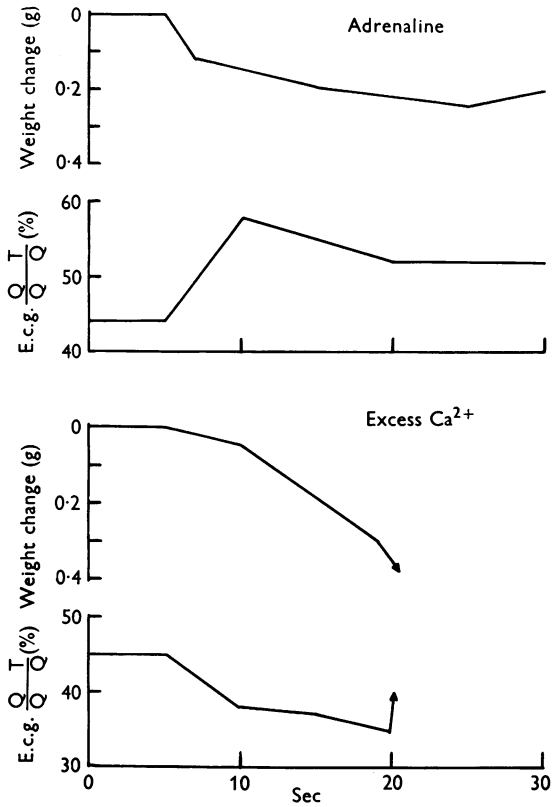

Fig. 6. Effect of adrenaline and of calcium on the Q–T interval of the e.c.g. during the periods of loss in weight.

Lowering perfusion pressure

When the perfusion is temporarily stopped there is a loss in weight, as might be expected from a partial emptying of the coronary vascular bed. Extravascular fluid is shown to be lost, however, by a dilution of the first effluent to be collected when the perfusion is restarted. This demonstrates that normal systoles are able to expel interstitial fluid and that the loss must ordinarily be masked by a filtration, outward from the capillaries, of new interstitial fluid when the perfusion pressure is adequate.

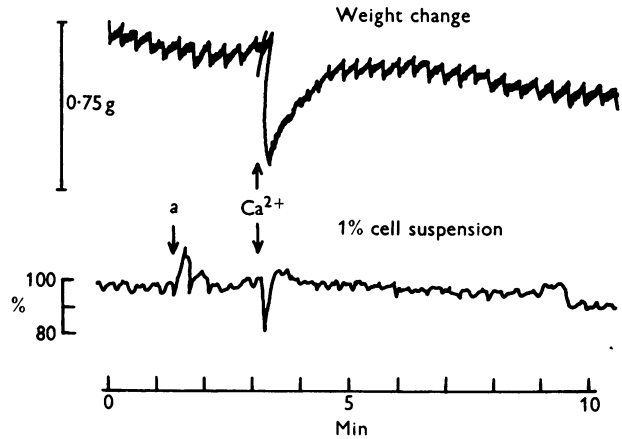

Fig. 7. Simultaneous tracings of weight change and colour of coronary effluent in a heart having spontaneous extra-systoles. Extra-systoles produce a sudden reduction in weight and a corresponding dilution of the effluent. A reduction in weight due to an injection of excess Ca ions is also shown. At *a* the calcium solution was mixed with the effluent and proved to be more concentrated than the perfusate. This made the record unsuitable for quantitative use but the dilution was still significant.

DISCUSSION

The experiments described show that losses of weight in the perfused isolated heart stimulated by adrenaline or excess calcium ions are due to the loss of extravascular fluid. Although we have no direct evidence as to whether intracellular water is concerned, the immediate loss must be from the interstitial fluid, and we are therefore concerned with the factors regulating the interstitial fluid volume in the heart. Since the hearts were perfused with Locke's saline in which there was no colloid to exert an osmotic pressure, the normal Starling hypothesis (Starling, 1896) cannot apply. The circumstances in which a loss of interstitial fluid occurs strongly suggest that the loss results from cardiac systole.

The consequences of ventricular systole on coronary flow have been much discussed since Scaramucci (1689), but there has been little or no consideration given to the effect of systole on the interstitial fluid balance in the heart. It has probably been assumed that such a balance would be of such a long time course as to be unimportant. The observation that in calcium rigor the heart can lose interstitial fluid at the rate of 1% of its total weight per second, and that significant changes occur with a single extra-systole, indicates a dynamic balance of much shorter time sequence and calls for a re-examination of this whole problem. It is already known (Johnson & Dipalma, 1939; Gregg & Eckstein, 1941) that during systole the intramural pressure in the heart rises to values greater than aortic systolic pressures. The raised intramural pressure of

systole must thus reverse the pressure gradient across the heart capillaries and (since there is no colloid osmotic pressure) the expulsion of interstitial fluid must be ascribed to a backward filtration. The results taken together suggest that in the Langendorff preparation there is a balance between filtration outwards during diastole and backward filtration during systole. That is, there is a temporal balance regulating the volume of interstitial fluid rather than a spatial one, as is usually assumed under Starling's hypothesis. This balance could be disturbed either by an encroachment of systole on diastole, or by an increased force of contraction altering the amount of fluid transferred by backward filtration during systole. A third variable could be a change in perfusion pressure which would affect the outward filtration rate.

Changes in perfusion pressure do cause changes in the weight of the heart in the direction expected, but it could be argued that this may be brought about by a passive distension of the coronary vessels (Osher, 1953). However, when the perfusion pressure is lowered sufficiently, a dilution of the effluent is seen, and this gives indirect evidence that filtration outward from the capillaries has fallen to a lower level than backward filtration from the interstitial space.

The possibility that backward filtration is a factor in the control of interstitial fluid has generally been rejected following Starling's (1896) investigations of the problem. However, it is interesting to recall that Starling found that increased tissue pressures did not retard capillary flow, and, in rejecting backward filtration in most sites of the body, gave it as his considered opinion that 'absorption by filtration is only possible in those regions of the body where a sudden rise in tissue pressure will not be propagated to the neighbourhood of the larger veins'.

It seems that these conditions are just those met with in the myocardium and that backward filtration is a new factor of considerable importance in this site. It may help to promote a rapid turnover of interstitial fluid which would have obvious advantages in facilitating the access of substrates and the removal of metabolites. The over-all reduction in interstitial fluid which occurs under adrenaline stimulation may assist the heart in achieving a lower end-systolic volume with less mutual interference by the shortened muscle fibres. It should, however, be emphasized that these studies were made on hearts perfused with saline solutions, and before the conclusions can be applied to conditions *in vivo* parallel studies with solutions containing protein and with whole blood will be required.

SUMMARY

1. The isolated perfused rabbit heart loses weight when stimulated by adrenaline or by excess calcium ions. Extra-systoles also cause a loss in weight.

2. The losses in weight have been shown to be due to losses of extravascular fluid.

3. The factors concerned are the increased force of contraction and possibly an encroachment of systole on diastole.

4. The observations suggest that backward filtration from the interstitial space occurs during systole and that this may be an important factor in the regulation of the interstital fluid volume in the heart.

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