STUDIES ON THE

PHYSIOLOGY OF BLOOD VESSELS

VOLUME 1

A Thesis submitted to the University of Glasgow

in candidature for the degree of Doctor of Philosophy

in the Faculty of Medicine

BY

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March, 1954

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University of Glasgow

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SEPARATE SUMMARY

Blood vessels are capable of responding selectively to influences of both local and general origin. In the first chapter of this thesis, the influence of ascorbic acid is reported on vascular responses in the cornea of guinea-pigs following on a standard heat lesion. The time to reach maximum oedema formation and the incidence and intensity of the vascularization, as noted by direct observation, were significantly greater in a scorbutic group of guinea-pigs than in a control group. The

influence of ascorbic acid on capillary permeability and on corneal vascularization is discussed. The ascorbic acid may act directly and locally at the site of healing in the cornea or indirectly by exerting an influence on the adrenal cortex or on its hormones.

Blood vessels in isolated tissues continue to respond to stimuli of local origin. Thus, using a perfused isolated preparation it is possible to study certain of the fundamental aspects of the functioning of the blood vessels in more detail.

In Chapter 2, a relatively simple technique is given for the perfusion under controlled conditions of the blood vessels of the isolated ear of the rabbit. The necessary equipment, some of which had to be devised for the purpose, is described. Changes in weight of the isolated ear give the net outward filtration (oedema formation). By mathematical analysis, it has been found possible to derive the gross outward filtration. The behaviour of the blood vessels in the isolated preparation is gauged by the rate of inflow of the perfusion solution.

Lowering the temperature of the perfusion solution from 38° to 16°C increased the flow through the blood vessels and reduced both net and gross outward filtration. It is suggested that, at 16°C, the perfusion solution largely passes directly from arterioles to venules by arterio-venous anastomoses while, at 38°C, the fluid passes through the capillary bed. It is considered that experiments designed to investigate permeability of capillaries in the rabbit's ear should be carried out with perfusion solution at 38°C. Those primarily designed to investigate vasomotor activity should be at a perfusion temperature maintained constant in the neighbourhood of 16°C.

Additional experiments of a preliminary nature were carried out in which the effects of altering the environmental temperature of the isolated ear, and of adding hyaluronidase to the perfusion solution were noted. The effects of alterations in the pressure, in oxygen content and in pH of the perfusion solution were also investigated in some experiments.

The course and relationships of the main arteries

and veins of the ear of the rabbit are described.

The effect of previous denervation with degeneration of the nerves was also investigated. Those experiments are reported in Chapter 3. In the absence of nerves the formation of oedema was somewhat more rapid. However, it is not possible to say how the capillaries reacted to denervation. Lowering the temperature of the perfusion solution from 38° to 16°C still reduced net outward filtration, irrespective of denervation.

After degeneration of the nerves to the blood vessels the effect of temperature on the inflow of the perfusion solution was greatly reduced. Yet, sudden lowering of the temperature of the perfusion solution from 38°C to 16°C still caused some increase in flow. Restoring the temperature to 38°C slightly reduced the inflow. It is suggested that, even in the isolated preparation, reactivity of the arterio-venous anastomoses is largely dependent on the presence of local nerves. However, local spasm of the arterial wall in response to trauma was, if anything, exaggerated by denervation.

In Chapter 4 the theoretical mathematical derivation of gross outward filtration through the capillary walls in the perfused isolated ear of the rabbit is given, together with the detailed evaluation of 2 of the analyses whose results are used in Chapter 2.

Finally, an improved constant pressure device is described in Chapter 5 and a detailed evaluation is made of the fluid mechanics involved. This device was used in the perfusion experiments on the rabbit's ear. Other applications of this constant pressure device in experimental physiology are described.

Throughout, a critical review of the relevant literature is given and the significance of the results obtained is discussed in relation to this literature.

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PREFACE

The work of such pioneers as Krogh, Dale, Lewis and Clark has demonstrated that the terminal ramifications of the arterial vascular tree are not merely a series of inert tubes which serve to bridge the gap between arteries and veins, but represent physiological units whose delicately balanced activity controls the distribution of blood to the tissues. This peripheral vascular apparatus, the terminal arterioles, precapillaries, capillaries and venules are collectively referred to as the capillary bed, a system of vessels which possesses a considerable degree of independence from the circulation at large and is capable of responding selectively to influences of both local and general origin.

In the first chapter of this thesis, the influence of ascorbic acid is reported on the appearance of oedema and on the formation of new capillaries in the cornea of guinea-pigs, consequent to a standard heat lesion. Both control and scorbutic guinea-pigs are studied. The mode of action of ascorbic acid on capillary permeability and on capillary vascularization is discussed. The action is considered either as a purely local effect in the cornea, or as a local manifestation of modification of the stress syndrome.

Rightly or wrongly, physiological and pharmacological observations on the behaviour of perfused blood vessels

in isolated tissues seem not to have been carried out with the same consideration for control of variable factors as have observations on the behaviour of tissues themselves in vitro.

The vascular bed of the isolated ear of the rabbit is a well-established preparation for the study of vascular responses. Chapter 2 describes an attempt to control and to standardize the technique used in perfusion of the blood vessels in the isolated ear of the rabbit. To this end the behaviour of the vessels is observed, not by recording the rate of outflow of the perfusate, but by measuring the rate of inflow. Thus a more accurate index is obtained of the behaviour of the arterial portion of the vascular bed. Moreover, a special constant pressure system which had been devised, is used, the tension of oxygen in the perfusion solution is enhanced and environmental influences are kept as steady as possible.

The blood vessels of the ear of the rabbit are known to respond delicately to the temperature of the animal's body. The present investigation shows that, even in the isolated preparation, this responsiveness of the ear vessels to changes in temperature of the fluid flowing through them, still persists. Moreover, the work described in Chapter 2 shows that

it is possible to obtain some concept of the differential behaviour of parts of the vascular tree in the isolated preparation at different temperatures of the perfusion solution.

Section, with subsequent degeneration, of the nerves to the blood vessels of the ear leads to alterations in the responses of the vessels to different temperatures of the perfusion solution. These changes are described in Chapter 3.

By mathematical treatment it is possible to obtain a picture of the movement of fluid from the vessels into the tissue spaces and from the tissue spaces back into the vessels. This analytical portion of the work is described briefly in Chapter 2. The mathematical derivation is given in detail in Chapter 4, together with the practical evaluation of 2 of the analyses whose results are used in Chapter 2.

In Chapter 5 a detailed evaluation is made of the fluid mechanics acting in an improved constant pressure device which was used in the perfusion experiments on the rabbit's ear. Other applications of this constant pressure device in experimental physiology are described.

Throughout all these experiments, I have been fortunate in having the collaboration of other workers, and I would particularly like to express my indebtedness to them.

CHAPTER 1

OEDEMA FORMATION AND VASCULARIZATION IN THE CORNEA OF THE GUINEA - PIG FOLLOWING INJURY BY HEAT

CONTENTS

INTRODUCTION

The influence of lack of ascorbic acid on the rate of healing of heat injuries to the cornea of guinea-pigs was studied by Campbell, Ferguson & Garry, (1949, 1950). The healing of superficial lesions, confined to the corneal epithelium, was not found to be impaired by a deficiency of ascorbic acid. On the other hand, we found that deeper lesions, involving the substantia propria of the cornea, healed more slowly and were structurally weaker in deficient guinea-pigs. This difference was presumably due to a defective formation of collagen in the scorbutic animals.

During this investigation I took the opportunity to observe the incidence and progress of oedema formation in, and of vascularization of, the cornea following a heat injury (Campbell & Ferguson, 1950a, b). These observations on oedema formation and vascularization were made both in normal controls and in scorbutic animals.

Although the cornea normally has no blood vessels, yet, under certain conditions, new vessels freely enter the substance of the cornea from the limbal plexus. Cogan (1949) believes that vascularization is always preceded by oedema of the cornea involving the tissues of the limbus.

METHODS

Animals. Female non-pregnant guinea-pigs were used. Their initial body weights were between 450 and 650 g.

Diet. The basal diet was crushed rat-cake cubes (Thomson, 1936) moistened with a little water. To supplement this diet 6 drops of cod liver oil were added daily to the cubes eaten by each animal.

Ascorbic acid. These rat cubes are free from ascorbic acid. To ensure that the cavies had a uniform initial level of saturation with ascorbic acid, I gave 20 mg ascorbic acid (Roche) orally in 0.5 ml. water once per day. The ascorbic acid tablets were ground to a fine powder in a mortar and pestle, and were suspended in the water immediately before administration to the guinea-pigs. The required volume of the suspension was then given orally from a pipette. This intake of 20 mg ascorbic acid was given for 21 days in all cavies in order to obtain tissue equilibrium (Jones, Bartlett, Ryan & Drummey, 1943)

Control animals. Control animals were injured after 21 days of saturation with vitamin C and the daily administration of 20 mg ascorbic acid was continued thereafter.

Scorbutic animals. After a preliminary 21 days of saturation with 20 mg ascorbic acid per day, the animals were given 0.5 mg ascorbic acid every second day for a further 21 days. The injuries were then made to the animals, the dosage of 0.5 mg ascorbic acid every second day being continued thereafter.

The scorbutic guinea-pigs were less active, ceased to gain weight and had an increased liability to respiratory infection.

Apparatus. The lesions were produced with a cautery made from a loop of 32 S.W.G. platinum wire. A predetermined constant voltage was fed to the cautery through a relay connected to the 1 sec contacts of a Palmer A.C. time clock. This circuit allowed the current to flow through the cautery for exactly 1 sec when required.

Technique. The cornea was anaesthetized by instilling into the conjunctival sac 2 drops of a 2% pontocaine hydrochloride solution. The subsequent operations did not cause discomfort since the corneal reflex was never elicited and the animals remained quiet. No signs of distress appeared after the operation and in no case did infection occur.

The cold cautery was pressed firmly and vertically on the cornea 2 mm from the limbus at 12 o'clock. The current

was allowed to flow for exactly 1 sec and the cautery was removed 1 sec later. This gave a standard heat injury similar to that used by Campbell & Michaelson (1949). Histological examination showed that the resulting lesion was 1 mm in diameter, and that the corneal epithelium and the anterior 2/3 of the substantia propria were destroyed. I gave each guinea-pig in both the control and the scorbutic groups a serial number. The order in which the corneal injuries were made was determined by the order in which these serial numbers appeared in a table of random numbers (Fisher & Yates, 1948)

All thermal injuries were carried out by Dr. F. W. Campbell, who was unaware whether a control or a scorbutic animal was being injured.

Examination of the injury. The degree of oedema formation and of vascularization was noted by me, at 8 hourly intervals day and night.

The observations were made with the aid of a binocular loupe and focal illumination. Once epithelial healing had occurred observations were continued at 24-hr intervals.

The method used to estimate the extent of corneal oedema was to note the corneal area which the oedema covered on a 12-hr dial drawing. The area of new vessel formation

was recorded in a similar fashion. The density of the corneal oedema and the density of the vascularization were recorded as 0, +, ++, or +++.

Although these methods of recording the oedema formation and the new vessel formation in the cornea were only approx-:imate, they were used since they allowed quick classification.

RESULTS

Oedema formation in the cornea. During healing, oedema formed in the cornea in all the control guinea-pigs and in all the deficient animals.

The time of onset and the time of maximum oedema formation are shown in table 1. The difference between the means of the times of onset is not significant. However, the scorbutic group of guinea-pigs did take significantly longer than the control group to reach the time of maximum oedema formation.

The figures could not be analysed for the time of disappearance of the oedema as some of the animals were killed for histological examination before the oedema had completely disappeared.

Vascularization of the cornea. During healing, vascularization of the cornea occurred in 9 out of 32 eyes in the control group of guinea-pigs, and in 19 out of 32 eyes of deficient animals (table 2).

The results were analysed by the χ - squared method ($\chi 2 = 5.1$). Such a result could occur by chance only less than 1 in 40 times, and it may therefore be presumed

that the greater incidence of vascularization in the scorbutic guinea-pigs is significant.

The onset of vascularization, the time of its maximum extent, and the time of disappearance all tended to be delayed in the scorbutic group compared with the control group of guinea-pigs (table 3).

D I S C U S S I O N

Nutrition of the cornea. Inadequate knowledge exists on the physiology of the fluid circulation within the cornea. It is known, however, that the cornea can obtain nourishment from 3 sources: firstly from the anterior chamber through the endothelium, secondly, directly from the capillaries of the limbus, or finally through the epithelium from the lachrymal fluid (Terry, 1939). Weekers (1940) considered that there was insufficient evidence to assess accurately the importance of each source in the normal metabolism of the cornea.

Ascorbic acid in the cornea. The cornea is known to contain high concentrations of ascorbic acid. Since the cornea is avascular, vitamin C may act as a hydrogen acceptor, and thus play an important part in the normal metabolism of the cornea.

There is some disagreement concerning the partition of ascorbic acid between the various layers of the cornea. Schmid & Bürki (1943), using a histochemical method, found the greatest concentration in the superficial layers of the corneal epithelium. The substantia propria had a lower content, although there was a high concentration in the

region of Bowman's and of Descemet's membranes.

Henkes (1946), on the other hand, who extracted and titrated ascorbic acid from various regions of the cornea, found no ascorbic acid in Bowman's membrane, a little in the corneal epithelium and a high concentration in the substantia propria and in Descemet's membrane. The sub-epithelial portion of the substantia propria contained the highest concentration of vitamin C. Pirie (1946), using microtitration, found the concentration of ascorbic acid to be greatest in the corneal epithelium.

Oxygen supply to the cornea. Bakker (1947) carried out experiments on rats in an air-tight chamber into which any desired gas mixture could be introduced. He concluded that the cornea in vivo did not obtain appreciable quantities of oxygen from the atmosphere. Bakker pointed out that a person asleep did not experience corneal changes, even although the cornea was cut off from atmospheric oxygen. However, there may well be a reasonably high oxygen tension in the closed conjunctival sac due to diffusion from the very rich vascular bed of the palpebral conjunctiva.

Langham (1951) carried out experiments in the rabbit with the cornea in vivo. He estimated the concentration of lactic acid in the cornea following exposure to gaseous

environments either of oxygen or of nitrogen. Langham concluded that *in vivo* the cornea may utilise oxygen directly from the air. His results also indicated that oxygen can diffuse through the layers of the living cornea in both directions.

Oedema formation in the cornea

It is impossible to establish from the present experiments whether the ascorbic acid present or absent in the 2 groups of guinea-pigs acted either directly at the site of healing in the cornea, or indirectly through the metabolism of the adrenal gland.

Local action of ascorbic acid on oedema formation in the cornea. Of importance for the present work is the fact that in experimental scurvy in guinea-pigs, Henkes (1946) found that ascorbic acid disappeared from the cornea in from 2 to 3 weeks, although the glutathione content remained unchanged. Nevertheless, the uninjured cornea shows no obvious change even in severe and prolonged scurvy in guinea-pigs or in human beings. Ascorbic acid is known to be necessary for formation of collagenous tissue in the repair of wounds in the cornea as in other tissues (Beattie,

1947). Thus, the injuries inflicted to the cornea may have unmasked locally a deficiency not otherwise apparent.

Possibly the first event leading to fibre formation in wounds is the deposition of a mucopolysaccharide about the fibroblasts in homogeneous form. This is followed by the formation of very fine argyrophil fibres embedded in the homogeneous material. Thickening of these fibres occurs, while the mucopolysaccharide also takes a fibrous form, matures and becomes no longer demonstrable histologically. Duke-Elder (1938) reviews the literature on corneal wound healing. Penney & Balfour (1949) were unable to demonstrate the deposition of mucopolysaccharide in wounds in scorbutic guinea-pigs. Moreover, intramuscular injection of ascorbic acid to the deficient animals resulted in the appearance within 12 hr of mucopolysaccharide in the wound.

Another possibility is that the reparative processes after injury make additional metabolic demands. The breakdown products of injury must be removed, new formation of tissues has to take place. The delay in these processes, consequent on a lack of ascorbic acid, may explain the delayed onset of, and slightly more marked oedema in the deficient animals.

It may be also that part of the oedema formation in the scorbutic animals may be attributed to a defect in the walls of the capillaries of the limbus or in the newly formed vessels invading the cornea. It has been found that normal vascular endothelial cells give strong alkaline phosphatase activity. Investigations by Danielli, Fell & Kodicek (1945) have shown that alkaline phosphatase is strikingly decreased in regenerating connective tissues of scorbutic guinea-pigs. Obviously the possible role of an alteration in the vascular endothelium in the causation of oedema awaits further clarification.

The experimental evidence of endothelial proliferation in scurvy, and the subsidence of capillary fragility after vitamin C administration, was considered by Klemperer (1948) to support strongly the hypothesis that a defect in the cement substance between the cells, and not in the endothelial cells themselves, is responsible for the haemorrhagic tendency in scurvy. Thus there is the possibility that an increased permeability of the cement substance was a factor contributing to the oedema formation in the scorbutic animals.

Ascorbic acid and the metabolism of the adrenal cortex. It was possible that ascorbic acid acted indirectly on the

oedema formation in the cornea by influencing glucocorticoid formation in the adrenal cortex.

Clayton & Prunty (1951) found that there is an increase in the activity of the adrenal cortex during scurvy in guinea-pigs. This work was confirmed by Bacchus & Heiffer (1953). These authors (Bacchus, Altszuler & Heiffer, 1952) also showed that ascorbic acid depressed the breakdown of cortical hormones to 17-ketosteroids. Simultaneous ascorbic acid treatment of adrenalectomized female rats receiving cortisone acetate, resulted in a decrease of urinary 17-ketosteroids and an increase of the urinary corticosteroids (Bacchus, 1952).

The mechanism of the control of the secretion from the adenohypophysis of adrenocorticotrophic hormone (ACTH) was reviewed by Harris (1951a, b). Administration of ACTH depletes the adrenal ascorbic acid in the guinea-pig and the rat (Sayers, Sayers & Woodbury, 1948). In the hypothysectomized rat, adrenal ascorbic acid is unaffected by stress, although the same degree of stress in a normal animal would lower the ascorbic acid concentration (Sayers, Sayers, Liang & Long, 1945). This finding suggests that physiological quantities of ACTH do influence the ascorbic acid content of the adrenal gland (Sprague, Power, Mason, Albert, Mathieson,

Hench, Kendall, Slocumb & Polley, 1950).

Clayton & Prunty (1951) considered that the balance of evidence favoured the view that adrenal cortical activity is increased in scurvy, and can be further increased, in the absence of vitamin C, by ACTH.

An increased adrenocortical activity is associated with a reduction in the concentration of the adrenal ascorbic acid (Lowenstein & Zwemer, 1946). However, Vogt (1948) was unable to detect a significant difference in ascorbic acid concentration between the blood entering and that leaving the adrenal gland under conditions of increased activity.

On the other hand, there is considerable evidence to suggest that ascorbic acid does not play an essential role in the metabolism of the adrenal cortex (Sayers, 1950). It is possible that some other constituent of the gland, possibly glutathione, can substitute for the vitamin in these metabolic processes of the adrenal which are concerned with the secretion of cortical hormones.

Indirect action of ascorbic acid via the adrenal cortex on oedema formation in the cornea. The heat injury to the cornea must have acted as a stress to the guinea-pigs in both the control and scorbutic groups. Selye (e.g. 1950)

has emphasized in biology, the importance of stress as the interaction between damage and defence. Following injury, the body requirement for ascorbic acid is greater (Beattie, 1947). This greater requirement is possibly due to a greater utilization of ascorbic acid in the adrenal cortex and in the liver, as well as to the greater needs of the tissue at the site of injury. The latter factor has already been discussed. Long (1950) considered that a preliminary release of adrenaline was at least a contributing factor in the secretion of ACTH following stress. Certainly adrenaline is effective in decreasing the adrenal ascorbic acid content, and in releasing adrenal cortical hormones (von Euler, 1951).

Scarborough (1944) demonstrated that the resistance of skin capillaries to rupture by negative pressures, rises sharply after many types of surgical operation. Robson & Duthie (1950) suggested that the rise in capillary resistance may be due to adrenocortical stimulation, by the release of endogenous adrenaline or some similar mechanism.

A detailed review of the effects of cortisone has been made by Carlisle (1950). If gluco-corticoids were released following the heat injury to the cornea, they would tend to inhibit the oedema which would be formed in this mesenchymal tissue (Mulinos & Christakis, 1953; Schaffenburg, Masson &

Corcoran, 1950).

In the present experiments such a factor, however, would operate in both groups of guinea-pigs. Moreover, it is very unlikely that the slight injury to the cornea would act as a sustained stressor. Since all the guinea-pigs had previously become accustomed to handling by me 3 times daily, it is not considered likely that the local examination of the eye would act as a significant stressing stimulus.

Nevertheless, the review of the literature has shown that there would be increased activity of the adrenal cortex in the guinea-pigs in the scorbutic group, relative to those in the control group. The finding in the present investigation that the scorbutic guinea-pigs did take significantly longer than the control group to reach the time of maximum oedema formation, would be consistent with a greater activity of the adrenal cortex in the scorbutic animals, thereby releasing more gluco-corticoids and thus delaying the oedema formation.

Vascularization in the cornea

The oedema, however formed, would result in opening up of the lamellae of the substantia propria. Thus the oedema may act mechanically permitting invasion of blood vessels.

Cogan (1949) believes that vascularization of the cornea never occurs without oedema of the corneal stroma. This suggestion had previously been made by Julianelle & Lamb (1934) and by Mann & Pullinger (1942). These authors consider that the presence of oedema fluid in the cornea opens up the spaces between the fibres of the substantia propria and this in turn may allow ingrowth of the limbal capillaries. Similarly, subsidence of the oedema may produce occlusion of some of these newly formed vascular channels. It is, however, difficult to believe that this physical factor is the only one operative in the formation of new vessels in the cornea. Thus in the present experiments the degree of oedema was no greater in the corneae that became vascularized.

Meyer & Chaffee (1940) and Bacsich & Riddell (1945) look at the problem from a somewhat different angle. They believe that avascular tissues, cornea, cartilage, and Wharton's jelly, normally contain a substance inhibiting

vascularization. One could postulate that injury to the cornea destroys or inhibits formation of this hypothetical substance, thus permitting invasion of capillary vessels.

Local action of ascorbic acid on vascularization in the cornea. There is the other possibility that the metabolites may act as a direct stimulus to new blood-vessel formation. Just as ariboflavinosis evokes spontaneous vascularization of the cornea, avitaminosis C may lead to vascularization when there is the extra metabolic demand following on injury. Since riboflavin is part of an oxidation enzyme system, Bessey & Wolbach (1939), and Johnson & Eckardt (1940) believe that anoxia is a stimulus for corneal vascularization.

Corneal wounds heal both by sliding of the epithelium (Friedenwald & Buschke, 1944; Mann, 1944) and by mitotic activity in the epithelium (Smelser & Ozanics, 1945). The increased cellular activity in response to the lesion will result in an increased oxygen demand. This may produce an area of relative anoxia around the lesion. This low oxygen tension may act as a stimulus to new vessel formation from the limbus if the lesion is sufficiently close to it. Moreover, due to the loss of vitamin C in its role as a hydrogen

acceptor, it may be that this resulted in a relatively greater local oxygen deficiency and hence more new vessels in the scorbutic animals.

Indirect action of ascorbic acid via the adrenal cortex on vascularization in the cornea. Certain gluco-corticoids such as Compound E, are known to depress granulation tissue formation in the rabbit (Ragan, Howes, Plotz, Meyer & Blunt, 1949). Moreover, cortisone has been shown to cause a diminution of vascularity in granulation tissue (Spain, Molomut & Haber, 1950).

Jones & Meyer (1950) reported that subconjunctival injections of cortisone acetate in the rabbit markedly inhibited the vascularization of the cornea which follows intracorneal injections of sodium hydroxide solution. Campbell (1952) showed that cortisone delays healing in our standard corneal injuries. He concluded that adrenocortical hormones acted on corneal vascularization not directly but indirectly, probably through their effects on tissue regeneration.

Thus it would seem likely that the greater vascularization of the cornea in the scorbutic guinea-pigs would be consistent, as was the slight difference in oedema formation,

with the view that it could be attributed to greater activity of the adrenal cortex in scurvy.

Moreover, it is said that stress, such as injury, in a biologically most illogical fashion, evokes the secretion of cortisone. The injuries inflicted on the corneae of the guinea-pigs may just possibly have acted as a stress. The cortisone thus liberated, especially in the scorbutic cavies, may have delayed healing leading to greater vascularization.

SUMMARY

1. A method is described for producing small standard heat injuries to the cornea.

2. These injuries were inflicted on control and scorbutic guinea-pigs.

3. The scorbutic group of guinea-pigs took significantly longer than the control group to reach the time of maximum oedema formation.

4. New vessel invasion of the cornea following the injury occurred with significantly greater frequency in the scorbutic group than in the control group.

5. The possible causal factors in oedema formation and in new vessel formation in the corneae of the control and scorbutic guinea-pigs are discussed in the light of these findings. The ascorbic acid present or absent in the 2 groups of guinea-pigs may have had an effect either locally at the site of healing in the cornea or indirectly through the metabolism of the adrenal gland.

CHAPTER 2

CAPILLARY FILTRATION AND VASOMOTOR ACTIVITY IN THE PERFUSED ISOLATED EAR OF THE RABBIT

CONTENTS

FURTHER WORK

Observations on the formation of oedema and on the new formation of blood vessels in the cornea of the guinea-pig after injury give but little scope for controlled investigation of the functioning of the smaller blood vessels.

At this time I was asked to supervise the work of an Honours Science undergraduate for the thesis he had to present at the end of his final year. My mind was still running on the problem of formation of oedema so I suggested to the candidate that he should find the effect of a fraction of "Dextran", a polysaccharide, on the rate of formation of oedema when perfusing the hind limbs of a frog by the Laewen - Trendelenburg technique (Trendelenburg, 1910). Using this method the rate of formation of oedema is assessed by weighing the isolated preparation. The candidate quickly found that the amphibian preparation was far from ideal for the purpose since the capillaries of the frog do not offer the same complete barrier to molecules of large size as they are supposed to do in mammals. Consequently I turned the student's attention to the effect of "Dextran" on the rate of formation of oedema when perfusing the isolated ear of the rabbit. This preparation was satisfactory for

his purpose.

The blood vessels of the isolated ear of the rabbit show a considerable degree of independence from the central nervous system and continue to respond to factors of local origin. I realised, consequently, that consistent results could be expected only by much more rigid control of the conditions of perfusion than is usually practised. Therefore the rest of my thesis is largely concerned with establishing control of variable factors in technique and in investigating the effect of one main variable, viz. temperature, on capillary filtration in the perfused isolated ear of the rabbit. In addition, by the use of an improved constant pressure device, it proved possible to make accurate measurements of inflow thus providing an assessment of vasomotor activity in the perfused isolated ear of the rabbit.

INTRODUCTION

Malpighi demonstrated the existence of capillaries in 1661, four years after the death of William Harvey, and now almost 3 centuries ago, but it has been mainly in the last 3 decades that the most rapid advances in knowledge of the functions of capillaries have been made (Krogh, 1922; Landis, 1926, 1927a, b; and Lewis, 1927). Lucké & McCutcheon (1932) reviewed existing knowledge of the permeability of the living cell to water and Pappenheimer (1953) has critically assessed the passage of molecules through capillary walls.

Boyd (1952) discussed arterio-venous anastomoses throughout the body. Grant (1930b) and Clark (1938) summarised much new knowledge on arterio-venous anastomoses in the rabbit's ear. These anastomoses are present in all parts of the rabbit's ear, but are most numerous on the dorsal surface of the thinner portion and are situated in the deeper layer of the skin and on the surface of the underlying perichondrium. Most of the arterio-venous anastomoses in the rabbit's ear are tortuous vessels, arranged in groups.

Vascular anatomy of the rabbit's ear

No adequate description of the course and relationships of the main arteries and veins of the rabbit's ear exists in the literature. The best is that given by Jackson (1939).

Functioning of differently structured vessels in the capillary bed

Starling's hypothesis (1896a, b) of capillary hydrostatic pressure versus colloid osmotic pressure, must be supplemented by taking into account the topographical arrangement and functioning of differently structured vessels in the capillary bed.

The capillary bed (fig. 1) possibly may consist of (1) preferential or main thoroughfare channels which are proximally muscular (metarterioles), and which, since they course from the terminal arterioles to the beginning venules, are sometimes designated arterio-venous bridges; (2) sphincteric offshoots from the main thoroughfare channels which control the flow of blood into the capillary network; and (3) the non-muscular, true capillaries which constitute the bulk of the bed and drain into the distal portions of the channels (Chambers & Zweifach, 1944; Zweifach, 1937, 1940). Gilding (1951) defined the true capillary as beginning at the point where the investment of connective tissue and muscle ceases,

and continuing until these structures reappear at the venous end of the vessel.

Chambers (1948) considered the preferential or thoroughfare channels as the basic structural components of the bed. He postulated that they maintained a consistent degree of outward filtration due to a high hydrostatic pressure, and that the network of capillaries was built about them and was accessory to them. The network characteristically served for inward filtration due to a low hydrostatic pressure. Two main features are controlled in this type of circulation. One is the possibility of a shift in the balance of outward and inward filtration within the capillary bed, either by keeping the blood flow predominantly in the preferential channels or by dispersing it throughout the true capillaries. The other feature is the ability of the capillary circulation to permit local tissue conditions to outweigh outside influences.

The controlling factor is vasomotion.

Vasomotion

Vasomotion is a term applied to relaxation and contraction of smaller blood vessels. Such movements appear to occur rhythmically and spontaneously, but they are also naturally

controlled by the vasomotor nervous system.

Clark & Clark (1932) constructed a transparent tissue observation chamber permitting visualisation of the smaller vessels of the ear of the intact rabbit. The arteries and arterioles were seen to exhibit spontaneous rhythmical contractions, which varied in phase from vessel to vessel, and even in different parts of the same vessel. These periodic movements greatly influenced the distribution of blood to different areas.

Large numbers of arterio-venous anastomoses were observed in the rabbit's ear (fig. 2) connecting the terminal arteriole to the collecting venule. These were actively contractile, but each with an individual rhythm (Clark & Clark, 1934a, b). No active contractility of the true capillaries was observed.

Holton & Holton (1952) recorded changes in light absorption of the rabbit's ear. They interpreted their results as due to changes in the total amount of blood in the rabbit's ear and made the further assumption that this was primarily dependent on capillary dilatation and constriction. The latter assumption is unjustifiable since dilatation or constriction of arterio-venous anastomoses would markedly alter the total amount of blood in the

rabbit's ear.

The term 'precapillary sphincter' has been used (Zweifach, Chambers, Lee & Hyman, 1948) to designate the immediate junctional component of the capillary offshoot which is encircled by 1 or 2 well-defined muscle cells. These muscle cells permit this strategically located component to maintain a sphincteric control of the blood flow into the capillary network.

As a result of the observations on the small vessels in the mesentery of dogs, Chambers & Zweifach (1944) drew attention to the importance of vasomotion of terminal arterioles and precapillary sphincters in the regulation of the flow of blood through the capillary bed, and in the maintenance of the processes of filtration and absorption across the capillary wall. They observed that an increase in temperature above 37.5°C resulted in dilatation and in decreased vasomotion.

Rhythmical active contraction of small vessels has also been studied in detail by Nicoll & Webb (1946) in the subcutaneous tissue of the living bat's wing. They noted the presence of either irregular or rhythmical vasomotion. Rhythmical activity involved the arteries, arterioles, precapillary sphincters and veins. Such rhythmical activity

survived denervation and degeneration of the vasomotor nerves.

To sum up, deficiency or absence of vasomotion shifts the balance of fluid exchange in favour of outward filtration, while enhanced vasomotion shifts the balance to inward filtration (Chambers, 1948). It follows that some types of oedema are the result of disturbances in the vasomotor mechanism, and do not necessarily involve alterations in the permeability of the capillary wall.

Intravascular environment

There is a comparative dearth of information concerning vascular responses to changes in temperature from within the vessel, compared with the multiplicity of observations which have been made on the effects of changes in external temperature. Lui (1894) was the first to study this problem. By perfusing the vessels of the isolated hind limb of the dog with physiological saline at different temperatures, he observed a transitory vasodilatation, followed by marked spasm, at a fluid temperature of 49°C. This rapid contraction of the vessel wall was not unexpected, since the very high temperature of the perfusion solution must have been lethal to the tissues causing coagulation.

Lui also observed that immediate vasodilatation occurred when the preparation was perfused with solution at 33°C.

Vascular responses to alterations in the temperature of Ringer-Locke perfusion solution between 10° and 40°C were first studied in the isolated rabbit's ear by Pissemski (1914). He recorded the occurrence of a slower rate of flow at the lower temperatures. More recently, however, Pappenheimer, Eversole and Soto-Rivera (1948), during similar experiments on the isolated hind limbs of the cat, recorded a higher rate of inflow at a constant temperature of 10°C than at a temperature of 40°C. These experiments of Pissemski and Pappenheimer et al, are not, however, irreconcilable since the blood vessels of the rabbit's ear are superficial in the skin, whereas in the hind limb of the cat, the bulk lie in the deeper tissues in muscles. It must be appreciated that, in spite of an apparent histological similarity of blood vessels throughout the body, the responses of a particular vascular bed to a particular stimulus cannot be regarded as an indication of the responses which would occur in another vascular bed to the same stimulus.

External environment

That raising or lowering the temperature of the external

environment of the vessels of the extremities results in an increase or decrease respectively in the rate of blood flow, has long been known (e.g. Hewlett, van Zwaluwenburg & Marshall, 1911; Carrier, 1922; Goldschmidt & Light, 1925). Mosso (1889) recorded, however, vasodilatation in the human arm immersed in water at 5°C. This he attributed to a paralysis of the smooth muscle of the vessel wall, resulting from the local action of the cold fluid on the vessel wall.

Piotrowski (1894) using isolated strips of arteries and veins noted dilatation when these vessels were cooled, and contraction when warmed.

A similar, but more critical investigation, by O'Connor & Edozien (1952) demonstrated that, when isolated strips of ox external carotid arteries in an atmosphere of O_2, 6% CO_2 and water vapour, were heated from a low temperature, contraction occurred at about 15°C. Above this temperature the vessel wall relaxed, but at about 34°C contraction of the vessel occurred again. The temperatures at which these contractions took place were influenced by the load on the arterial wall. These authors assume that the tone of the muscle fibres in blood vessels is a surface tension effect and that variations in tone with change of temperature are a consequence of the influence of monomolecular layers of

fatty acids on surface tension. O'Connor & Edozien consider that the responses to environmental temperature of peripheral blood vessels are predominantly local responses independent of the central nervous system and that vascular responses in isolated tissue are typical of the responses of blood vessels *in vivo*. This hypothesis receives no general support.

Lewis (1926) postulated that the minute vessels, the terminal arterioles, capillaries and small venules, responded mainly to the local metabolic needs of the tissues, while the tone of the arteries and muscular arterioles was mainly under nervous control. Subsequently, Lewis (1930) demonstrated that the finger immersed in cold water (10°C) had a more rapid flow of blood than when immersed at a temperature about 20°C. This phenomenon was, however, restricted to certain well defined areas where arterio-venous anastomoses were demonstrable histologically.

Hoyer (1877) showed convincingly that arterio-venous anastomoses in the rabbit were confined mainly to outlying parts of the body, such as the ears and tip of the snout. He suggested that they played a part in maintaining the warmth of these parts when exposed to cold.

In recent years more detailed examination of these

vessels has shown that they vary in diameter from 6 to 60 microns, and consist of 3 functionally different parts (fig. 2). A proximal or arterial portion which varies in diameter with variations of the parent vessel, an intermediate segment which is the narrowest and most muscular part of the vessel and is independently contractile, and a wide thin-walled distal or venous portion. These vessels are either direct short communicating channels, or pursue a lengthier and more tortuous course from the arterial to the venous circulation. The vessels are accompanied by a very rich nerve supply.

Grant (1930b) suggested that, at least in the intact rabbit, arterio-venous anastomoses appeared to play a part in the regulation of body temperature. Grant & Bland (1931) suggested that possibly in man as well, arterio-venous anastomoses constituted a defence mechanism for the protection of the extremities against exposure to extreme cold. Grant, Bland, & Camp (1932), using direct microscopy, demonstrated a consistent pattern of response to alterations in body temperature, and to alterations in local or general environmental temperature. They noted that these vascular changes were largely independent of the nervous system, since they persisted in the ear deprived of both sensory and sympathetic

nerves. However, van Dobben-Broekema & Dirken (1950a) using a modified Clark's chamber found that the capillaries, but not the arterio-venous anastomoses, of the rabbit's ear consistently responded to heating the rabbit's body by dilatation.

The present concept of the possible functions of arterio-venous anastomoses (Hürlimann & Bucher, 1950) is that they increase venous pressure, take part in the regulation of temperature and protect the capillary networks against stress.

Perfused isolated preparation of the ear of the rabbit

Because of its high vascularity and of the relative absence of voluntary muscle, the perfused isolated ear of the rabbit has been used for a long time to study the actions of physiological factors and of pharmacological agents on the peripheral vessels (von Anrep, 1912; Armin & Grant, 1953; Pissemski, 1914). In recent years the rabbit's ear has served as a useful preparation for the assay of vasoconstrictor substances, such as adrenaline and nor-adrenaline (Burn, 1950; Burn & Robinson, 1951). It must be remembered however, that the rabbit's ear is a very specialised organ.

It is an important mechanism in the rabbit for the control of body temperature. Care must therefore be taken in the interpretation of the responses of the vessel of the rabbit's ear to stimuli, as these responses need not be typical of those in the vessels of the peripheral circulatory system in general when exposed to similar conditions. Nevertheless, it is unlikely that capillary permeability, per se, will be different in the rabbit's ear from that in other parts of the circulation.

Although many results have been published recording the effects of particular stimuli on the isolated ear of the rabbit, there is little unanimity of opinion as to the physical conditions under which these perfusion experiments should be carried out. The most striking divergence of opinion concerns the choice of temperature of the perfusion solution, and the regulation of environmental conditions. Page (1942) advised most elaborate precautions to ensure that the perfusion solution had a temperature constant at 37°C. He also regulated carefully the environmental temperature. Burn (1952) and Burn & Robinson (1951) used perfusion solutions at room temperature, without mention of controlling the environmental conditions. On the other hand, West (1950) indicated that the temperature of the perfusion solution is

not of great importance. Consequently he advised perfusion of the ear with unheated solution 'since perfusion at 37°C does not seem to have any special advantage.'

Does this conflict of opinion perhaps signify that the vessels of the isolated ear, as opposed to the vessels of the intact ear, (Grant, 1930a), remain in precisely the same state irrespective of the temperature? This does not seem at all likely since Landis, Wood & Guerrant (1945) demonstrated clearly the rapidity with which the vessels of the isolated ear of the rabbit reacted to sudden cold draughts.

The present work was planned to study the effects of various factors on the vascular responses of the isolated ear of the rabbit. The factors which were altered, under controlled conditions, were the temperature, pressure, oxygen content and pH of the perfusion solution, the temperature of the environment of the ear and the presence or absence of hyaluronidase in the perfusion solution. To study the effects of these factors on the vascular responses of the isolated ear of the rabbit, the rate of net outward filtration of fluid from the capillaries into the interstitial spaces of the ear, oedema formation, was measured by weighing the ear, and the rate of inflow of perfusion solution was recorded. Thus alterations in capillary filtration, area of the capillary

bed and net peripheral resistance could be demonstrated.

The isolated preparation was chosen instead of the intact ear since less knowledge was available about the isolated ear, and since it was considered that the more accurate control of a large number of the experimental variables which was possible in the isolated preparation, would yield greater knowledge of capillary and vasomotor responses. It has been concluded (Ferguson & Levinson, 1952a, b) that the responses to temperature of blood vessels in the perfused isolated ear of the rabbit seem to be similar in character to those in the intact ear.

METHODS

Histological

Injection mass. Burt (1928) pointed out that latex of the rubber tree is normally of milky consistency, but coagulates to form rubber in the presence of dilute organic acids, e.g. acetic acid, or in the presence of potassium oxalate. Moreover, the latex can be coloured by the addition of aniline pigment. Since latex is coagulated by dry material and by vibration, any pigment to be added must be wet to start with, and the rubber latex must not be violently agitated. As a precaution against large aggregates of pigment, it is wise to strain the coloured latex through muslin.

Coloured rubber latex is an excellent injection mass for demonstration of either the arterial, or by retrograde injection, the venous vascular anatomy. In addition, by appropriate dilution with distilled water, it is possible to inject the vascular system using a mass which will fill even the capillary vessels.

The general technique is to wash out the animal's blood with 0.85% NaCl solution containing a trace of sodium bicarbonate, and to follow this up by the appropriately

coloured injection mass. A small pad of cotton wool soaked in acetic acid, rapidly seals off any leaks from cut or torn vessels. The animal is subsequently totally immersed in a dilute acid bath to coagulate the latex in situ in the vascular tree.

Rubber latex solution in water is available from:
(1) I.C.I. Ltd., Dyestuffs Division, Blackley, Manchester
(2) Revertex Co., Ltd., Arthur St., London
(3) Typke & King, Birtley, Co. Durham.

Aniline pigments are available from I.C.I Ltd. Monolite fast scarlet in RNVS paste was used for the injection of the arterial system and monastral fast blue in BVS paste for the venous system.

In the present investigation ofthe vascular anatomy of the rabbit's ear, latex neoprene 601A was used, after it had been coloured by means of dyes, and diluted with distilled water until the desired viscosity was obtained. Injections of a rather viscous neoprene were made at a pressure of 100 mm Hg into the left and right ventricles of intact rabbits, in order to outline the arterial and venous patterns respectively in the rabbit's ear.

Perfusion of the isolated ear under controlled environmental conditions was carried out at a perfusion solution

temperature of 16°C for ½ hr, then neoprene of low viscosity and at the same temperature was perfused for a few min.

When the vessels were seen to have become filled with the dye, the injection was stopped. The ear was placed in a solution containing 10 ml. 40% formalin, 5 ml. glacial acetic acid and distilled water to 100 ml. A few hr later the skin, subcutaneous and perichondrial tissue were dissected free from the cartilage, and the injected vessels in the skin were examined under a binocular microscope. Hongo & Luck (1953) give the details of a better method of clearing tissues injected with coloured latex which does not make the specimen excessively hard while still not destroying the rubber. The present injections were carried out before this paper appeared.

Attempts were also made to perfuse the isolated ear with neoprene at 38°C. As can be seen under the heading 'Results', these attempts failed.

Experimental

The composition, pH and temperature of the Ringer's perfusion solution, the perfusion pressure and the temperature of the air surrounding the ear were all controlled

at chosen values. Two indices were used as a measure of the vascular responses:

(1) the weight of the ear. This was found at 15 min intervals and gave the rate of formation of oedema. Oedema is an index of the net outward filtration, which depends on many factors. These factors are the degree of capillary permeability, the extent of the capillary area, the perfusion pressure, the interstitial pressure, the composition of the perfusion solution and possibly vasomotion. Mathematical analysis permitted an evaluation of the effect of these factors together, except the interstitial pressure, on net outward filtration. Interstitial pressure was evaluated separately. In some experiments the interstitial pressure was measured experimentally.

(2) the rate of inflow of the perfusion solution as an index of the net peripheral resistance. This was measured also at intervals of 15 min.

Composition of the perfusion solution. A Ringer's fluid was used as the perfusion solution. The composition was 0.9 NaCl, 0.04KCl, 0.025 $CaCl_2$, 0.005 $MgCl_2$, 0.05 $NaHCO_3$ and 0.005 NaH_2PO_4% (W/V). Expressed in terms of molarity this is 0.154 $\underline{\underline{M}}$ - NaCl, 0.0054 $\underline{\underline{M}}$ - KCl, 0.00225 $\underline{\underline{M}}$ - $CaCl_2$,

0.000052 M - $MgCl_2$, 0.00596 M - $NaHCO_3$ and 0.000417 M - NaH_2PO_4. This solution was freshly made about 1 hr before each experiment.

In a number of the perfusions, hyaluronidase (4.5 mg/l.; 1 mg = 100 Benger units) was added to the solution just before the experiment commenced since the enzyme is rapidly destroyed by dilution in salt solutions (Chain & Duthie, 1940).

pH of the perfusion solution. The pH of the solution was adjusted to between 7.3 and 7.4 by the addition of 0.1 N-HCl. The solution was oxygenated and pressurized by a mixture of 95% oxygen and 5% carbon dioxide which did not cause any change in pH value. If the solution was oxygenated and pressurized by 100% oxygen, the pH rose markedly to 8.0 when the solution was heated to 38°C. Consequently, unless otherwise stated, the mixture of 95% oxygen and 5% carbon dioxide was used.

In some experiments the pH of the solution was adjusted to other chosen values by the addition of 0.1 N-HCl or 0.1 N-NaOH, or the solution was pressurized by nitrogen gas.

Perfusion pressure and rate of inflow. The solution was delivered to a glass cannula at a constant pre-determined

pressure during the perfusion experiments by using an improved constant pressure device (Chapter 5) applied to 2 100 ml. burettes (Ferguson & Garry, 1952). The perfusion technique was such that the solution was constantly in equilibrium with a gas mixture of 95% oxygen and 5% carbon dioxide at a pressure considerably in excess of one atmosphere. It was hoped thus to diminish the degree of anoxia which must be present to a considerable extent in preparations perfused in conventional manner with aqueous solutions. As the solution left either burette, its place was taken by the gas mixture at any desired pressure above atmospheric pressure.

The rate of inflow was read directly on the burettes, the readings being taken at 15 min intervals, timed accurately by means of a stop watch. The 2 burettes, A and B, (fig. 3) were filled from below from a reservoir of perfusion solution, W. While one burette was discharging, its companion was refilled with perfusion solution from the reservoir, W.

A 10 l. reservoir, U, was filled, initially by displacement of water, with a mixture of 95% oxygen and 5% carbon dioxide. When required further gas mixture was passed into U under pressure from a cylinder. If necessary

the pressure in U could be decreased by turning a 3-way oblique bore stopcock E, thus allowing gas to escape through a valve V. U was connected to the side limbs of both the burettes, A and B. During the discharge of solution from either burette, the solution was oxygenated under pressure by the gas bubbles entering the burette from U. Thereby the solution was constantly in equilibrium with the gas mixture of 95% oxygen and 5% carbon dioxide at a pressure considerably in excess of one atmosphere.

In a few experiments, the perfusion solution was pressurized by pure nitrogen gas instead of the oxygen/carbon dioxide mixture.

The pressure in the reservoir, U, did not fall rapidly owing to its relatively large capacity, consequently the discharge of solution from the burettes was readily maintained at any constant desired pressure. The pressure of the perfusion solution immediately proximal to the cannula was measured on a mercury manometer, M, and maintained constant by adjusting the pressure in the gaseous reservoir, U. In all experiments, unless otherwise stated, the pressure in the manometer was maintained at 100 mm Hg to allow for the drop in pressure which took place across the cannula. The drop in pressure across the cannula varied depending on

the inflow rate. The pressure in the central artery of the ear just beyond the tip of the cannula thus varied, in almost all the experiments, between 60 and 70 mm Hg. This is approximately the normal value of the mean blood pressure, since Grant & Rothschild (1934), using a pressure capsule, estimated that the systolic blood pressure in the central artery of the ear of the intact rabbit normally lies between 70 and 90 mm Hg.

By allowing the solution from the burettes and cannula to discharge freely into air at several different pressures, it was ensured that the maximal rate of discharge of the apparatus was greater than the maximum rate of inflow during actual perfusion experiments. This also served to check that the highest perfusion pressure used during actual perfusion was lower than the critical pressure above which the rate of discharge from the cannula would decrease. Dodge & Thompson (1937) stated that the production of turbulence in a system increases and may double the resistance to flow. Turbulence will occur when the pressure and hence the velocity of flow is increased above a critical level and this would explain the decrease in flow through a perfusion apparatus consequent upon increase in pressure above a critical value. It may also be pointed out that turbulence is produced in

varying degrees if the apparatus is complicated by the introduction of impediments, even though they consist merely of angulation or change in calibre of the tube system.

Calibration of the cannula. With a very few exceptions the same cannula was used throughout the whole series of experiments. The cannula was made from glass tubing of 2 mm internal diameter. A short length of bicycle valve tubing was used to connect the cannula to the perfusion apparatus. The dimensions of the cannula were measured on a vernier microscope. The cannula (fig. 4) was gradually tapered since Melrose & Shackman (1951) have shown that the flow at several pressures through a gradually tapering cannula exceeded that through a non-tapering one of the same inlet and outlet diameters. The pressure-flow characteristics of the cannula were determined both by noting the manometric drop in pressure resulting from selected flow rates, and by flow measurements with free discharge resulting from selected pressures, assuming full pressure recovery from the velocity head.

In the first method (fig. 5) the cannula, C, was allowed to discharge into a wide bore rubber tube at chosen rates of

flow, controlled by a screw clip, S, near the distal end of the wide bore rubber tube. The lateral pressures, proximal and distal to the cannula, were measured by two mercury manometers, M_1 and M_2 respectively. The upstream manometer, M_1, was kept at a constant pressure of 100 mm Hg and the pressure registered on M_2 noted for the chosen rates of flow.

The pressure drop across the cannula orifice can be shown to equal the difference in pressure in mm Hg between the two manometers minus 1/13.6 times the distance in mm between the two menisci in the proximal limbs of the manometers. This theoretical relationship is derived as follows (fig. 5):-

H_1 and H_2, head of mercury registered in M_1 and M_2;

A and B, selected points;

r_1, length of water column from A to C;

r_2, length of water column from C to B;

$R = r_1 + r_2$ = distance between the two menisci in the proximal limbs of the manometers;

If P_A and P_B = pressure at selected points A and B

P_1 and P_2 = pressure proximal and distal to cannula orifice;

D_m and D_w = density of mercury and water (g/cm^3);

Therefore $P_A = D_m \cdot H_1$ (g/cm^2)

and $P_1 = P_A - D_w \cdot r_1$ (g/cm^2)

$$\underline{P}_B = \underline{D}_m \cdot \underline{H}_2 \quad (g/cm^2)$$

$$\text{and } \underline{P}_2 = \underline{P}_B + \underline{D}_w \cdot r_2 \quad (g/cm^2)$$

$$\underline{P}_1 - \underline{P}_2 = (\underline{P}_A - \underline{D}_w \cdot r_1) - (\underline{P}_B + \underline{D}_w \cdot r_2) \quad (g/cm^2)$$

$$= \underline{D}_m \cdot \underline{H}_1 - \underline{D}_m \cdot \underline{H}_2 - \underline{D}_w \cdot r_1 - \underline{D}_w \cdot r_2 \quad (g/cm^2)$$

$$= \underline{D}_m(\underline{H}_1 - \underline{H}_2) - \underline{D}_w(r_1 + r_2) \quad (g/cm^2)$$

$$(\underline{P}_1 - \underline{P}_2)/\underline{D}_m = \underline{H}_1 - \underline{H}_2 - \underline{D}_w \cdot \underline{R}/\underline{D}_m \quad (cm)$$

i.e. pressure drop across cannula orifice = $\underline{H}_1 - \underline{H}_2 - 1/13.6 \cdot \underline{R}$ (cm).

The second method of calibration used was to allow the cannula to discharge freely into air, while the pressure registered in the manometer immediately proximal to the cannula (fig. 3) was adjusted to various chosen levels. The rates of flow/min at different pressures were noted. In order that fluid would flow from the cannula there had to be pressure available:-

(1) to produce the kinetic energy of the flow

i.e. velocity head = $v^2/2g$

(2) to overcome the friction in the cannula.

In this method of calibration, the pressure to produce the kinetic energy of the flow would be almost entirely lost, since the velocity of the fluid discharging into air when compared to the velocity of flow through the cannula was almost entirely zero. During actual perfusion experiments, however, as the internal diameter of the cannulated ear

artery was not much larger than the internal diameter of the cannula, the pressure recovery due to velocity head would be considerable, making the calibration values for pressure drop across the cannula too high. A correction was applied for this in the cannula calibration by this method, by assuming that there would be little velocity head loss during the perfusion experiments. Consequently the calculated figure for pressure losses due to velocity head at the different rates of inflow were subtracted from the calibrated pressure losses.

Thus the drop in pressure across the cannula for different rates of inflow was obtained. By subtracting this pressure drop from the reading on the manometer, M, (fig. 2), which recorded the pressure proximal to the cannula, the downstream pressure for each experiment could be calculated. The pressure in the central artery of the ear just beyond the tip of the cannula was taken as the downstream pressure. In almost all the present experiments, the downstream pressure was of approximately the normal value of the mean blood pressure.

Temperature of the perfusion solution. The perfusion solution was heated by an electro-thermal heating tape, H,

(fig. 3) insulated by knitted glass yarn. The surface loading of the tape was approximately 2½ W/sq. in. tape, carrying a maximum current of 1 A. This tape was coiled round the curved tube, T, through which the perfusion solution flowed after leaving the burettes. An air trap, S, was incorporated into T near its distal end, to collect any gas coming out of solution due to heating of the perfusion fluid. The heating tape was connected in series with a variable resistor, G, and with an A.C. ammeter, F, having a range 0 - 1 A and a knife edge pointer. The temperature of the perfusion solution could thus be controlled accurately.

The temperature of the perfusion solution was recorded by a copper-eureka thermo-couple, J, inside a fine glass capillary wiich passed through the valve tubing, K, immediately proximal to the cannula. The thermo-couple junctions were made using low resistance solder. Cort (1952, personal commun.) suggested that better junctions would be achieved by carbon arc welding of the junction under liquid paraffin.

The small projecting ends of the glass capillary, containing the registering thermo-couple junction, and the valve tubing round it, were heat insulated from the local environmental temperature by covering them by a liberal coating of rubber solution. Aiken (1951) gave details of

a wire enamel for the same purpose.

The thermo-couple recorded the temperature on a string galvanometer to within 0.1°C. Calibration of the thermo-couple unit was carried out every 10 days initially, but subsequently al less frequent intervals. During the course of an experiment in which it was desired to use a constant temperature of the perfusion solution, only slight alteration of the variable resistor was occasionally required. Deliberate raising or lowering of the temperature of the perfusion solution was achieved by variation of the resistor, but several minutes elapsed before the temperature at J stabilised at the new level.

Environmental temperature of the ear. A 2 gallon polished metal tin, with a small aperture in the bottom, was hung over the rabbit's ear after cannulation in order to maintain constant the local environment. The environment of the ear could thus be maintained at any chosen temperature, with a high relative humidity and minimal air movement. A thermometer, N, (fig. 3) held in a rubber stopper in the top of the tin, registered the temperature of the air surrounding the ear. The ear could be observed, and its environmental temperature read, through cellophane windows cut in the

front and back of the tin.

The environmental temperature of the ear was raised by heating the tin from without by means of 100 W black electric bulbs, or lowered by keeping ice on the top of the tin and on a ledge inside the tin. Stabilisation of the environmental temperature took several min. The relative humidity of the air inside the tin was naturally high, due to the presence of fluid dripping from the perfused ear, while the air movement was relatively slight.

Cannulation of the central artery of the rabbit's ear. Adult rabbits of either sex were used. Their ears were all approximately of the same size. At least 1 hr prior to the removal of the ear, the rabbit was given an intramuscular injection of heparin (1000 units/kg. body wt.). This ensured that clotting did not take place in the small vessels of the ear following death (Abrahams, 1950; Godlowski, 1951). Inadvertent ommission of this procedure on 4 occasions caused, following cannulation, delay in the start of the flow of the perfusion solution and, throughout the perfusion, the rate of flow was grossly diminished. This apparently minor precaution could be of major significance

in the interpretation of results.

At this time, also, the hair over the base of the ear dorsally, was clipped short to facilitate the subsequent dissection.

An hour later the animal was killed by a blow over the back of the neck, care being taken to hold the ears forward to protect them from injury.

The central artery of the ear (the dorsal auricular artery) was exposed by a longitudinal incision in the skin of the dorsal surface close to the base of the ear. At the base of the ear the artery, central vein and auricular nerve lie close together. The artery is medial, and the nerve lateral to the vein. The artery was dissected free for about 3 cm from the point where it emerges from the deep surface of the levator auris muscle.

The artery was ligated proximally and cut proximal to the ligature. Then the ear was removed from the head of the rabbit and suspended, by a thread through its distal end, from the attachment, L, (fig. 3) to the pan of the sliding weight double beam balance, R. The artery was cannulated and the cannula tied in. The point of insertion of the cannula is shown in fig. 6. As soon as the cannula was tied in, the ear was weighed and the perfusion pressure

in the system raised to the desired level. Approximately 5 min elapsed between killing the animal and the commencement of the perfusion.

Before the perfusion would start satisfactorily, it was usually necessary to recut the ends of the 3 main veins at the base of the ear, in order to permit the perfusate to drip away. A filter funnel, Q, below the ear ensured that all the fluid dripping from the ear was trapped, and either led to waste or collected for analysis, as desired.

Rate of oedema formation. This was recorded by weighing the ear on the beam balance at 15 min intervals. The mode of suspension permitted throughout the experiment an accurate estimate of the weight of the isolated ear preparation without cessation of the perfusion. The assumption was made that the specific gravity of the oedema fluid did not vary significantly throughout the experiments.

Interstitial pressure. These measurements were carried out during some of the perfusion experiments by inserting a lumbar puncture needle shaft into the subcutaneous plane of the perfused rabbit's ear. The needle shaft was connected by a length of polythene tubing of 2 mm internal bore, to a

vertical glass tube of the same calibre, acting as a water manometer. The whole system was filled with perfusion solution before the start of the experiment and the polythene tubing temporarily occluded by a screw clip.

After the ear had been suspended, the artery cannulated and the perfusion commenced, the needle shaft was carefully inserted into the cut base of the ear in the subcutaneous plane until the needle tip was about half way up the ear. The fluid meniscus in the manometer was then brought to the level of the needle tip, the screw clip released, and the first reading taken shortly afterwards. This initial reading was arbitrarily used as zero interstitial pressure. During these experiments in which the interstitial pressure was recorded, it was not possible to record the rate of oedema formation of the ear, since movements of the balance during weighings caused alteration in the position of the needle in the ear.

Weight of the ear cartilage. Ears were removed from rabbits of different breeds, ages and body weights. The total weight of the isolated ear was measured, then the ear cartilage from each was dissected out and weighed.

Observations. Each perfusion experiment was continued for 5 hr during which time the following readings were taken at intervals of 15 min: (1) volume of inflow; (2) wt. of preparation or interstitial pressure; (3) temperature of the perfusion solution; (4) environmental temperature; (5) pressure of the perfusion solution. The 15 min interval between recordings was chosen as the shortest convenient time to permit one experimenter to control the experimental conditions and to observe and record all readings.

Oxygen content of perfusion solutions

All estimations of dissolved oxygen were made by Winkler's method (Cumming & Kay, 1945). This method depends on the action of the dissolved oxygen on manganese hydroxide, resulting in the formation of higher hydrated oxides which are then dissolved in hydrochloric acid in the presence of potassium iodide.

The iodine equivalent to the dissolved oxygen was thus liberated and the iodine was titrated with standard sodium thiosulphate.

Calculation:-

Volume of oxygen at N.T.P. in 1 l. of soln. = $\frac{V_1 \times 0.05}{1000} \times 5592 \times 2$ ml.

where V_1 = vol. sodium thiosulphate used in titration

0.05 = normality of sodium thiosulphate solution

5592 ml = vol. occupied by 8 g oxygen at N.T.P.

In practice, volumes of 500 ml. solution were titrated at one time and in every case 3 samples were estimated and the mean result obtained.

Estimation was made of the oxygen content of perfusion solution exposed to the atmosphere.

The oxygen content of the perfusion solution at 16°C and at 38°C was also estimated when pressurised under experimental conditions, either by a gaseous mixture of 95% oxygen and 5% carbon dioxide or by gaseous nitrogen. These experiments were carried out by collecting the solution, which would normally have entered the ear after leaving the cannula, in a beaker under a layer of liquid paraffin. The flow of the perfusion solution through the apparatus was decreased, by a screw clip placed between the manometer and the cannula, to the rate which would have resulted if an isolated ear had been perfused for 5 hr with solution at the chosen temperature.

Estimation was made of oxygen uptake by the isolated

rabbit's ear. Perfusion experiments were carried out under the standard conditions except that both the isolated ear and the perfusate as it dripped from the cut surfaces were kept in an environment of nitrogen (fig. 7). The perfusate was immediately collected under liquid paraffin at hourly intervals during the experiments and its oxygen content estimated. The amount of oxygen taken up by the ear could thus be found by the oxygen difference between perfusion solution and perfusate under any specified conditions. It can be seen under the heading 'Discussion, Anoxia' that certain criticisms of this technique exist.

Mathematical derivation of the gross outward filtration

The conventional methods of perfusing the blood vessels of the ear give but a meagre picture of all the events taking place in the vessels and in the tissue spaces. During the course of the perfusion, fluid must be leaving the capillaries and entering the tissue spaces of the ear. This is the gross outward filtration. Much of this fluid returns to the vessels, assisted in part by the rising interstitial pressure. A certain amount leaks from the

cut surfaces at the base of the ear. The fluid which remains in the tissue spaces as oedema fluid is the net outward filtration, which can be directly measured by increase in weight.

The cumulative net outward filtration from the capillaries was recorded as the increase in weight of the ear in each experiment, but the rate of gross outward filtration across the capillary membrane could not be measured experimentally.

The rate of accumulation of oedema fluid may be expressed (Donaldson, Ferguson, Levinson & Silvey, 1953) by:-

$$\frac{dw}{dt} = \alpha(1 - e^{-\gamma t}) - \beta w \quad \ldots\ldots\ldots(1)$$

where t = time,

w = cumulative net outward filtration.

The expression $\alpha(1 - e^{-\gamma t})$ represents the gross rate of outward filtration across the capillary membrane.

The parameter α represents the ultimate value of gross outward filtration

and γ describes its rate of change.

The expression $\alpha(1 - e^{-\gamma t})$ thus takes into account capillary permeability, effective filtration force and the spontaneously recurring periods of relaxation and contraction of smaller blood vessels, vasomotion.

The parameter β describes the extent to which oedema fluid already formed retarded further gross outward filtration.

In perfusion experiments at a solution temperature of 16°C it was noted that the rate of oedema formation was almost linear. This suggested that the gross rate of outward filtration remained steady and that it would be justifiable to adopt the simplification $\gamma = \infty$, whence

$$\frac{dw}{dt} = \alpha - \beta w \quad \ldots\ldots\ldots\ldots (2)$$

Values for the parameters α and β were determined (Chapter 4) using the method of least squares to give the best fit for w in relation to t, whence

$$w = \frac{\alpha}{\beta}(1 - e^{-\beta t}) \quad [w = 0 \text{ when } t = 0] \quad \ldots(3)$$

However, at 16°C the increase in weight of the ear due to the initial filling of the blood vessels with perfusion solution, unduly predominated in relation to the low rate of gross outward filtration. This was particularly intrusive where values of β are small. This was allowed for by the introduction of a correction factor C. Accordingly, in this case the general solution

$$w = \frac{\alpha}{\beta} + Ce^{-\beta t} \quad \ldots\ldots\ldots\ldots (4)$$

of the differential equation was used (Chapter 4) and values for α, β and C were determined.

In experiments when the temperature of the perfusion solution was 38°C, since fitting the original formula was difficult (Chapter 4) the simplification $\gamma = \beta$ was used, whence

$$w = \frac{\alpha}{\beta}(1 - e^{-\beta t}) - \alpha t e^{-\beta t} \quad [w = 0 \text{ when } t = 0] \quad ...(5)$$

Since a good fit was obtained by this simplification, an even better fit would be obtained without it.

It was thus possible to derive the gross outward filtration rate across the capillary membrane by evaluation of α at 16°C and of $\alpha(1 - e^{-\beta t})$ at 38°C.

RESULTS

Vascular anatomy of the rabbit's ear

Arterial supply. The main vessel of supply to the rabbit's ear is the dorsal auricular artery (central ear artery)(fig. 6). This vessel enters the base of the ear by passing deep to the levator auris muscle. The artery becomes more superficial and lies near the junction of the lateral and middle thirds of the dorsal surface. It then passes towards the tip of the ear giving off small branches in its course. About 4/5 of the way from the base of the ear it terminates by dividing into medial and lateral divisions, which form an arcade.

The medial division of the dorsal auricular artery runs to the medial margin of the ear, down which it passes giving off numerous branches which form a network over the medial 2/3 of the dorsal aspect. The medial division gives off branches which supply a part of the ventral surface, and from it also arises a small artery which passes down towards the base of the ear between the medial division and the dorsal auricular artery.

The lateral division of the dorsal auricular artery pursues a similar course down the lateral margin, except that

near its origin, it gives off a large branch which passes to the tip of the ear forming an arterial arcade there. The lateral division also gives off small branches which curve round the lateral margin of the ear to supply part of the ventral surface.

In addition, the ventral surface is supplied by the ventral auricular artery.

Venous drainage. The medial 2/3 of the dorsum of the ear drains in its distal 2/3 into 2 main veins, one passing down the medial margin and the other almost parallel but immediately lateral (fig. 6). About 2/3 of the way towards the base of the ear, the latter vessel divides. One branch joins the vein at the medial margin to form the medial marginal vein, one of the 3 main veins of the ear.

The second branch inclines in a lateral direction crossing superficial or deep to the dorsal auricular artery to join the central vein, which lies along the lateral aspect of the artery. The central vein is formed by tributaries draining the middle third of the dorsal aspect of the ear and only becomes relatively large following this junction.

The lateral third of the dorsum is drained by the lateral marginal vein, the third main vein, which is situated

near the margin of the ear. All 3 main veins unite at the base of the ear.

The ventral surface of the ear is drained by small veins which incline towards the edges to join the dorsal marginal veins. The ventral auricular vein drains the base of the ventral surface of the ear.

At the most convenient site for cannulation of the artery at the base of the dorsal surface of the rabbit's ear, the dorsal auricular artery, the central vein and the dorsal auricular nerve lie in close proximity, with the artery the most medial structure and the nerve the most lateral.

Arterio-venous anastomoses. Viscous neoprene, such that it was of too great viscosity to enter capillaries, was injected at a temperature of 16°C into the left ventricle of a recently killed rabbit whose heart was still contracting. Both the arterial and venous systems in the ears were well injected. This suggests that there must be wide-bore shunts between arterioles and venules, the arterio-venous anastomoses.

On the other hand, neither the venous systems of the heart nor mesentery contained the viscous neoprene, although both these arterial systems were well injected. This seems

to suggest that in these regions there were no patent arterio-venous anastomoses in this preparation.

Neoprene perfusion of the isolated rabbit's ear

Neoprene at 16°C. In 4 experiments, coloured latex neoprene of low viscosity was perfused into the dorsal auricular artery. The injected specimens were all examined microscopically. Capillary vessels were not found to be injected on histological examination, in spite of the fact that both the arterial and venous systems were well filled. It was not possible to demonstrate the existence of injected patent arterio-venous anastomoses with certainty since it was difficult to identify arteries as distinct from veins.

Neoprene at 38°C. Three unsuccessful attempts were made to perfuse the isolated ear with neoprene at a temperature of 38°C. Difficulty was experienced in controlling the temperature of the injection mass. The neoprene set prematurely and so completely blocked the cannula orifice. These experiments were abandoned since they did not give promise of being reasonably practical.

Cannula dimensions

The dimensions of the cannula (fig. 4) measured on a vernier microscope were: internal diameter of shank = 2 mm approximately, internal diameter at the orifice = 0.39 mm, external diameter at the orifice = 0.58 mm, and length of terminal constriction = 8.5 mm. This one cannula was used, with a very few exceptions, during all the experiments.

Cannula calibration for pressure drop

Manometric pressure drop at selected flow rates. The head of mercury was maintained constant at 100 mm in the upstream manometer, i.e. the manometer proximal to the cannula. The same level of pressure was maintained during all the standard perfusion experiments. The head of mercury in mm in the downstream manometer, the manometer distal to the cannula, was measured at various rates of flow (table 4). The pressure drops at these flow rates were calculated (table 4) and the results graphed (fig. 8).

Flow measurements with free discharge at selected pressures. Table 5 shows the values obtained during calibration, assuming no significant recovery of pressure from

the velocity head, i.e. the head of pressure due to velocity of flow. These results are graphed in fig. 8. Table 6 details the theoretical evaluation of velocity head loss over the range of inflow rates which occurred during the perfusion experiments. The resultant theoretical pressure drops due to cannula friction loss, assuming full pressure recovery from the velocity head, were graphed against the flow rates (fig. 8).

Fig. 8 shows that close correspondence was obtained between the 2 methods of calibration. The line of best fit was drawn by inspection. Thus the pressure drop across the cannula was obtained for various rates of flow.

The physiological information required is not pressure drop, but remaining perfusion pressure, i.e. the downstream pressure or the pressure in the central artery of the ear just beyond the tip of the cannula. Consequently another graph had to be constructed for experiments in which the pressure in the upstream manometer immediately proximal to the cannula was maintained constant at 100 mm Hg. From this constant pressure the different pressure drops across the cannula at particular flow rates were subtracted. It was then possible to graph directly downstream pressure against flow rates for the standard series of experiments.

It will be seen from below that there was a more rapid rate of inflow during perfusion with solution at 16°C, and hence a greater pressure drop across the cannula, than during perfusion at 38°C. Thus the downstream pressure during perfusion with solution at 16°C was lower than that at 38°C. At both temperatures, however, the downstream pressure approximated to the normal value of the mean blood pressure.

Oxygen content of perfusion solutions

The perfusion solution contained only 5.4 ml. oxygen/l. when exposed to atmospheric air at a room temperature of 20°C. However, the oxygen content of the solution normally entering the artery in experiments was found to be 13.4 ml. dissolved oxygen/l. perfusion solution at 16°C and 10.5 ml. at 38°C (table 7). The value of exposing the perfusion solution to the oxygen and carbon dioxide mixture is thus obvious.

When pressurized by nitrogen the perfusion solution contained only 4.5 ml. dissolved oxygen/l. at 16°C and 4.2 ml./l. at 38°C (table 7).

Oxygen uptake by the isolated rabbit's ear. Four ears

were perfused at 16°C and 4 at 38°C. When the perfusion was carried out at 16°C, the amount of oxygen taken up during the first 2 hr was slightly more than for the rest of the experiment (table 8). The results (table 9) show, however, that there was less oxygen present in the solution at the higher temperature before it entered the ear. During the actual perfusion of the vessels there appeared to be slightly less oxygen used by the isolated ear at 38°C than at 16°C. The greatest consumption of oxygen took place during the first and the second hr of the perfusion (tables 8 & 9). Thereafter there was a decrease in the oxygen uptake by the ear.

Expression of results from perfusion

A total of 131 perfusion experiments was carried out. Fourteen of these experiments failed for technical reasons. A number of these have been omitted from description since they were additional control experiments in which the results of minor variations in perfusion conditions merely confirmed those detailed under the subdivisions of results and discussion.

Oedema formation

Initial ear weight. It was noted in preliminary perfusion experiments that although the perfused isolated rabbits' ears initially varied in weight over a range of 2 g, the amount of oedema which finally developed in ears under similar conditions of perfusion fell within a very narrow range. Moreover, the heavier ears did not invariably develop the greater amount of oedema at the end of the perfusion. The heavier ears increased very slightly more in weight during the first few min of the perfusions due to the greater capacity of the vascular tree.

Weight of ear cartilage. In a control group of 8 rabbits' ears of varying ear weights, the weight of the avascular cartilage varied only over the small range 22 - 30% of the total weight of the isolated ears.

Cumulative weight of oedema formation. The oedema was recorded by weighing the ear at 15 min intervals. The oedema formation was thus the cumulative amount by which the isolated ear preparation increased above its initial weight during perfusion due to net outward filtration. The

cumulative weight of oedema formation was graphed in the figures at intervals of 15 min.

Mean cumulative weight of oedema formation. Isolated rabbits' ears each initially weighing about 9 g, and of similar size, were used during the main experimental series. These ears of similar initial weights perfused under similar perfusion conditions developed, within narrow limits, similar final weights, and also similar cumulative weights at any one period of time throughout the experiment. It was thus considered justifiable to calculate the mean cumulative weights in the case of ears perfused under similar stimuli.

Since cumulative weights and consequently mean cumulative weights taken at 15 min intervals for the 5 hr perfusions all showed a consistent tendency throughout for the weights to be different under different sets of perfusion conditions, it was not considered to be necessary to calculate the standard deviations of cumulative weights, or the standard errors of the mean values or to determine at what time following the commencement of the perfusion one oedema formation was significantly different from another.

Interstitial pressure

The initial reading was arbitrarily used as zero pressure.

The interstitial pressure at any period of time following the commencement of the perfusion was the amount by which the pressure in the isolated ear preparation was increased cumulatively above the zero pressure. Readings were taken at 15 min intervals.

Inflow

The inflow was recorded as ml. perfusion solution leaving the cannula during the preceding 15 min and is graphed against time in the figures. Thus inflow signifies the increment or decrement of inflow/15 min and not the cumulative inflow.

Under any one set of standard conditions the results, from experiment to experiment, varied very slightly. As in the case of oedema formation, it was considered justifiable to use increments of inflow without calculation of the standard deviations of the observations, to use mean increments of inflow without determining the standard errors of the mean values and not to determine at what times following the commencement of the perfusion that one inflow volume was significantly different from another.

There was no necessity to convert the values for increment

or mean increment of inflow/15 min into ml./unit of vol. of ear/min, since, during the main experimental series, isolated rabbit's ears each initially weighing about 9 g were used.

Calculation of the net peripheral resistance of the isolated ears in dynes.sec.cm^{-5} (Wiggers, 1950) was undertaken in a number of experiments using the downstream pressure and inflow. Since the resultant graph of the peripheral resistance was found to be almost a mirror image of the curve of rate of inflow, these calculations were abandoned.

Effect of temperature on rate of oedema formation, on rate of inflow and on interstitial pressure in the isolated ear of the rabbit

Effect of constant temperature of the solution during perfusion

Oedema formation at 16°C. The mean values of 2 such experiments are illustrated in fig. 9. After the first 15 min of the perfusion, the rate of oedema formation was practically constant giving a straight line. The ears, at the end of 4 hr, had about double their original weights.

Mathematical analysis of the oedema formation, w, gave

values of $\alpha = 0.53$, $\beta = 0.02$, $C = -26.94$. Table 10 shows that the observed and calculated values of w are an excellent fit. Chapter 4 gives the detailed analyses. The gross outward filtration across the capillary membrane, α, was derived and is illustrated in fig. 10.

Inflow at 16°C. At this temperature, an initial spasm of the artery occurred. During the first few min of all experiments there was invariably a slow rate of inflow, which accompanied a visible spasm of the cannulated artery (fig. 9). After the visible spasm passed off, usually within 10 min, the rate of inflow remained practically constant for about $3\frac{3}{4}$ hr. Thereafter the inflow decreased very slightly. During the course of 5 hr perfusion approximately 4,200 ml. of perfusion solution passed through the vessels of the ear as a whole. No oscillation was noted of the galvanometer spot which recorded the temperature of the solution entering the artery.

Oedema formation at 38°C. Four such experiments are illustrated. Figure 9 shows that the rate of oedema formation increased rapidly until about the end of the second hr, after which the rate rapidly decreased. The absolute amount of oedema formation after perfusion for 5 hr at 38°C was about

4 times that which accumulated at the lower temperature of 16°C. The ears were grossly swollen having roughly 5 times their original weight at the end of 5 hr.

Mathematical analysis of the oedema formation, w, gave values of $\alpha = 11.00$, $\beta = 0.29$. Fig. 11 and table 10 show that the observed and calculated values of w are a very good fit. Chapter 4 gives the detailed analyses. The gross outward filtration, $\alpha(1 - e^{-\beta t})$ was derived and is illustrated in fig. 10.

The mathematical analyses also showed that the ultimate value of gross outward filtration at 16°C was 0.53 while at 38°C it had risen by the factor of 20 to 11.00. Similarly, the extent to which oedema fluid already formed retarded further net outward filtration rose from 0.02 at 16°C by a factor of 15 to 0.29 at 38°C.

Inflow at 38°C. During the first few min of the experiment there was a slower rate of inflow, which was due to a visible spasm of the cannulated artery (fig. 9). In this case the initial spasm of the artery was less marked. Thereafter the rate of inflow rose to a maximum by the end of the first 30 min of the perfusion. Having reached its peak, the rate of inflow then decreased markedly. About 2 hr after the

start of the perfusion the flow began to increase gradually and continued to do so until the end of the perfusion. Throughout, however, the rate of inflow at 38°C was considerably less than the rate of inflow at 16°C.

During perfusion experiments at a temperature of 38°C it was observed that the galvanometer spot, which recorded the temperature of the solution just before it entered the artery from the cannula, oscillated over a range of temperature of about 0.75°C. Each cycle took about 40 sec. It is probable that these oscillations were produced by rhythmical variations in the calibre of the arterial tree.

Interstitial pressure at 38°C. Three experiments were carried out and the results from one are shown in fig. 12. During the first ¾ hr of the experiment there was only a slight rise in interstitial pressure, but this was succeeded by a rapid rise which continued for 1 hr, during which period the most rapid rise in the rate of oedema formation would also be taking place. Thereafter the rate of increase of the interstitial pressure became very much less rapid, and the pressure began to oscillate slowly over a range of several mm water. From 3½ hr onwards the interstitial pressure showed a tendency to decrease, but the oscillations in pressure

continued.

The results for the rate of inflow in these experiments at 38°C (fig. 12) followed the characteristic pattern already described. Yet although the rate of inflow decreased markedly after the first ½ hr of the perfusion, the value for the interstitial pressure was still low at this time and only after 1 hr had elapsed was there any significant rise in interstitial pressure.

Oedema formation at 24°C. One such experiment is illustrated. In general, the results at this temperature resembled the experiments at 16°C. The rate of oedema formation was more irregular than that at either 16° or at 38°C (fig. 14). The cumulative wt. at 5 hr lay approximately midway between that at the other two temperatures of 16° and 38°C.

Mathematical analysis gave $\alpha = 1.19$, $\beta = 0$, $C = 0.2$. Thus the ultimate value of gross outward filtration at 24°C was greater than that at 16°C only by a factor of 2, since α rose to 1.19 from a value of 0.53.

Inflow at 24°C. During the first 1¾ hr of the perfusion, the rate of inflow was very irregular and rapid (fig. 14) but

subsequently the rate of inflow decreased fairly steadily.

Oedema formation at 32°C. Only two experiments were carried out. In general, the results at this temperature resembled the experiments at 38°C. During the first 1¾ hr of the perfusion, the rate of oedema formation was almost the same as the rate when the ear was perfused with solution at 38°C (fig. 15). Thereafter the rate of oedema formation became less rapid.

Inflow at 32°C. The rate of inflow at this temperature (fig. 15) was much slower than that at 38°C. After an initial rise in the flow during the first ½ hr of the experiment, the rate of inflow became less rapid until about 3 hr, after which it remained fairly constant.

Oedema formation at 45°C. Four experiments were carried out with the perfusion solution at the unphysiological temperature of 45°C. The rate of oedema formation was a little less rapid for the first hr than when isolated ears were perfused with solution at 38°C (fig. 9). Following this, a rapid increase in the rate of oedema formation took place. There was a definite decrease in the rate after

2¾ hr, then a marked rise occurred which continued until the end of the experiment. The final amount of oedema developed by these preparations was very much more than at 38°C. During the perfusions at 45°C, one or more large blebs developed which occupied almost the whole of the ventral surface of each ear, while several smaller vesicles appeared on the dorsal aspect (plate 1).

Inflow at 45°C. The rate of inflow was similar to that when solution at 38°C was used to perfuse the ear, but the rates of flow were considerably higher throughout (fig. 9). However, the rate of inflow at 45°C was lower than at 16°C. Slight oscillation was noted of the galvanometer spot recording the temperature of the solution entering the artery.

Effect of variations in the temperature of the solution during perfusion

Perfusion of the ear was commenced with the perfusion solution either at 16° or at 38°C. An hour or so later the temperature was changed rapidly to 38° or 16°C as the case might be. After a period the temperature of the

perfusion solution was returned to its original value. This complete cycle was repeated once more.

Oedema formation. Six experiments were carried out. The results from one of these are shown in fig. 13. Perfusion of the ear was commenced with solution at a temperature of 38°C for 1¼ hr, after which, without interruption of the flow, the perfusion solution temperature was lowered to 16°C. As a rule, about 8 min elapsed before the solution at the cannula reached this temperature. A concomitant lowering of the ear environmental temperature took place from 21° to 16°C. After 1 hr at this temperature, the perfusion solution temperature was raised once more to 38°C, but was lowered again temporarily from after 3 hr till after 3¾ hr from the start of the perfusion.

During perfusion of the ear with solution at a temperature of 16°C, there was a decrease in the rate of formation of oedema fluid, but this rate increased again when the solution was reheated to 38°C (fig. 13). The relative decrease in the rate of oedema formation was much more marked during the first than the second cycle of cooling.

Inflow. A definite increase in the rate of inflow

occurred when the ear was being perfused at 16°C, and the inflow decreased rapidly when the temperature of the solution was raised to 38°C (fig. 13). This cycle of heating and cooling the solution was repeated, and it was seen that, in contrast to the effect on the rate of oedema formation, the period of cooling following the second period of heating increased the rate of inflow relatively a little more than after the first episode, and raised it until it almost reached the level attained by the cool solution 2 hr previously. During the final period of heating the rate of inflow started to rise after ½ hr, but this coincided with the increase in inflow rate, which had already been observed to occur, during perfusions which had been carried out at a constant solution temperature of 38°C throughout.

Interstitial pressure. Two perfusions were carried out. The results of one experiment are shown in fig. 16. The perfusion was commenced with solution at 38°C and cycles of alternate cooling and heating were instituted.

The interstitial pressure did not appreciably increase until after ¾ hr, although the rate of inflow had fallen markedly from its maximum after ½ hr (fig. 16). When cooling was commenced, a rapid rise in the rate of inflow followed,

but the interstitial pressure continued to increase at only a slightly lower rate. Moreover, this slowing of the rate of increase of interstitial pressure was observed to have started before the solution was cooled at all.

When the solution was again heated to a temperature of 38°C the rate of increase of the interstitial pressure was temporarily quite marked, although the decrease in the inflow rate was, at this period, relatively small. Following this, there was a considerable decrease in the interstitial pressure, but, at this time, the rate of inflow also decreased rapidly.

The cycle was repeated and the interstitial pressure continued to fall, but, this time it was accompanied by a marked increase in the rate of inflow. There was an appreciable rise in the interstitial pressure when heating was recommenced, but this rise was of very short duration and the level fell soon afterwards. This part of the perfusion coincided with the normal downward trend of the interstitial pressure during the experiments performed with the perfusion solution at a constant temperature of 38°C.

Oedema formation and inflow at 16° & 38°C following 4½ hr at 38°C. Two experiments were carried out. Figure 17 shows the results of one of these experiments. A decrease

in the rate of oedema formation occurred when the solution was cooled to 16°C following perfusion at 38°C for 4¼ hr. The rate increased again when the perfusion solution was reheated to 38°C.

The rate of inflow had started to increase after 4¼ hr perfusion at a constant solution temperature of 38°C (fig. 17). On cooling rapidly to 16°C for ½ hr there was an increased rate of flow but the response was relatively slight. A slight decrease in the inflow rate occurred when the perfusion solution was again heated to 38°C.

Appendix. Effect of various factors on rate of oedema formation, on rate of inflow and on interstitial pressure in the isolated ear of the rabbit

Effect of perfusion with solution containing hyaluronidase

From preliminary experiment, using perfusion solution containing hyaluronidase, it was observed that the oedematous ear was markedly fluctuant. The subcutaneous space consisted of a large communicating sac filled with oedema fluid under low pressure.

Oedema formation and inflow at 38°C with hyaluronidase. Three experiments were carried out with perfusion solution containing hyaluronidase and compared with the results of perfusion using solution without hyaluronidase. It was observed that, during the first hr, when hyaluronidase was present in the solution there was a slightly more rapid rate of formation of oedema (fig. 18). However, this rate then decreased quite quickly and by the end of the experiment the cumulative increase of weight by the perfused ears was no more than 2/3 of that attained by the preparations which were perfused with solution which did not contain hyaluronidase (fig. 18). No bullae were observed. Mathematical analysis of the oedema formation gave $\alpha = 4.75$, $\beta = 0.21$, showing that the ultimate value of gross outward filtration at 38°C had fallen by more than half from 11.00 to 4.75 when the perfusion solution contained hyaluronidase. However, the extent to which oedema fluid already formed retarded further net outward filtration only fell very slightly from 0.29 to 0.21 when the solution contained hyaluronidase.

Although, in general, the rate of inflow did not fall to as low a level as it did in the experiments without hyaluronidase, there was still a rapid decrease in the rate of inflow which started after ½ hr and continued until 1½ hr

(fig. 18). Thereafter the rate of inflow increased but was more irregular than in the corresponding period during perfusion experiments in which hyaluronidase was not present.

Interstitial pressure and inflow at 38°C with hyaluronidase. One experiment was carried out with perfusion solution containing hyaluronidase and compared with the results of perfusion using solution without hyaluronidase (fig. 19). Interstitial pressure measurements during the perfusion experiments at 38°C gave a maximum of only 41 mm water compared with 183 mm for perfusion under the same conditions except that the solution did not contain hyaluronidase. In addition, the rise in interstitial pressure was more gradual.

The inflow rates showed the same general pattern as before (fig. 19).

Oedema formation at 38° & 16°C with hyaluronidase. Two experiments were carried out. One of these is graphed in fig. 20, together with oedema formation resulting from the use of solution without hyaluronidase. The amount of oedema fluid formed was very much less than that which developed when no hyaluronidase was added to the solution. However, in both sets of conditions, the rate of oedema formation decreased,

when the temperature of the perfusion solution was lowered from 38° to 16°C, and increased, when the solution temperature was raised to 38°C once more. At the end of both cycles of cooling the cumulative amount of oedema fluid decreased slightly when hyaluronidase was present in the perfusion solution.

Inflow at 38° and 16°C with hyaluronidase. Two such experiments were carried out. Although in general the rate of inflow was slightly higher than in the experiments in which no hyaluronidase was added, the inflow responses to heating and cooling the solution were similar and just as marked (fig. 21).

Effect of downstream pressure of the solution during perfusion

The downstream pressure is the pressure in the central artery just distal to the tip of the cannula. It is evaluated by subtracting the pressure drop across the cannula from the pressure measured immediately proximal to the cannula.

In all the experiments reported so far, the downstream pressure was approximately 60 mm Hg during perfusion with solution at a temperature of 16°C and approximately 70 mm Hg

with solution at 38°C. The values are in the normal range of the mean blood pressure.

Oedema formation with pressures at 50, 60 & 70 mm Hg and with a temperature of 16°C. Two isolated ears were perfused at a calculated mean downstream pressure of 50 mm Hg, and 3 ears at 70 mm Hg. The results were compared with the standard series at a calculated mean downstream pressure of 60 mm Hg.

At the lowest downstream pressure of 50 mm Hg, the rate of oedema formation was very slightly less rapid than at the intermediate pressure of 60 mm Hg (fig. 22). At the highest downstream pressure of 70 mm Hg, the rate of oedema formation increased more rapidly than it did during the experiments carried out at the intermediate pressure of 60 mm Hg. This increase in formation of oedema took place throughout the entire perfusion, although the difference in the rates of increase was more marked during the last 2 hr of the experiment.

Mathematical anasysis of the oedema formation gave at

50 mm Hg: $\alpha = 0.51$, $\beta = 0.01$, $C = -35.47$

60 mm Hg: $\alpha = 0.53$, $\beta = 0.02$, $C = -26.94$

70 mm Hg: $\alpha = 0.91$, $\beta = 0.03$, $C = -39.72$

Thus during perfusion with solution at a temperature of 16°C although raising the downstream pressure from 50 to 60 mm Hg had little effect on the ultimate value of gross outward filtration (α), yet increasing the downstream pressure a further 10 mm Hg to 70 mm almost doubled the ultimate value of gross outward filtration. However, none of these alterations in downstream pressure had any significant effect on the extent to which oedema fluid already formed retarded further net outward filtration (β).

Inflow with pressures at 50, 60 & 70 mm Hg and with a temperature of 16°C. It was found that the lower the downstream pressure, the less rapid was the rate of inflow (fig. 23).

Oedema formation with pressures at 53, 71 & 83 mm Hg and with a temperature of 38°C. Two isolated ears were perfused at a calculated mean downstream pressure of 53 mm Hg, and 3 ears at 83 mm Hg. The results were compared with the standard series at a calculated mean downstream pressure of 71 mm Hg.

At the lowest downstream pressure of 53 mm Hg there was a decrease in the rate of oedema formation during the first

1½ hr of the experiment compared to the rate at the intermediate pressure of 71 mm Hg (fig. 22). From then, until about 2¾ hr after the perfusion commenced, the rate of increase was the same as that when the downstream pressure was 71 mm Hg. During the last 2¼ hr of the perfusion, there was an increase in the rate of formation of the oedema compared to the intermediate pressure experiments.

At the highest downstream pressure of 83 mm Hg, there was an increase in the rate of formation of oedema during the first 1¼ hr of the perfusion. Thereafter the rate decreased and was less than either the intermediate (71 mm Hg) or lowest (53 mm Hg) downstream pressure experiments.

Mathematical analysis of the oedema formation gave at

53 mm Hg: $\alpha = 9.43$, $\beta = 0.23$

71 mm Hg: $\alpha = 11.00$, $\beta = 0.29$

83 mm Hg: $\alpha = 12.00$, $\beta = 0.38$.

Thus during perfusion with solution at a temperature of 38°C equal increments in downstream pressure give equal increments in the ultimate values of gross outward filtration (α). Increase in downstream pressure increased the extent to which oedema fluid already formed retarded further net outward filtration (β). The gross rates of outward filtration

for these experiments calculated as $\alpha(1 - e^{-\beta t})$ are shown in fig. 25.

Inflow with pressures at 53, 71 & 83 mm Hg and with a temperature of 38°C. The curves of inflow rates were all of essentially similar configuration (fig. 24). The lower the downstream pressure, the less rapid was the rate of inflow.

Oedema formation at 70 mm Hg downstream pressure & 16°C temperature and at 71 mm Hg downstream pressure & 38°C temperature. From fig. 26 it can be seen that in 2 series of experiments in which the preparations were perfused with solution at either 16° or 38°C using calculated mean downstream pressures which were essentially identical, the rate of oedema formation was very much lower at the lower temperature. Moreover, the curve of oedema formation was of a different character.

Inflow at 70 mm Hg downstream pressure & 16°C temperature and at 71 mm Hg downstream pressure & 38°C temperature. Except for the rate of inflow at ½ hr following the commencement of the perfusion, the rate of inflow was higher at the lower temperature, and the curve was of a different character (fig. 26).

Effect of oxygen content of the solution during perfusion

A group of preliminary experiments were carried out.

N at 16°C. Two experiments were performed at 16°C using nitrogen to pressurize the solution. This reduced the oxygen content of the solution from 13.4 ml. dissolved oxygen/l. perfusion solution to 4.5 ml./l. The only difference from the normal curve of oedema formation was a slight increase in the rate during the perfusion (fig. 27).

The rates of inflow compared to the normal curve at this temperature showed a decrease in the first half of the perfusion (fig. 27).

N at 38°C. Three isolated ears were perfused at 38°C with solution pressurized by nitrogen. This reduced the oxygen content of the solution from 10.5 ml. dissolved oxygen/l. perfusion solution to 4.2 ml./l. It was found that less oedema developed after 5 hr perfusion with this solution than when solution of a higher oxygen content was used (fig. 27). It was observed, however, that there was a slightly more rapid rate of oedema formation during the first ¾ hr of experiment. Thereafter the rate decreased more abruptly

than when the higher oxygenated solution was used. On examination of the ear after each perfusion, small bullae were seen under the surface of the skin.

The rates of inflow during these experiments were much lower than when the solution was more highly oxygenated (fig. 27).

Effect of pH of the solution during perfusion

A group of preliminary experiments were carried out.

One perfusion experiment was carried out at a pH of 7.6 to 7.8 and a temperature of 16°C. The cannula used, however, was of slightly different pressure-flow characteristics from the cannula used throughout the remainder of the perfusions. Control perfusions for this cannula were carried out for oedema formation and inflow at a perfusion solution pH of 7.3 to 7.4. These non-standard results have not been displayed in a figure.

At a pH of 7.6 to 7.8, the rate of oedema formation was slightly more rapid during the first 1½ hr of the perfusion. The rate of inflow was not significantly different from that of the standard pH.

One perfusion was carried out at a pH of 7.6 to 7.8 and a temperature of 38°C. There was a slightly more rapid rate of oedema formation, but the shape of the curve was similar (fig. 28). The rates of inflow, although following the normal pattern of that produced with the perfusion solution at a pH of 7.3 to 7.4, were less rapid at corresponding times during the first part of the perfusion and more rapid thereafter (fig. 28).

One experiment was carried out at a pH of 6.6 to 6.8 and a temperature of 16°C. The rate of oedema formation was very slightly more rapid than when the isolated rabbit's ear was perfused with solution at a pH equal to 7.3 to 7.4. No significant difference was observed in inflow.

One experiment was carried out at a pH of 6.6 to 6.8 and a temperature of 38°C. A marked rise in the rate of oedema formation took place during the first ½ hr of the experiment, the ear attaining 2/3 of its final weight in this short time (fig. 28). After this, however, the rate of oedema formation became much slower than the standard. The rate of inflow did not differ very markedly from that obtained at a pH equal to 7.3 to 7.4 (fig. 28).

One perfusion was carried out at a pH of 4.0 and a

temperature of 16°C. Oedema formation was quite rapid during the first 3 hr of the perfusion and then markedly decreased (fig. 29). The rate of inflow was slow, especially towards the end of the perfusion (fig. 29).

One perfusion was carried out at a pH of 4.0 and a temperature of 38°C. The rate of oedema formation was more rapid than normal for the first ¾ hr of the perfusion and then decreased quickly (fig. 30). After 2 hr, however, the weight of the ear increased so very rapidly within a few min that the thread suspending the ear cut through the tissues, thus terminating the experiment. The rate of inflow was erratic, and much less rapid, than when the perfusion was carried out with the solution at a pH of 7.3 to 7.4 (fig. 30).

Effect of chosen constant temperature of the environment during perfusion

During preliminary experiments in a room thermostatically controlled at 20°C, it was found that even a slight temporary draught of cold air caused a marked variation in the rates of inflow and oedema formation.

When the temperature of the perfusion solution was at 16°C, the temperature of the air within the metal shield surrounding the ear tended to become stabilized at 16°C also. When the perfusion solution was at 38°C, the temperature of the surrounding air was in the neighbourhood of 21°C. In the experiments so far described any deviation from 16°C or from 21°C was corrected.

In the present series, during perfusion with solution at 16°C, the temperature of the environment was raised to 32°C. When the temperature of the perfusion solution was 38°C, the environmental temperature was lowered from 21° to 16°C.

Oedema formation and inflow at environmental temp. 16°C with perfusion solution at 16°C. The results for oedema formation and inflow at a solution temperature of 16°C where the environmental temperature was 16°C, have already been detailed earlier in this chapter (fig. 9). In summary, the rate of oedema formation was almost linear, the cumulative amount of oedema fluid slight, and $\alpha = 0.53$, $\beta = 0.02$, $C = -26.94$. The rate of inflow was high and almost constant throughout. For comparison these results have been regraphed in fig. 31.

Oedema formation and inflow at environmental temp. 32° with perfusion solution at 16°C. Two experiments were carried out at a solution temperature of 16°C in which the environmental temperature had been raised to 32°C (fig. 31). The rate of oedema formation was slightly more rapid throughout than during experiments with the normal environmental temperature.

Mathematical analysis of the oedema formation, w, gave values of $\alpha = 0.65$, $\beta = 0$, $C = 1.80$ when the environmental temperature was raised to 32°C. This means that the gross outward filtration across the capillary membrane during perfusion at a temperature of the solution of 16°C rose only very slightly from 0.53 to 0.65 when the temperature of the environment was raised from 16° to 32°C.

The rate of inflow decreased slightly during the course of the experiments and the variations in the curve of rate of inflow were larger than when the environmental temperature was at its normal of 16°C with the perfusion solution at 16°C.

Oedema formation and inflow at environmental temp. 21°C with perfusion solution at 38°C. The results for oedema formation and inflow at a solution temperature of

38°C where the environmental temperature was 21°C have already been detailed earlier in this chapter (fig. 9). In summary, the rate of oedema formation was most rapid during the 1¼ hr following the first ½ hr of the experiment. The rate of inflow decreased rapidly after the first ½ hr of the perfusion, but increased again gradually after about 2 hr. For comparison these results have been regraphed in fig. 31.

Oedema formation and inflow at environmental temp. 16°C with perfusion solution at 38°C. Two experiments were carried out at a solution temperature of 38°C in which the environmental temperature had been lowered to 16°C (fig. 31). It was found that the rate of oedema formation at the lower environmental temperature was significantly less for the first half of the experiment. During the second half of the perfusion, however, although the curve flattened out a little, the rate of oedema formation was actually greater than during the similar stage of experiments carried out in the warmer environment of 21°C. The rate of oedema formation was somewhat irregular.

The rates of inflow showed little change, tending to follow the same type of curve as was obtained at the higher

environmental temperature of 21°C although they were more irregular (fig. 31). The initial inflow reading was much lower than in the normal curve.

DISCUSSION

The external ears of the rabbit are relatively large structures whose function is to play an important part *in vivo* in the regulation of body temperature. Alterations in the nature of the capillary bed and in the rate of blood flow through the ear affect the rate of heat elimination.

Vascular anatomy of the rabbit's ear

In spite of several studies on the functioning of blood vessels in the rabbit's ear, no adequate description of the course and relationships of the main arteries and veins in the ear was found in the literature.

Arterio-venous anastomoses

Since the injection of neoprene at 16°C into the left ventricle of the rabbit's heart, or into the dorsal auricular artery, had been found to result in filling of both arterial and venous systems of the ear even although capillaries were not injected, it was considered that this could only be explained by the presence in the rabbit's ear of

arterio-venous anastomoses which were patent at 16°C.

The experiments with neoprene at a temperature of 38°C were abandoned since they did not give promise of being reasonably practical.

Heparin

The mucopolysaccharide heparin has a rapid action solely on the clotting substances already present in circulating blood (Wright, 1953). Thus it was reasonable to assume that the heparin would have no effect on the subsequent perfusion.

General heparinization of the rabbit was carried out using intramuscular administration an hr before death. Clotting of the blood was very obviously delayed and I attribute the uniformity of the results in perfusion to avoidance of clotting of blood in any of the vessels of the ear before the start of the perfusion. It may well be that such a precaution should be adopted before attempting perfusion of the isolated mammalian heart.

The disadvantages to regional heparinization (Murray, Jaques, Perrett & Best, 1937; De Takats, 1950) of the ear were many.

Although protamine is used clinically to counteract heparin (Tuchman & Moolten, 1950), Swyer (1948) found that heparin inhibited hyaluronidase activity. Under the conditions of the present experiments this action would be relatively unimportant. Fabinyi & Szebehelyi (1949) found that histamine opposes the inhibition of hyaluronidase by heparin.

Net outward filtration (oedema formation)

It is universally accepted that the internal layer of the capillary wall is formed by a continuous layer of endothelial cells. The other constituents of the capillary wall are the intercellular cement substance and an external basement membrane reinforced by a fibrillar network. The latter component of the capillary wall seems to be closely related to the intercellular substance of the connective tissue, being composed of a homogenous ground substance and fibres.

There are many monographs and review articles on permeability (e.g. Brooks & Brooks, 1941; De Robertis, Nowinski & Saez, 1948; Teorell, 1949). Two main theories are currently held concerning the mode of transfer of substances through capillary walls to the extracellular extravascular spaces. It may be that substances leave the

capillaries fundamentally by passage through the intercellular cement (Leading article, 1951b). The 'lipoid-sieve' theory of permeability, however, depends on the presence of lipoids composing the cell membrane, through which substances can pass owing to their membrane solubility (Collander & Bärlund, 1933). The lipoid-insoluble substances can pass through pores in the membranes, rendering the rate contingent to their molecular size.

Before ascribing the greater or lesser passage of a given substance to a change in permeability, the conditions under which the passage occurred must be scrutinized. Landis (1946) pointed out that conclusions concerning capillary permeability are sometimes arrived at without considering the many simple physical forces which are concerned in the movement of dissolved substances through the capillary walls.

The principal factors which affected the rate of net outward filtration in the perfused isolated rabbit's ear and which were under experimental control or measurement were:

(1) Capillary permeability:

duration of survival following isolation

temp. & duration of perfusion

degree of capillary anoxia

perfusion solution pH

(2) Effective filtration force:

filtration area

extent of capillary dilatation

degree of bye-pass through a.-v. a.

filtration pressure

perfusion solution downstream pressure

upstream pressure

perfusion solution osmotic pressure

perfusion solution viscosity

(3) Vasomotion.

Provisional results have been obtained on the effect of all these factors with the exception of the perfusion solution osmotic pressure and viscosity. With regard to the perfusion solution osmotic pressure, the use of a solution of crystalloids inevitably resulted in the formation of the gross amounts of oedema fluid which were recorded in the present perfusion experiments. It was shown by Bayliss that crystalloids readily diffuse across the capillary membrane thus leading to rapid oedema formation. More recent workers (e.g. Hevesy & Jacobsen, 1940; Cowie, Flexner & Wilde, 1949) have merely confirmed this by more complicated

techniques. Moreover, the development of oedema may have been facilitated by 'washing' away the endocapillary layer of protein which Danielli (1940) and Chambers & Zweifach (1947) have postulated as a lining to the capillaries. However, part of the explanation of the gross oedema may lie in the work of Zweifach (1940) who put forward evidence to show that in perfusion of the vessels of the mesentery and of the tongue of the frog with a solution which did not contain either red cells or suspended carbon, there was a tendency for the flow to be restricted to the a.-v. capillaries. This results in a greater outward movement of fluid from the capillaries. However, such a mechanism does not necessarily hold true in the case of perfusion of the blood vessels of the rabbit's ear.

In liquids the coefficient of viscosity decreases as the temperature rises, because it is more dependent on the cohesive forces which are decreased by a rise of temperature, than it is on the increase, as the velocity increases, of the transfer of momentum caused by molecules escaping from the faster to the slower layers of the fluid (Jameson, 1914).

Increase of the temperature of water from 16° to 38°C decreases its viscosity from 0.01142 to 0.00657 c.g.s. (Kaye & Laby, 1948). Pappenheimer (personal commun., 1952) recently

observed that a decrease in temperature from 38°C to 15°C in Ringer's fluid will appreciably decrease the rate at which the fluid passes across the capillary membrane, due to the altered viscosity. If this preliminary work is substantiated it would signify that in the present investigation at temperatures of 16° and 38°C, the increase in viscosity of the perfusion solution at the lower temperature may be a factor of considerable importance in reducing the rate of oedema formation.

The Laewen-Trendelenburg (Trendelenburg, 1910; Hyman & Chambers, 1943) *in vitro* technique was first used for the study of blood vessels in the isolated hind limb of frogs, and was used for the assessment of rate of formation of oedema in the present series of perfusions.

The ears contained a varying percentage of cartilage. This tissue being avascular would not contribute at all to the formation of oedema in the ear, yet the weight of the preparation and consequently the results were partly based on the weight of the cartilage. The results in a control group showed that this tissue varied over the range 22 - 30% of the initial weight of the ear in a group of varying ear weights. This range would probably be decreased in a group of ears of the same initial weight. Consequently variation

in the weight of cartilage was unlikely to be significant in the main series of perfusion experiments. It was decided to express oedema formation as the observed cumulative weights of increase above the initial ear weight.

Mathematical derivation of the gross outward filtration

The equation was the simplest mathematical model which represented the physiological changes thought to be taking place. The calculated values of net outward filtration were found to fit the observed values so well, under so many different perfusion conditions, that it was considered that the differential equation could be used to interpret the events taking place in the blood vessels of the ear. The constants, which gave good fits for the experimental data, had calculated values which differed for each particular set of perfusion conditions. Thus the relative magnitude in different experiments of the gross outward filtration and interstitial pressure were derived.

The value of the equation was limited, however, since it was neither possible further to analyse the component $\alpha(1 - e^{-\gamma t})$ into its constituent terms of capillary permeability, effective filtration force or vasomotion, nor to analyse βw

in terms either of the interstitial pressure or of the loss of oedema fluid from the cut base of the ear. The equation had no predictive value.

Net peripheral resistance (inflow)

Little justification is required for the use of inflow rather than the more commonly used outflow measurements, particularly in series of experiments in which a variable amount of oedema fluid is formed in response to different perfusion conditions. Inflow is an accurate measure of the volume of perfusion solution flowing through the blood vessels of the ear. On the other hand, measurement of outflow is not an accurate or an immediate indication of the net peripheral vascular resistance of the vascular bed undergoing perfusion. Outflow measurements would depend not only on the venous flow, but also on the leak of oedema fluid from the cut base of the ear, which must vary at different periods throughout the perfusion. Outflow recorders (e.g. Stephenson, 1948) require volume calibration since they do not yield direct readings obtainable from the constant pressure burettes (Chapter 5) as used for inflow measurements.

Peripheral resistance is dependent upon:

(1) effective viscosity of the fluid

(2) lengths of the vessels

(3) individual and collective cross-sectional areas of the vessels. These areas are in turn dependent on the extravascular pressure provided by the interstitial pressure, on metabolic processes, on constrictor and dilator substances and on any vasomotor impulses which are arising within the isolated preparation. In turn, the interstitial pressure depends upon the absolute amount and upon the rate of formation of oedema fluid in the tissues

(4) altered intraluminal pressure.

Thus inflow measurements can be taken as an index of the calibre of the vessels perfused only if it is appreciated that this yields information as to the summated changes in vasomotor tone of the isolated preparation and provided also that the viscosity and pressure of the perfusion solution remain constant.

O'Connor & McKeever (1950) considered that perfusion of isolated tissues with Ringer's solution yielded inconsistent results. Consequently a haemoglobin-saline solution (e.g. Smyth, 1942) might have been used with advantage, but whole blood would have been of less value since Burton (1952) has pointed out that due to plasma skimming, the higher the velocity of flow of blood, the lower will be the effective

viscosity. However, unlike blood, the viscosity of a solution of crystalloids does not vary with the dimensions of the vessels along which the fluid flows (Pappenheimer & Maes, 1942).

An increased viscosity would tend to decrease the rate of inflow. In liquids the coefficient of viscosity is increased as the temperature is lowered. Since in the isolated ear of the rabbit the inflow rate was considerably more rapid at the lower temperature, it is obvious that the factors producing this higher inflow rate were sufficiently marked to mask the decreased inflow due to increased viscosity at the lower temperature.

Increase in peripheral resistance consequent upon vasoconstriction from whatever cause, would *per se* decrease the inflow. The decrease in inflow would increase the downstream pressure, resulting in turn in a smaller decrease in the rate of inflow. The net result, therefore, was a less marked decrease in inflow during vasoconstriction and conversely a less marked increase during vasodilatation, than if constant downstream pressure could have been maintained. Something like this very probably occurs *in vivo* where vasodilatation decreases the diastolic blood pressure and, as a consequence, the mean blood pressure.

A change of the ratio of flow dilated to flow constricted will indicate the direction of change of vasomotor tone, and roughly its magnitude. However, since this ratio is not the same at all perfusion pressures for a given change of vasomotor tone, it cannot be used as a quantitative expression for the magnitude of the change (Green, Lewis & Nickerson, 1943; Green, Lewis, Nickerson & Heller, 1944).

Appreciation of all these limitations in perfusion of the isolated ear of the rabbit nevertheless permits the conclusion that alterations in the effective calibre of the vessels, and hence in the peripheral resistance or 'upstream pressure', will be roughly demonstrated in magnitude by alterations in the rate of inflow.

Inspection of the results of the present experiments shows the extremely rapid inflows of perfusion solution obtained in all experiments, being of the order of 110 ml./100 ml. ear vol./min. It is not logical to assume that the flow of blood in the ear of the rabbit ever approaches this value. Unfortunately there is no information about the normal blood flow in the rabbit's ear. The resting circulation in the hand in normal humans is about 9 ml./100 ml. hand vol./min; in the forearm it is about 2 ml./100 ml. limb vol./min (e.g. Abramson & Fierst, 1942; Hewlett & van

Zwaluwenburg, 1909). However, anatomically the vascular supply of the rabbit's ear is particularly rich relative to the volume of the ear.

Arterial spasm

Consequent to the trauma inevitably inflicted during cannulation, arterial spasm of varying degree and duration inevitably developed.

Hunter (1835) considered arterial spasm to be part of the natural mechanism for preventing bleeding. Grant (1930a) described, as a result of trauma, a localised fusiform dilatation with spasm at each end, in the dorsal auricular artery of the rabbit. An intact sympathetic nerve supply is unnecessary for the maintenance of arterial spasm in rabbits with periarterial stripping of the vessel (Kinmonth, 1952). Spasm of the proximal arteries can be induced only by direct trauma (Leading article, 1952).

Burton (1952) considered the problem from an entirely new viewpoint, that of the critical closing pressure. The critical closing pressure like 'resistance' is a measure of vasomotor tone, but unlike 'resistance' is independent of viscosity. The critical closing pressure increases with

increasing tension in the wall, i.e. vasomotor tone, and with decreasing size of the vessel. The simple, yet adequate explanation of arterial spasm suggested by Burton was that it occurred when the critical closing pressure was higher than the available blood pressure.

Temperature of the perfusion solution

It was decided to investigate the factors involved in producing the uniform and repeatable differences in rates of oedema formation and of inflow when the rabbit's ear was perfused under constant conditions with solution at temperatures of 16°C and 38°C respectively. The former temperature was chosen since it was an approximation throughout the year to the temperature of Ringer's solution in the laboratory. Thus the same constant temperature could readily be achieved in all experiments. Moreover, many other workers have perfused the isolated ear of the rabbit with fluid at this temperature.

On the other hand, 38°C is about the normal blood temperature of the rabbit. Thus, in the intact rabbit, blood of this temperature will be circulating through the vessels of the ear. When the environment is hot the vessels of the

ear are widely dilated, in an attempt to keep down the body temperature.

It has been seen in the results that at the lower temperature, the rates of oedema formation were less, but the rates of inflow more rapid than at the higher temperature. In the present experiments on isolated ears, at a temperature of 16°C during the course of 5 hr a volume of 30% more perfusion solution passed through the vessels of the ear as a whole than during perfusion at 38°C. It might have been expected that the lower flow at the higher temperature would have resulted in less oedema. Yet, the accumulated oedema following 5 hr perfusion was approximately 4 times as great at the higher temperature. Mathematical treatment, moreover, showed that the ultimate value of gross outward filtration at 38°C was 20 times that at 16°C. Similarly the extent to which oedema fluid already formed retarded further gross outward filtration rose by a factor of 15 at the higher temperature.

In addition, altering the temperature of the perfusion solution from 16°C to 38°C resulted in a lower rate of inflow and a higher rate of oedema formation. The responses in oedema formation had been anticipated. However, the responses of the rates of inflow to changes in the temperature

of the perfusion solution were unexpected since Pissemski (1914), who studied the responses of the vessels of the ear of the rabbit to alterations in temperature of Ringer-Locke perfusion solution between 10° and 40°C, had found a slower rate of flow at the lower temperatures. Similarly Harris (1941) stated dogmatically in a practical text-book of experimental physiology that, in the perfused ear of the rabbit a more rapid rate of inflow occurs at 38°C due to vasodilatation. No adequate explanation of the difference in the results obtained by Pissemski and by Harris from the present series of experiments can be offered. It should be noted, however, that these authors carried out their experiments under less adequately controlled conditions.

Recently, Pappenheimer, Eversole & Soto-Rivera (1948) recorded that, during perfusion of the hind limb of the cat with blood cooled from 40° to 25°C, there occurred a decrease in the rate of flow. Further cooling, however, below 25°C caused a more rapid blood flow, which increased progressively as the blood temperature was lowered. From observations of the temperature of the skin of the paw and of the muscles of the leg, they suggested that the blood stream had been diverted from the small vessels in the skin into larger channels in the muscles.

Some part of the rapid initial inflow recorded in all the present experiments, and presumably partly masked by the arterial spasm, was used to fill the vascular systems of the rabbit's ear.

These different rates of oedema formation and inflow would be consistent with the interpretation that the vascular tree of the isolated ear of the rabbit when perfused with solution at 38°C was different from that when the ear was perfused with solution at 16°C.

It was considered desirable to carry out a few preliminary experiments at other temperatures, e.g. 24°, 32°C. The rates of inflow were slowest at the temperature range intermediate between 16° and 38°C, yet while the flow was slower at 32°C than at 38°C, the rate of oedema formation was the same during the first part of the perfusions, suggesting that the capillary bed was well dilated at both temperatures. Although the cumulative weight following 5 hr perfusion at 24°C lay approximately midway between that at the temperatures of 16° and 38°C, mathematical analysis showed that, in fact, the ultimate value of the gross outward filtration at 24°C was only greater than that at 16°C by a factor of 2, since α rose to 1.19 from a value of 0.53. It seemed reasonable to attribute the slower rates of inflow at

temperatures intermediate between 16° and 38°C to contraction of the smooth muscle of the walls of arteries and arterioles.

The gross oedema and marked vesiculation which resulted when the perfusion solution temperature was 45°C was attributed to damage to, and abnormal permeability of, the vascular endothelium, particularly that of the capillaries, caused by the very high and quite unphysiological temperature of the perfusion solution. The slightly more rapid rate of inflow at this temperature than at 38°C was possibly due to heat paralysis with dilatation of the vessels. To a very minor extent, the greater rate of formation of oedema together with the resultant greater rate of loss of oedema fluid from the cut end of the rabbit's ear, may have contributed to the slightly more rapid inflow.

The topography of the capillary bed in the ear of the rabbit has not been studied in detail, but Clark & Clark (1934a, b) and Clark (1938) using their transparent observation technique, have observed numerous anastomoses varying in size from 5 to 60μ in the rabbit's ear. They recorded that, under normal conditions, these vessels displayed periodic contractions and dilatations, while the arteries and arterioles exhibited similar movements but with a different rhythm. Very low temperatures abolished these rhythmical

movements, as did temperatures between 38°C and 45°C.

During experiments on isolated segments of mesenteric artery of the dog, Bürgi (1944) showed that, at a temperature of 38°C, rhythmical contraction and dilatation of the vessels occurred. Nichol & Burton (1950) found that, when adrenaline in Ringer's solution was perfused through the isolated rabbit's ear at room temperature, marked oscillation in the rate of flow occurred.

In the present experiments, the rhythmical oscillation of the galvanometer spot which recorded the temperature of the solution entering the ear occurred at 38°C, was slight at 45°C and was not observed at 16°C. Since the oscillation in temperature must have been due to alteration in the rate of inflow, this was interpreted as indicating the presence at a solution temperature of 38°C of a rhythmical alteration in the calibre of the blood vessels of the isolated ear, i.e. vasomotion, just as gut *in vitro* shows rhythmical pendular movements.

Although no evidence of vasomotion was found by this technique at 16°C, yet it was at this temperature that a decreased rate of oedema formation resulted. Thus the results contradict any hypothesis that relatively less vasomotion at 38°C had resulted in the more rapid rates of oedema formation

at the higher temperature.

Heating the ear of the intact rabbit produced capillary dilatation, and cooling to a temperature of 16°C tended to open up the arterio-venous anastomoses (van Dobben-Broekema & Dirken, 1950a; Grant, 1930b). Therefore it seemed possible that, if the isolated ear of the rabbit were perfused with solution at 16°C, the solution might be diverted from the capillary bed, through larger channels and so bypass the area of filtration. Such a response would cause an increase in the rate of flow and a decrease in the oedema formation at this temperature.

Burton (1952) suggested that the mechanism of opening of vascular shunts, for example arterio-venous anastomoses, in parallel with the resistance of the capillary bed would depend on their critical closing pressure. Due to tension in their wall, shunts are endowed with a high critical closing pressure. Closure of the capillary bed during perfusion with solution at a temperature of 16°C might raise the pressure at the junction of the shunt with the artery and result in opening of the shunt.

Appendix

The results showed clearly that alterations in the rate of

capillary net outward filtration, and in the rate of inflow, took place in the perfused isolated ear of the rabbit in response to alterations in the temperature of the perfusion solution. To summarise the results again. In the present experiments on isolated ears, mathematical treatment showed that the ultimate value of gross outward filtration at 38°C was twenty times that at 16°C. Yet at a temperature of 16°C, during the course of 5 hr, approximately 4,200 ml. of perfusion solution passed through the vessels of the ear as a whole. The corresponding figure at 38°C, however, was approximately only 3,200 ml.

The differences might be due to alterations in the perfused vascular bed of the ear, but might equally well be secondary effects due to an altered physiological state of the ear, e.g. different degree of survival of isolated preparations, different selective survival of the arterioles or capillaries, different upstream pressure consequent upon the level of interstitial pressure from the oedema formation.

Alternatively the differences might be due to secondary physical effects of one or more of the concomitants which were inherent in the carrying out of the perfusions at solution temperatures of 16° and 38°C. These concomitants were the altered downstream pressure, oxygen availability,

and pH, and the altered environmental temperature.

Preliminary experiments have been carried out in an attempt to assess provisionally the importance, in modifying the results obtained, of an altered physiological state of the ear and of the physical concomitants of the perfusion technique. Many additional experiments will require to be carried out before the tentative conclusions advanced in the remainder of this chapter could be definitely substantiated.

As a generalization the further results showed without exception that factors, which would be expected to affect the rate of net outward filtration did, in fact, produce a more marked response on the rate of oedema formation during perfusion at a solution temperature of 38°C than at 16°C. The inference was that at the higher temperature, the active capillary bed was very extensive, whereas at 16°C the bed was reduced in size and the surface area of the capillaries was markedly diminished.

Survival of the perfused isolated ear of the rabbit. When the ear was perfused with solution constantly at 38°C, the rate of inflow was considerably less rapid, and more irregular, than during perfusions with solution constantly at

16°C. Moreover, the oedema formation and interstitial pressure were considerable only at the higher constant temperature. The results also show that if during a perfusion at a temperature of 38°C, the solution temperature was lowered to 16°C, a marked increase in the rate of inflow occurred together with a slowing of the rate of oedema formation. When the temperature of the solution was elevated once more to 38°C, a marked reduction in the rate of inflow took place, together with a rise in the rate of oedema formation. Even more marked, however, were the alterations observed in the rate of inflow in response to these temperature changes, when hyaluronidase was added to the perfusion solution.

It was arbitrarily decided that the isolated ear was still surviving throughout all these experiments since it gave vascular responses to thermal stimuli.

During experiments with the solution at 38°C, if no alteration was made in the temperature until the last hr of the perfusion, these vascular responses to temperature stimuli were not so markedly obtained. The decreased response of the ear to thermal stimuli seemed to indicate that, when the isolated ear of the rabbit was perfused continuously at 38°C, cell death intervened after approximately 4 hr, but

that this had not occurred by 5 hr when perfusion was carried out at a solution temperature of 16°C.

Interstitial pressure. Undoubtedly the filtration rate was influenced by the value of interstitial pressure which, in turn, depended on the absolute amount, and rate of formation, of oedema fluid. Thus more extensive filtration area, combined with increased permeability, during perfusion at 38°C would result in a more rapid rise in interstitial pressure. This might have caused an earlier compression of venules thus producing an increased upstream pressure (peripheral resistance), and consequently a less rapid rate of inflow. The increased venous pressure would initially also increase the rate of accumulation of oedema fluid, but eventually the increased interstitial pressure would reduce the net transfer of fluid to the tissue spaces.

The present investigation was not designed to produce direct evidence for or against the existence of such a mechanism. In man, the effect of raising the venous pressure has been investigated by means of the pressure plethysmograph. Drury & Jones (1927) and Krogh, Landis & Turner (1932) concluded that increased venous pressure led to an increase in the rate of filtration from the capillary lumen to the tissue

spaces. Landis & Gibbon (1933) showed, by a similar method, that, if at a given venous pressure the ability to filter fluid from the capillaries to the tissues diminished, then it was because of some force in the tissue spaces opposing capillary pressure, assuming that the permeability of the capillaries does not alter.

McMaster (1946a, b) recorded interstitial pressure directly in the subcutaneous tissue of mice and of men and confirmed the effects of increased venous pressure shown by pressure plethysmography. Thus from the literature it would appear quite possible that the inflow at the two temperatures used in the present experiments might be subordinate to the accumulation of oedema fluid and thus to the interstitial pressure.

Although it was found that the phase of very rapid oedema formation coincided with the most rapid rise in the interstitial pressure, yet the rate of inflow began to decrease long before this rise commenced and indeed at a time when the interstitial pressure was still low. However, there is no evidence from these experiments that a very slight increase in interstitial pressure might not be adequate to produce some degree of venous obstruction and hence a decreased inflow. It may be relevant that it was found that the rate of inflow

ceased to fall at the same time as the highest interstitial pressure was recorded, and the interstitial pressure fell shortly after the rate of inflow began its terminal increase.

At the beginning of this reduction in the interstitial pressure, the pressure level commenced to oscillate over a range of about 12 mm water. This oscillation continued for the remainder of the experiment, while the mean interstitial pressure pursued a gradually decreasing level.

Two factors may be responsible for this 'oscillatory decline'. McMaster (1946a, b) described a 'breaking point', defined as a critical level of interstitial pressure beyond which the connective tissues were broken apart. In the present experiments, if the 'breaking point' were exceeded, the resultant coalescing of tiny pockets of fluid would cause a reduction in the interstitial pressure and thus temporarily favour increased net outward filtration, consequently producing oscillations in the interstitial pressure. It would be of interest to take continuous, instead of intermittent, ear weight records to support or refute this hypothesis.

Moreover, if the 'breaking point' were exceeded, there would be an increased leak of oedema fluid from the cut base of the ear. Both factors operating together would result in an 'oscillatory decline'.

The use of any isolated preparation must result in speculation as to when any particular type of cell in the preparation is no longer viable. It may be that during perfusion of the isolated rabbit's ear with solution at a temperature of 38°C, that the connective tissue is particularly liable to yield to pressure forces after about 2½ hr, thus resulting in the 'oscillatory decline'. In similar fashion, decreased vascular tone at this time would result in a reduced upstream pressure (peripheral resistance), thus the rate of inflow would gradually become more rapid.

Although no experimental observations were carried out it seems reasonable to expect that the interstitial pressure which would develop at a solution temperature maintained constantly at 16°C throughout the perfusion would be considerably lower than at 38°C.

Hyaluronidase. In 1929 Duran-Reynals demonstrated that an aqueous extract of mammalian testes yielded a substance which facilitated the spread of intradermal injections. He named this substance the 'spreading factor'. Later Chain & Duthie (1940) proved that this was actually an enzyme which acted on hyaluronic acid, an important constituent of connective tissue. They therefore called the enzyme hyaluronidase,

which was grouped (Tauber, 1949) as one of the mucolytic enzymes, which catalyse the depolymerization of mucopolysaccharides.

Hyaluronic acid is a polymeric disaccharide of glucuronic acid and N-acetyl glucosamine, with a molecular weight at about 200,000 to 500,000 (Dalgaard-Mikkelsen & Kvorning, 1948). Hyaluronic acid is not antigenic (McClean, 1942) and is probably formed in the liver (Leading article, 1951a).

Methods of estimation of the potency of hyaluronidase enzyme preparations are given by McClean & Hale, 1941; Dalgaard-Mikkelsen & Kvorning, 1948; Chain & Duthie, 1940; Fulton, Marcus & Robinson, 1948.

No general agreement has been reached in the literature on the effect of hyaluronidase on capillary permeability. Meyer (1946) found no evidence of increased vascular permeability when hyaluronidase was administered intravenously to rabbits, but when injected into the connective tissues hyaluronidase brought about transudation from the capillaries. This finding was corroborated by Chambers & Zweifach (1947), and Zweifach & Chambers (1950), who observed that, when hyaluronidase was applied topically to the omental capillaries of the frog, petechial haemorrhages occurred. They concluded

that since they found that hyaluronidase extracts did not produce a gradual, over-all change in the porosity of the capillary, but rather an abrupt rupture and extravasation at irregular loci along the vessel, it would seem to suggest that this reaction did not represent the type of change regulating vascular permeability under physiological conditions. Intravenous administration led to no change in capillary permeability.

The permeability of the connective tissue throughout the body is controlled by the state of the interfibrillar ground substance (Woodin, 1950). Chambers & Zweifach (1947) gave evidence for the presence of mucopolysaccharide in the connective tissue sheath of capillaries. They suggested that the hyaluronidase, by acting on the hyaluronic acid in the connective tissue and in the pericapillary sheath, increased the fragility rather than the permeability of the capillaries and thus caused haemorrhage and increased transudation of fluid into the tissues. Scarborough (1941) described a method for determining experimentally the capillary resistance by using a 'negative pressure principle'.

Elster (1949) and Elster, Freeman & Dorfman (1949) opposed this school of thought by claiming to have demonstrated an increased capillary permeability in rats following intravenous

injection of this enzyme. They criticised the results of Chambers & Zweifach (1947) on the grounds that an insufficient quantity of the enzyme had been given intravenously (Elster, 1950). However, this conclusion of Elster's was based on measurements of decreased plasma volume, and on an increased passage of the dye T-1824 from the capillaries into the tissue spaces. Such methods are themselves open to the criticism that they can furnish only indirect evidence of an alteration in capillary permeability. Similar indirect observations of increased capillary permeability in rabbits on intravenous injection of testicular extract, together with dyes, were made by Duran-Reynals (1942).

The results of the present experiments in which hyaluronidase was added to the perfusion solution at 38°C show that, during the first part of the perfusion there is an increase in the rate of formation of oedema, which thereafter decreases rapidly. Those results, however, similarly to those of Elster, can be explained either as due to an increase in capillary permeability, or alternatively as due to passage of hyaluronidase into tissue spaces early in the perfusion. This would permit it to act on the hyaluronic acid in the supporting connective tissue and in the pericapillary sheath and thus increase the net outward filtration.

The subsequent rapid decrease in the recorded rate of oedema formation was caused by a very marked leak of oedema fluid from the cut base of the ear, due to the spreading action of the hyaluronidase. Mathematical analysis showed that the addition of hyaluronidase to the perfusion solution stabilised the gross outward filtration rate over the experiment, making it higher initially and lower ultimately than in perfusions at the same temperature of 38°C without the addition of hyaluronidase.

No bullae were noted in the perfused isolated ears although MacCardle, Baumberger & Herold (1943) postulated that, since hyaluronic acid bound water by holding it as a gel, hyaluronidase might produce bullae by hydrolising the hyaluronic acid leaving the water of the gel free as blister fluid at the site of the bulla. It is relevant to note that no hyaluronidase was found in the bullous fluid of patients with pemphigus (Grais & Glick, 1948) although Grais (1949) was able, in some of the cases of advanced pemphigus which he investigated, to produce bullae by intradermal injections of hyaluronidase.

It may be that the hyaluronidase may have affected the permeability of the lymphatic capillaries (Hudack & McMaster, 1932) whatever its effects on the permeability of the blood

capillaries. Increased lymphatic permeability might tend to increase the rate of net outward filtration due to the increased lymphatic drainage lowering the interstitial pressure. There is no certainty that such a postulated increase in filtration from blood capillaries would be recorded by the technique used, however, since increased flow in lymph vessels would result in a greater loss of fluid from the cut base of the isolated ear.

Hyaluronidase increases the diffusion rate of substances through connective tissue by reducing the viscosity of the intercellular substance. If the enzyme, when added to the perfusion solution, was capable of diffusing out of the vessels, then it would prevent any marked increase in interstitial pressure by its action on the hyaluronic acid in the interstitial tissue.

The results indicate the importance of the interstitial pressure in producing the different inflow readings at the 2 temperatures under consideration. In the present experiments at 38°C, addition of hyaluronidase reduced the interstitial pressure to about 20% of its value in experiments without the addition of hyaluronidase, yet the rates of inflow did not differ materially. This suggested that the lower rate of inflow at a temperature of 38°C was not due to the higher

interstitial pressure, if it was assumed that the small increase in interstitial pressure during the first part of the experiment was insufficient to cause partial venous obstruction and so account for the initial sharp decline in the rate of inflow.

In the experiments using hyaluronidase in which the temperature of the perfusion solution was changed intermittently between 38° and 16°C, the slight increase in the absolute amount of oedema fluid recorded at the end of both cycles of cooling was attributed to the spreading action of hyaluronidase on the connective tissue of the subcutaneous tissue of the ear, allowing the oedema fluid to leak away with greater ease from the cut base of the ear. This permitted more fluid to drain away from the tissue spaces than accumulated there as a filtrate from the capillaries.

Downstream pressure of the perfusion solution. The downstream pressure, or effective perfusion pressure, is the pressure of the solution in the artery just beyond the tip of the cannula. The importance of this measurement has not been generally recognised in perfusion experiments. Workers have generally based calculations on what was the perfusion pressure in the system proximal to the cannula.

Yet even more inaccurate were the readings of Hyman (1944) on innervated hind limbs of rats and frogs, and those of Nichol, Girling, Jerrard, Claxton & Burton (1951) on the isolated ear of the rabbit, who accepted as the perfusion pressure, readings on a manometer recording in the apparatus a considerable distance proximal to the cannula. Disregard of the pressure loss due to bends, constrictions, and the length of tubing leading to the cannula, and disregard of the pressure drop across the cannula must completely invalidate calculations based on such pressure readings.

Perfusion of the ear with a constant, instead of with a pulsatile pressure had the disadvantage of increasing the accumulation of tissue fluid by decreasing lymph flow (Parsons & McMaster, 1938; McMaster & Parsons, 1938; McMaster, 1942). In retrospect, it is considered that, due to the abnormally high net outward filtration which occurred with the use of a crystalloid perfusion solution, some form of pulsatile pressure instead of a constant pressure could, with advantage, have been applied to the side limbs of the burettes. A constant pressure was applied, however, since the steady pressure facilitated the calibration of the pressure-flow characteristics of the cannula and thus the calculation of the effective perfusion pressure or downstream pressure. In addition, the

apparatus thus remained simple and manageable by 1 experimenter. In marked contrast is the complicated pulsatile pressure apparatus designed by Holgate (1949) for the same purpose.

Although the perfusion pressure immediately proximal to the cannula was maintained constant no matter the rate of inflow, the downstream pressure during perfusion with solution at 16°C was lower than that during perfusion at 38°C by approximately 10 mm Hg. The reason was that the more rapid rate of inflow at the lower temperature caused a greater pressure drop across the cannula. This lower pressure might have been wholly or partly the cause of the slower rate of oedema formation at the lower temperature.

Landis (1927a, b) demonstrated quantitatively on the mesenteric capillaries of the frog that the rate of filtration from the capillaries was dependent upon the intraluminal pressure, and not on an increased permeability due to mechanical stretching of the vessel wall as had been postulated by Krogh (1922). Gilding (1941) criticised the technique used by Landis on the grounds that the mesenteric vessels exposed on the microscope stage did not represent a physiological preparation. Experiments on isolated hind limbs of rats (Hyman, 1944) and of dogs (Pappenheimer & Soto-Rivera, 1948) also demonstrated that the rate of filtration was dependent

on the perfusion pressure. The latter workers demonstrated that the rate of oedema formation could be increased by raising either the arterial or the venous pressure, and that by suitable variations in both these pressures, an infinite number of pairs of values could be obtained at which no alteration in weight of the limb occurred. This work might be criticised on the grounds that, by increasing the arterial pressure, capillaries which were previously closed would then be forced open. The primary effect of increased arterial pressure on capillary filtration would then be masked by a simultaneous increase in the area of filtration. However, Krogh (1920) claimed that, if the capillaries were in a contracted state, increasing the arterial pressure would not force these vessels open, a finding subsequently confirmed by Carrier (1922).

The results of the present experiments contribute additional evidence that raising or lowering the downstream pressure at both temperatures leads to an increase or decrease respectively in the rate of oedema formation and rate of inflow. A more accurate representation of the effect of altering the downstream pressure at 38°C was obtained by calculation for each pressure of $\alpha(1 - e^{-\beta t})$ which represented the gross rate of outward filtration across the capillary membrane.

At 38°C the increased rate of oedema formation at the highest pressure apparently occurs only during the first part of the perfusion. The final weight of the preparation after 5 hr perfusion is actually less than when lower pressures were used. The anomaly is due to the initial high net outward filtration rate causing a marked increase in interstitial pressure, thus subsequently opposing filtration from the capillaries and resulting in the early reduction in rate of oedema formation. In addition, the high interstitial pressure would result in an earlier and greater leak of oedema fluid at the cut base of the ear.

The slower but more prolonged increase in the development of oedema at the lowest pressure was attributed to the slow increase in interstitial pressure, which was a direct result of the less rapid filtration rate.

The effects on oedema formation were confirmed by mathematical analysis of α and β at the different values of downstream pressure at the 2 temperatures. The effect of raising the temperature from 16° to 38°C at the same downstream pressure of approximately 70 mm Hg was to increase the ultimate value of gross outward filtration by a factor of 12 from 0.91 to 11.00.

It is concluded that the downstream pressure is a factor

regulating both the rate of oedema formation and the rate of inflow. However, when experiments were performed at solution temperatures of 16° and 38°C but with the same downstream pressure it was apparent that the rates of oedema formation and of inflow still remained vastly different. Thus a different downstream pressure is not the principal factor responsible for the different vascular responses at 16° and 38°C.

Anoxia. Harris (1941) maintained that there was no necessity to oxygenate the solution used for perfusion of the isolated ear of the rabbit, yet Landis (1928) using his capillary micro-injection technique, demonstrated on amphibia that anoxia increased capillary permeability. Independent workers (Maurer, 1940; Saslow, 1938) using other techniques, arrived at a similar conclusion to that of Landis.

Pochin (1942) studied the effect of ischaemia of 18 hr duration on rabbits' ears and noted massive oedema formation within 2 hr of release of the circulation, but in these experiments there was the added complication of accumulation of metabolites in the tissues. Hopps & Lewis (1947) on the other hand found no effect on capillary permeability, as measured by the passage of antibody globulin or T-1824, when guinea-pigs

were exposed to an environment with a low oxygen tension. Their results are debatable due to their indirect methods of estimating capillary permeability. In summary, from the literature, there is little doubt that anoxia increases the permeability of capillary endothelium.

Any mammalian isolated preparation perfused with an aqueous solution instead of either blood or a haemoglobin-saline perfusion solution (Smyth, 1942; Taylor & Hastings, 1939) must be markedly anoxic. However, if the metabolism of the isolated preparation is slow, the degree of anoxia may not be marked. It is probable that the isolated ear of the rabbit was metabolising less actively during perfusion with solution at a temperature of 16°C than at 38°C. On the other hand, the tissue may be able to transfer oxygen more readily at the higher temperature.

At ordinary temperatures and pressures, water is capable of dissolving only about 0.5% of its own volume of oxygen. As the temperature is raised part of this is liberated. At any given temperature the volume of gas absorbed is sensibly dependent on the pressure, so that the weight absorbed is proportional to the pressure. Consequently the use of the constant pressure burettes, together with a gas mixture at a pressure greater than atmospheric, to provide a downstream

pressure approximating to arterial blood pressure, resulted in higher oxygen tensions in the perfusion solution than would have been obtained by the use of a high hydrostatic pressure.

In the present experiments the quantity of oxygen in solution was estimated. Since both the pressure and temperature were known, the oxygen tension could have been calculated. This was not done, however, since the technique used to collect the perfusate for estimation of its oxygen content is open to marked criticism. It can be seen from fig. 7 that the perfusate, in the interval between dripping from the ear and being collected under liquid paraffin, was exposed to an environment of nitrogen. During this interval oxygen loss to, and nitrogen gain from, the environment must have taken place. Moreover, it is possible that the error due to gaseous exchange was more marked during perfusion with solution at a temperature of 38°C than at 16°C. Consequently the values obtained for oxygen uptake by the isolated ear of the rabbit perfused with solution at different temperatures, are suspect.

It did not prove practical to eliminate this error by immersing the cut base of the ear below liquid paraffin. Such a technique would have invalidated the readings of weight of

the ear due to the flotation effect, and the readings of inflow due to increased resistance to venous outflow.

At a temperature of 38°C, there was less oxygen dissolved in the perfusion solution than at the lower temperature (table 7) and, moreover, the tissues constituting the ear would be more actively metabolising at the higher temperature. Thus the isolated ears perfused at the higher temperature were probably relatively more anoxic and their greater capillary permeability might have been due solely to the greater degree of anoxia.

However, in the present investigation, when perfusion experiments were carried out at a temperature of 16°C using solution pressurized by nitrogen, giving a very low oxygen content, instead of by 95% oxygen and 5% carbon dioxide, there was no significant difference either in the rate of oedema formation or of inflow. Thus the oxygen content of the solution over this range of oxygen availability was not a main factor in determining the difference in the rates of oedema formation between 16° and 38°C.

The increase in capillary permeability due to increasing the anoxia at 38°C could not be due to alteration in pH, since this was unchanged in the perfusion solution. It could be postulated that the slow rate of inflow and the reduced amount

of oedema fluid formed during the last 4 hr of these experiments resulted from an increase in tone of the smooth muscle of the vessel walls, as a direct effect of anoxia. An alternative explanation, which these experiments would tend to offer some evidence in support of, is that since the pressure of the solution would be reduced as it flowed through the vessels of the ear, gaseous emboli might block the small vessels (Karsner, 1950; Duff, Greenfield & Whelan, 1953). The occurrence of numerous subcutaneous bullae, associated with a lowered absolute amount of oedema fluid could be attributed to pressure disruption of the connective tissue space caused by nitrogen which had diffused across the capillary membrane and accumulated in the extra-cellular extravascular space.

While there was slightly less dissolved oxygen at 38°C than at 16°C, even at the higher temperature there was still some oxygen remaining in the perfusate. These experiments indicate that the greater anoxia at the higher perfusion temperature is not a main factor in determining the greater rate of oedema formation and lower inflow at this temperature. In general, they suggest that the tissues of the ear are by no means indifferent to the oxygen content and tension of the perfusion solution even at the abnormally low levels used during these experiments.

pH of the perfusion solution. Gaskell (1880), Bayliss (1901) and Hooker (1911) all observed that in the blood vessels of the frog a slightly alkaline perfusion solution caused a marked increase in vascular tone, whereas acid solutions produced vasodilatation. However, Leake, Hall & Koehler (1923) concluded that the vessels of the whole frog were maximally dilated at the normal blood pH level and that any deviation from this resulted in vasoconstriction.

In the present investigation on an isolated ear of a mammal, it can be concluded from the results that during perfusion with solution at a temperature of 38°C and a pH intentionally raised slightly, slight increase in net outward filtration occurred and some degree of vasoconstriction. It was consequently also necessary to carry out perfusion of the ear with solution at 16°C and at a pH equal to about 7.7; the increased alkalinity might appreciably affect oedema formation or inflow at one or both temperatures. It can be concluded that increasing the alkalinity of the solution at 16°C does not eliminate the differences in oedema and inflow which occurred when the rabbit's ear was perfused at 16° and 38°C.

Roy & Brown (1880) were among the earliest workers to

observe that the accumulation of acid metabolites in the tissues led to capillary dilatation. Chambers & Zweifach (1940) have demonstrated that increases in acidity within the limits of viability (pH 7.0 to 7.6) caused an appreciable increase in permeability of frog mesenteric capillaries. In the isolated ear of the rabbit, it was possible that, since the perfusion solution was slightly less alkaline at 16°C than it was at 38°C, the responses at 16°C might be simulated at 38°C merely by increasing the acidity of the solution. The results during perfusion at a pH of 6.6 to 6.8 indicate a marked increase in net outward filtration particularly at 38°C, but no unequivocal effect on the rate of inflow.

In summary, deviation in either direction from the normal pH of 7.3 to 7.4 resulted in a more rapid rate of oedema formation at both 16° and 38°C but more so at the higher temperature. Increased acidity of the perfusion solution had a more marked effect on oedema formation than had an increased alkalinity. Increased alkalinity produced a slower rate of inflow.

Landis (1934) using the capillaries of the frog's mesentery found that it was only when the pH of the perfusion solution was lowered below the normal physiological minimum

level to 4.0 that signs of endothelial cell damage appeared. In view of this work, it was decided to perfuse the isolated ear of the rabbit with solution of pH equal to 4.0. Furthermore, the effect of perfusing the ear with solution at the lowest physiological level of pH 6.6 to 6.8 could then be compared to the effect of perfusing the solution at a very unphysiological pH. In the present investigation on the rabbit's ear vessels, perfusion at pH 4.0 provoked spasm of the vessels and then very markedly increased net outward filtration causing the development of gross oedema associated with extensive bullae. The oedema which developed during perfusion with solution at a pH 4.0 and a temperature of 16°C was, however, slight compared to that which developed at 38°C even at a pH about 6.7.

It is logical to conclude that the slight change in pH of the perfusion solution between 16° and 38°C was not responsible for producing the different rates of oedema formation and inflow at these 2 temperatures. Moreover, when alteration in capillary permeability has been caused by variation in the oxygen content or the pH of the perfusion solution, this has always occurred during perfusion of the rabbit's ear vessels at a temperature of 38°C. Very little change could be demonstrated using the technique at a temper-

ature of 16°C. This consequently supports a hypothesis of a different perfusion bed at these two temperatures.

Environmental temperature. It is to expected that the temperature of the perfusion solution would be the dominant factor in the responses of blood vessels in the perfused isolated ear of the rabbit. However, during some preliminary perfusion experiments carried out in a constant temperature room before the metal shield to surround the ear had been designed, I noticed that the blood vessels of the isolated ear responded by vasoconstriction to the slight draught of cold air which entered the room on opening the door. It may well be that the environmental temperature of the ear also played a part in the control of the blood vessels even although the ear was isolated.

Using the standard apparatus described, the local environmental temperature of the ear was influenced mainly by the temperature of the perfusion solution. Thus the ear environmental temperature was readily maintained constant throughout the standard perfusions at a temperature of 16°C using solution at 16°C, and at a higher environmental temperature of 21°C using solution at 38°C.

In other words, in the experiments already described,

during perfusion of the ear with solution at 38°C, the environmental temperature was higher than when the ear was perfused with solution at 16°C. It was thus necessary to determine the vascular responses obtained by varying the temperature of the environment while maintaining the perfusion solution constant at both the standard constant temperatures.

The rabbits used in the pressent investigation were born and reared in an animal house with a controlled environmental temperature between the limits of 17° and 22°C throughout the year. During the perfusion experiments the local environment of the ear was kept constant at 21°C when perfused with solution at 38°C, and at 16°C when perfused with solution at 16°C. The ear was thus more or less within its accustomed environmental temperature range.

Oedema formation. Part of the increased rate of oedema formation in those experiments in which the environmental temperature of the isolated ear was artificially raised might have been caused by the presence of ultra-violet light from the use of black electric bulbs to raise the ear environmental temperature. Ultra-violet light may increase cell permeability by destruction of the cellular membrane (Heilbrunn & Mazia, 1936; Partington, 1953).

Yet the ear was protected from any ultra-violet light by the metal shield and the 2 experiments reported in which the ear environmental temperature was raised by this means gave results which followed the same general trend of all the other perfusion experiments.

The present results (summarised in table 11) on the isolated ear of the rabbit showed that raising the environmental temperature increased the rate of oedema formation, while cooling had the opposite effect as confirmed by mathematical analysis of the ultimate values of gross outward filtration. However, the vascular responses of the isolated ear, when perfused at 16° or at 38°C, could not be mimicked either by raising the environmental temperature during the perfusion with solution at 16°C, or by lowering the temperature of the environment during the perfusion with solution at 38°C.

Van Dobben-Broekema & Dirken (1950a) showed that when the body temperature of the rabbit was increased by elevating the environmental temperature, the capillaries in the ear consistently responsed by dilatation. Increased capillary filtration rate on raising the environmental temperature and decreased rate on lowering the temperature had previously been shown by Brown & Landis (1947) on frogs, by Berdan

(1949) in the aqueous humour of the rabbit, and by Kitchin (1953) in the human arm. These authors concluded that their results were due to alterations in the filtration area and to consequent changes in intracapillary pressure.

The present results may also be postulated to depend on an increased dilatation, or on an increase in the number, of functioning capillaries at the higher environmental temperature. At a low environmental temperature, the capillary bed is correspondingly reduced in capacity.

Inflow. Only a slight reduction in the rate of inflow was found at a perfusion solution temperature of 16°C when the environment was warmed to 32°C. No significant difference was found in the rates of inflow at a perfusion solution temperature of 38°C when the environment was cooled from 21° to 16°C. The fact that at an environmental temperature of 16°C and a solution temperature of 38°C, the initial inflow reading was much lower than in the normal curve, indicated that the initial vascular spasm was greater under these conditions.

Mosso (1889) and Berti (1910) using perfused isolated organs subjected to variations in their environmental temperature, showed that, when the blood vessels were cooled to below blood temperature, they contracted. More recent

experiments by O'Connor & Edozien (1952) on isolated arterial strips, showed that when cooled below 15°C the smooth muscle of these preparations relaxed. Warming to above 15°C resulted in contraction which gradually passed off with the rising temperature. O'Connor attributed the contraction as due to alterations in the surface tension of a monomolecular layer of fatty acids present on the muscle fibrils.

Spealman (1945) discovered that, in the case of the human hand, the blood flow at 5°C was more rapid than at 15°C. As a result of his own observations, and of those of Lewis (1930) who recorded an increase in flow of blood to the finger tip when this digit was immersed in water at about 15°C, Grant (1930b) suggested that one of the functions of the numerous arterio-venous anastomoses present in extremities is to prevent local injury when the extremities are exposed to severe cold. Clark (1938) criticised this on the grounds that, when fingers are exposed to very low temperatures for some length of time, they become frozen. This is not a justifiable criticism, since their protective value may have been overcome at this extreme of temperature. Theoretical and experimental investigations of temperature movements in the skin have been carried out by Hensel (1950).

Grant (1930b) and Grant, Bland & Camp (1932) observed under the microscope the effect of varying the environmental temperature on the arterio-venous anastomoses in the ear of the intact rabbit and in the denervated ear of the otherwise intact rabbit. They found that, when the ear was subjected to a moderate degree of heat in the region of 37°C, the majority of the arterio-venous anastomoses were closed. Above this temperature, the arterio-venous anastomoses dilated. However, lowering the temperature of the environment of the ear to about 15°C caused dilatation of these arterio-venous anastomoses, and of the smaller arteries and veins. The capillaries remained in a constricted state. As a result of all these vascular responses, there was an increase in the flow of blood through the ear at the lower temperature of 15°C. When the temperature was raised above 15°C, the first vessels to close were the arterio-venous anastomoses, then the capillaries began to dilate.

It is known that arterio-venous anastomoses play an important part in regulating blood flow through the ear of the intact rabbit. Arterio-venous anastomoses are present in very large numbers in the ear of the rabbit, which itself is a relatively large structure, and which

acts as an efficient thermal radiator at high environmental temperatures, since both the capillaries and arterio-venous anastomoses become dilated. I now suggest that the reactivity of arterio-venous anastomoses persists in the surviving ear for a considerable period.

In the light of these observations, a possible explanation for the results of the present work may be that at 16°C the fluid perfusing the isolated ear is largely diverted from the capillary bed proper into other vessels, possibly arterio-venous anastomoses. It must be pointed out, however, that most investigations on the effect of environmental temperature on the peripheral circulation have been carried out on extremities which retained their connection to the rest of the body (e.g. Hemingway & Lillehei, 1950; Gibbon & Landis, 1932), whereas the effect of environmental temperature on the peripheral ear vessels in the present experiments was being studied on an isolated preparation.

General discussion

The conclusions drawn in these investigations concerning the capillary permeability and area in the perfused isolated ear of the rabbit must depend on the indirect observations

of net outward filtration, interstitial pressure and inflow.

The experimental observations reveal that when the temperature of the perfusion solution was raised to 38°C from 16°C, an increased rate of filtration into the tissue spaces occurred. It seems reasonable to consider this to be due mainly to capillary dilatation since van Dobben-Broekema & Dirken (1950b) have shown in the denervated ear of the otherwise intact rabbit, that the vessels which constantly dilate in response to increased temperature are capillaries.

There can no longer be any doubt that a reduction in the temperature of the perfusion solution from 38° to 16°C produced a marked increase in the rate of inflow, which was largely independent of any secondary effect due to altered physiological state of the ear or physical concomitant inherent in the carrying out of the perfusions at the different temperatures.

The temperature of the perfusion solution influenced the behaviour of the blood vessels of the isolated ear of the rabbit. The mechanism producing the increased rate of inflow at 16°C was possibly that little solution was passing through the capillary bed proper, the bulk of the solution being diverted, away from the area of filtration

and absorption, through dilated arterio-venous anastomoses thus increasing the rate of flow of solution from arteries to veins. The diversion of perfusion solution was possibly further assisted by the closure of the true capillary vessels at 16°C. Perfusion with solution at a temperature of 38°C may have caused constriction of the arterio-venous anastomoses and dilatation of the capillaries. The perfusion fluid would then be forced to flow through the dilated capillary bed proper, resulting in the increased rate of filtration and oedema. If this be true, an increased rate of gross outward filtration is obviously to be expected. Any delay in the return of fluid from the tissue spaces to the blood vessels would then cause oedema.

It may be that the environmental temperature may play a part in the control of the blood vessels even although the ear is isolated. The effect seemed to be chiefly on the number of functioning capillaries and possibly on the type of capillary vessel perfused. In the present investigation, the temperature of the perfusion solution was, of necessity, dominant. However, these experiments provided some evidence that the blood vessels of the ear were still sensitive to the temperature of the surrounding air.

It was concluded that the vessels of the isolated

preparation, similar to those of the intact ear, showed vascular reactions to temperature.

The temperature may exert its influence directly on the smooth muscle of the arteries, arterioles and arterio-venous anastomoses. Presumably, too, capillaries are sensitive to temperature. Intervention of surviving local nervous elements is not excluded and further investigation of this question is described in Chapter 3.

In the use of the perfused isolated ear of the rabbit as a test preparation for vascular responses, inflow rather than outflow measurements should be made and the temperature of the perfusion fluid and of the ear environment must be maintained at constant values. Allowance should be made for the pressure drop across the cannula and the pH of the perfusion fluid should ideally be maintained constant irrespective of the temperature or of the method of oxygen-ation of the fluid. If a solution of electrolytes is used as a perfusion fluid, gross oedema of the tissues is inevitable

Although this preparation is far from physiological, and while we do not understand the complexity of the vascular responses, it is nevertheless abundantly clear that the temperature of fluid perfusing the vessels must be carefully controlled, and chosen with reference to the problem to be

investigated.

Experiments designed to investigate permeability of capillaries in the isolated rabbit's ear are probably better carried out with perfusion fluid maintained constant at a temperature of 38°C. Those primarily designed to investigate vasomotor activity should be perfused with fluid maintained constant at a temperature of 16°C.

SUMMARY

1. Vascular responses of the perfused isolated ear of the rabbit to alteration in the temperatures of the perfusion solution and the environment are investigated. The effect of hyaluronidase is considered, as are the effects of alterations in the pressure, oxygen content and pH of the perfusion solution.

2. A simple technique is described for the controlled perfusion of the isolated ear of the rabbit. Changes in capillary permeability, area of capillary bed and net peripheral resistance are gauged by net outward filtration, interstitial pressure and inflow. The gross outward filtration is derived by mathematical analysis.

3. Lowering the temperature of the perfusion solution from 38° to 16°C reduces both net and gross outward filtration and increases inflow. This effect on inflow is largely independent of accumulation of oedema fluid and of level of pressure in the tissue spaces.

4. It is suggested that, at 16°C, the perfusion fluid passes largely directly from arterioles to venules by arterio-venous anastomoses, while, at 38°C, the fluid passes through the capillary bed.

5. Raising the environmental temperature of the isolated ear increases, and lowering decreases, the rate of net outward filtration.

6. The significance of these results is discussed. The vascular responses to temperature of the perfused isolated ear of the rabbit are suggested to be similar in character, for some considerable time, to the reactions of the blood vessels in the intact ear.

7. It is suggested that experiments designed to investigate permeability of capillaries in the rabbit's ear are probably better carried out with perfusion solution at 38°C. Those primarily designed to investigate vasomotor activity should be at a perfusion temperature maintained constant in the neighbourhood of 16°C.

8. The course and relationships of the main arteries and veins of the rabbit's ear are described.

CHAPTER 3

CAPILLARY FILTRATION AND VASOMOTOR ACTIVITY IN THE PERFUSED ISOLATED DENERVATED EAR OF THE RABBIT

CONTENTS

INTRODUCTION

The experiments described in Chapter 2 proved that the blood vessels in the surviving perfused isolated ear of the rabbit gave complex vascular responses to alterations in the temperature of the perfusion solution. Do the local nerves surviving in the isolated ear play a part in these responses? It was obviously desirable to extend such work to denervated blood vessels. The isolated ear of the rabbit which had previously been subjected to denervation and the nerves allowed to degenerate, was perfused with solution at different temperatures. It was considered that this would provide evidence of the effect of denervation on net outward filtration and also evidence for the part played by the surviving local nervous elements in the vascular responses to temperature of the normally innervated but isolated ear of the rabbit.

In a preliminary note Ferguson & Levinson (1953) concluded that in the denervated ear there is an increased capillary permeability and also a diminished response of the larger vessels to differences in the temperature of the perfusion solution.

A noticeable trend of physiological opinion has been

to revise the view that the central nervous system sends out vasodilator impulses, and to refer such vasodilatation to a peripheral and chemical stimulus. Thus McDowall (1935, 1938) pointed out that the so-called 'vasomotor centres' in the medulla might really be the apices of reflex arcs and that subsidiary vasomotor centres might exist in the spinal cord, or at least that vasomotor reflex arcs might occur in the cord. At that time McDowall limited the vasodilator system to:-

(1) sympathetic dilators primarily to striated muscles

(2) parasympathetic dilators distributed to special localised regions

(3) dorsal root dilators mainly to the skin and viscera.

He pointed out, however, the normal peripheral vasodilator action of carbon dioxide on capillaries.

In 1953 Armin, Grant, Thompson & Tickner provided evidence for the presence of a sympathetic cholinergic nerve mechanism in the central artery of the rabbit's ear. They suggested that, normally, acetylcholine was continuously synthesized and released, independently of central nerve stimulation, and tended to keep the artery in a state of dilatation.

Cannon & Rosenblueth (1949) pointed out that when

smooth muscle was normally stimulated or inhibited by cholinergic fibres, denervation of the muscle resulted in an exaggerated response to acetylcholine or applied substances, whether the cutting had been of penultimate neurons or ultimate neurones. By the term 'denervated' Cannon & Rosenblueth referred to severed ultimate effector neurones, i.e. post-ganglionic denervation in the case of sympathetic neurones. 'Decentralised' on the other hand referred to severed penultimate neurones, i.e. preganglionic denervation in the case of sympathetic neurones.

METHODS

Denervation of the ear of the rabbit

Nerve supply to the rabbit's ear. Feldberg (1926) and Grant, Bland & Camp (1932) gave detailed descriptions of the sympathetic and sensory nerve supplies to the rabbit's ear.

Denervation of the rabbit's ear. It is possible to cut the 'sympathetic' nerves alone by excision of the superior cervical and stellate ganglia. Alternatively the procedure may be carried out by excision of the superior cervical ganglion, with section of the vertebral ramus of the stellate ganglion and removal of as much as is accessible of the stellate ganglion from the ventral aspect of the neck (Armin et al., 1953; van Dobben-Broekema & Dirken, 1950b; Lewin & Schilf, 1927).

On the other hand 'denervation' of the rabbit's ear, i.e. section of both sympathetic and sensory nerves, is carried out by excision of the superior cervical ganglion and portions of the ventral and dorsal auricular nerves. Such interference leaves intact the motor innervation of

the external auricular muscles by the posterior facial nerve, a branch of the seventh cranial nerve. The branches of the posterior facial nerve lie between the ventral and dorsal auricular nerves at the base of the ear.

Operative procedure for unilateral denervation of the blood vessels of the rabbit's ear. Twenty-two adult rabbits of either sex were used. Their ears were of approximately the same size.

Denervation of one ear in each rabbit was carried out. General anaesthesia was induced by intravenous injection of pentobarbitone sodium (25 - 30 mg/kg body wt.) and maintained when necessary by intraperitoneal injection of pentobarbitone sodium (15 - 30 mg/kg body wt.).

Using aseptic precautions the superior cervical ganglion on one side was exposed, usually through a mid line incision in the neck. The ganglion was situated on the inner side of the external carotid artery and was partly hidden by this vessel. The ganglion was excised widely.

In the first 4 experiments while the denervation procedure was being completed the excised tissue was examined histologically, following CO_2 freeze cutting technique

and haemalum and eosin staining, to ensure that it contained ganglion cells. This information was thus available before the end of the operation. In the subsequent experiments it was not considered necessary to adopt this precaution.

A separate skin incision was made at the base of the ear on the same side and a length of 1 cm was resected from each of the ventral and dorsal auricular nerves.

Procaine penicillin G(300,000 i.u. in aqueous solution) was administered intramuscularly on the day of the operation and on the 2 subsequent days. None of the wounds became infected and recovery of the rabbits was uneventful in all cases.

Methylene blue technique for demonstrating of nerves in the rabbit's ear (method as used by F. W. Gairns, after Schabadasch)

This was used to check the presence or absence of nerves in both ears of rabbits. Two of the 22 rabbits which had been subjected to denervation of one ear 21 days beforehand, were asphyxiated by coal gas and immediately perfused through a cannula inserted into the left ventricle first with Ringers' solution and then with a solution of methylene blue.

The formula of the methylene blue solution was 0.8% anhyd. NaCl, 0.15% anhyd. $MgBr_2$, 0.2% $C_6H_{10}O_6$ (W/V). The pH was in the range 5.5 - 6.2. 0.15 g methylene blue BX Grüber was added to this solution and dissolved at a temperature of 50°C.

The perfusion was carried out at a temperature of 37°C for 10 - 15 min. Small pieces of the innervated ear were excised and examined microscopically until optimum staining of nerves was obtained. Both ears were then completely removed from the rabbit and the epidermis and superficial layers of the dermis stripped off, while leaving as much as possible of the subcutaneous tissue in place on the perichondrium, particularly on the dorsal surface.

The perichondrium with subcutaneous tissue was then cut into squares with an area of approx. 4 sq. cm and fixed for 24 hr at 4°C in a solution of 3% ammonium molybdate containing a few drops of osmic acid. The pieces were washed in running water overnight and then flattened and blotted between filter papers. Dehydration was carried out by placing the pieces on a wire gauze platform in a tightly stoppered jar which was filled with ethylene glycol monoethyl ether and which contained some anhydrous calcium chloride. 24 hr was allowed and 2 changes were used before

clearing the pieces in benzyl benzoate and mounting in neutral canada balsam, taking care that the dorsal surface was uppermost. The specimens were permanent if stored in the dark.

No difficulty was experienced using this technique although McGregor (1953) pointed out that it was notoriously difficult to produce consistent and uniform results in the vital staining of nerve fibres with methylene blue. McGregor achieved very considerable improvement in uniformity of staining of nerve plexuses of the ear in rabbits by adding hyaluronidase to the solution of the dye immediately before injection, in the proportion of 1,000 'Benger' units to 20 ml. of the dye solution.

The photomicrographs shown in plates 2 to 7 were treated by the method of after-toning, in which the black of the developed print was changed to blue. This colour is not likely to be permanent (Mitchell, 1953). The solutions for the blue-toning bath were:-

(1) Ferricyanide bath

Potassium ferricyanide, 1 g

Sulphuric acid, conc., 2 ml.

Water, up to 500 ml.

(2) Iron solution

Ferric ammonium citrate, 1 g

Sulphuric acid, conc., 2ml.

Water, up to 500 ml.

Solutions (1) & (2) were made up by first dissolving the salts in the water and then adding the sulphuric acid slowly. The working bath was made by mixing equal parts just before use. The developed prints were thoroughly freed from hypo, and were somewhat light in depth. They were immersed for approx. 1 min until the blue colour was reached, placed immediately in running water for approx. 1 min or until the yellow colour had gone from the whites, and hung up to dry after wiping or blotting off all drops of water.

Perfusion of the isolated ear of the rabbit

Experiments on perfusion of the denervated ear were carried out using the remaining 20 rabbits which had been subjected to unilateral denervation of the ear. These experiments were done 21 - 24 days following the denervation in each case. In addition 4 innervated ears were perfused.

The technique and apparatus used was that already

described in Chapter 2. Perfusions were carried on for 3½ or 4 hr. The rate of formation of oedema was taken as an index of the net outward filtration and the rate of inflow of the perfusion solution was used as an index of the net peripheral resistance.

Ringer's solution was used as the perfusion solution. The formula was 0.9 NaCl, 0.04 KCl, 0.025 $CaCl_2$, 0.0005 $MgCl_2$, 0.05 $NaHCO_3$ and 0.005 NaH_2PO_4 % W/V. This solution was freshly made about 1 hr before each experiment. The pH was adjusted to between 7.3 and 7.4, and the solution was oxygenated and pressurized by a mixture of 95% oxygen and 5% carbon dioxide. This did not cause any significant change in pH value. The perfusion solution was delivered to the cannula at a pressure maintained constant at 100 mm Hg.

The temperatures of both the perfusion solution and the environment were either both maintained at 16°C or alternatively the temperature of the perfusion solution was raised to 38°C when the environmental temperature was maintained constant at 21°C.

RESULTS

Denervation of the ear of the rabbit

Denervation of one ear was carried out successfully in all 22 rabbits. The temperature of the skin of the ear on the denervated side rose almost immediately after the operation.

Plate 8 shows a photomicrograph of one of the superior cervical ganglia excised and examined histologically while the operative procedure was being completed. Numerous nerve ganglion cells with large nuclei are demonstrated, proving that the excised tissue was from a ganglion.

Plates 2 to 7 are photomicrographs showing in one of the 2 rabbits the microscopical appearances following methylene blue perfusion. One of the ears of the rabbit had been denervated 21 days previously. As was to be expected, motor end-plates were demonstrated in the auricular muscles of both denervated and innervated ears, while there was a complete absence of nerves to the blood vessels on the denervated side.

Effect of perfusion solution temperature on rate of oedema formation and rate of inflow in the denervated and innervated ear of the rabbit

Twenty perfusion experiments were carried out on denervated rabbits' ears and 4 experiments, additional to those detailed in Chapter 2, on innervated ears. All these perfusion experiments were successful. A few of them have been omitted from description since they were additional control experiments in which the results of minor variations in perfusion solution temperature stimuli were confirmatory to those detailed under the subdivisions of results and discussion.

The pattern of development of net outward filtration and inflow in the innervated rabbit's ear perfused with solution at a constant temperature of 16° or 38°C or with solution varied between these temperatures during the course of the perfusion has already been described in Chapter 2. These results from Chapter 2 were used as a standard of reference confirmed by the results from perfusion of the 4 normal ears in the present series.

Expression of results from perfusion

Oedema formation. The oedema formation was the cumulative amount by which the ear preparation increased above its initial weight during perfusion. The readings were obtained by weighing the ear at 15 min intervals. The cumulative weight of oedema formation was graphed against time in the figures.

Inflow. The inflow was recorded as ml. perfusion solution leaving the cannula during the preceding 15 min. Thus inflow signifies the increment of inflow/15 min and not the cumulative inflow.

Effect of solution at a constant temperature of 16°C during perfusion

Oedema formation. Six experiments using denervated ears were carried out. The mean results are shown in fig. 32. The results from 2 experiments with innervated ears at 16°C are also given in the same figure.

After the first 30 min of the perfusion, the rate of oedema formation was practically constant in the denervated

preparations and only very slightly more marked than in the innervated preparations. In both cases at 16°C, the rate of oedema formation was very much less rapid than it was at 38°C, and the absolute amount of oedema formation was only about ¼ of that which accumulated in the denervated and the innervated rabbits' ears perfused at a solution temperature of 38°C.

Inflow. At this temperature, an initial spasm of the artery occurred which was more marked and took a longer time to pass off in the denervated preparations at 16°C than in the innervated preparations at the same temperature (fig. 32).

In denervated ears at 16°C after the first ½ hr of the perfusion the inflow remained fairly steady during the next 3 hr, but was very slightly more irregular and was at a rate well below the level in innervated preparations at the same temperature. During this period, the rate of inflow at 16°C in the denervated rabbit's ear was approximately midway between the extremes of inflow characteristic of the innervated preparations perfused at 16° and 38°C.

Compared with innervated ears, the rate of inflow was

reduced by approximately 20% in denervated ears.

Using a perfusion solution temperature of 16°C, no oscillation of the temperature of the solution entering the artery was noted either in the denervated or in the innervated preparations.

Effect of solution at a constant temperature of 38°C during perfusion

Oedema formation. Six experiments with denervated ears were carried out. Three of these are illustrated in fig. 32. Four experiments with innervated ears at 38°C are also regraphed in the same figure.

Oedema formed more rapidly in the denervated than in the innervated ears at the same temperature of 38°C, until about the end of the first 1½ hr of the perfusion. Thereafter the rate decreased more rapidly in the denervated preparations. The total amount of oedema formation after 3½ hr perfusion was similar in both cases, and was about 4 times greater than the amount which accumulated using perfusion solution at a temperature of 16°C.

Inflow. The visible spasm of the cannulated artery

was more marked but of the same duration in the denervated preparations than in the innervated preparations (fig. 32). The initial spasm of the arteries in both denervated and innervated ears was of shorter duration at 38° than at 16°C.

Thereafter the rate of inflow showed the same pattern throughout the perfusion in both types of preparation, with a maximum by the end of the first 30 min of the perfusion and a minimum about 2 hr. However, the rate of inflow in the denervated ears did not show this pattern alteration so markedly but was more regular and at a rate well above the level in innervated preparations at the same temperature of 38°C.

Moreover, the rate of inflow, apart from the initial fluctuation, was of the same order, although more irregular, in denervated preparations perfused with solution either constantly at 38° or constantly at 16°C. Inspection of fig. 32 and calculation of the mean inflow over the 3½ hr of perfusion showed that the mean inflow to the denervated ears was only very slightly greater at 38° than at 16°C.

During perfusion experiments at 38°C it was observed that the temperature of the solution entering the artery of the innervated ears oscillated over a range of about 0.75°C, each cycle taking about 40 sec. Such oscillations

are probably caused by rhythmical variations in the calibre of the blood vessels. Oscillation was not observed in denervated ears during perfusion at this temperature.

Effect of variations in the temperature of the solution during perfusion

Perfusion of the ear was commenced with the perfusion solution either at 16° or at 38°C. An hour or so later the temperature was changed to 38° or 16°C as the case might be. After a period, the temperature was returned to its original value. This complete cycle was repeated once more.

Oedema formation. Eight experiments were carried out on denervated ears, 4 on innervated ears.

A typical result is shown in fig. 33. Perfusion was commenced with solution at a temperature of 38°C for 1¼ hr, after which, without interruption of the flow, the perfusion solution temperature was lowered to 16°C. A concomitant lowering of the ear environmental temperature took place from 21° to 16°C. After 1 hr at this temperature, the perfusion solution temperature was raised once

more to 38°C, but was lowered again temporarily from after 3 hr till after 3½ hr from the start of the perfusion.

Alterations in the rate of formation of oedema in response to change in temperature of the perfusion solution were very similar in both denervated and in innervated preparations (fig. 33). During perfusion of the ear with solution at a temperature of 16°C, there was a decrease in the rate of formation of oedema fluid but this rate increased again when the solution was reheated to 38°C.

Inflow. In both types of preparation an increase in the rate of inflow occurred when the ear was perfused at 16°C, and a rapid decrease occurred when the temperature of the solution was raised to 38°C. However, when alterations were made in the temperature of the solution from 38° to 16°C or vice versa, the response in terms of inflow was more delayed and less marked in the denervated ears than in the innervated preparation (fig. 33).

DISCUSSION

Denervation of the ear of the rabbit

Using an intra-vital methylene blue technique Millen (1948) made observations on the general innervation of blood vessels. He noted that nerve endings from a single fibre might supply a relatively short segment of a small artery with a dense and intimate innervation, a longer segment of an arteriole with a less intimate supply and a still greater length of capillary vessel with a loose investment of nerve fibres. In other words, as the blood vessels got smaller their nerve supply became more generalized. Florey showed in his well known film that the capillaries were occluded by swelling of the endothelial cells on stimulation of the nerves to the ear.

Grant (1930b) described a very rich distribution of the perivascular sympathetic nervous plexus to the numerous arterio-venous anastomoses present in the rabbit's ear. Boyd (1952b) published a more detailed description of the innervation of peripheral arterio-venous anastomoses. He observed both non-myelinated fibres and also quite heavily myelinated fibres innervating arterio-venous

anastomoses, possibly pressor receptors.

Thus denervation of the rabbit's ear might well cause marked changes in the functioning of all the blood vessels of the ear, including the arterio-venous anastomoses.

Undoubtedly histological examination following methylene perfusion is the best test, where it is practical, for the adequacy of a denervation procedure. No other single test receives general support. Thus Grant (1935) found that the vessels of the denervated ear of the otherwise intact rabbit did not respond to changes in body temperature, even after the vascular tone had returned. Indeed Armin et al. (1953) used this absence of response as evidence for complete interruption of the sympathetic nerves to the rabbit's ear.

On the other hand, van Dobben-Broekema & Dirken (1950b) found that the vessels of the denervated ear of the rabbit still responded to heating and cooling of the body, although the reactions were more gradual than the sudden 'flushing' of the normal ear. These authors stated that the arterioles and capillaries still responded to temperature stimuli and that in a number of cases the larger vessels also reacted. The inconsistency of these results with those of Grant and his co-workers remains to be explained.

Using a very gross difference in temperature, the present experiments provide little evidence for persistence of sensitivity to temperature of the blood vessels of the ear after denervation. In Chapter 2 I suggested that the arterio-venous anastomoses are chiefly responsible for the marked influence of temperature on the flow of fluid through the blood vessels of the isolated ear of the rabbit. Consequently it is now suggested that denervation probably impairs to a marked degree the responsiveness of arterio-venous anastomoses to temperature.

Perfusion of the isolated ear of the rabbit

Oedema formation. The results show that oedema fluid developed somewhat more rapidly in the denervated ears both at a perfusion solution temperature of 16° and 38°C, than in the innervated ear of the rabbit.

The increased rate of oedema formation found in the denervated preparations might have been caused by increase either in the capillary permeability, or in the effective filtration force or alternatively by decreased vasomotion. Of these, increased permeability of cell walls has hitherto been suggested as the principal factor. Thus Gabbe (1926)

showed in guinea-pigs that section of the sympathetic supply resulted in the readier passage of colloidal dyes through the walls of the capillaries in smooth muscle compared with the control non-operated side, and concluded that the section of the sympathetic supply had made the muscle capillaries more permeable. Engel (1941) using studies of penetration of dye through synovial membrane concluded tentatively that the permeability of capillaries might be decreased following denervation.

That the increased permeability following denervation was not localised only to capillary endothelial cells was demonstrated by Lyman (1942). He claimed that he was able to rule out the disturbing influence of modified circulation, and showed that the rate of penetration of radioactive potassium into denervated rat's gastrocnemii muscle cells was greater than into control muscles.

In their review Cannon & Rosenblueth (1949) concluded that the larger penetration of substances into denervated than into normal cells, the more rapid effects of chemical agents on denervated than on normal elements, and the efficacy of doses of chemical substances smaller than the usual on cells which had been denervated, all were consistent with the theory that deprivation of nerve impulses increased

the ease of passage of substances through cell walls.

In the present experiments, however, the denervation might also have resulted in an increased effective filtration force, due to an increase either in the filtration area or filtration pressure. Thus the capillary filtration area might have been relatively increased at both perfusion solution temperatures in the denervated ears compared with the corresponding temperatures in the innervated preparations.

A lower rate of inflow at 16°C was found in the denervated preparations relative to the innervated ears and might have suggested a relative decrease in the bye-pass through arterio-venous anastomoses. This would result in a greater downstream pressure consequent upon the decreased pressure drop across the cannula. These factors would cause a greater net outward filtration at a perfusion solution temperature of 16°C in the denervated preparations, but cannot be considered to play a significant part since the greater oedema formation was also observed at 38°C, where the opposite set of conditions would hold, and these factors would correspondingly tend to mask the greater net outward filtration actually recorded at this temperature.

Since the oscillation in temperature of the perfusion solution entering the ear must have been due to alteration

in the rate of inflow, this was interpreted as possibly indicating the presence at a solution temperature of 38°C of vasomotion in the innervated preparations. Nevertheless the possible absence of vasomotion, as recorded indirectly by this method, at 38°C in the denervated preparations cannot be considered to explain the whole of the greater net outward filtration in these ears, since vasomotion was not recorded at 16°C in either type of preparation although the difference in net outward filtration was still found.

It was concluded that denervation resulted in an increased capillary permeability but the possibllity that it also resulted in an increased capillary filtration area must also be considered.

The distinctive pattern of the development of the oedema at either temperature was not affected by denervation. Consequently it was concluded that raising the temperature of the perfusion solution to 38°C still resulted in an increased dilatation, and/or increase in the number, of functioning capillaries irrespective of the denervation. Lowering the solution temperature to 16°C was concluded to result in a decreased dilatation, and/or a decrease in the number, of functioning capillaries irrespective of the denervation. Thus denervation had had no marked effect

on the ability of capillaries to respond to changes in temperature. These results were attributed to a direct action of temperature on the capillary vessels. These conclusions were supported by the further results that alterations in the rate of change in oedema formation in response to rapid alterations in solution temperature were similar in denervated and in innervated preparations.

Inflow. In the present experiments, alterations in the effective calibre of the vessels, and hence in the peripheral resistance or 'upstream pressure' were roughly demonstrated in magnitude by alterations in the rate of inflow.

The vascular spasm which occurred following cannulation at the beginning of the perfusion at a solution temperature of either 16° or 38°C was more marked in denervated rabbits' ears than in innervated preparations. Moreover at 16°C the spasm took a longer time to pass off in the denervated preparations. This demonstrated the ability of the vascular smooth muscle following denervation to respond to direct trauma by sustained contraction. These results confirmed the work of Kinmonth (1952) who found that an intact sympathetic nerve supply was unnecessary for the maintenance of arterial spasm in rabbits after stripping off the outer coats of the vessel.

In innervated ears the rate of inflow was found to be more rapid when the temperature of the perfusion solution was 16°C than when it was 38°C (Chapter 2). However, in denervated ears with the solution either at 16° or 38°C, there was little difference in the rates of inflow at either constant temperature, both being approximately midway between the extremes of inflow characteristic of the innervated preparations. The finding that the mean inflow to the denervated ears was very slightly greater at 38° than at 16°C may possibly be attributed to flow through a greater cross-sectional area of the capillary bed at the higher temperature. The difference in mean inflow was too slight to be likely to be due to a diversion of solution through larger vessels e.g. arterio-venous anastomoses. This would be consistent with the work of Clark (1938) who found that when the nerves to arterio-venous anastomoses were cut, the anastomoses remained dilated for 10 - 14 days, after which the muscle acquired tonicity and the lumen was thereafter maintained at a narrow calibre.

The effective calibre of the blood vessels of the denervated rabbit's ear was not entirely unaltered by marked temperature stimuli. This was shown by the fact that inflow responses to rapid alterations in solution temperature from

38° to 16°C or vice versa were still present, although they were more delayed and less marked, in the denervated preparations than in innervated ears. This tended to support the work of van Dobben-Broekema & Dirken (1950b) who found that the vessels of the denervated ear of the rabbit were still responsive to heating and cooling the rabbit's body although the reaction was of a more gradual type than the sudden 'flushing' of the normal ear.

Pinkston (1934) carried out observations on the ear vessels of rabbits subjected to experimental fever. He found that vasoconstriction in a febrile response only occurred in 50% of sympathectomised ear vessels, compared with 100% of normal vessels. Moreover, the blood vessels of the sympathectomised ear did not dilate so completely as those of the normal ear when there was a general peripheral vasodilatation.

Another inconsistency, however, exists in the report by Ahmad (1953) who demonstrated in the hand in a few sympathectomised human subjects that vasodilatation occurred at a plethsmograph water temperature of 25°C and vasoconstriction at 41°C. The responses, which were better elicited when the subjects were warmed by indirect heating and were of a transient nature, were shown by apparently normal blood

vessels. Ahmad was not able to offer any entirely satisfactory explanation for the mechanism of production of these results, nor did he report a large number of cases.

General discussion

It was concluded from the present experiments that temperature had little direct action on the smooth muscle of denervated arterio-venous anastomoses in the isolated rabbit's ear. In more general terms the presence of the sympathetic nervous system was needed for calibre responses of vessels to temperature stimuli although not for the development of vascular spasm to direct trauma. It was also considered justifiable to conclude that since the presence of a sympathetic nervous system was needed for calibre responses of vessels to temperature stimuli then the responses which did occur in isolated ears, not previously subjected to denervation, were dependent on the presence of the peripheral nervous elements.

Cannon, Rosenblueth & Ramos (1945) demonstrated that when one of the elements was severed in a functional chain of neurons, the ensuing total or partial denervation of some of the subsequent elements in the chain caused a

supersensitivity of all the distal elements, including those not denervated, and effectors if present, to the excitatory or inhibitory action of chemical agents and nerve impulses. The supersensitivity was greater for the links which immediately followed the cut neurones and decreased progressively for more distal elements. The increased sensitivity might fall into one or more of the following types:-

(1) superduration of response - prolonged time course, but amplitude unchanged

(2) hyperexcitability - lowered threshold

(3) increased susceptibility - normal amplitude responses to lessened stimuli above threshold level

(4) superreactivity - augmented capacity of tissue to respond.

Supersensitivity has been demonstrated more commonly to chemical agents than to nerve impulses. Thus sensitisation to adrenaline in the denervated ear vessels of the rabbit has repeatedly been shown (e.g. Meltzer & Meltzer, 1903a, b; Lichtwitz & Hirsch, 1910; White, Okelberry & Whitelaw, 1936; Burn, 1950) and to adrenaline, acetylcholine, histamine, pituitrin, and ergotoxine (e.g. Grant, 1930a, 1935; LeCompte, 1941).

Barcroft (1952) pointed out that there was an undoubted resemblance between the time courses of the recovery of vascular tone in the hand and foot following sympathectomy and those of the development of adrenaline sensitivity in the rabbit's ear and cat's nictating membrane. Yet the generally accepted opinion was summed up in the words of Cannon & Rosenblueth (1949) who concluded that the super-sensitivity evoked by denervation involved a plurality of causes and a plurality of mechanisms, both of which might differ in different structures and with different modes of denervation. They suggested that sensitization following denervation might be due to the removal of a restraining nervous influence, or to inactivity and consequent metabolic changes or to a trophic non-nerve-impulse influence.

Burn & Robinson (1952) carried out sympathectomy of the blood vessels of the foreleg of the cat. They found that denervation resulted in an early fall in amine oxidase in the blood vessels. The fall in amine oxidase was greatest at about 10 days, with a subsequent return towards normal. Burn & Robinson suggested that amine oxidase at the postganglionic terminations in the blood vessels plays a similar part to cholinesterase at cholinergic nerve endings.

On the other hand in an important contribution to the

problem Armin et al. (1953) found that acetylcholine disappeared from the dorsal auricular artery of the rabbit's ear about 3 days after sympathetic nerve section. They concluded that this resulted, at least in part, in the heightened reactivity of the denervated artery to constrictor influences. The artery exhibited both amine oxidase and cholinesterase activity, the latter being approximately half the activity shown by the normally innervated artery. Following section of both the sensory and sympathetic nerves the amine oxidase activity was found to persist in this preparation virtually undiminished while the cholinesterase activity disappeared.

Thus the sensitivity of the rabbit's dorsal auricular artery following denervation would be explained by Armin et al. on Cannon & Rosenblueth's classification as due to the removal of a restraining (nervous) impulse, that of the continual synthesis and release of acetylcholine, which before denervation tended to keep the artery in a state of dilatation.

Nevertheless in the present experiments the denervated rabbit's ear instead of showing sensitization to temperature stimuli was found to give delayed and markedly reduced vascular responses to temperature stimuli which in the

isolated preparation resulted in either vaso-constriction or vaso-dilatation. Thus it was less likely, although not impossible, that the effect of denervation on vascular smooth muscle was to result in directly decreased sensitivity of the vascular muscle cells to temperature stimuli. Consequently it was concluded that the perfused innervated rabbit's ear involved peripheral nervous elements.

SUMMARY

1. By appropriate section of nerves the blood vessels of the ears of 22 rabbits are deprived of their innervation. Methylene blue perfusion and histological examination are used to determine the completeness of the denervation of the rabbit's ear.

2. After 21 to 24 days, the ears are removed from the rabbits. Vascular responses of the isolated denervated ear of the rabbit perfused with Ringer's solution under controlled conditions are investigated to alterations in the temperature of the perfusion solution. Changes in capillary permeability, area of capillary bed and net peripheral resistance are gauged by net outward filtration and inflow.

3. At perfusion solution temperatures of both 16° and 38°C net outward filtration occurs more rapidly in denervated rabbits' ears than in innervated preparations, and more rapidly at 38° than at 16°C. Alterations in the rate of net outward filtration in response to rapid alterations in solution temperature are similar in both preparations. Denervation probably results in an increased capillary permeability but the possibility that it also results in

an increased capillary filtration area is discussed. It is concluded that the number and the state of dilatation of functioning capillaries is decreased at the lower temperature and increased at the higher temperature by a direct action of temperature on the capillary vessels, irrespective of denervation.

4. In denervated ears with the solution either at 16° or 38°C, there is little difference in the rates of inflow at either constant temperature, both being approximately midway between the extremes of inflow characteristic of innervated preparations of the rabbit's ear. Inflow responses to rapid alterations in solution temperature from 38° to 16°C or vice versa are still present in the denervated preparations but are more delayed and less marked than in innervated ears.

Thus the rate of flow of fluid through the vessels of the denervated ears is little affected by such a gross difference in temperature. This is in marked contrast to the behaviour of the blood vessels in ears with intact nerve supply, the flow at 16°C being 1/3 greater than at 38°C, probably due to opening up of arterio-venous anastomoses at the lower temperature. Denervation markedly impairs this action.

It is concluded that an intact sympathetic nervous system is needed for effective calibre responses of blood vessels to temperature stimuli and it is suggested that the calibre responses to temperature stimuli in the perfused innervated ear of the rabbit involve peripheral nervous elements.

5. Vascular spasm in response to mechanical trauma occurs at either perfusion solution temperature in both denervated and innervated preparations of the rabbit's ear. It is concluded that an intact sympathetic nerve supply is unnecessary for the onset and maintenance of arterial spasm.

CHAPTER 4

MATHEMATICAL DERIVATION OF CAPILLARY GROSS OUTWARD FILTRATION IN THE PERFUSED ISOLATED EAR OF THE RABBIT

C O N T E N T S

INTRODUCTION

Even in an isolated preparation, when the blood vessels are perfused, factors similar to these in the intact animal undoubtedly operate. Fluid leaves the capillaries, enters the tissue spaces, and returns to the vessels at the venous end of the capillary bed.

When perfusion is carried out with a solution of electrolytes, due to the absence of colloids, and probably to impairment of the state of the capillary walls, fluid tends to accumulate rapidly in the tissue spaces. This is oedema, what I have called "net outward filtration". There is no direct knowledge, however, of the volume of fluid which leaves and then re-enters the vessels.

The present chapter is an attempt, from experimental data, to derive mathematically the total volume of fluid leaving the capillaries, the "gross outward filtration." The difference between "net" and "gross" outward filtration could then be used to give the volume of fluid returning to the vessels.

Mr. Donaldson and Dr. Silvey of the Mathematics Department of the University kindly undertook to attempt to design a mathematical model which would represent the net outward filtration. I suggested that the net outward filtration

be considered in terms of an expression to represent the gross outward movement of fluid minus an expression to represent the fluid which returned to the vessels. Together we discussed the different shapes of the observed curves of rates of accumulation of net outward filtration under different experimental conditions. Each curve was considered in terms of alteration of one or both of the expressions gross outward movement of fluid and the return of fluid to the vessels. A formula was eventually decided upon. Different methods of fitting the formula to the experimental data under any one set of experimental conditions were then tried.

PERFUSION OF THE ISOLATED EAR OF THE RABBIT WITH RINGER'S SOLUTION

The rate of accumulation of net outward filtration, that is the rate of formation of oedema, may be expressed (Donaldson, Ferguson, Levinson & Silvey, 1953) by:-

$$\frac{dw}{dt} = \alpha (1 - e^{-\gamma t}) - \beta w \quad \ldots\ldots\ldots\ldots (1)$$

where t = time, w = cumulative net outward filtration;

$\alpha(1 - e^{-\gamma t})$ represented the rate of gross outward filtration across the capillary membrane, thus taking into account capillary permeability, effective filtration force and vasomotion; the parameter α represented the ultimate value of gross outward filtration and γ described its rate of change. The parameter β described the extent to which oedema fluid already formed retarded further net outward filtration.

PERFUSION AT SOLUTION TEMPERATURE 16°C

Since the net rate of outward filtration remained steady throughout perfusion at this temperature (figs. 9, 22, 26, 27 & 31), this corresponded to the simplification $\gamma = \infty$, whence

$$\frac{dw}{dt} = \alpha - \beta w \quad \ldots\ldots\ldots\ldots\ldots (2)$$

The method of least squares was used, and values of α and β chosen to make the sum of squares

$$\sum \left[\frac{dw}{dt} - \alpha - \beta w\right]^2 \quad \ldots\ldots\ldots\ldots (3)$$

as small as possible.

This led to the following 2 equations

$$\sum \frac{dw}{dt} - n\alpha + \Sigma w\beta = 0 \quad \ldots\ldots\ldots\ldots (4)$$

$$\sum w\frac{dw}{dt} - \Sigma w\alpha + \Sigma w^2\beta = 0 \quad \ldots\ldots\ldots (5)$$

$$\therefore \; n\alpha - \Sigma w\beta = \sum \frac{dw}{dt}$$

$$\Sigma w\alpha - \Sigma w^2\beta = \sum w\frac{dw}{dt}$$

$$\therefore \; n\Sigma w\alpha - (\Sigma w)^2\beta = \Sigma w\sum \frac{dw}{dt}$$

$$n\Sigma w\alpha - n\Sigma w^2\beta = n\sum w\frac{dw}{dt}$$

$$\therefore \; \beta = \frac{n\sum w\frac{dw}{dt} - \Sigma w\sum\frac{dw}{dt}}{(\Sigma w)^2 - n\Sigma w^2} \quad \ldots\ldots\ldots\ldots\ldots (6)$$

$$\alpha = \frac{\sum\frac{dw}{dt} + \Sigma w\beta}{n} \quad \ldots\ldots\ldots\ldots\ldots (7)$$

It was found in practice that, in general, better fits were obtained by summing only over central values of t, otherwise the end values were unduly predominant.

$$\frac{dw}{dt} = \alpha - \beta w \quad \ldots\ldots\ldots\ldots (2)$$

$$\therefore \; \frac{d}{dt}(e^{\beta t}w) = \alpha e^{\beta t}$$

$$\therefore we^{\beta t} = \frac{\alpha}{\beta}(e^{\beta t} - 1)$$

$$\therefore w = \frac{\alpha}{\beta}(1 - e^{-\beta t}) \quad \left[w = 0 \text{ when } t = 0\right] \quad .....(8)$$

The exponential $e^{-\beta t}$ decreases with time towards zero.

Evaluation of w was made by substitution of the calculated values of α and β in (8). The degree of closeness of fit of the experimental and calculated values of w was noted.

α was then graphed against t in order to display the gross outward filtration rate across the capillary membrane at a perfusion solution temperature of 16°C.

The steps in the evaluation were:-

t	w	$\frac{dw}{dt}$	$w\frac{dw}{dt}$	w^2
1	Experimental results	$\frac{1}{2}(w_2 - w_0)$		
2				
3				

19				
	Σ	Σ	Σ	Σ

Evaluations of $\beta = \dfrac{n\sum w\dfrac{dw}{dt} - \Sigma w\sum \dfrac{dw}{dt}}{(\Sigma w)^2 - n\Sigma w^2}$ .....(6)

and $\alpha = \dfrac{\sum \dfrac{dw}{dt} - \Sigma w\beta}{n}$ ..... (7) were made.

t	βt	$e^{\beta t}$	$e^{-\beta t}$	$1 - e^{-\beta t}$	$\frac{\alpha}{\beta}(1 - e^{-\beta t})$	αt
1		From table of natural logaritims				
2						
3						
- - - -						
19						

Equation (1) took no account of the increase in weight of the ear due to the filling of the blood vessels with perfusion solution. Thus, in practice, when t = 0, the value for the net outward filtration will not pass through zero on the ordinate.

$$\left(\frac{dw}{dt} - \alpha + \beta w\right)^2$$

$$\left[\frac{dw}{dt} - \alpha + \beta(w + 1)\right]^2$$

$$\sum\frac{dw}{dt} - n\alpha + \beta\sum w + n\beta = 0$$

$$\sum(w + 1)\frac{dw}{dt} - \alpha\sum w - n\alpha + \beta\sum(w + 1)^2 = 0$$

$$\therefore \sum w\frac{dw}{dt} - \alpha\sum w + \beta\sum w^2 + \beta\sum w = 0 \quad \ldots\ldots\ldots(9)$$

$\therefore$ if β was small, a greater correction was needed.

During perfusion of the isolated rabbit's ear with solution at a temperature of 16°C, the increase in weight of the ear due to filling of the blood vessels with perfusion solution was unduly predominant since oedema developed comparatively slowly. This was allowed for by introducing a correction factor C.

$$\frac{dw}{dt} = \alpha - \beta w \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

$$\therefore \alpha = \frac{dw}{dt} + \beta w$$

$$\frac{d}{dt}(e^{\beta t}w) = \alpha e^{\beta t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(10)$$

$\therefore$ solution:- $$e^{\beta t}w = \frac{\alpha}{\beta}e^{\beta t} + C$$

$$C = \frac{1}{n}\Sigma C_r$$

whence $$C_r = (w_r - \frac{\alpha}{\beta})e^{\beta tr} \quad \ldots\ldots\ldots\ldots(11)$$

The best value of C was calculated by using central values of t, whence

$$w = \frac{\alpha}{\beta} + Ce^{-\beta t} \quad \ldots\ldots\ldots\ldots\ldots\ldots(12)$$

The steps in the evaluation were:-

t	$w_r - \frac{\alpha}{\beta}$	$(w_r - \frac{\alpha}{\beta})e^{\beta t}r$
3		
4		
5		
⋮		
10		
		Σ

$C = \frac{1}{n}\Sigma C_r$ was evaluated.

Then $w = \frac{\alpha}{\beta} + Ce^{-\beta t}$ was calculated by substitution of the

chosen values of α , β , and C in equation (12) and the degree of closeness of fit of experimental and calculated values of w was noted.

The values for a worked example, that of oedema formation at 16°C in expt. no. 24, 47, are shown in tables 12, 13 and 14.

PERFUSION AT SOLUTION TEMPERATURE 38°C

Since fitting the original formula was difficult the simplification $\gamma = \beta$ was used, whence

$$\frac{dw}{dt} = \alpha(1 - e^{-\beta t}) - \beta w \quad \ldots\ldots\ldots\ldots(13)$$

Since a good fit was obtained by the simplification an even better fit would be obtained without it.

Values for the parameters α and β were determined.

$$\frac{dw}{dt} = \alpha e^{-\beta t} - \alpha e^{-\beta t} - \alpha\beta t e^{-\beta t}$$

$$\text{i.e. } \frac{dw}{dt} = \alpha\beta t e^{-\beta t} \quad \ldots\ldots\ldots\ldots\ldots\ldots(14)$$

Let $k = \alpha\beta$

$$\frac{dw}{dt} = kte^{-\beta t} \quad \text{.....(15)}$$

Logarithms were taken in order to obtain a linear equation of the constants.

$$\log\frac{dw}{dt} = \log k + \log t - \beta t \quad \text{.....(16)}$$

Let $A = \log k$

$B = \beta$

$$\log \frac{dw}{dt} = A + \log t - tB \quad \text{.....(17)}$$

The method of least of two squares was used, and values of A and B chosen to make the sum of squares

$$\sum\left[\log \frac{dw}{dt} - A - \log t + tB\right]^2 \quad \text{.....(18)}$$

as small as possible.

This led to the following 2 equations

$$\sum \log \frac{dw}{dt} - nA - \Sigma \log t - \Sigma tB = 0 \quad \text{.....(19)}$$

$$\sum (t \log\frac{dw}{dt}) - \Sigma tA - \Sigma t \log t - \Sigma t^2B = 0 \quad \text{....(20)}$$

$$\therefore \quad nA - \Sigma tB = \Sigma \log \frac{dw}{dt} - \Sigma \log t$$

$$\Sigma tA - \Sigma t^2B = \Sigma (t \log \frac{dw}{dt}) - \Sigma t \log t$$

$$\therefore \quad n\Sigma tA - (\Sigma t)^2B = \Sigma t \, \Sigma\log \frac{dw}{dt} - \Sigma t \, \Sigma\log t$$

$$n\Sigma tA - n\Sigma t^2B = n\sum(t \log \frac{dw}{dt}) - n\Sigma t \log t$$

$$B = \frac{\Sigma t\sum \log\frac{dw}{dt} - n\sum(t \log\frac{dw}{dt}) + n\Sigma t \log t - \Sigma t\Sigma \log t}{n\Sigma t^2 - (\Sigma t)^2}$$

.....(21)

A was evaluated by substitution.

α and β were evaluated by substitution for A and B

$$A = \log\alpha - \log\beta .$$

$$\frac{dw}{dt} = \alpha(1 - e^{-\beta t}) - \beta w \quad .....(13)$$

$$\frac{dw}{dt} + \beta w = \alpha(1 - e^{-\beta t})$$

$$\frac{d}{dt}(e^{\beta t}w) = \alpha(e^{\beta t} - 1)$$

$$e^{\beta t}w = \frac{\alpha}{\beta}(e^{\beta t} - 1) - \alpha t$$

$$w = \frac{\alpha}{\beta}(1 - e^{-\beta t}) - \alpha t e^{-\beta t} \quad [w = 0 \text{ when } t = 0] \quad ..(22)$$

Evaluation of w was made by substitution of the calculated values of α and β in (22) and the degree of closeness of fit of the experimental and calculated values of w was noted.

$\alpha(1 - e^{-\beta t})$ was then graphed against t in order to

display the gross outward filtration rate across the capillary membrane at a perfusion solution temperature of 38°C.

The steps in the evaluation were:-

t	w	$\frac{dw}{dt}$	$\log\frac{dw}{dt}$	$t \log\frac{dw}{dt}$	t^2	log t	t log t
1			From table of natural logarithms				
2							
3							

19							
Σ			Σ	Σ	Σ	Σ	Σ

Evaluations of B, β, A, and α were made by substitution in (21) et seq.

t	βt	$e^{\beta t}$	$e^{-\beta t}$	$1 - e^{-\beta t}$	$\frac{\alpha}{\beta}(1 - e^{-\beta t})$	$\alpha(1 - e^{-\beta t})$	αt	$\alpha t e^{-\beta t}$	w
1									
2									
3									

19									

During perfusion of the isolated rabbit's ear with solution at a temperature of 38°C, the increase in weight of the ear due to filling of the blood vessels with perfusion solution was not unduly predominant since oedema developed rapidly. However a correction factor might have been applied if required.

$$\frac{dw}{dt} = kte^{-\beta t} \quad \text{.....(15)}$$

$$\frac{dw}{dt} = \alpha(1 - e^{-\beta t}) - \beta w$$

$$\frac{d}{dt}(e^{\beta t}w) = \alpha(e^{\beta t} - 1) \quad \text{.....(23)}$$

$\therefore$ solution:- $e^{\beta t}w = \frac{\alpha}{\beta}e^{\beta t} - \alpha t + C$

$$C_r = (w_r - \frac{\alpha}{\beta})e^{\beta t_r} + \alpha t_r \quad \text{.....(24)}$$

An average correction might have been calculated for the points used in fitting α and β, whence

$$w = \frac{\alpha}{\beta} - (\alpha t - C)e^{-\beta t} \quad \text{.....(25)}$$

The steps in the evaluation would have been:-

t	$w_r - \frac{\alpha}{\beta}$	$(w_r - \frac{\alpha}{\beta}) e^{\beta t_r}$	αt_r	C_r
3				
4				
5				
⋮				
12				
				Σ

Then $C = \frac{1}{n} \Sigma C_r$ would have been evaluated.

Then $w = \frac{\alpha}{\beta} - (\alpha t - C) e^{-\beta t}$ would have been calculated by substitution of the chosen values of α, β and C in equation (25) and the degree of closeness of fit of experimental and calculated values of w noted.

It was found in several analyses of experimental results at a solution temperature of 38°C that the slight additional accuracy of closeness of fit obtained by the use of the correction factor did not justify the labour involved.

The values for a worked example, that of oedema

formation at 38°C in expt. no. 38, 39, 40, 41, are shown in tables 15 and 16.

CHAPTER 5

IMPROVED CONSTANT PRESSURE DEVICE

C O N T E N T S

I N T R O D U C T I O N

The perfusion or infusion of liquids under constant pressure is a common requirement in experimental physiology. The science of fluid mechanics dates its origin from an important discovery of Torricelli. Torricelli enunciated in his 'De Motu Gravium Naturaliter Accelerato, 1643', that the velocity of a fluid passing through an orifice in the side of a reservoir is the same as that which would be acquired by a body falling *in vacuo* from the vertical height, measured from the surface of the fluid in the reservoir to the centre of the orifice. This proposition, known as the theorem of Torricelli, is expressed by $v = (2gH)^{\frac{1}{2}}$. Upon it rests the whole theory of water actuated by the force of gravity.

Mariotte made many experiments illustrating the truth of this theorem, the results of which were published after his death, in 1686.

LABORATORY CONSTANT PRESSURE DEVICES

Mariotte's bottle with central tube

Mariotte (1718) reported some simple hydrodynamic experiments using a bottle with a hole near the bottom. A tube,

open to the atmosphere, entered through the stoppered top of the bottle (fig.34).

If the bottle was filled with water and the tube reached below the level of the hole, water ceased running out when the tube was empty as far as the level of the middle of the hole. The bottle then remained full of water. On the other hand, if the end of the tube was higher than the level of the top of the hole, then Mariotte observed that atmospheric air entered through the tube. The air rose through the water to the stoppered top of the bottle while, at the same time, the water continued to discharge through the hole.

Since Mariotte either omitted or failed to make the observation that the water discharged at the same rate no matter the level of the water in the bottle, he probably did not realise that he had devised a constant pressure bottle. Mariotte also reported the results of observations using holes of different sizes in the bottle, although he made no mention of using central air tubes of different bores.

Theoretical treatment of Mariotte's bottle with central tube

Mariotte's explanation of the factors involved was definitely inadequate. Consideration of the conditions led to the following evaluation.

Water discharging through orifice in bottom.

$Q = C_d a \sqrt{2gh_1}$

where Q = quantity of water discharged (cm^3/sec): obtained by measurement.

C_d = coefficient of discharge of orifice.

a = cross sectional area of the outer or smallest section of the orifice (cm^2): obtained by measurement.

h_1 = height of lower end of central tube above orifice (cm): obtained by measurement.

$\sqrt{2gh_1}$ = velocity equivalent to the static head h_1.

Hence C_d = could be evaluated as $Q/a\sqrt{2gh_1}$.

Air entering by central tube.

$Q = v.\pi d^2/4$

where Q = air entry (cm^3/sec).

= quantity of water discharged through orifice in bottom (cm^3/sec): obtained by measurement.

v = mean velocity of air entering central tube (cm/sec).

d = diameter of central tube (cm): obtained by measurement.

Hence v could be evaluated as $4Q/\pi d^2$.

The flow of air below the critical velocity in a straight, narrow bore, circular tube is stream-lined, viscous non-turbulent flow. Hence $p = 32\mu lv/d^2$, where

δp = difference between atmospheric pressure and pressure at lower end of central tube due to friction (dynes/cm^2)

μ = viscosity of air (dynes-sec/cm^2): obtained from tables

l = total length of central tube (cm): obtained by measurement

v = mean velocity of air entering central tube (cm/sec)

d = diameter of central tube (cm): obtained by measurement.

Hence δp could be evaluated. In practice, the difference between atmospheric pressure and the pressure at the lower end of a central tube of a Mariotte's bottle would be found to be slight.

Mariotte's central tube applied to a burette

Such a constant pressure bottle consisting of a burette with central air tube was used in the frog heart apparatus devised by Professor Kronecker of Berne in the second half of the 19th century (McKendrick, 1892) and by van Wijngaarden (1926).

Van Wijngaarden desired to obtain measured volumes of fluid delivered at constant pressure. His apparatus consisted of a standard 100 ml. graduated burette which was filled with fluid and closed with a one-holed stopper (fig.35,A). A glass tube passed through the rubber stopper and the bottom of this central tube was below the lowest graduation of the burette. On opening the burette tap, air bubbles rose through the fluid in the burette from the lower end of the central tube.

Van Wijngaarden selected the bore of the central tube so that the desired outflow rate was obtained. This was unnecessary since variations in delivery rate could have been obtained more readily by altering the height of the burette above the cannula tied into the preparation. Moreover, the inflow readings, since they were read from a burette with standard calibrations, were inaccurate due to the volume taken up by the central tube. Nor was it

possible to calibrate the burette specially in order to allow for the central tube, since different tubes were used to obtain the desired outflow rate.

Burn (1928) advised the use of van Wijngaarden's constant pressure device as the best available apparatus for measuring inflow volumes. He specified that the glass tube which passed through the burette stopper should be drawn out, immediately below the stopper, into a long capillary. This modification considerably increased the accuracy of the volume readings when using a standard calibrated burette.

Other laboratory constant pressure devices

Only 2 other laboratory methods of obtaining a constant fluid pressure appear to have been described in the literature. Shanks (1923) used a constant pressure perfusion cannula (fig.43, A) in which the perfusion fluid on reaching a certain level in the cannula ran off to waste through a side tube. Shanks suggested its use only for perfusion of the isolated heart, and he failed to realise the possibility of a wider application of the principle. In fact, the cannula could be attached to the preparation by a short length of rubber tubing instead of directly, thus permitting a choice of level of constant pressure. Nevertheless, the device suffered from the disadvantage that the cannula could

not be used to measure inflow unless specially calibrated, and that even in Shanks' unmodified cannula, the fluid dead space involved in testing drug action was considerable. A final disadvantage was that it was necessary frequently to adjust the rate of the fluid delivery to the cannula in an attempt to reduce to a minimum the waste of fluid through the overflow side tube.

Herbst (1950) described an excellent constant pressure apparatus (fig.36). The liquid was stored in the bottle A, from which it flowed through the filter B, into the reservoir C. A constant level was maintained at D: air was admitted to A through the rubber tube E whenever the level in C fell sufficiently. The orifice F admitted the necessary air. The liquid passed through tube G to the orifice H. The rate of flow was controlled by the vertical distance between D and H and by the dimensions of H.

Except for its slightly cumbersome attachments, and the time involved in cleaning and refilling the component parts consequent upon a change in the nature of the fluid, this constant pressure apparatus is extremely useful.

DISADVANTAGES OF THE CENTRAL TUBE IN THE MARIOTTE CONSTANT PRESSURE DEVICE

It was obvious that the Mariotte principle was the best, and offered the most convenient device, to use in order to obtain a constant head of pressure for physiological experiments.

Yet the central air tube was not only not necessary but was also an actual disadvantage. This was especially so if it was desired to measure accurately the volume of fluid leaving a standard calibrated burette under constant pressure.

Moreover, when filling or refilling a classical Mariotte bottle, there resulted a pressure initially in excess of the constant head desired for the perfusion or infusion. This initial excess pressure was due to the entry of fluid up the central air tube, as a result of the insertion of the stopper causing an increase in the pressure of the air contained above the fluid in the bottle. On refilling it was noted occasionally that there was a variation in the constant pressure level due to failure to reinsert the stopper and central tube to exactly the previous level. Moreover, the lower end of the central air tube might lie so far up the reservoir that frequent refills were required.

Yet another disadvantage of use of the Mariotte bottle with central tube was that, to obtain constant pressure of the order of mammalian blood pressure, the required high hydrodynamic pressure had to be provided by the awkward positioning of the reservoir bottle at a considerable height from the bench level.

Finally, oxygenation of the fluid, delivered at constant pressure, had to be achieved either by oxygenation before the experiment commenced or by a special side limb attached to the reservoir bottle (fig.48, B).

IMPROVED MARIOTTE CONSTANT PRESSURE DEVICE

All that was required to overcome the disadvantages of the central tube, and yet retain all the advantages of the Mariotte principle, was to have a stoppered fluid reservoir, burette or bottle, with 2 orifices which were at different levels below the surface of the fluid and as near to the bottom as possible (fig.37). The reservoir would then deliver fluid at constant pressure from the lower orifice, while air entered the upper orifice and bubbled through the fluid.

This apparatus had the additional advantages of simplicity and also economy, since, due to the fixed site of the air entry orifice close to the fluid discharge orifice, all but the very last of the fluid in the flask was delivered at constant pressure.

STAGES IN DEVELOPMENT OF THE IMPROVED CONSTANT PRESSURE BURETTE

Stage 1

The device was made from a 50 ml. or 100 ml. graduated burette complete with pinchcock or with straight bore glass stopcock. The upper end of the burette was closed with a stopper. A narrow bore glass tube ran parallel to the burette (fig.35,B). The lower end of the tube was fused through the burette below the graduations and just above the cock. The upper end of the parallel glass tube was open to the atmosphere.

It was easy to fuse the parallel glass tube on to the standard laboratory article catalogued as 'burette, with side tube for filling fitted below graduations'. A cork wired between the burette and side limb near the top made the glass junction near the cock less liable to breakage.

To prepare the apparatus for use, the stopper in the upper end of the burette was replaced by a filter funnel, through which the burette and side limb were filled with fluid to above the level of the zero graduation. The stopper was then replaced, the stopcock opened and fluid run out until the side limb was empty, air was bubbling into the burette and the level of fluid in the burette had reached the zero calibration. Finally the burette was clamped to an upright so that the side limb entrance into the burette was at the required head of pressure above the cannula.

Stage 2

The addition of a 3-way T-bore stopcock (fig.35, C) to the side tube below the level of the burette junction facilitated the filling, emptying and washing out of the burette and side limb from below. During filling and washing the stopper at the top of the burette still had to be removed. Nevertheless the additional stopcock largely obviated the necessity of unclamping the burette from its established pressure height during filling, or of disconnecting at the cannula on changing perfusing fluids. Moreover, any error in inflow measurements after refilling was abolished, since the fluid on the side tube was run off via the additional stopcock, before recommencing the infusion.

Stage 3

The presence of another 3-way T-bore stopcock, at a junction situated above the zero level, permitted the filling, emptying and washing of the burette and side limb from below without removal of the stopper at the top of the burette. It was noted as a possibility, in this stage of the development, that in research work requiring the rapid interchangeable use of 2 or 3 constant heads of pressure, additional 3-way T-bore stopcocks could be added at the appropriate heights on the side limb, each with a junction to the burette. Such additional junctions would make the use of wired corks to strengthen the apparatus unnecessary.

The advantages of such a complication were slight.

Stage 4

By a fortunate accident the parallel tube of a constant pressure burette was broken off at the point where it had been fused on to the standard side tube (fig.35,D). It was noted that a constant pressure device was available simply by purchasing a catalogued burette fitted for filling, below the graduations and at right angles to the long axis of the burette, with a side tube of 3-4 mm internal diameter (Ferguson & Garry, 1952). If very rapid delivery of fluid was desired from such a standard burette it might be necessary to cut

off the terminal drawn out portion of the delivery tip of the burette.

Subsequent to the demonstration of our device Redonnet (1952) described a constant pressure burette in which the air entry orifice consisted of a capillary passing through a stopper and inserted into a special dilatation on the burette, blown below the lowest graduation (fig.38). Similarly to van Wijngaarden he modified the outflow rate under constant pressure by using capillaries of different bore. However, the capillary side limb is broken frequently during use of the burette. Replacement of the capillary side limb was claimed by Redonnet to be easier and more rapid. Yet this was irrelevant since in the Ferguson & Garry burette a break in the short stout side air tube is an extremely rare occurrence.

In our burette filling and refilling has been carried out through the stoppered end, while the side air tube A, (fig.39), was closed temporarily by the finger or by a clip on a short length of rubber tubing. The stopper was removed and the burette filled from above through a filter funnel. This was inconvenient if a high constant hydrostatic pressure was being used. Once the stopper was replaced the side air tube was reopened, so that a small quantity of fluid

might be ejected through it before the burette reading was noted.

Filling the burette through the side tube with fluid from a pressurized reservoir was next attempted. However, this resulted each time in an inaccuracy in the inflow readings due to the unmeasured volume of fluid temporarily lodging in the side tube.

Filling from below upwards through a T-bore tap placed below the cock of the burette was the most convenient, particularly since this permitted the application to the fluid in the burette of any desired gaseous pressure greater than atmospheric pressure through the side tube. This method was finally adopted.

Stage 5

A final modification attempted consisted of merely blowing a small hole below the lowest graduation of the burette. This hole acted as an air inlet. As before the upper end of the burette was closed with a stopper.

This constant pressure burette, although less fragile than any of the others constructed, suffered from 2 disadvantages. It was always necessary to supply closure of the air inlet orifice by hand during the process of filling nor was there any side tube available by which pressures greater

than atmospheric could be conveniently applied. For these reasons the final modification given under Stage 4 was adopted.

WORKING PRINCIPLES OF THE IMPROVED CONSTANT PRESSURE BURETTE

The working principles of the burette with side tube are those which govern the Mariotte bottle.

Using atmospheric pressure applied to side tube

The burette (fig.40, 1), open at the top, was filled with fluid from below through C, up to the graduation mark zero, the side air tube A being closed. Tap B was then turned off. The lateral pressure at X was now equal to atmospheric pressure + the pressure due to the head of the column of fluid above point X in the burette. The head was the height of the level of the free surface of the water above the point considered and signifies energy per unit weight. The pressure-intensity is the same in all directions in a fluid at rest. The air inlet was treated as a small orifice since its dimensions were small in comparison with the head of water above its centre, so that at any point in its area the head could be taken as equal to that at its centre without sensible error.

The rubber tube attached to a stopper already *in situ* in the top of the burette was then closed off by a screw clip (fig.40, (2)). When the burette was closed off at the top, and the side air tube A was opened, there was an outward movement of the air/water interface at A. Alternatively if the top of the burette was closed by actually inserting a stopper then, on opening A, a spurt of a very little fluid occurred out of A. This was due to the insertion of the stopper compressing the air above the meniscus, thus bringing the pressure of the air to a little above atmospheric and altering the lateral pressure at X further from the equilibrium value. Due to the spurt of fluid out of the side air tube A, the meniscus level in the burette fell very slightly until, assuming negligible surface tension effects, the gaseous pressure above was reduced to below that of the atmosphere to the value $P - g\rho_{\omega}h$, where P = barometric pressure: h = height of fluid column between the meniscus and A. Thus a state of equilibrium existed between the atmospheric pressure, P, acting inwards and the outward lateral pressure, p, at A. The outward lateral pressure, p, was equal to the subatmospheric pressure exerted by the air contained in the burette, $P - g\rho_{\omega}h$, + the hydrostatic pressure of the column of fluid, $g\rho_{\omega}h$. Thus movement of the

air/water interface at X was zero since p equalled P.

When the tap of the burette was opened there was an effective pressure at point C which was produced by the hydrostatic pressure of the column of fluid XC above atmospheric pressure. This equalled $P + g\rho_w h_1$, where h_1 = height of fluid column between A and C. Consequently the burette started to empty.

For water to be discharged from C, $g\rho_w h_1$ must be equal to or greater than $2T/r$

where T = surface tension of water and air

r = internal radius of air inlet,

i.e. the largest diameter which would prevent flow was given by $2.73.2 / 980.1.h_1 = 0.3 / h_1$ cm.

e.g. for h_1 = 5 cm, the smallest diameter of C is theoretically just greater than 0.06 cm. Since $Q = C_d.a.\sqrt{2gh_1}$, for flow through orifices with free discharge

where Q = quantity discharged per second,

a = cross sectional area of the outer or smallest section of aperture C,

it was possible to obtain the coefficient of discharge, C_d, for the orifice C. For practical purposes the head due to to velocity of approach would not be appreciable, but the more viscous the liquid in the burette, the smaller would be

the value of C_d (Smith, 1886).

Thus after fluid had discharged from the burette, and before the bubble entered, the pressure of the air enclosed in the increasing space above the fluid was decreased, as also was the hydrostatic pressure of the fluid at A. The resultant unbalance of equilibrium produced at the air/fluid interface in A caused air to enter the burette through the side tube when $p = P - 2T/r$ where T = surface tension of water and air: r = internal radius of air inlet. Air entered the burette until the equilibrium state was re-established. Air can exist as a bubble in the fluid, due to the surface tension of the fluid at its free surface with the air. Since the fluid was not gas-free, the air bubble was not extinguished by being dissolved. The air bubble rose in the fluid due to its buoyancy, until it reached the surface of the liquid.

Table 17 shows the theoretical order of pressures involved when air entered, or should water have discharged, through side air tubes of various diameters of a constant pressure burette. Thus with a side tube of 0.10 cm diameter, air would enter the constant pressure burette most readily while water would have had marked difficulty in leaving.

The part played in the equilibrium state by the hydrostatic column of fluid would gradually decrease as the burette emptied, thus causing the air contained at a subatmospheric pressure in the burette above the fluid, to approach nearer and nearer to atmospheric pressure.

This cycle of a pressure range near the equilibrium value, was maintained as long as tap B was kept open and the fluid meniscus was still above the level of the side tube. Thus the delivery of the fluid from the burette took place at what was essentially a constant pressure. The pulse pressure was observed to be of the order of 2 mm water, which is even less than the pulse pressure in a conventional Mariotte bottle.

In general, an orifice in a horizontal plane results in bubbles of a smaller size than an orifice in a vertical plane. However, the inverted nature of the interface in the case of the central air tube of a Mariotte bottle resulted in bubbles of a larger size than in the case of the vertical orifice of the side air tube of the improved device. The bubbles at the vertically orientated orifice had the appearance shown in plate 9, just before release.

Consequently the pulse pressure in the 'constant' pressure delivery of a burette with side air tube varied

over a smaller range than that of a burette with central tube. Moreover, by grinding the orifice of the side air tube at 45° instead of square, so that the orifice pointed upwards, there was a consequent further decrease in the pulse amplitude. This possibly limited eddy formation at high discharge rates, since the bubbles had considerably less chance of being sucked down temporarily towards the fluid discharge orifice.

If the fluid level fell below the side tube, the hydrostatic pressure of the column of fluid would be less than XC (fig.40) and consequently delivery would be at a decreasing pressure.

The constant pressure at which fluid was delivered was determined by the height of the side air tube, A, above the preparation. It should be noted that, as with other constant pressure methods, the loss of head due to friction, and eddy formation as fluid passed through tubing, was not appreciably affected by the amount of pressure to which the interior walls of the tube were subjected. Nevertheless, no theoretical calculation of any degree of accuracy could be made for the perfusion pressure actually obtained. This pressure must always be measured just immediately proximal to the cannula, since it would vary depending upon the length, internal bore, etc., of the tubing leading from the constant

pressure apparatus to the cannula.

Using a constant gaseous pressure, greater than atmospheric pressure, applied to side tube

The improved constant pressure burette was filled as before. The burette was then closed off from the atmosphere at the top. Due to the higher level of inward pressure on the gas/fluid interface, a volume of gas entered into the burette through the side gas tube A, when this was opened. Gas rose through the burette, until the pressure of the gas in the burette was slightly less than the chosen constant pressure greater than atmospheric pressure.

A state of equilibrium was thus rapidly established between the constant pressure of the gaseous reservoir acting inwards through the gas side tube, and the outward lateral pressure. The outward lateral pressure was equal to the pressure, greater than atmospheric pressure which was exerted by the gas contained in the burette + the pressure due to the column of fluid in the burette. Thus the movement at X (fig.40) would again be zero, although the forces in equilibrium had higher absolute values than when the gas entering the side limb was air at atmospheric pressure.

When the tap B (fig.40, 2) was opened, the effective pressure was produced by the hydrostatic pressure of the

column of fluid XC + the constant gaseous pressure greater than atmospheric pressure, which was applied to the side tube. The re-establishment of an equilibrium state involved similar principles. As the burette emptied the gas, contained in the burette above the fluid, increased nearer and nearer to the chosen constant gaseous pressure, greater than atmospheric pressure.

Thus, using a constant gaseous pressure applied to the side tube, it was possible to deliver fluid from a burette at any desired constant pressure above atmospheric pressure. This fluid was saturated under pressure by the gas or gases used.

LABORATORY METHODS PROVIDING A CONSTANT GASEOUS PRESSURE, GREATER THAN ATMOSPHERIC PRESSURE

Lockett (1952) applied an almost constant pressure, greater than atmospheric, by the awkward positioning of a large capacity Mariotte reservoir bottle, A, with central tube, at a considerable height above the bench level (fig.41).

When the tap of the improved constant pressure burette, E, was opened, water, under a high chosen head of constant pressure, flowed through tube B into the large capacity air

reservoir, C. Air was thus displaced, at high constant pressure, through tube D into the side tube of the burette E. Reservoir C was occasionally drained of the collected water, before the water level had risen significantly. Reservoir A was refilled as required.

The disadvantages were the troublesome height at which the heavy water reservoir A had to be suspended, and secondly the fact that air was the only convenient gas with which to fill the gas reservoir C.

Two methods were employed by me in order to apply a constant gaseous pressure, greater than atmospheric pressure, to the side limb of the burette, so that a constant pressure of the order of mammalian blood pressure would be obtained. The first consisted of a gas cylinder, which was connected when required, to a gaseous reservoir of large capacity relative to the burette capacity. The pressure in the gaseous reservoir was adjusted as required. This method, which was the more satisfactory, was used during the perfusion experiments of the isolated ear of the rabbit (Chapters 2 & 3).

An alternative method was to connect the gas cylinder, through an Adams variable pressure reducing regulator, directly to the side tube of the burette. The Adams

regulator was a single stage regulator, employing a rubber diaphragm and toggle lever cut-off principle. The static outlet pressure could be selected. In practice an appreciable variation in flow pressure took place and this second method was abandoned.

THEORETICAL FORCES ACTING IN THE IMPROVED CONSTANT PRESSURE DEVICE

The laws governing many of the phenomena of the mechanics of fluids in motion can be formulated. However, the difficulties to be overcome, before all the disturbing factors can be taken fully into account, are very great. These difficulties are chiefly analytical. Thus hydrodynamics provides mathematical treatment for perfect fluids in motion while hydraulics is an experimental science involving the introduction of empirical formulae and tables of coefficients (Jameson, 1944).

Experience, based on the results of experiment, forms the only safe guide. In the case of theoretical calculations, the best that can be done is to modify the results, obtained on the assumption that the fluid in motion is perfect, by the introduction of some empirical constant, which

shall involve the effect of every disregarded factor. The theoretical results are only approximations to the truth, since water is not a perfectly non-viscous, inelastic fluid, whose particles when in motion always follow sensibly parallel paths.

The effect of the air, bubbling up through the fluid, in the improved constant pressure burette prevented the steady stream line motion, which would otherwise have been set up. Using phenylamine (aniline) or methylene blue, it was found possible, by means of an extremely long Pasteur pipette, to inject a drop of either of these substances at 2 levels in the burette fluid column. The presence of stream lined flow was demonstrated by this method during discharge from a standard calibrated burette without side tube. This was contrasted with the turbulent fluid flow set up by air entering from the side tube during fluid discharge from a constant pressure burette.

With steady motion of a fluid, an expression for the velocity, in terms of the applied forces, is obtained by equating the work done on the mass to the increase of energy in the potential, pressure and kinetic form, together with the loss by dissipation, as by internal friction, which converts mechanical energy into heat.

However, unsteady, sinuous motion of the mass of fluid took place in the constant pressure device. It was impossible to determine the kinetic energy possessed by the fluid eddies in virtue of their rotation. Consequently it was only possible to consider the motion theoretically from the point of view of production of momentum, since the momentum of the fluid forming a vortex is unaltered by its motion of rotation. However, the fluid momentum having no immediately physiological significance in the present work, further theoretical speculations devoid of experimental verification were abandoned.

LIMITATIONS OF ORIFICE DIMENSIONS OF THE IMPROVED CONSTANT PRESSURE DEVICE

The reservoir discharged fluid at constant pressure from the lower orifice, while air entered the upper orifice and bubbled through the fluid, provided that the following conditions were satisfied:-

(A) the bore of the upper orifice, the air side tube, had a value below a certain maximum which would produce an unstable air/fluid junction at A (fig.40). If this condition was not satisfied fluid discharged from both orifices

while air was entering the upper orifice. In addition, the bore of the upper orifice must have a value above a certain minimum which, due to inadequate air entry through the upper orifice, would limit the rate of discharge from the lower orifice.

(B) the bore of the lower fluid discharge orifice had a value below a certain maximum at which the quantity of fluid discharged would have been greater than the quantity of air which could enter the upper orifice in unit time. In addition, if the lower orifice were too large, then theoretically, air would enter it at the same time as fluid discharged. In practice, this would never happen since few rates of flow in physiological experiments, e.g. flows in Starling's heart-lung preparation, require large orifices. Moreover, in physiological experiments the lower discharge orifice is always connected by an unbroken column of fluid to the preparation under experiment.

(A) Air entry orifice

Relation of volume of bubble to diameter of air entry orifice.

Pattle (1950b) pointed out that the production of small bubbles by an orifice required that the following conditions be satisfied to a certain extent:

(1) an irregular or obstructed orifice, or one with side entrance

(2) sufficient resistance to air flow in the tube leading to the orifice

(3) a flow rate so small, or a liquid of such a nature, that bubbles did not reunite with one another, or with the bubble growing on the orifice

(4) a liquid which had zero contact angle with the orifice.

At the air/water junction a ratio existed between the volume of the bubble to the diameter of the orifice at which it was formed. It was obvious that if the bubble had a larger diameter than the bore of the side tube, then the water meniscus would be stable; if the bubble diameter equalled the orifice diameter, an unstable equilibrium of the air/water junction existed; if the bubble diameter was smaller than the orifice diameter, the interface would be unstable and water would escape past the orifice.

Figure 42 gives plots of the relationship of the bubble diameter to the orifice diameter. Line 5 represents the unstable state in which the two diameters are equal. Values plotted to the right of line 5 are unstable states in which the bubble diameter is less than the orifice diameter. Values plotted to the left of line 5 are correspondingly

stable states.

Datta, Napier & Newitt (1950) carried out a very thorough series of investigations, (fig.42, curve 1), in which they found a value for the ratio of volume of the bubble to the diameter of the orifice at which it was formed. This value centred about 0.33 cm^2 at slow rates of bubble formation. Datta et al used orifices constructed by grinding square the ends of glass capillary tubing of several internal diameters between 0.022 and 0.519 cm. They also found that the volume of bubbles formed at a given size of orifice, decreased with increasing rate of formation to a minimum value and then increased, until a random size distribution set in.

Newman & Whelan (1952) found a theoretical value of the ratio of 0.231 cm^2 for air/water at 20°C, provided that the plane of the orifice was horizontal (fig.42, curve 3). By blowing bubbles at a very slow rate, Newman & Whelan were able to observe a further variation in the ratio at very slow speeds. They constructed orifices of glass capillary tubing with diameters ranging from 0.0131 to 0.4744 cm and found a value of the ratio of volume of bubble/diameter of orifice = 0.19 cm^2. This value of the ratio was found with all except the smallest two orifices, of diameter 0.0131 and 0.020 cm (fig.42, curve 4). As soon as the rate of formation

of individual bubbles increased to the point where they could no longer be observed to cling momentarily to the orifice, the value of this ratio rose to about 0.26 cm^2 (fig.42, curve 2). It was probably this effect which prevented observation of the value of 0.19 cm^2 with the smallest two orifices in their experiments.

The ratio value of 0.19 cm^2 is very close to that reported by Coppock & Meiklejohn (1951) for very slow rates of bubble formation in homogeneous liquids or mixtures of liquids. These authors also suggested an equation which described the relation between the size of bubble formed at a circular orifice, the diameter of the orifice, the surface tension and the liquid density. This equation has little to recommend it, however, since it necessitates calibration under experimental conditions, in order to determine the numerical value of an empirical constant, which is related to the rate of bubble formation.

Since in perfusion or infusion experiments a very slow rate of bubble formation may be required, or the flow may require to be stopped completely for a period of time, it was decided to use curve 4, fig.42, as the limiting values for internal diameter of the air entry orifice. Thus if more rapid flows took place, with a consequent greater

average bubble diameter, the only result would be a greater stability of the air/water interface with a negligible increase in the small pulse pressure of the effectively constant delivery pressure.

Using curve 4, it can be seen that with a ratio of volume of bubble/diameter of orifice = 0.19 cm^2, the air/water equilibrium becomes unstable at an orifice diameter of 0.6 cm when the average bubble diameter becomes 0.604 cm. Thus the maximum orifice diameter must be less than this, say 0.5 cm, when the average bubble diameter becomes 0.570 cm, i.e. the interface would be stable. Further inspection of curve 4 showed that decrease in orifice diameter below a value of approximately 0.15 cm would result in no further increase in the stability of the air/water interface, but rather in a slight decrease in its stability.

The conclusion reached at this stage was that the internal diameter of the side air tube should lie in the range 0.2 to 0.5 cm.

Velocity of air flow in side tubes of various internal diameters. It is known that at the fluid discharge orifice:

$$Q = C_d \cdot a \cdot \sqrt{2gh}$$

where C_d = coefficient of discharge = 0.60 approx.

a = cross sectional area of water discharge orifice

d = diam. of water discharge orifice = 0.15 cm approx.

h = distance between air entry and water discharge orifices = 5 cm approx.

$\therefore \quad \underline{Q} = 0.6 \times 3.142 \times (0.15)^2/4 \times \sqrt{2 \times 981 \times 5}$

$= 1.1$ ml./sec

In practice, calibration of an improved constant pressure burette of these dimensions gave a discharge of 1.2 ml./sec.

The flow of air in the side tube must equal the flow of air in the constant pressure reservoir. Thus in the side tube, air volume (as cylinders) x velocity must equal in the reservoir, air volume (as spheres) x velocity.

Assuming a close approximation of side tube diameter to bubble diameter

$\therefore \quad 2\pi r^3 \times \underline{V}_p = 4/3\pi r^3 \times \underline{V}_R$

where $\underline{V}_p$ and $\underline{V}_R$ are the velocity of the air in the side tube and in the reservoir respectively

and r is the internal bore of the side tube.

Hence $\underline{V}_p = 2/3\underline{V}_R$

but in side tube $\underline{Q} = a \times \underline{V}_p$

where $\underline{Q}$ = quantity per unit time passing orifice

a = cross sectional area of the side limb

$\therefore \quad \underline{Q} = \pi r^2 \times 2/3\underline{V}_R$

$\therefore \quad \underline{Q} = \pi d^2/6 \times \underline{V}_R$

Hence $d^2V_R = 6Q/\pi$

e.g. where d = .2 cm

$V_R = 6 \times 1.1/3.142 \times .04 = 52.5$ cm/sec

Table 18 gives the values of actual air velocity for various internal diameters of the side air tube.

A general expression for the lower critical velocity in a straight parallel tube, applicable to any fluid and any system of units is

$$V_C = 2000\mu/d\rho$$

The expression $vd\rho/\mu$ is known as the Reynolds number, a non-dimensional quantity which constitutes a criterion of similarity of motion for all fluids. Thus for air at 18°C:

viscosity = 182.7 micropoises (Hodgman, 1948)

= 182.7×10^{-6} dyne-sec/cm^2 (poises)

density (moist air at 760 mm Hg pressure) = 1.2130×10^{-3} (g/cm^3) (Hodgman, 1948).

Hence $V_C = 2000 \times 182.7 \times 10^{-6} / 1.2130 \times 10^{-3} \times d$

= 301.2/d cm/sec (where d is in cm)

Table 18 gives the values of critical air velocity for various internal diameters of the side air tube, together with the ratio of actual air velocity/critical air velocity. It is obvious that air turbulence will not occur at any of

the tube diameters given in the table.

At this stage in the investigation it was decided to carry out further experiments using side air tubes of internal diameter 0.3, 0.4 and 0.5 cm.

Practical calibration of optimum bore of air entry orifice. Observations were made at very slow and at rapid outflow rates from a 60 cm length of glass tubing of 2.5 cm diameter, used as a model of a burette, or from an aspirator bottle. Both the burette model and the aspirator bottle could be fitted as required with constant pressure side air tubes having internal diameters of 0.3, 0.4 and 0.5 cm.

In the case of the 0.5 cm bore side tube, fitted either to the burette model or the aspirator bottle, it was noted that, if the side tube was displaced slightly from the horizontal, or if the outflow rate was very slow, and still more, if the outflow was stopped for a period, that the air/water interface visibly became unstable and that the interface tended to move outwards along the side tube. However, even in the case of 'dirty' glass the interface was stable under all conditions in the case of side tubes of either 0.3 or 0.4 cm bore and this size was finally adopted. Subsequently 2 years of general use of constant pressure devices in laboratory work has provided no indications for

altering this specification of the bore of the side air tube.

Practical calibration of bubble diameter. Calibration of bubble diameter was carried out using one side tube of 0.3 cm diameter and one side tube of 0.4 cm diameter. The simple principle was to count the number of air bubbles formed at the orifice an a unit of time, and to measure the volume of the collected air bubbles over the same unit of time.

The apparatus shown in fig. 43 was set up. A constant pressure cannula, A, (Shanks, 1923) was connected by means of rubber tubing, B, to an air reservoir consisting of an aspirator bottle, C, with a double neck. In the case of calibration of the ratio of volume of bubble/ diameter of orifice at atmospheric pressure, no water was delivered to the cannula A.

In calibration at higher pressures, the level of constant pressure at which air was displaced from the reservoir C, aling the tubing D to the side tube inserted into the water reservoir E, could be chosen by selecting the height of A above C. Calibration at extremely slow inflow rates was made by constricting the tubing B with a screw clip. When required tube B was clamped and the air which had been

collected and measured in the calibrated collecting tube, F, was ejected through the tap in the top of F. The fluid collected in C was run off from time to time before a significant alteration had occurred in the level of fluid.

The number of bubbles was counted over a period of time, depending upon the rate, until a volume of about 2 ml. had been collected. It proved impossible to count the number of bubbles with any degree of accuracy at rates above the order of 4 ml./min. Moreover, above this rate the rapidly changing meniscus of the collecting tube could not be read accurately.

Table 19 shows the results obtained. Each calculated bubble diameter is the mean of 6 observations. Slightly larger bubbles formed at the 4 mm than at the 3 mm orifice, and at the fast compared with the slow rate of formation. The ratios of volume of bubble/diameter of orifice were slightly larger than those obtained by Newman & Whelan (1952) (fig.42, curves 2 & 4), but this was not surprising since only routine care was taken in the treatment of the orifices used in the present observations.

It was concluded from these experiments that air entry orifices made by side tubes of either 3 or 4 mm diameter tubing were the best for constant pressure devices.

Shape of air entry orifice. It has already been shown that a flat horizontal plane orifice was adequate, but that the orifice of the side air tube could be improved slightly by grinding it at an angle of 45° pointing upwards. Either of these simple types of orifice constructed from 3 or 4 mm internal diameter tubing was satisfactory since the maximum rate of air entry was not found in practice during physiological experiments to act as a limiting factor to the rate of discharge from the lower orifice.

Consequently it was unnecessary in practice to secure still greater rates of gas entry by using a returned mouthpiece, e.g. a trumpet shaped mouthpiece, on the air inlet orifice. However, trials were carried out returning the mouthpiece for some distance into the burette, as in the Borda mouthpiece. Thus flow over the face containing the orifice was prevented, and the pressure on this face would then approximate to that due to the statical head alone. However, the returned mouthpiece would result in marked eddy formation further preventing stream line motion and thus presumably decreasing the coefficient of discharge.

(B) Fluid discharge orifice

In the case of an improved constant pressure device with a connecting rubber tube of the same diameter as the fluid

discharge orifice, the effluent stream, after forming a vena contracta, always re-expanded to fill the tube. The vena contracta in the case of a small circular orifice is at a distance from the orifice equal to about 0.498 times the diameter (Gibson, 1948).

A table of values of C_d, the coefficient of discharge, for circular orifices was given in Hamilton Smith's 'Hydraulics' (1886) for different values of head above the centre of the orifice. Experiments by Judd & King (1906) indicated that if an orifice was less than about 2.5 in. diameter, perfect contraction was impossible. The degree of imperfection became more marked as the diameter increased. This was true however high the head. Consequently in the present apparatus the imperfection is not due to the small head, inherent in the design of the improved constant pressure device when discharging under atmospheric pressure applied to the side tube.

Theoretically the coefficient of discharge is the same for a given orifice under a given head, whatever be the direction of discharge. However, the true value of the coefficient of discharge will depend entirely on the circumstances governing the flow to the particular orifice. Consequently, if small orifices are to be used for measuring

purposes they should be calibrated as nearly as possible under the conditions likely to obtain in use. Any interference with the free production of stream lines curtails the full flow to the orifice, and tends to prevent the full contraction of section at the vena contracta. Thus the turbulent fluid flow found to be set up in the constant pressure burette with side tube will decrease C_d and thus Q by an incalculable degree. Consequently each constant pressure device should be calibrated under the existing experimental conditions for its own coefficient of discharge.

OTHER APPLICATIONS IN EXPERIMENTAL PHYSIOLOGY OF THE IMPROVED CONSTANT PRESSURE DEVICE

(A) Small volumes under constant pressure

Intravenous infusion of fluids or injection of drugs in amphibia and mammalia. The constant pressure burettes provided a convenient source of small constant heads of hydrodynamic pressure for constant rate infusion of known volumes of fluids. They have been used routinely in this department, over the past 18 months, both in research and in teaching experiments in amphibia and mammalia.

Intravenous infusion of saline and various plasma substitutes have been delivered from the burettes at any rate over a wide range and for any desired period of time. The constant pressure burettes have also been used for 'washing in' following the intravenous injection of drugs, and for the continuous slow injection of drugs in volumes greater than that conveniently handled by a Thorp motor driven ram injector.

I have myself used improved constant pressure burettes for these various purposes in some 70 experiments.

Perfusion of a frog's heart through Green's cannula. In such experiments, perfusion of the frog's heart is carried out at pressures of the order of 2 cm water. Consequently the temporary increase in pressure upon refilling the bottle due to fluid entering the central air tube of a Mariotte bottle, is extremely significant since it may amount to a temporary pressure increase of the order of 100%. This variation in pressure is practically eliminated by using the improved device.

Originally, I prepared simple 200 ml. capacity glass bottles complete with constant pressure side tubes, as suitable for this experiment to be carried out by undergraduates in practical classes. Subsequently two constant

pressure burettes joined by a Y piece below the fluid discharge orifice have been used. Fig.44 shows one burette in use. When it was desired to record the venous inflow on the kymograph record, air was injected into the float of the volume recorder which was then connected to the side air tube of the constant pressure burette.

Cystometry. A constant pressure automatic inflow apparatus was built for filling the bladder in cystometry (fig.45). The infusion liquid flowed from an improved constant pressure burette through thin walled, small diameter, rubber tubing to a double surface and spiral condenser (fig.45). The temperature of the ingoing fluid, as registered on a thermometer placed just before the cannula, was maintained at 37°C by warm water circulated through the condenser jacket by a single Dale-Schuster perfusion pump.

The inflow was periodically interrupted by a clamp which compressed the thin walled rubber tubing. The clamp was operated by a solenoid activated by a 6 volt accumulator. A rotary key, which was driven by a constant speed motor, made and broke the circuit at predetermined intervals. The same motor was used to drive the pump. The periods of inflow were signalled on kymographic and optical records.

The complete apparatus was carried on a 'Dexion Angle' frame, 22 x 14 x 40 in., mounted on 3 in. diameter castors. When required the chassis could be wheeled rapidly into position beside the experimental preparation and the bladder cannula connected to the condenser.

The constant pressure apparatus has been used by me for 29 cystometric studies on decerebrated dogs, cats and rabbits. The effect of variation in constant filling pressure is shown in fig.46 on bladder filling and emptying of the decerebrate cat and in fig.47 on bladder pressure of the decerebrate greyhound.

Passage of fluid at constant pressure through intestinal loops. No experiments of this nature have been carried out by me while using the improved constant pressure device. The work of Gregory (1950), however, emphasises the necessity of using such a device.

(B) Large volumes under constant pressure

Improved constant pressure aspirator bottle. When the rapid delivery of relatively large volumes of fluid was required under high constant pressure, or alternatively, when relatively large volumes were required at low rates of delivery over a prolonged period of time, obviously a burette was inconveniently small to act as the improved constant

pressure fluid reservoir. An aspirator bottle with moulded outlet and vial neck was used (fig.34). The bottle was of appropriate capacity, and the neck was closed with a stopper while the outlet near the bottom was closed by a second stopper carrying 2 glass tubes.

Any required delivery pressure was achieved by connecting the upper of the 2 glass tubes to another large aspirator bottle pressured with O_2 or O_2/CO_2 mixture from a cylinder. The gas reservoir had a side tube to leak off any excess pressure which developed. This eliminated the necessity of suspending the reservoir high above the bench level and also simultaneously provided maximal oxygenation of the fluid. The high oxygenation of the fluid was obtained since bubbles of oxygen under pressure continually passed through the fluid, and also the fluid surface was left in contact with oxygen under a pressure, greater than atmospheric pressure.

Perfusion of mammalian coronary arteries. Perfusion of the coronary arteries of the excised mammalian heart is generally carried out using Gunn's cannula. The apparent coronary flow (Wiggers, 1909) is recorded by a drop counter recording from a drip chamber on the input side (Baker, 1951). The constant pressure desired may range from 30 to 90 mm Hg.

These pressures are usually achieved using a large Mariotte reservoir with central air tube (fig.48, A). The fluid is aerated or oxygenated in a side limb B after it leaves the reservoir.

The relatively low oxygen tension achieved is raised appreciably by attaching to the gas distribution tube by a non-porous surround, either a porous ceramic diffuser or a sintered plate. These diffusers have relative uniformity of pore size, pore distribution and permeability and produce a more even distribution of smaller diameter bubbles. Gas is more efficiently dissolved from smaller bubbles than from large. The efficiency of an aerator is the ratio of oxygen dissolved to oxygen supplied when the liquid is free from oxygen (Pattle, 1950 a). Irregularities in the orifices of the pores of an aerator allow bubbles to be detached without the aid of gravity (Pattle, 1950 b).

Each diffuser has a certain 'bubbling pressure' below which gas will not penetrate the diffuser into the liquid being treated, due to the normal resistance of the material itself to gas flow. Further, there are many pores through which no gas ever passes and which remain filled with stagnant liquid, even when gas bubbles are escaping freely from the diffuser under normal working conditions. As a result,

comparatively high gas pressures may be necessary and there occurs a gradual clogging of the diffuser material which may ultimately reduce the rate of diffusion below the working minimum. This latter effect results from the deposition of solid material, either chemically or by bacterial growth, within the liquid contained in the idle pores, followed by encroachment on the pores through which gas would normally flow.

Fortunately it was unnecessary to calibrate the changing pressure/flow characteristics of the diffuser while immersed in the perfusion fluid, since due to the existence of other pressures losses in the perfusion apparatus, it is always necessary to measure the actual pressure at the Gunn's cannula throughout the experiment.

Easier control of the pressure, together with appreciable higher oxygenation of the fluid without the use of a diffuser, was found to be achieved simply by applying the improved constant pressure device (fig.48, C, D) to an aspirator bottle.

Histological perfusion apparatus. The improved constant pressure device was also of value in the intact animal either for the introduction or injection masses e.g. neoprene, or for histological perfusion, e.g of methylene blue,

at any desired constant pressure. Everett (1935) has described a gravity feed histological perfusion apparatus which however results in awkward positioning of the reservoir. Moreover the apparatus suggested by Everett would result in the use of inaccurate pressures for histological perfusion, since the pressure is measured in the system at a point far from the cannula.

Experiments involving the use of the improved constant pressure device for the injection of neoprene and methylene blue into the ear of the rabbit have been described in Chapters 2 & 3.

Two experiments were also carried out on cats to perfuse the whole animal with methylene blue using the following method. Heparin, 200 unit/kg body weight was given by intramuscular injection, 10 minutes before the induction of anaesthesia. Nembutal 32.5 mg/kg body weight was then administered by intraperitoneal injection. This anaesthetic caused slight generalised vasodilatation. A tracheal cannula was tied in place, the sternum split up the mid-line and artificial respiration commenced. The internal mammary artery and vein were cut between ligatures on both sides and the chest opened widely.

The right external jugular vein was cannulated in a craniad direction and, in addition, a siliconed polythene cannula was passed down the vein in a caudad direction through the superior vena cava into the right atrium.

600 ml. heparinised isotonic saline (1 unit heparin/ml.) had meantime been maintained at 38°C in a small aspirator bottle. About 10 ml. of this heparinised saline were injected through the polythene cannula into the right atrium and the remainder oxygenated at a pressure of 100 mm Hg, an approximation to the mean blood pressure, was perfused from a constant pressure reservoir into a siliconed glass cannula passing through the wall of the left ventricle. The cat's blood was thus washed out by the heparinised saline and escaped through the right atrial and right external jugular venous cannulae.

The perfusion was continued by adding methylene blue solution at 38°C to the constant pressure reservoir. Chapter 3 gives details of the preparation of the methylene blue solution. The same constant pressure of 100 mm Hg was used for the perfusion of the methylene blue solution. Carleton (1926) used a pressure of 70 to 80 mm Hg for the preliminary washing out with saline, and a pressure of 150 mm Hg for the methylene blue solution, but he omitted

to give any reason for the choice of these pressure levels. The quantity of methylene blue required was determined by observation of the progressive staining in each experiment. Finally both the atrial and jugular cannulae were occluded and the left ventricular infusion stopped.

24 or 48 hr chromatogram columns. The improved constant pressure device applied to large capacity aspirator bottles has been used, during the past year in the Biochemistry Department of this University, for running chromatograms over long periods of time. The simplicity of the device results in easy cleaning of the apparatus.

A slight disadvantage has been noted in that although the constant pressure does not alter with temperature change during the night, yet about 8.30 a.m. the rise in temperature of the air confined above the fluid in the reservoir results in spilling of a small volume of fluid out through the air inlet tube as the air expands. This trivial disadvantage has been readily overcome by suspending a 25 ml. beaker below the air inlet tube to collect the spill.

STUDIES ON THE PHYSIOLOGY OF BLOOD VESSELS

VOLUME 2

A Thesis submitted to the University of Glasgow in candidature for the degree of Doctor of Philosophy in the Faculty of Medicine

BY

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March, 1954

Institute of Physiology,
University of Glasgow

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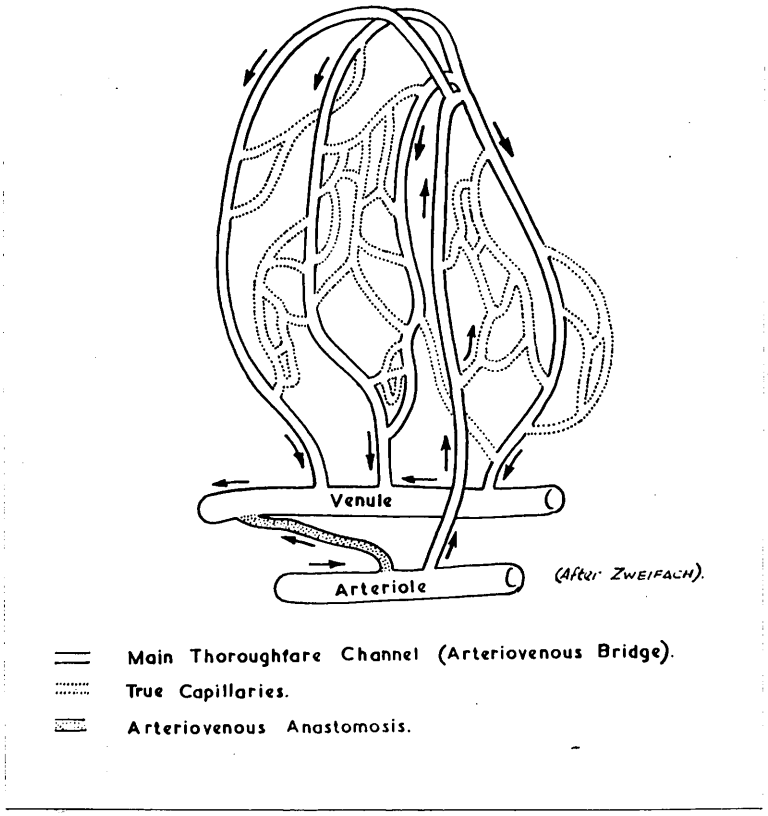

Fig. 1. Diagram of capillary bed showing main thoroughfare channels, true capillaries and an arterio-venous anastomosis.

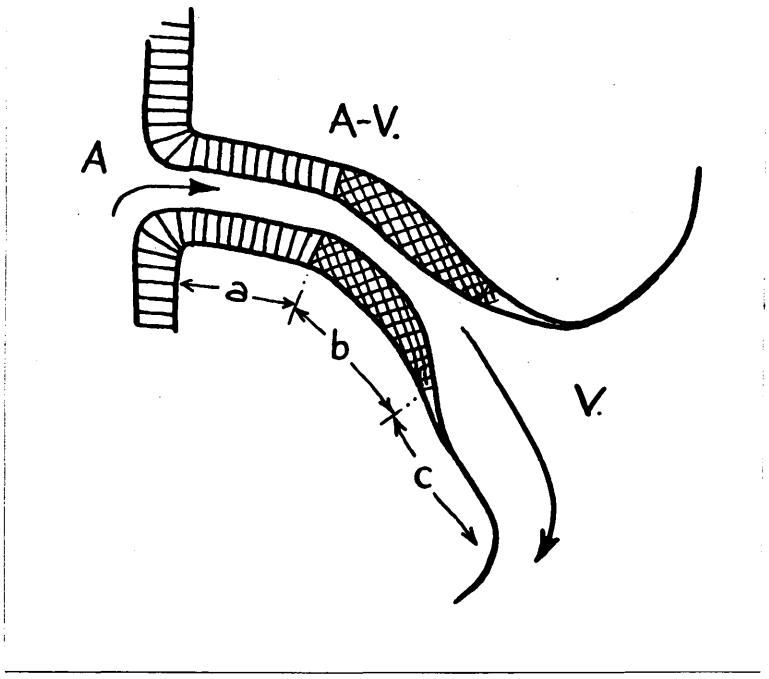

Fig. 2. Diagram of an arterio-venous anastomosis showing the three structurally different parts of the vessel. A, arterial vessel; V, venous vessel; A-V, arterio-venous anastomosis; a, arterial segment of arterio-venous anastomosis; b, intermediate segment; c, venous segment.

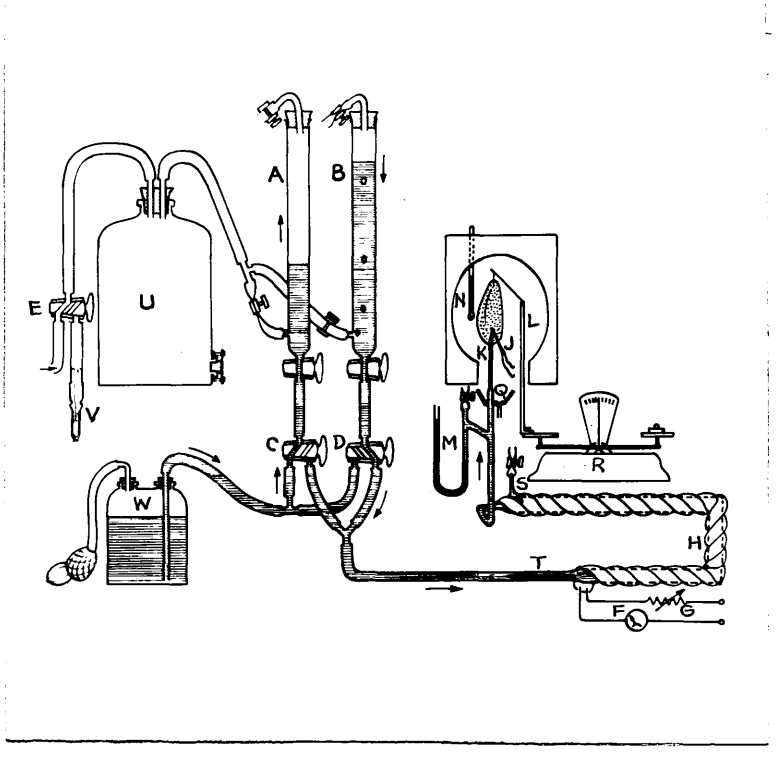

Fig. 3. Isolated ear perfusion apparatus. A, B, constant pressure burettes filled from below with perfusion solution; C, D, E, 3-way oblique bore stopcocks; F, ammeter; G, variable resistor; H, heating tape; J, thermocouple wires; K, bicycle valve tubing terminating in arterial cannula; L, attachment to pan of balance for suspending ear; M, mercury manometer immediately proximal to the cannula; N, thermometer recording environmental temperature inside metal tin; Q, filter funnel; R, balance; S, air trap; T, curved tube; U, 10 l. pressure reservoir for 95% oxygen, 5% carbon dioxide mixture from a cylinder; V, escape valve for adjustment of pressure in U; W, Woulff bottle reservoir of perfusion solution.

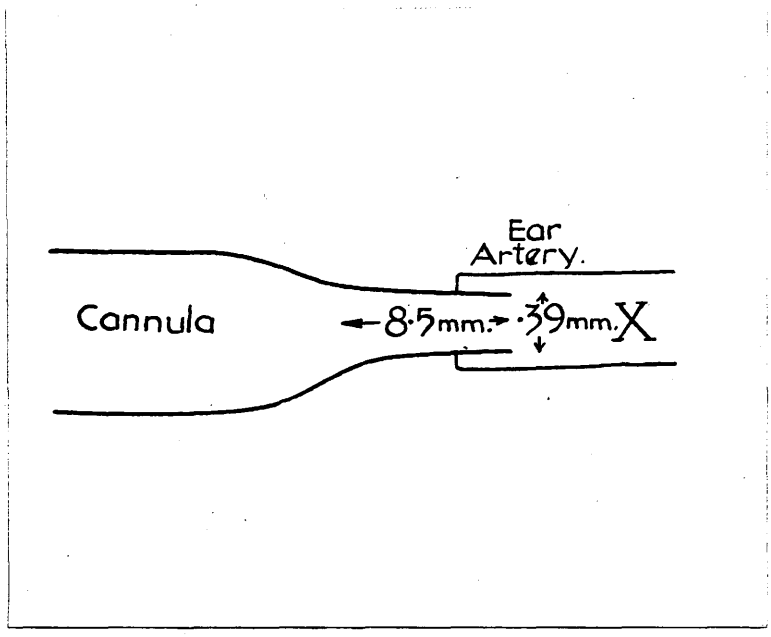

Fig. 4. Diagram of glass cannula in ear artery showing dimensions of cannula. X, point in artery just beyond the tip of the cannula for which the theoretical downstream pressure can be calculated.

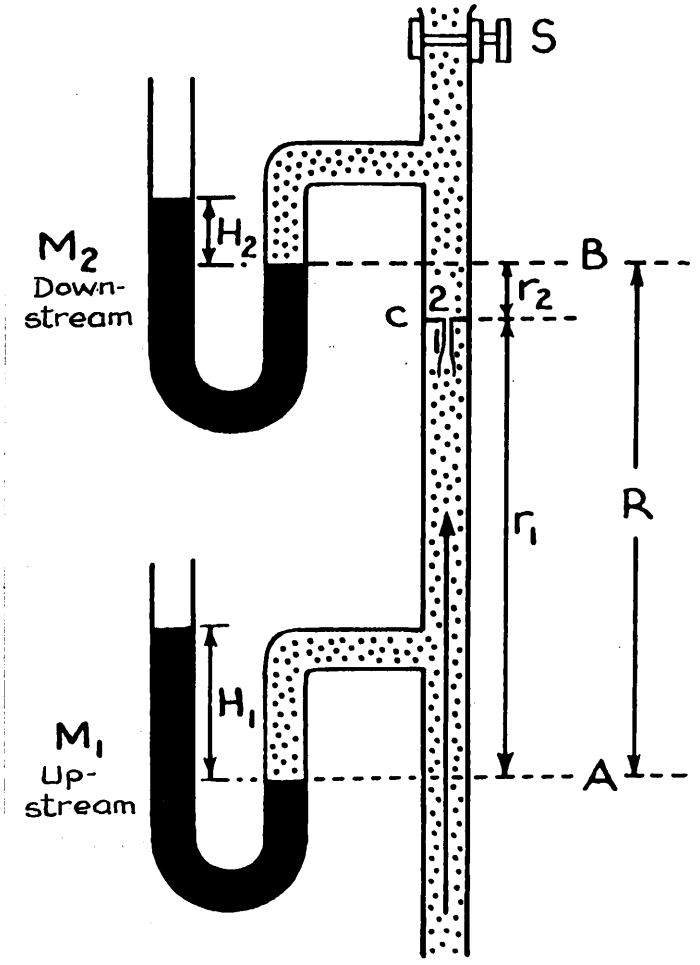

Fig. 5. Apparatus for calibration of perfusion cannula for pressure drop by controlling the flow rate. C, perfusion cannula orifice in lumen of wide bore rubber pressure tubing; 1 and 2, proximal and distal to cannula orifice; S, screw clip; M_1 and M_2, upstream and downstream manometers; H_1 and H_2, head of mercury registered in M_1 and M_2; A and B, selected points; r_1, length of water column from A to C; r_2, length of water column from C to B; $R = r_1 + r_2$.

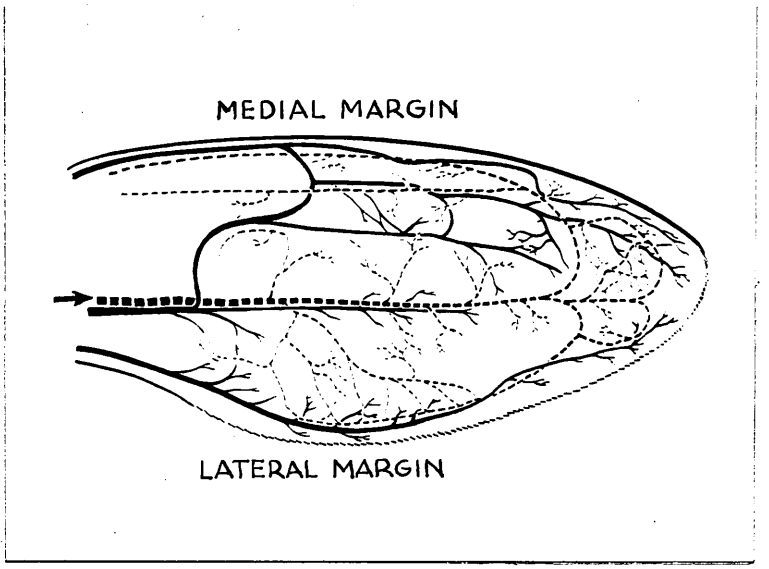

Fig. 6. Diagram of vascular anatomy of the dorsal surface of the rabbit's ear. Arterial system:-----. Venous system: ——— . Arrow shows point of insertion of the cannula into the dorsal auricular artery. The artery, central vein and dorsal auricular nerve lie in close proximity at this point with the artery the most medial structure and the nerve the most lateral.

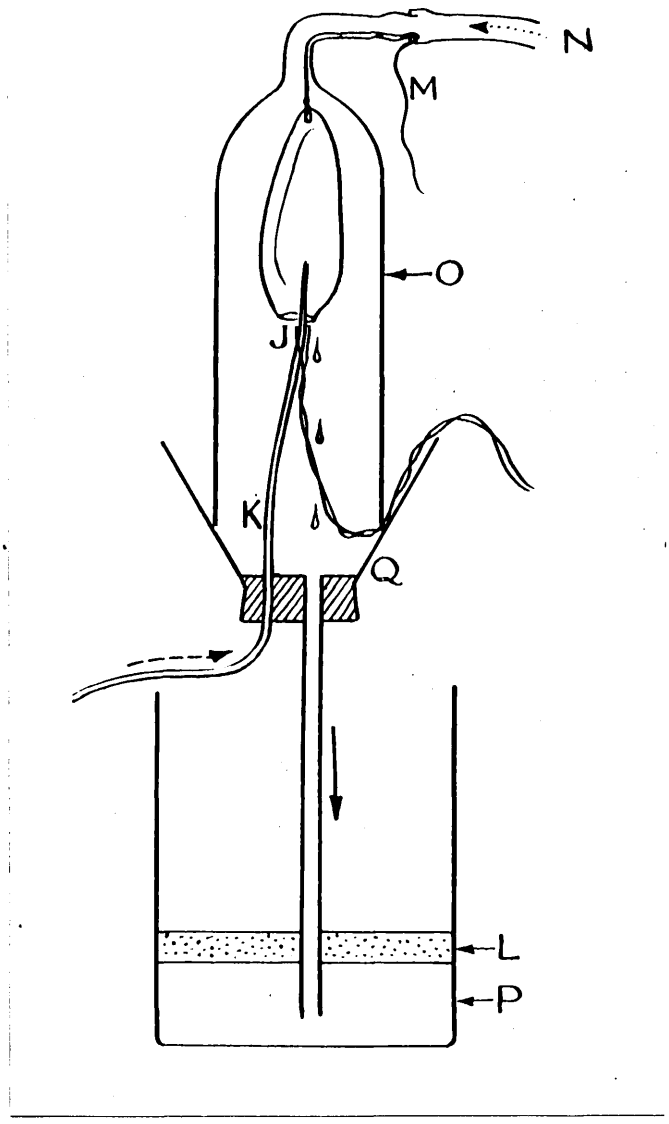

Fig. 7. Modified perfusion apparatus permitting perfusion of ear under anoxic conditions and collection of perfusate for estimation of oxygen content. J, thermocouple; K, valve tubing terminating in cannula; L, liquid paraffin; M, thread suspending ear; N, nitrogen; O, inverted isolated organ bath; P, perfusate; Q, filter funnel.

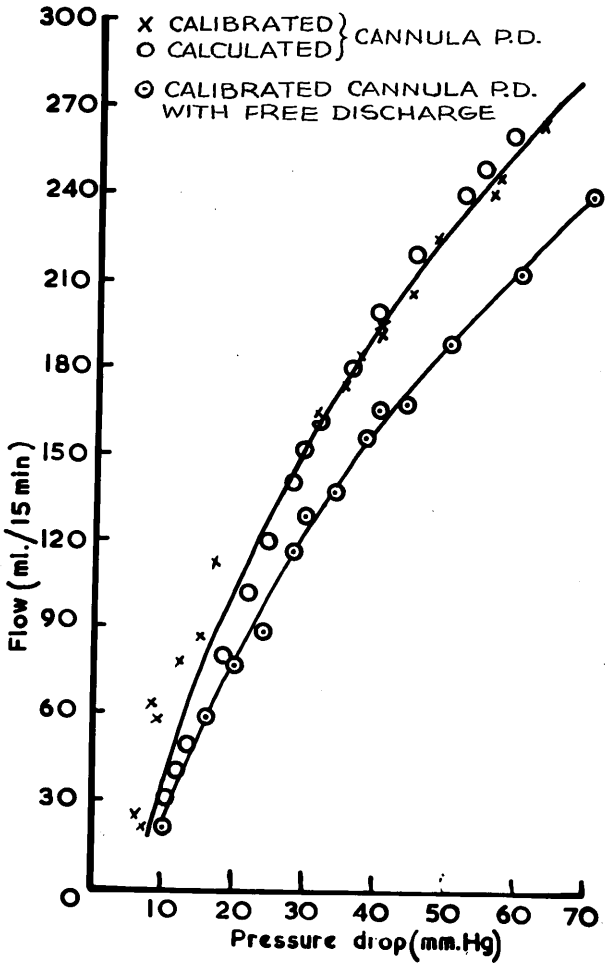

Fig. 8. Cannula calibration giving the pressure drops (P.D.) across the cannula over the range of inflow rates which occurred during the perfusion experiments. X : calibrated P.D. during selected flow rates; ⊙ : calibrated flow rates at selected pressures, with free discharge; O : flow rates at calculated theoretical P.D. due to cannula friction loss, assuming full pressure recovery from the velocity head.

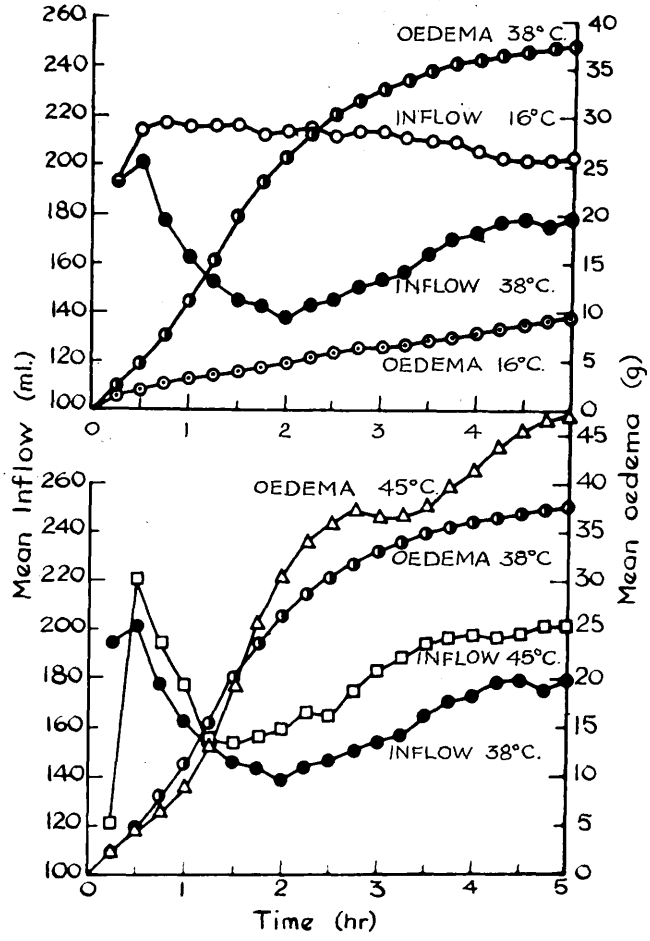

Fig. 9. Mean oedema formation and mean inflow in isolated rabbits' ears perfused at a solution temp. of 16°, 38° and 45°C respectively. The interpretation of the words oedema and inflow in this and in all subsequent legends to figures and tables has the significance detailed under expression of perfusion results in the text. n = no. of ears perfused, (expt. no.). 16°C, n = 2, (24, 47): oedema, ⊙ ; inflow, ○ . 38°C, n = 4, (38, 39, 40, 41): oedema, ◑ ; inflow, ● . 45°C, n = 2, (45, 46): oedema, △ ; inflow, □ .

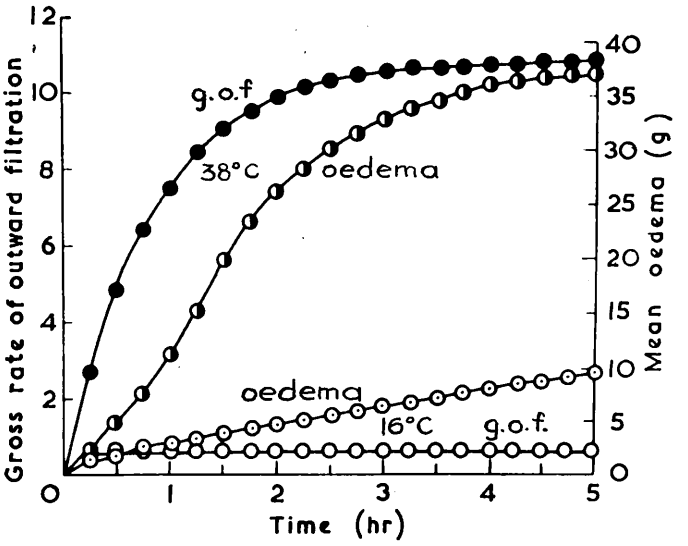

Fig. 10. Derived gross rate of outward filtration (g.o.f.) across the capillary membrane and observed mean oedema formation with perfusion solution at 16° and 38°C. n = no. of ears perfused, (expt. no.). 16°C, n = 2: (24, 47): gross outward filtration, $\propto$, O ; net outward filtration, oedema, ⊙ . 38°C, n = 4: (38, 39, 40, 41): gross outward filtration, $\propto (1 - e^{-\beta t})$, ● ; net outward filtration, oedema, ◑ .

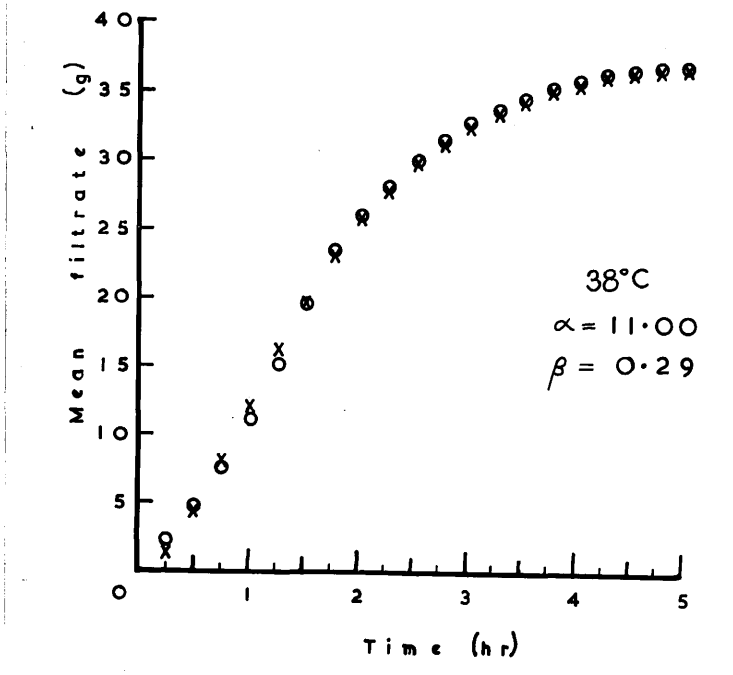

Fig. 11. Observed and calculated values for cumulative oedema formation (mean filtrate) in isolated rabbits' ears perfused at a solution temp. of 38°C, (expt. no. 38, 39, 40, 41). Observed values, O: calculated values, X, where $w = 37.93(1 - e^{-0.29t}) - 11.00te^{-0.29t}$.

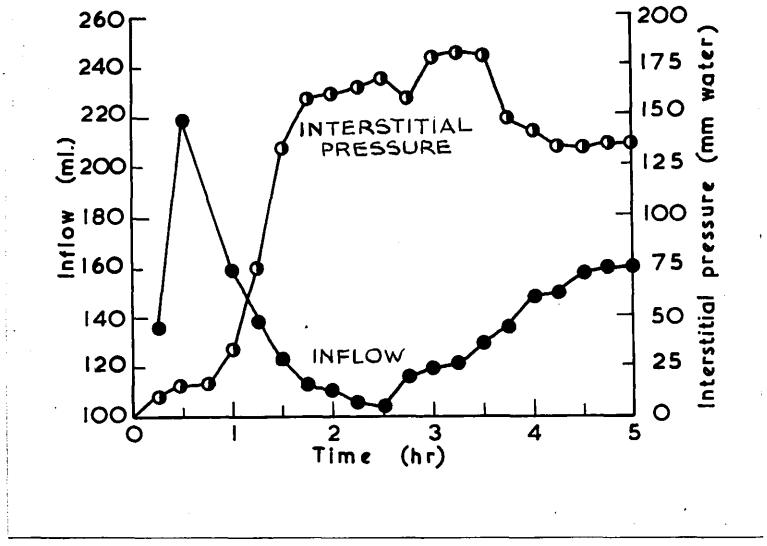

Fig. 12. Interstitial pressure and inflow with perfusion solution at 38°C. One ear perfused, (expt. 53). Interstitial pressure, ◑ . Inflow, ● .

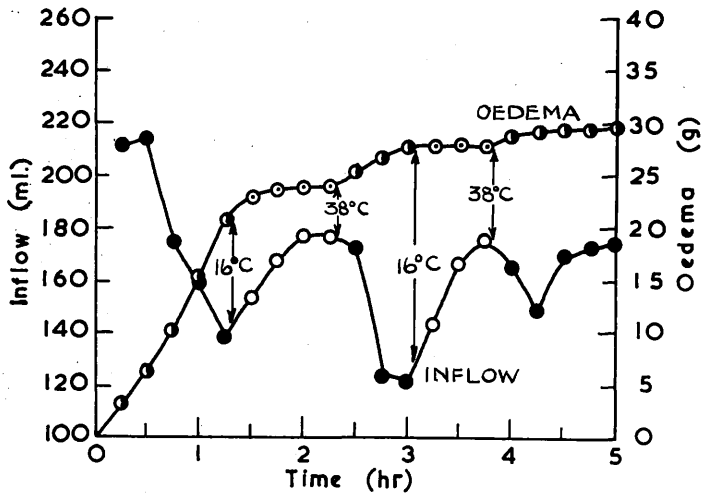

Fig. 13. Oedema formation and inflow with perfusion solution changed intermittently from a temp. of 38° to 16°C and vice versa. One ear perfused, (expt. 63). 38°C: oedema, ◑ ; inflow, ● . 16°C: oedema, ⊙ ; inflow, ○ .

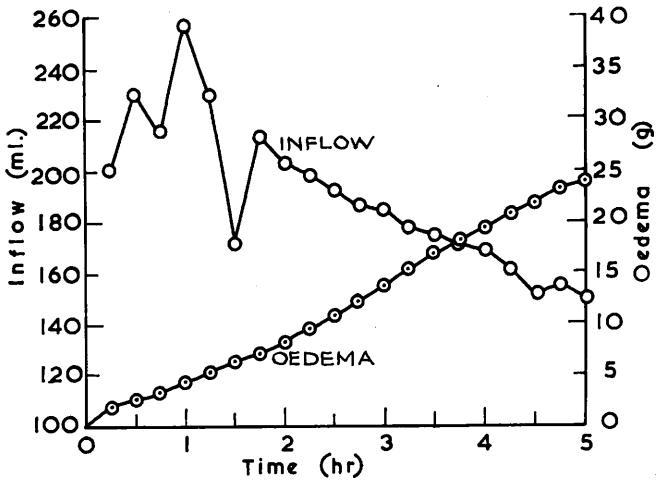

Fig. 14. Oedema formation and inflow with perfusion solution at 24°C. One ear perfused, (expt. 73). Oedema, ⊙ . Inflow, O .

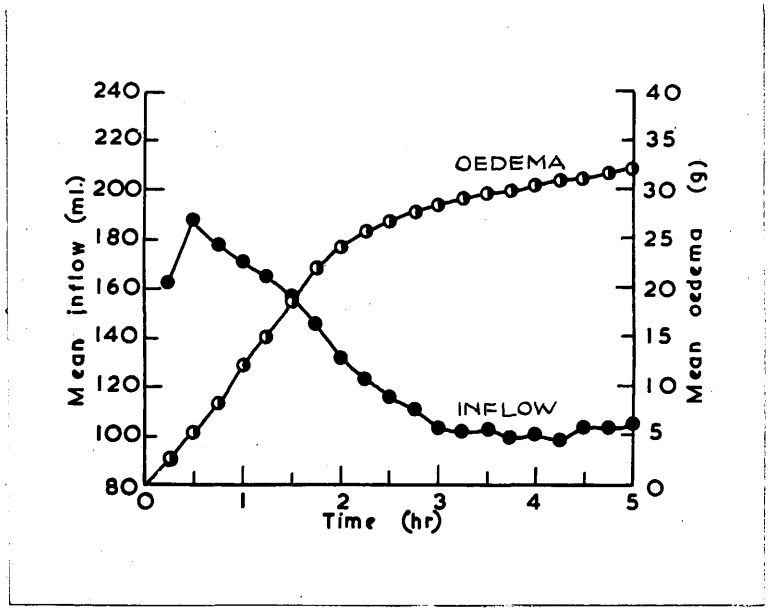

Fig. 15. Mean oedema formation and mean inflow with perfusion solution at 32°C. Two ears perfused, (expt. 71, 76). Oedema, ◑ ; inflow, ● .

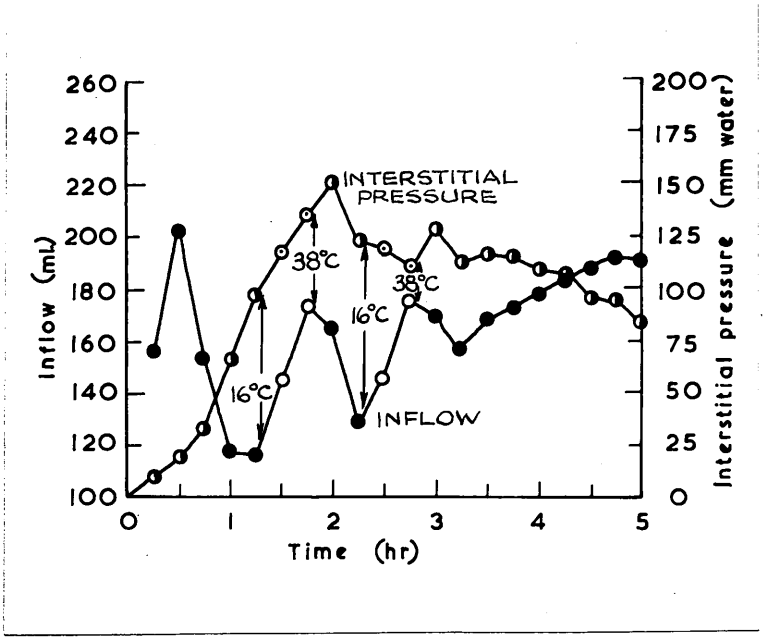

Fig. 16. Interstitial pressure and inflow with perfusion solution changed intermittently from a temp. of 38° to 16°C and vice versa. One ear perfused, (expt. 56). 38°C: interstitial pressure, ◑ ; inflow, ● . 16°C: interstitial pressure, ⊙ ; inflow, ○ .

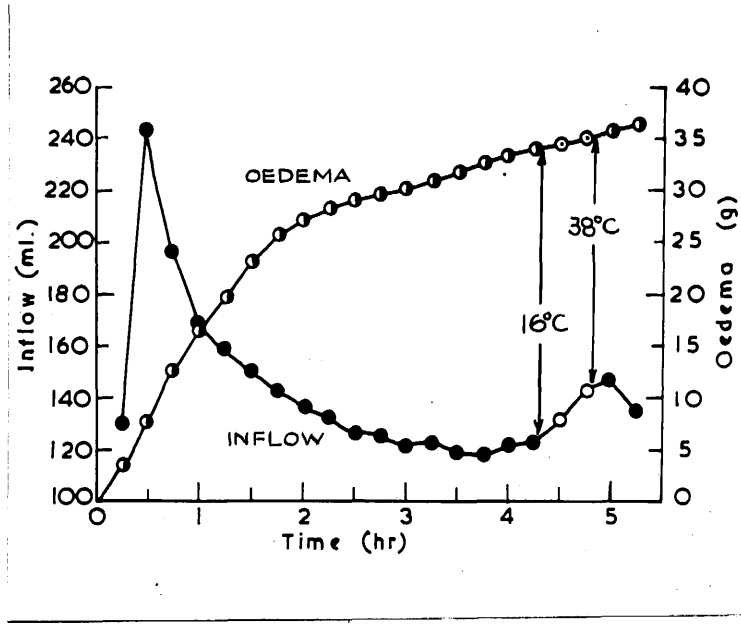

Fig. 17. Oedema formation and inflow with perfusion solution temp. changed to 16°C and then to 38°C following 4½ hr perfusion at a constant temp. of 38°C. One ear perfused, (expt. 70). 38°C: oedema, ◑ ; inflow, ● . 16°C: oedema, ⊙ ; inflow, ○ .

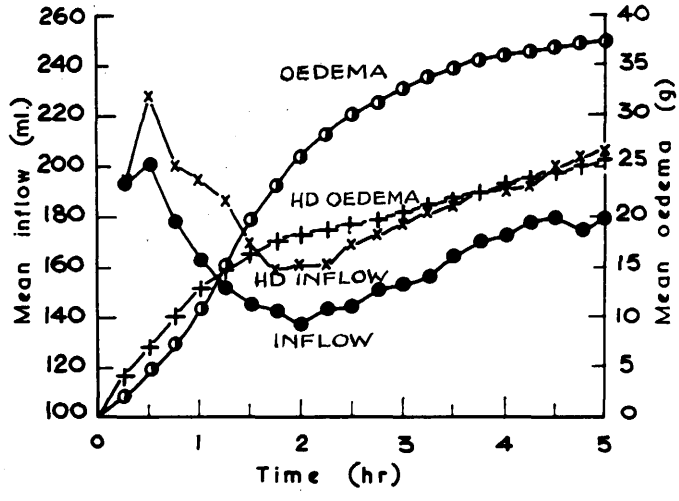

Fig. 18. Mean oedema formation and mean inflow with perfusion solution at 38°C containing hyaluronidase (HD) compared with previous values without hyaluronidase. n = no. of ears perfused, (expt. no.). With hyaluronidase, n = 3, (36, 37, 64): oedema, + ; inflow, × . Without hyaluronidase, n = 4, (38, 39, 40, 41): oedema, ◑ ; inflow, ● .

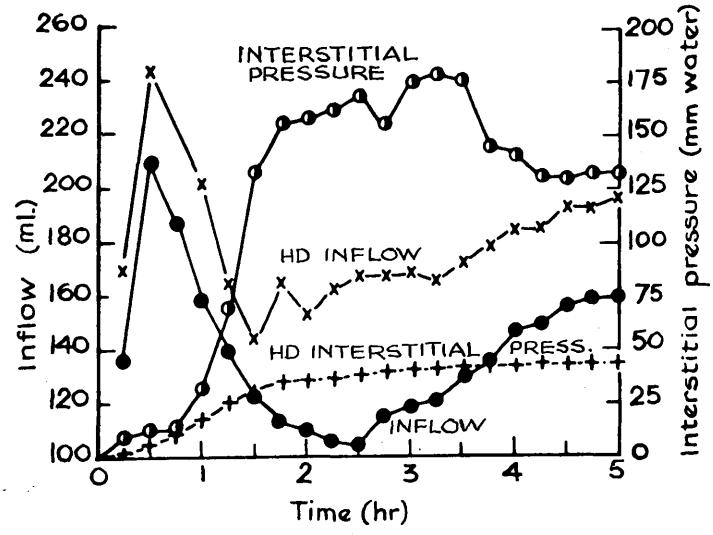

Fig. 19. Interstitial pressure and inflow with perfusion solution at 38°C containing hyaluronidase (HD) compared with previous values without hyaluronidase. One ear perfused in each case, (expt. no.). With hyaluronidase, (65): interstitial pressure, + ; inflow, × . Without hyaluronidase, (53): interstitial pressure, ◑ ; inflow, ● .

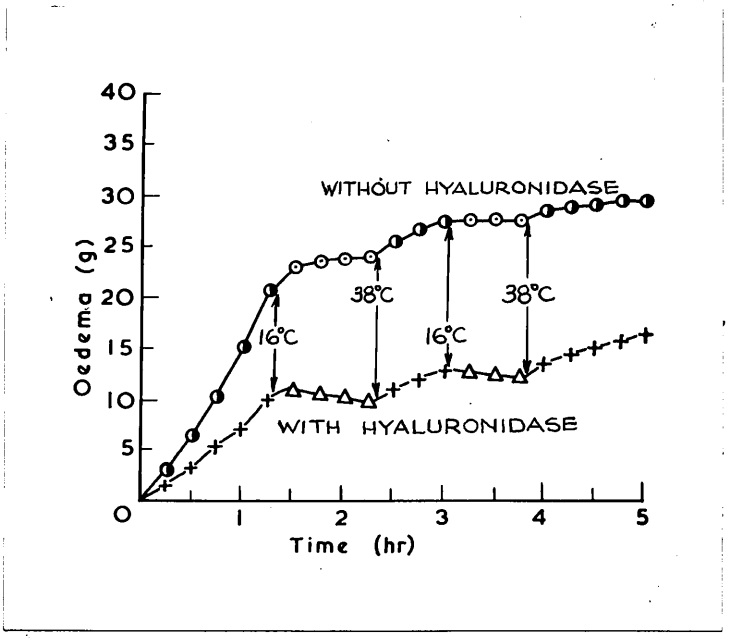

Fig. 20. Oedema formation with perfusion solution changed intermittently from a temp. of 38° to 16°C and vice versa. Values with solution containing hyaluronidase compared with values without hyaluronidase. One ear perfused in each case, (expt. no.). With hyaluronidase, (68): 38°C, + ; 16°C, △ . Without hyaluronidase, (63): 38°C, ◑ ; 16°C, ⊙ .

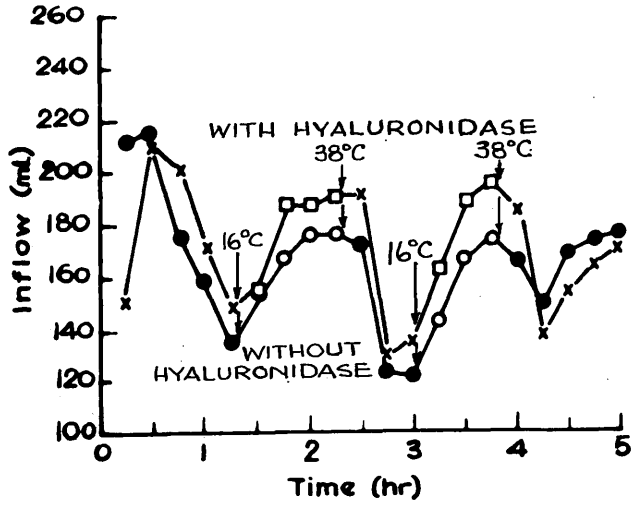

Fig. 21. Inflow with perfusion solution changed intermittently from a temp. of 38° to 16°C and vice versa. Values with solution containing hyaluronidase compared with values without hyaluronidase. One ear perfused in each case, (expt. no.). With hyaluronidase, (68): 38°C, × ; 16°C, □ . Without hyaluronidase, (63): 38°C, ● ; 16°C, ○ .

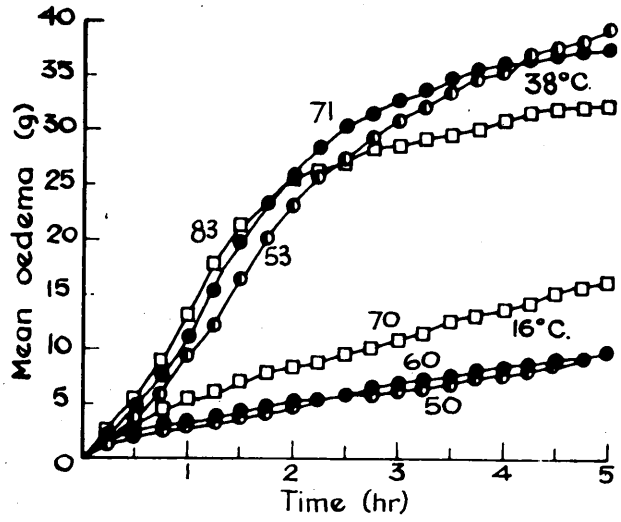

Fig. 22. Mean oedema formation with perfusion solution at 16°C and 38°C. Lower and higher mean downstream pressures compared with previous intermediate values. n = no. of ears perfused, (expt. no.). 16°C: pressure 50 mm Hg, n = 2, (23, 55), ◐ ; pressure 60 mm Hg, n = 2, (24, 47), ● ; pressure 70 mm Hg, n = 3, (21, 22, 43), □. 38°C: pressure 53 mm Hg, n = 2, (19, 50), ◐ ; pressure 71 mm Hg, n = 4, (38, 39, 40, 41), ● ; pressure 83 mm Hg, n = 3, (20, 25, 42), □ .

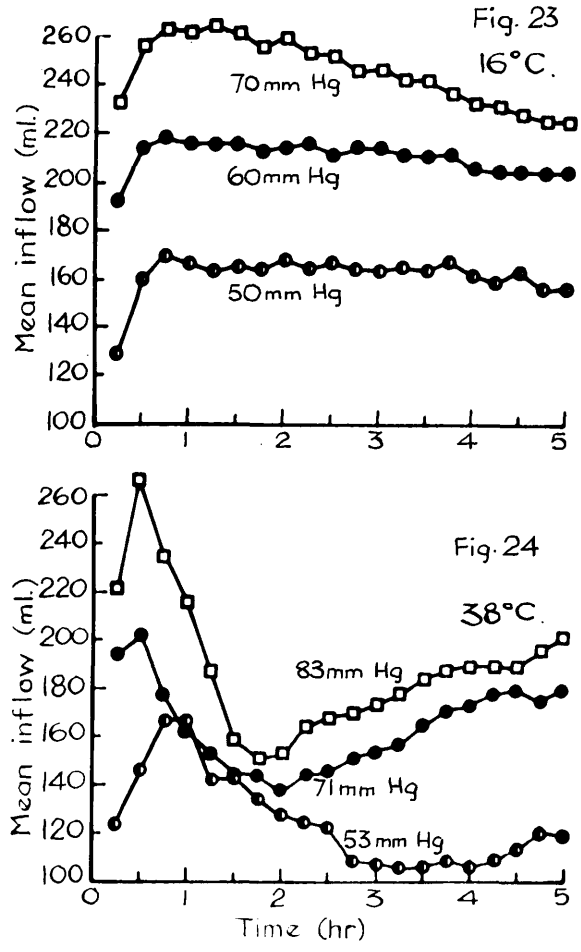

Fig. 23. Mean inflow with perfusion solution at 16°C. Mean downstream pressures of 50 and 70 mm Hg compared with previous values at a mean downstream pressure of 60 mm Hg. n = no. of ears perfused, (expt. no.). Pressure 50 mm Hg: n = 2, (23, 55), ◐. Pressure 60 mm Hg: n = 2, (24, 47), ●. Pressure 70 mm Hg: n = 3, (21, 22, 43), □.

Fig. 24. Mean inflow with perfusion solution at 38°C. Mean downstream pressures of 53 and 83 mm Hg compared with previous values at a mean downstream pressure of 71 mm Hg. n = no. of ears perfused, (expt. no.).
Pressure 53 mm Hg: n = 2, (19, 50), ◐.
Pressure 71 mm Hg: n = 4, (38, 39, 40, 41), ●.
Pressure 83 mm Hg: n = 3, (20, 25, 42), □.

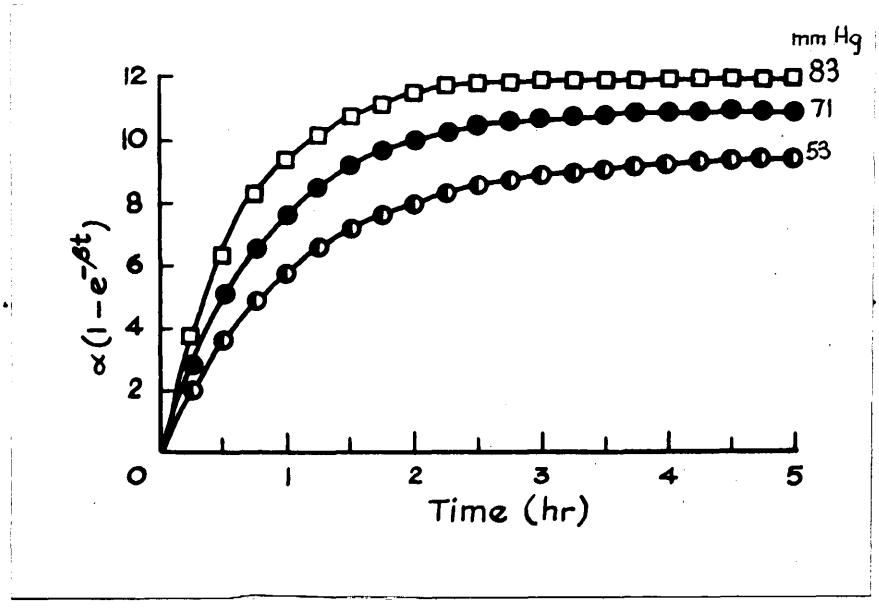

Fig. 25. Calculated gross rate of outward filtration across the capillary membrane derived from experiments with perfusion solution at 38°C graphed in fig. 22. n = no. of ears perfused. Pressure 53 mm Hg: n = 2, ◐ . Pressure 71 mm Hg: n = 4, ● . Pressure 83 mm Hg, n = 3, □ .

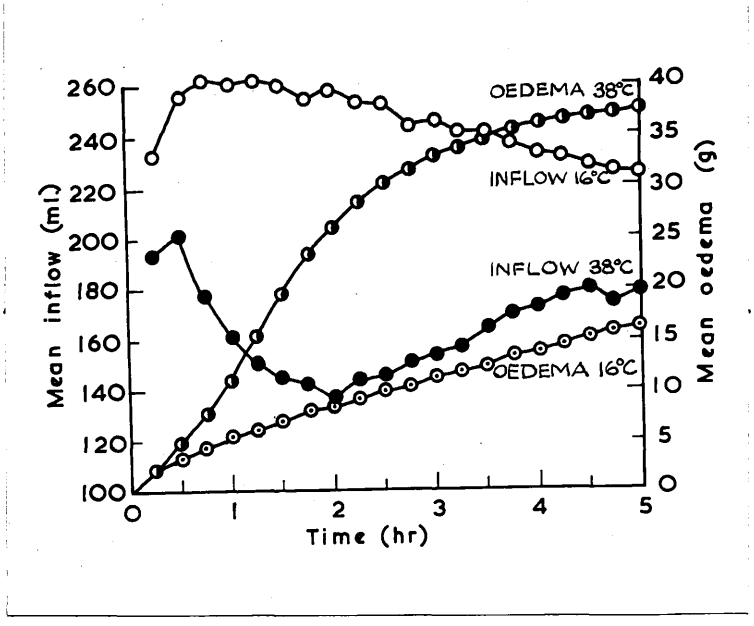

Fig. 26. Mean oedema formation and mean inflow with perfusion solution at 16° and 38°C. The same mean downstream pressure. n = no. of ears perfused, (expt. no.). 16°C, pressure 70 mm Hg, n = 3, (21, 22, 43): oedema, ⊙ ; inflow, O . 38°C, pressure 71 mm Hg, n = 4, (38, 39, 40, 41): oedema, ◑ ; inflow, ● .

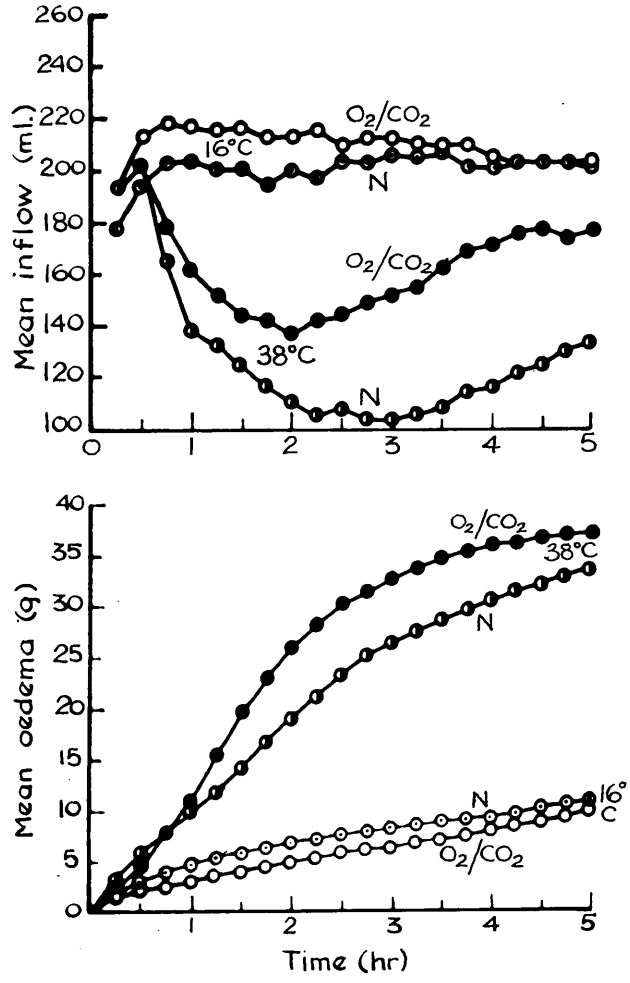

Fig. 27. Mean oedema formation and mean inflow with perfusion solution at 16° and 38°C. Pressurized by N and by 5% CO_2 in O_2. n = no. of ears perfused, (expt. no.). N: 16°C, n = 2, (66, 67), ⊙; 38°C, n = 3, (60, 61, 62), ◑. O_2/CO_2: 16°C, n = 2, (24, 47), O; 38°C, n = 4, (38, 39, 40, 41), ●.

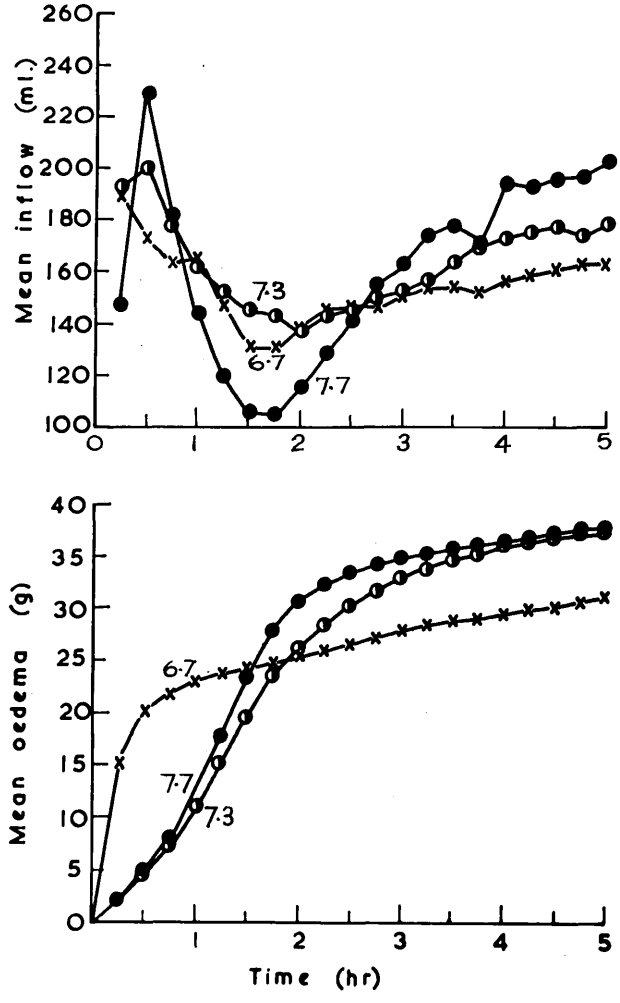

Fig. 28. Mean oedema formation and mean inflow with perfusion solution at 38°C. pH of 7.7 and 6.7 compared with previous values at a pH of 7.3. n = no. of ears perfused, (expt. no.). pH 7.7, n = 1, (57): ●. pH 7.3, n = 4, (38, 39, 40, 41): ◑. pH 6.7, n = 1, (29): ×.

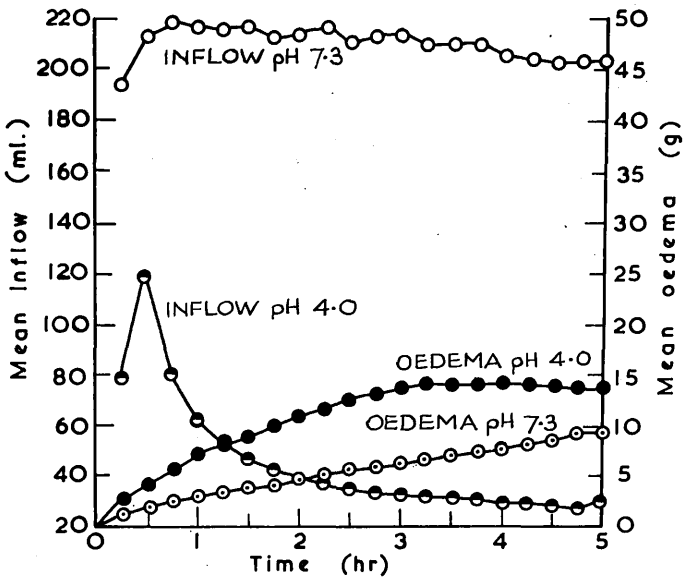

Fig. 29. Mean oedema formation and mean inflow with perfusion solution at 16°C. pH of 4.0 compared with previous values at a pH of 7.3. n = no. of perfused, (expt. no.). pH 4.0, n = 1, (26): oedema, ● ; inflow, ⊖ . pH 7.3, n= 2, (24, 47): oedema, ⊙ ; inflow, O .

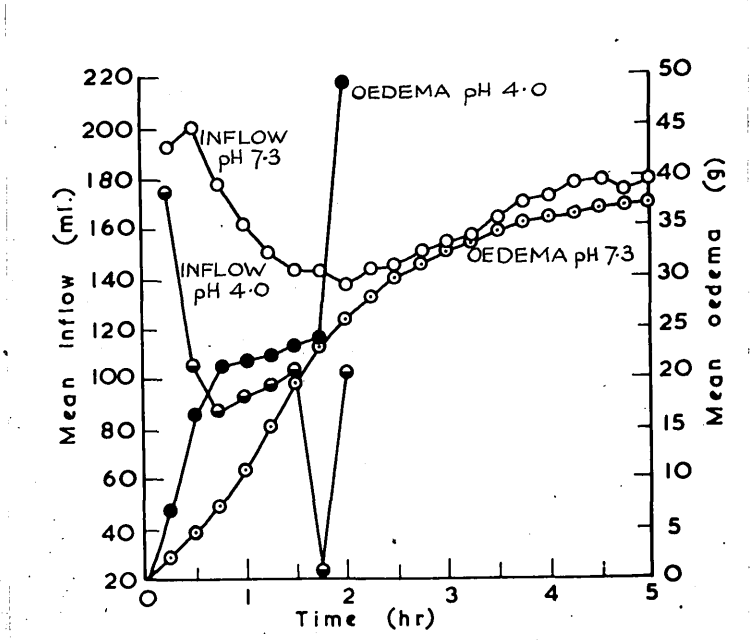

Fig. 30. Mean oedema formation and mean inflow with perfusion solution at 38°C. pH of 4.0 compared with previous values at a pH of 7.3. n = no. of ears perfused, (expt. no.). pH 4.0, n = 1, (27): oedema, ● ; inflow, ◒ . pH 7.3, n = 4, (38, 39, 40 41): oedema, ⊙ ; inflow, ○ .

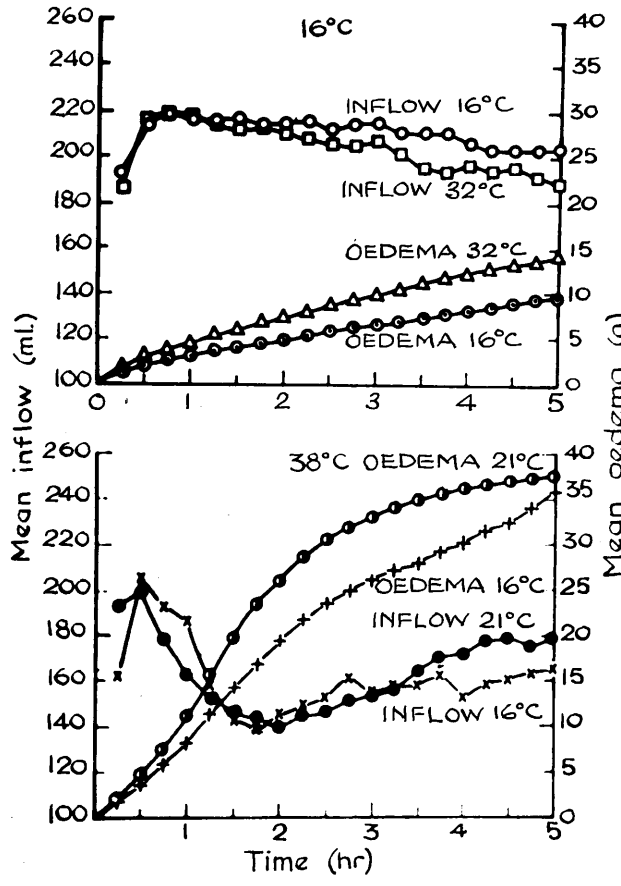

Fig. 31. Mean oedema formation and mean inflow with perfusion solution at 16° and 38°C. Chosen values of ear environmental temp. n = no. of ears perfused, (expt. no.). Solution 16°C: environment 16°C, n = 2, (24, 47), oedema ⊙ , inflow O ; environment 32°C, n = 2, (35, 48), oedema △ , inflow □ . Solution 38°C: environment 21°C, n = 4, (38, 39, 40, 41), oedema ◑ , inflow ● ; environment 16°C, n = 2, (32, 33), oedema + , inflow × .

FIGURES 32 & 33

CHAPTER 3

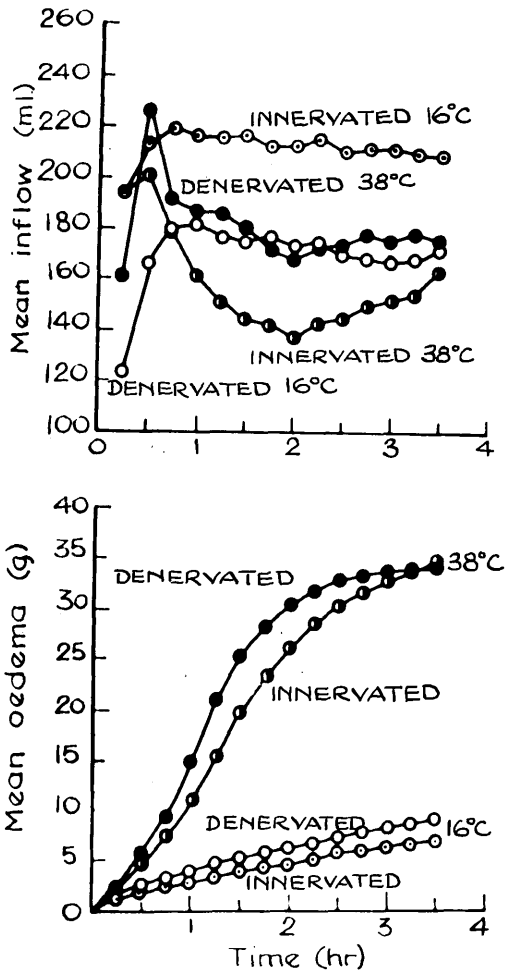

Fig. 32. Mean oedema formation and mean inflow in denervated and in innervated rabbits' ears with perfusion solution at 16° and 38°C. The interpretation of the words oedema and inflow in this and the following legend has the significance detailed under expression of perfusion results in the text. n = no. of ears perfused, (expt. no.). Denervated: 16°C, n = 6, (97, 98, 100, 101, 102, 103), ○ ; 38°C, n = 3, (91, 92, 93), ● . Innervated: 16°C, n = 2, (24, 47), ⊙; 38°C, n = 4, (38, 39, 40, 41), ◑ .

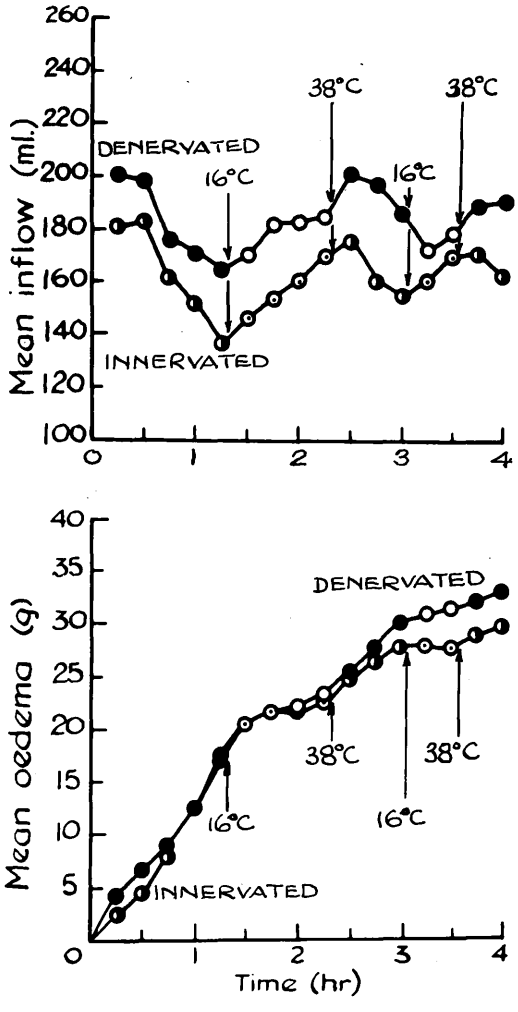

Fig. 33. Mean oedema formation and mean inflow in denervated and in innervated rabbits' ears with perfusion solution changed intermittently from a temp. of 38° to 16°C and vice versa. n = no. of ears perfused, (expt. no.). Denervated, n = 2, (96, 104): 38°C, ●; 16°C, ○. Innervated, n = 1, (108): 38°C, ◑; 16°C, ⊙.

FIGURES 34 to 48

CHAPTER 5

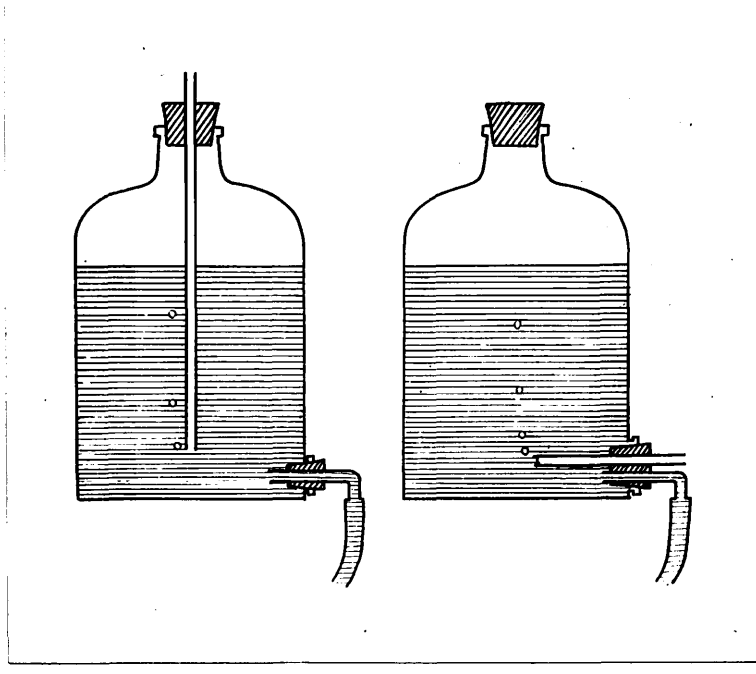

Fig. 34. Constant pressure devices applied to an aspirator bottle. Mariotte (1718): central tube; Ferguson & Garry (1952): short side tube.

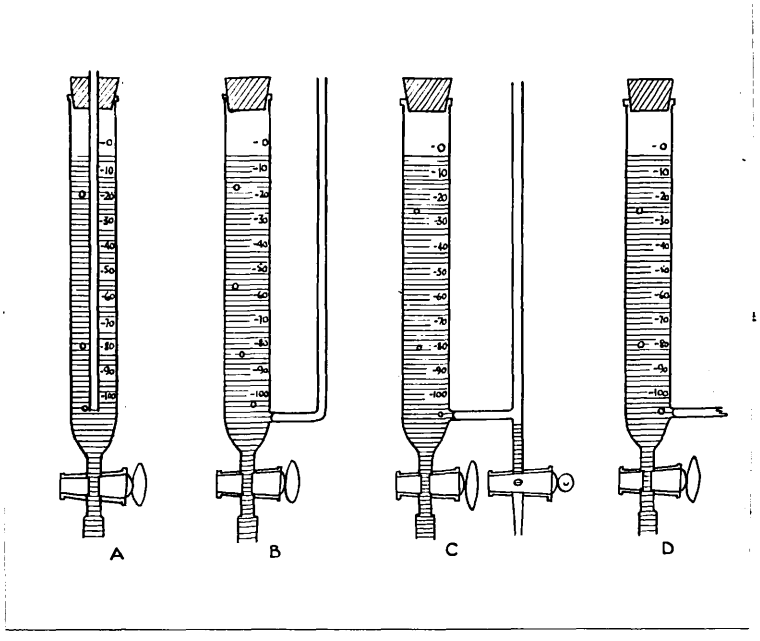

Fig. 35. Main stages in development of improved constant pressure burette based on the Mariotte bottle principle. A: stoppered burette with central tube (van Wijngaarden, 1926). B: Stoppered burette with parallel tube: lower end of tube fused into burette below graduations. C: burette as in B: additional 3-way T-bore stopcock. D: stoppered burette with short side tube (Ferguson & Garry, 1952).

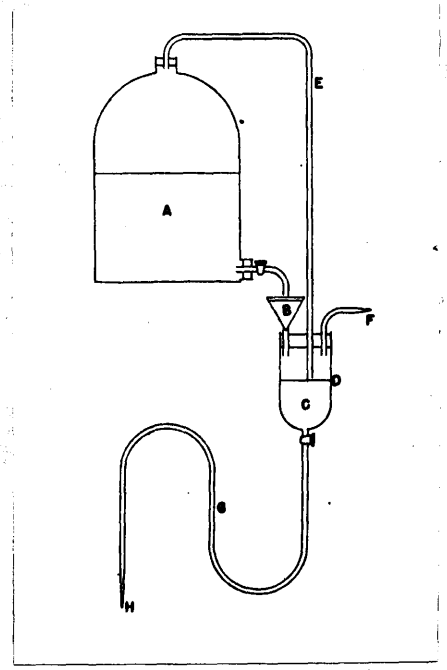

Fig. 36. Apparatus to deliver liquid at constant rate (after Herbst, 1950). A, bottle; B, filter; C, reservoir; D, constant level; E, G, rubber tubes; F, air inlet; H, orifice.

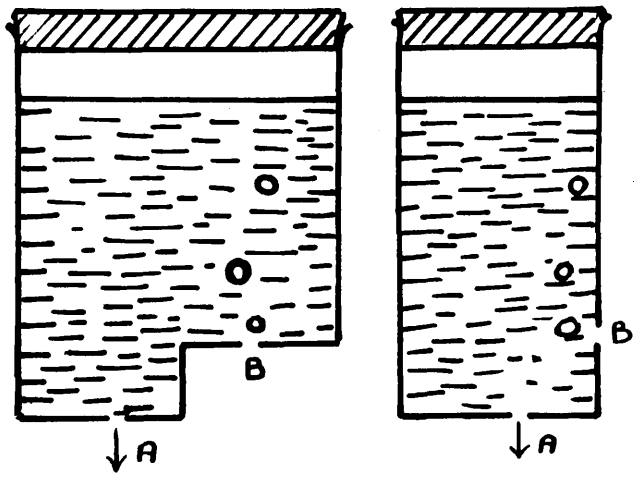

Fig. 37. Principle of improved Mariotte constant pressure device. Stoppered reservoir containing fluid. Two orifices at different levels in reservoir. A, lower orifice; B, upper orifice.

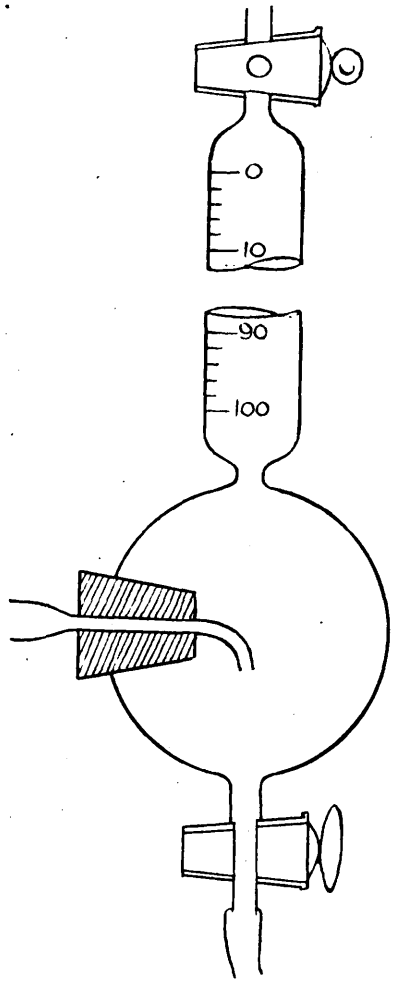

Fig. 38. Constant pressure burette (after Redonnet, 1952). Air entry orifice consists of a capillary passing through a stopper and inserted into a special dilatation blown below the lowest burette graduation.

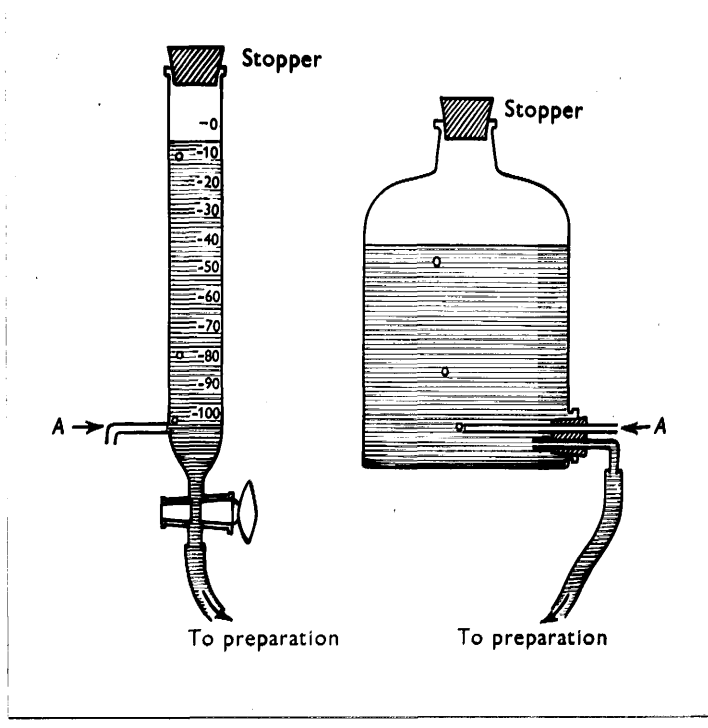

Fig. 39. Improved constant pressure device applied to a burette and to an aspirator bottle. A, side air tube; level from which constant pressure is measured.

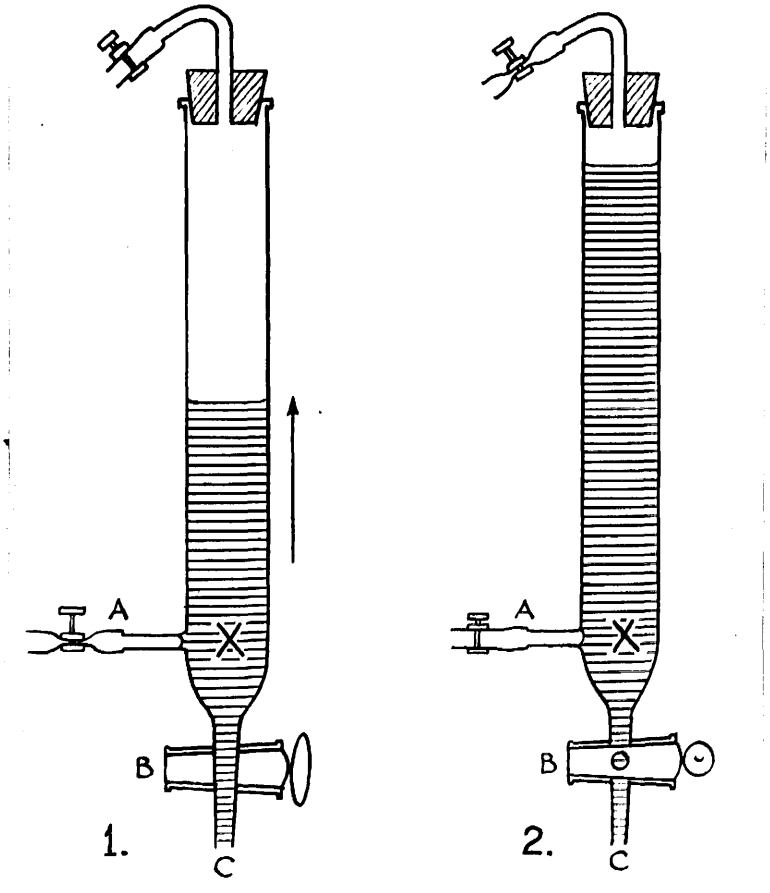

Fig. 40. Improved constant pressure burette: (1) filling (2) ready for discharge. A, side air tube leading to air entry orifice; B, stop cock; C, tip of burette, fluid discharge orifice.

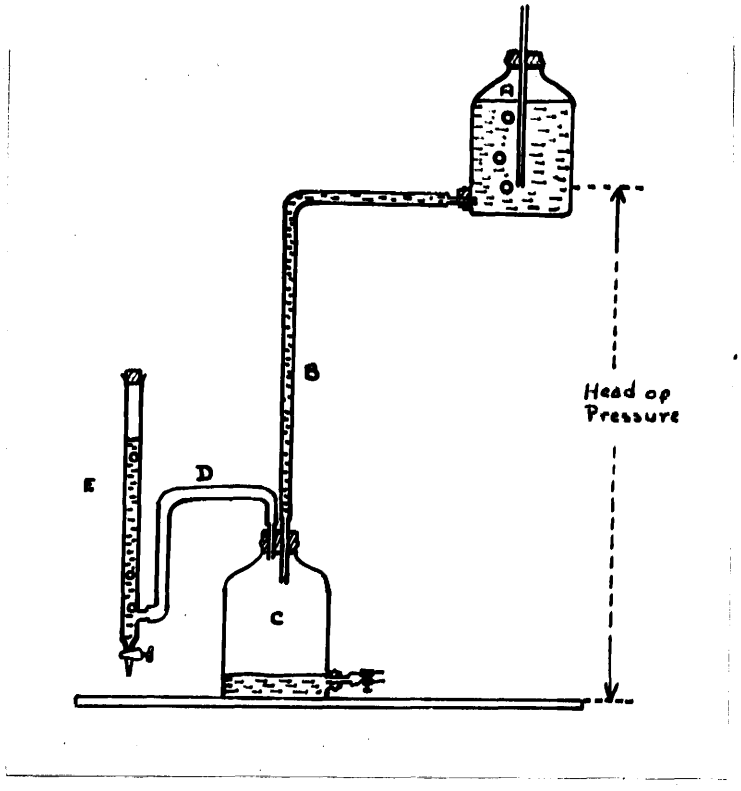

Fig. 41. Apparatus to deliver atmospheric air at a constant pressure greater than atmospheric to the side tube of a constant pressure burette (after Lockett, 1951). A, large capacity Mariotte reservoir bottle with central tube, high above bench level; B, D, rubber tubes; C, large capacity air reservoir; E, constant pressure burette with side air tube.

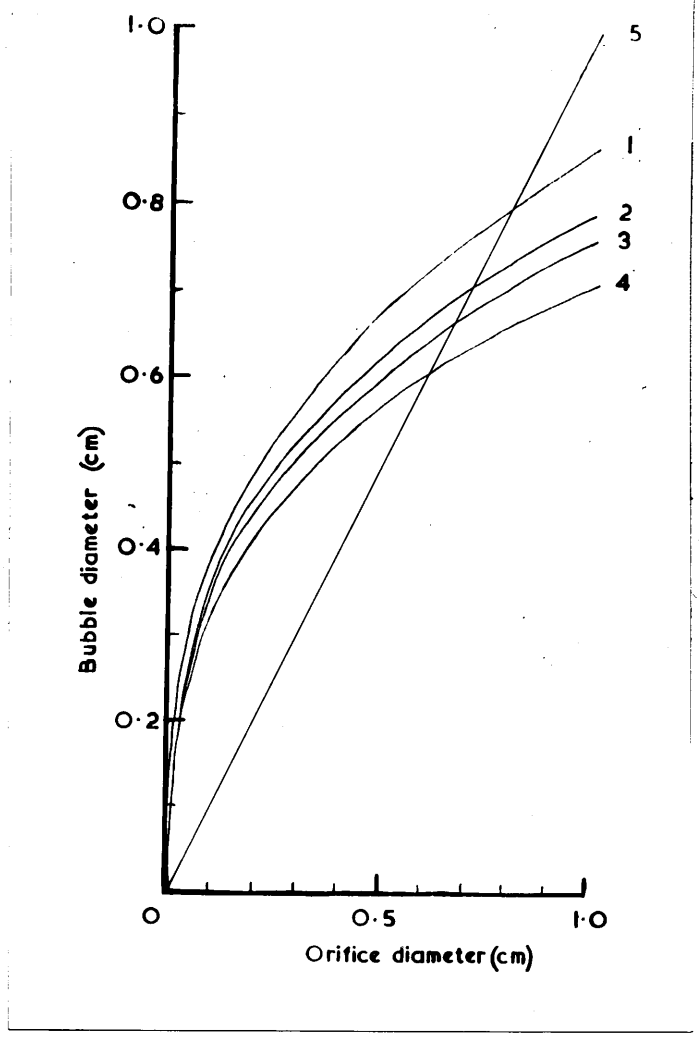

Fig. 42. Relation of vol. of bubble to diameter of orifice at which it is formed (air in water at 20°C). 1 : ratio centring about 0.33 cm^2, formation rate slow (Datta, Napier & Newitt, 1950). 2 : ratio about 0.26 cm^2, formation rate such that bubbles no longer cling momentarily to orifice. 3 : ratio about 0.23 cm^2, plane of orifice horizontal. 4 : ratio about 0.19 cm^2, formation rate very slow. (Curves 2, 3, & 4 calculated from Newman & Whelan, 1952). 5 : along this line bubble diameter equals orifice diameter.

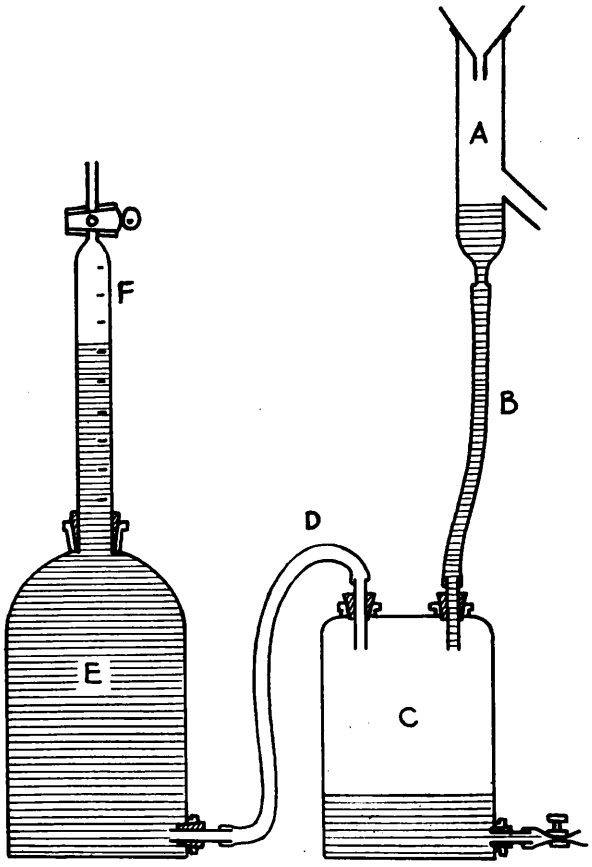

Fig. 43. Apparatus for determining the relation of bubble volume to orifice diameter. A, constant pressure cannula (Shanks, 1923); B, D, connecting tubing; C, air reservoir; E, water reservoir; F, calibrated collecting tube with tap.

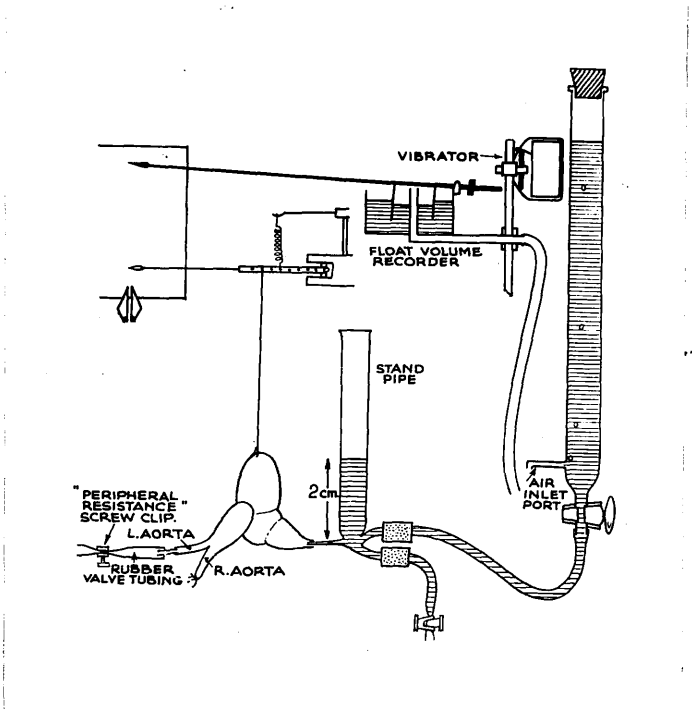

Fig. 44. Perfusion of a frog's heart through Greene's cannula using a constant pressure burette. When it is desired to display venous inflow on the kymograph record, the tubing attached to the float volume recorder is connected to the air inlet port.

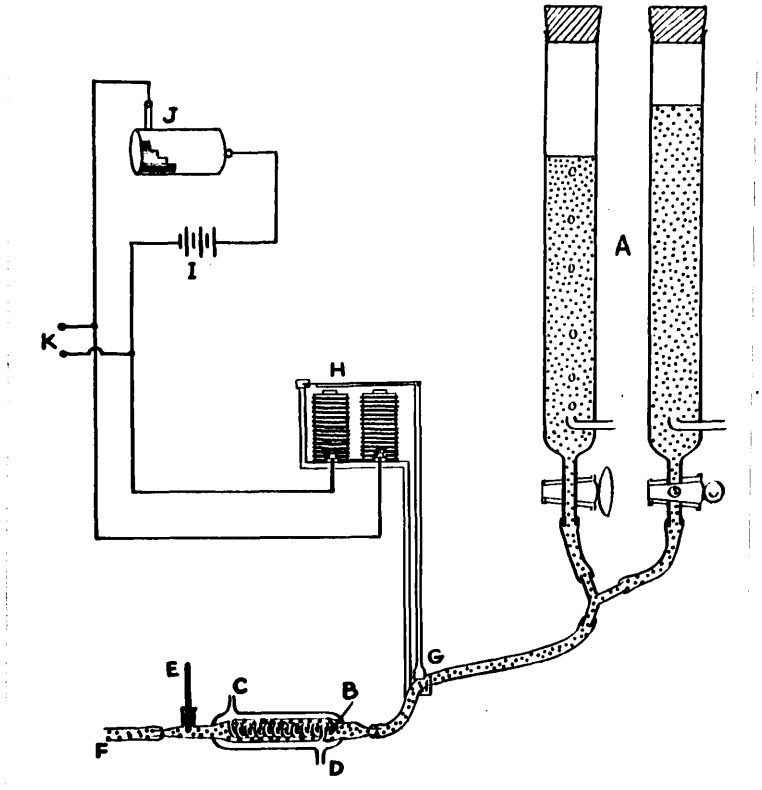

Fig. 45. Automatic inflow apparatus for cystometry. A, improved constant pressure burettes; B, double surface and spiral condenser; C, inlet for warm water from circulating pump; D, outlet for water; E, thermometer; F, connection to bladder cannula; G, clamp; H, solenoid; I, accumulator; J, rotary key; K, leads to kymographic and optical signals.

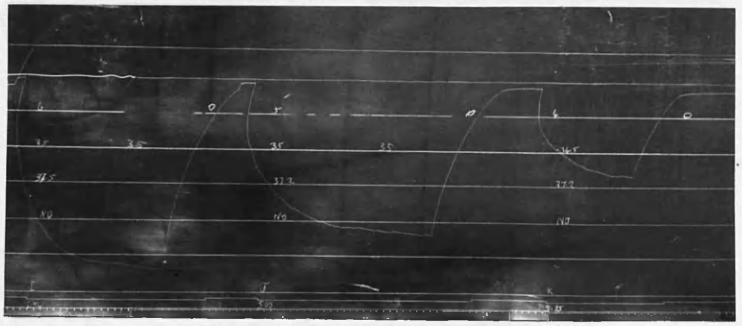

Fig. 46. Effect of variation in constant filling pressure on bladder filling and emptying of decerebrate cat. Kymograph record. Filling = downstroke. Depression of signal signifies filling with Tyrode solution at 35°C. I = 6 cm water constant pressure: J = 5 cm water constant pressure: K = 4 cm water constant pressure. Vol. calibration 1 unit = 10 ml.: time calibration 1 unit = 30 sec. Expt. no. S 19.

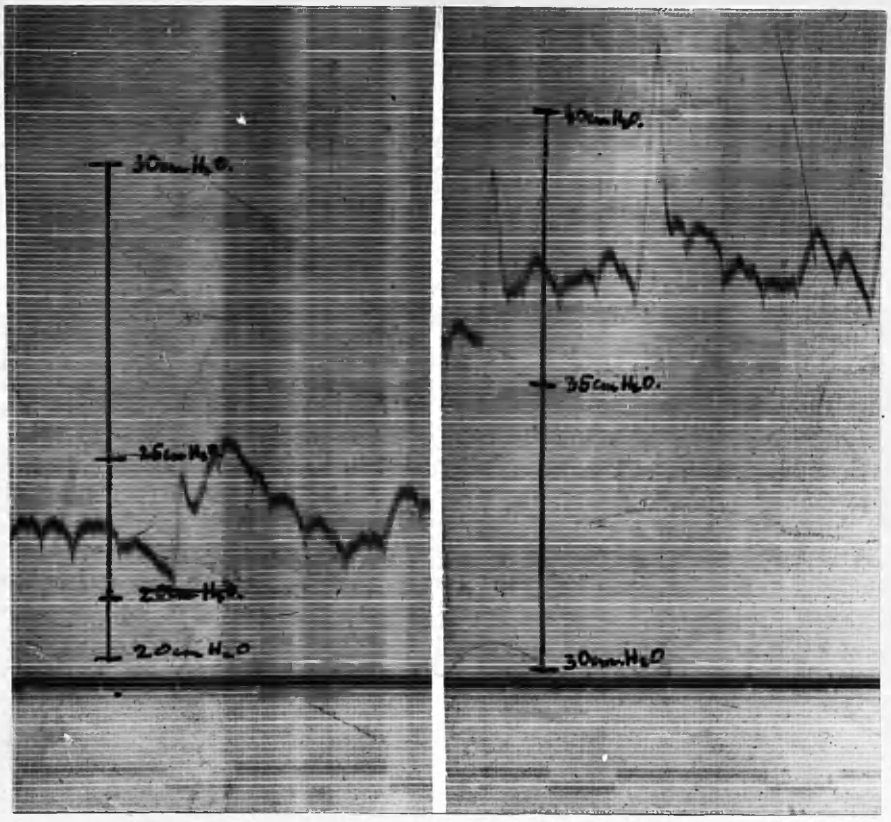

Fig. 47. Effect of variation in constant filling pressure on bladder pressure of decerebrate greyhound. Camera record. Depression of lower signal signifies filling with Tyrode solution at 37°C. 50 ml. solution in bladder in both records. Left hand record, constant filling pressure 30 cm water: right hand record, constant filling pressure 40 cm water. Upper signal, time calibration 1 unit = 10 sec. Expt. no. A 23.

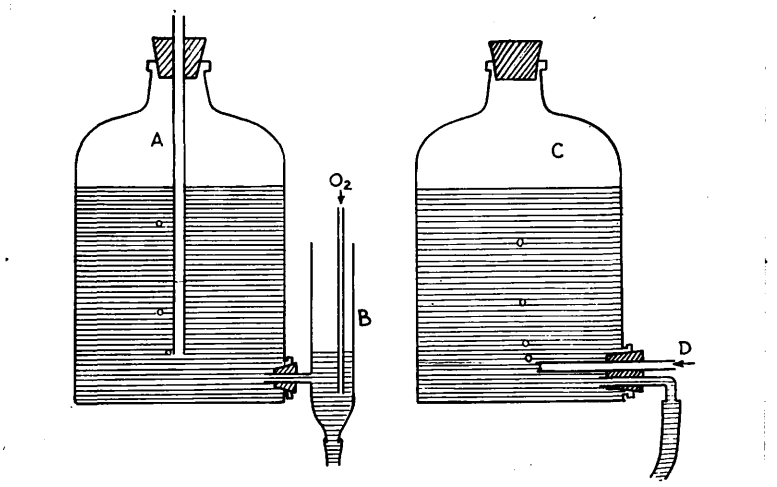

Fig. 48. Reservoirs for use with Gunn's cannula for perfusion of the coronary arteries of the excised mammalian heart. A, aspirator bottle with central constant pressure tube; B, side limb oxygenator; C, improved constant pressure device applied to aspirator bottle; D, high constant pressure gas source.

PLATE 1

CHAPTER 2

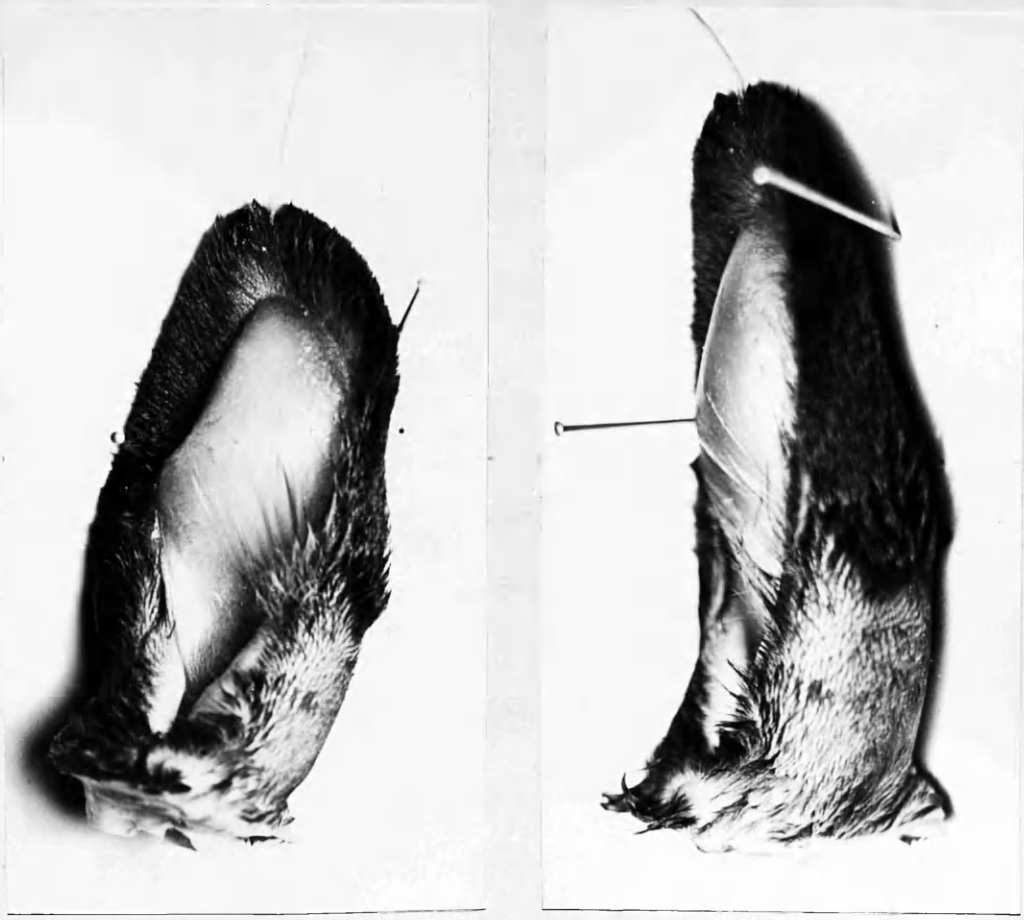

Plate 1. Photograph. Large bleb in isolated ear of rabbit perfused at a solution temp. of 45°C for 5 hr (preparation 46).

PLATES 2 to 8

CHAPTER 3

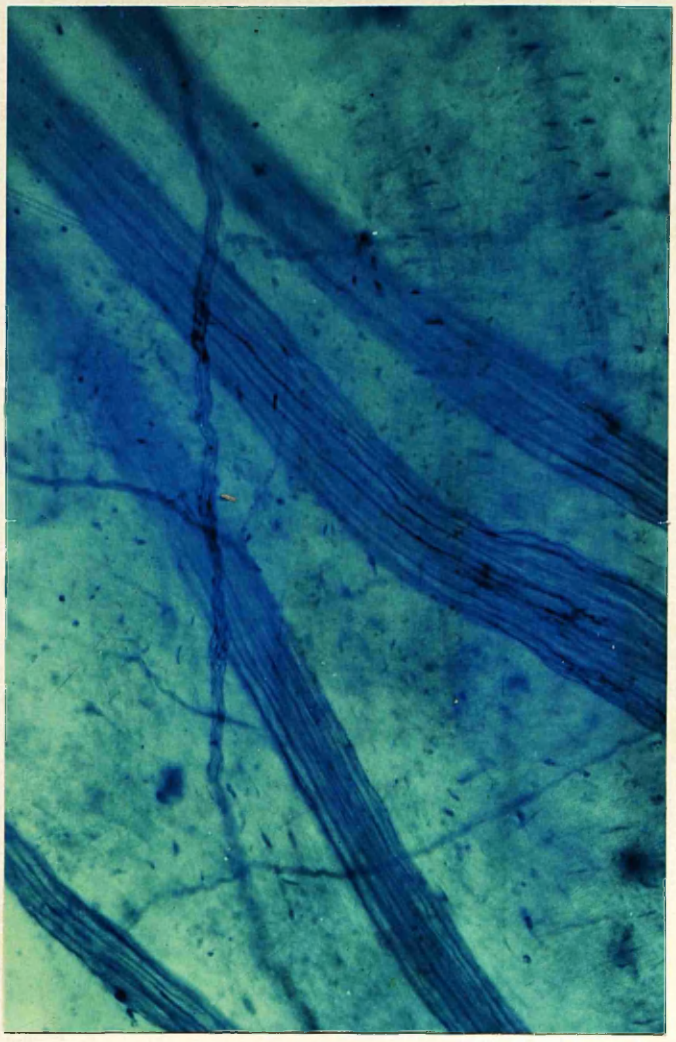

Plate 2. Photomicrograph. Normally innervated ear - rabbit. x 150. Mixed nerve bundles. Methylene blue by perfusion. Blue toned print.

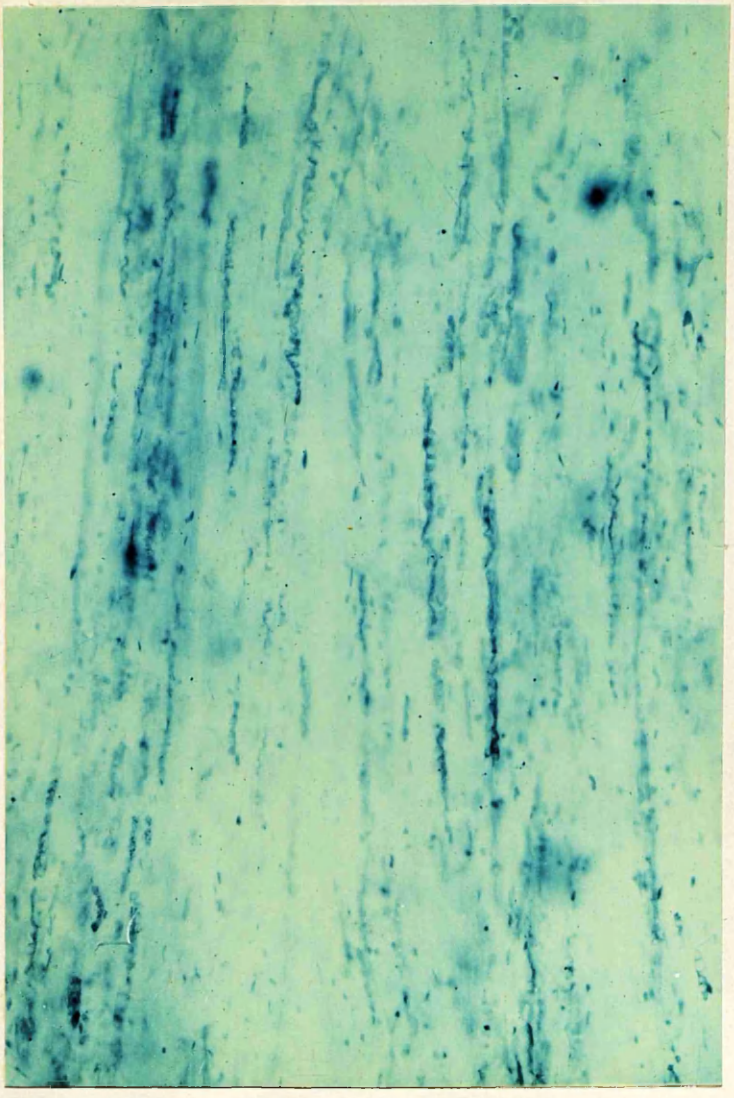

Plate 3. Photomicrograph. Denervated ear - rabbit.
x 150. Mixed nerve bundles 21 days after denervation.
Methylene blue by perfusion. Blue toned print.

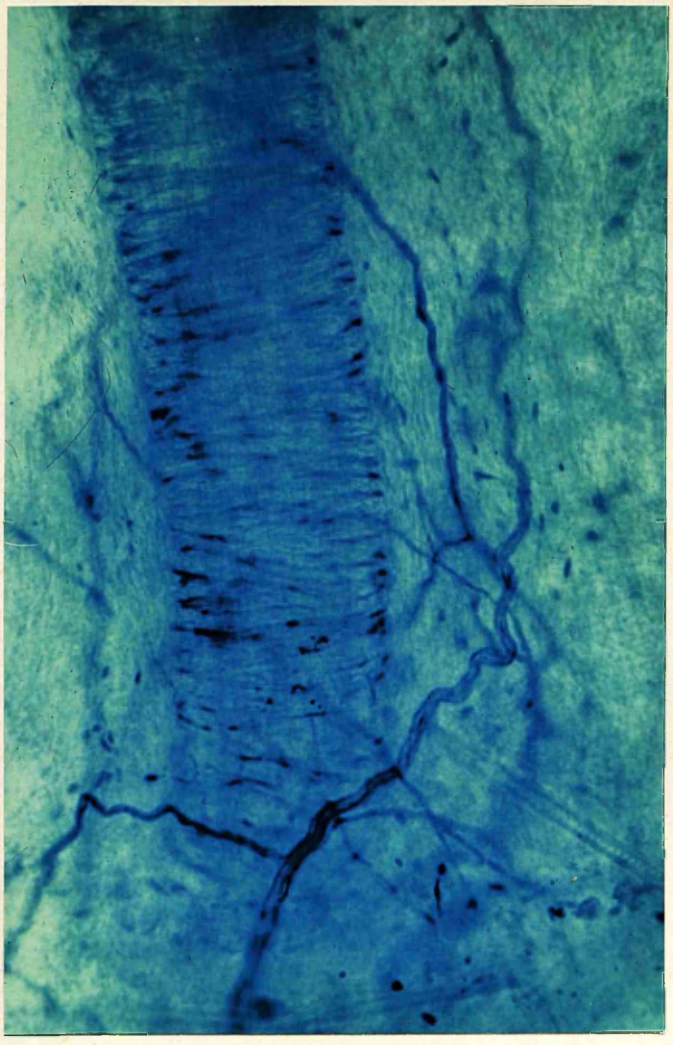

Plate 4. Photomicrograph. Normally innervated ear - rabbit. x 150. Small artery showing nerve supply. Methylene blue by perfusion. Blue toned print.

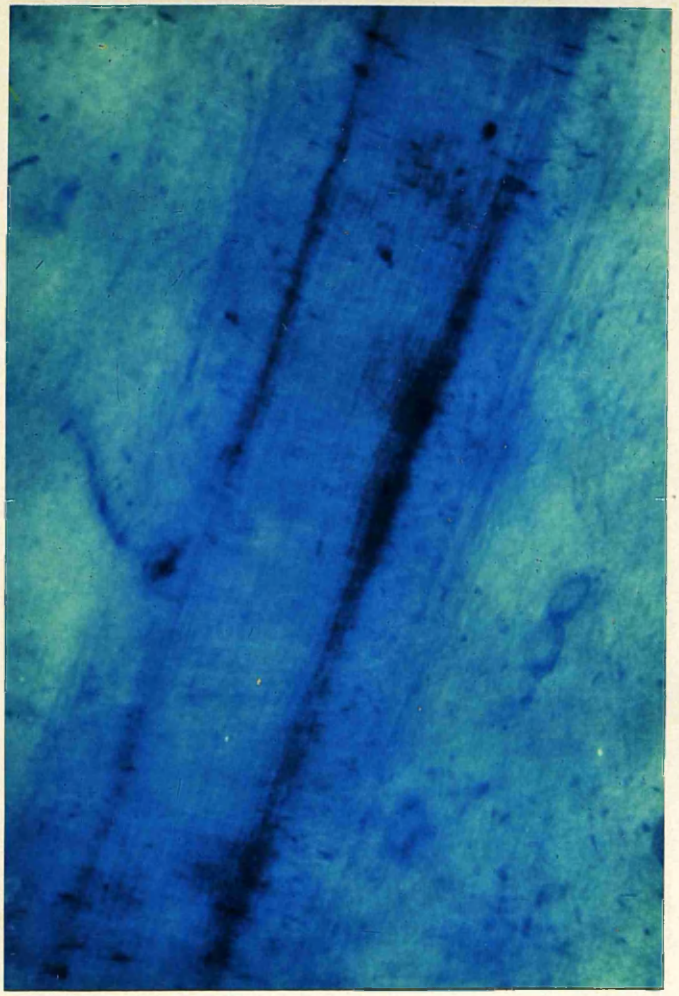

Plate 5. Photomicrograph. Denervated ear - rabbit. x 150. Artery showing absence of nerve supply 21 days after denervation. Methylene blue by perfusion. Blue toned print.

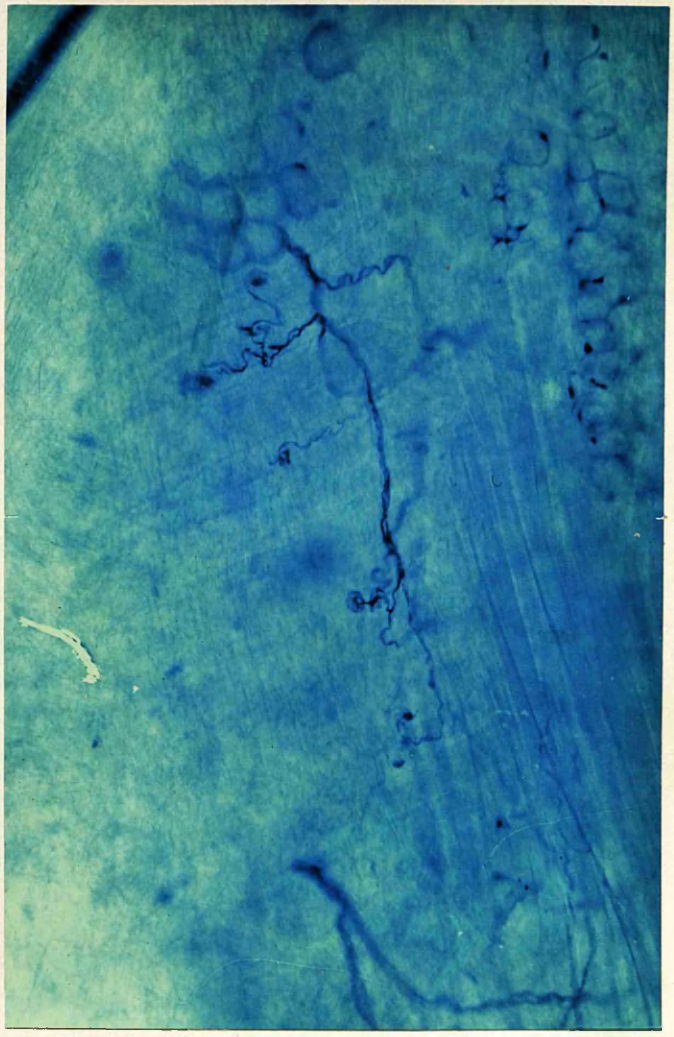

Plate 6. Photomicrograph. Normally innervated ear - rabbit. x 150. Motor end plates showing branching and final knob-like terminations of the branches in the muscle fibre. Methylene blue by perfusion. Blue toned print.

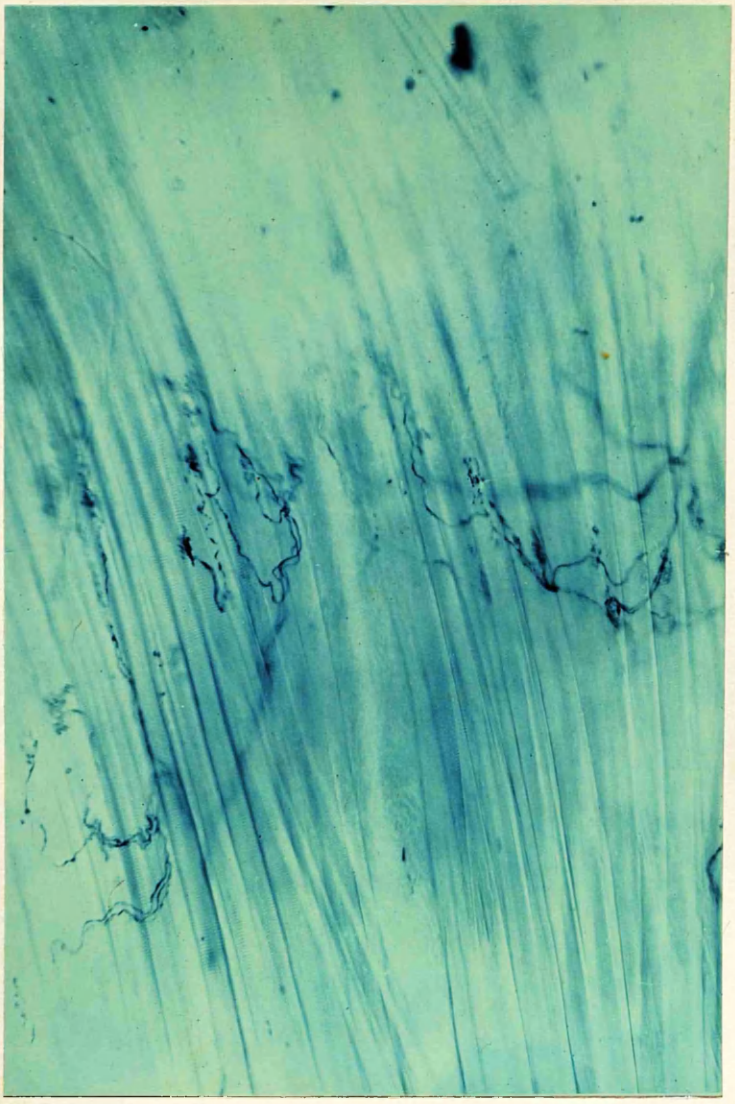

Plate 7. Photomicrograph. Denervated ear - rabbit. x 150. Motor end plates showing branching and final knob-like terminations of the branches in the muscle fibre. Twenty one days after denervation. Methylene blue by perfusion. Blue toned print.

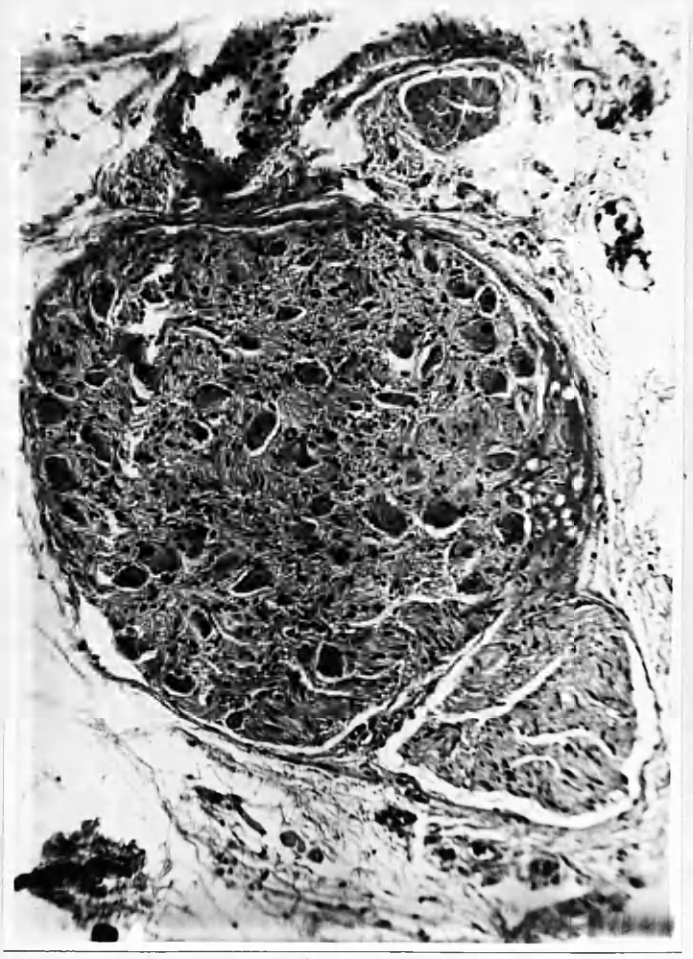

Plate 8. Photomicrograph. Superior cervical ganglion - rabbit. x 160. Numerous nerve ganglion cells with large nuclei: a few heavily myelinated fibres and a large number of finely myelinated fibres. H. & E.

PLATE 9

CHAPTER 5

Plate 9. Bubble growth just before release at a vertically orientated orifice. Photograph. Surface tension forces are operative only around a portion of the perimeter, the buoyancy forces tending to drag the bubble upwards across the plane of the orifice (after Datta, Napier & Newitt, 1950). The effect is seen to a greater degree at the orifice of the central tube of a Mariotte's bottle.

TABLES 1 to 3

CHAPTER 1

Oedema formation	Mean time (hr)		Difference in means (hr)
	Control (32 eyes)	Scorbutic (32 eyes)	
Onset	17.3 (±2.53)*	16.5 (±1.83)*	0.8
Maximum	25.8 (±2.78)	42.8 (±6.22)	17.0

* Standard error of the mean

Table 1. Comparison of the progress of oedema formation in the cornea in the control and scorbutic groups of guinea-pigs.

Eyes	No. of vascularized corneae	No. of nonvascularized corneae
Control	9	23
Scorbutic	19	13

$\chi^2 = 5.1 \quad P < 0.05$ (Significant)

Table 2. Comparison of the incidence of vascularization of the cornea in the control and scorbutic groups of guinea-pigs.

Vascularization	Mean time (hr) Control (9 eyes)		Scorbutic (19 eyes)		Difference in means (hr)
Onset	33.7	(±8.30)π	52.6	(±8.79)π	18.9
Maximum	64.9	(±6.99)	79.6	(±9.31)	14.7
Disappearance	134.2	(±33.44)	146.5	(±21.56)	12.3

π Standard error of the mean

Table 3. Comparison of the progress of vascularization of the cornea in the control and scorbutic groups of guinea-pigs.

TABLES 4 to 11

CHAPTER 2

F	H_2	R	P.D.
21	81	159	7
24	82	168	6
59	78	170	9
63	79	170	8
78	75	184	12
87	73	173	15
113	68	175	17
165	56	181	31
174	52	183	35
185	50	184	37
192	47	186	40
194	47	186	40
204	44	187	42
225	38	190	48
240	30	194	56
246	29	195	57
264	22	198	63
267	19	200	67
293	15	202	70

Table 4. Calibration of cannula by manometric pressure drop resulting from selected flow rates. $\underline{F}$: flow in ml./15 min; $\underline{H}_1$: head of mercury constant at 100 mm in upstream manometer; $\underline{H}_2$: head of mercury in mm in downstream manometer; $\underline{R}$: length of water column in mm from $\underline{A}$ to $\underline{B}$; P.D.: pressure drop across cannula = $\underline{H}_1 - \underline{H}_2 - \frac{\underline{R}}{13.6}$ mm Hg.

P	F
10	20
16	59
20	77
24	89
28	117
30	129
34	138
38	156
40	166
44	168
50	189
60	212
70	240
80	262
90	286

Table 5. Calibration of cannula by flow measurements with free discharge resulting from selected pressures, assuming no significant pressure recovery from the velocity head. P: pressure in mm Hg required to overcome friction in cannula and to produce the kinetic energy flow; F: flow in ml./15 min calculated from measurements made over 1 min.

Flow (ml/15 min)	Flow (ml/min)	Velocity (cm/sec)	Velocity Head Loss (mm Hg)
30	2.0	28.1	0.3
40	2.7	37.5	0.5
50	3.3	46.8	0.8
60	4.0	56.2	1.2
80	5.4	75.1	2.1
100	6.7	93.5	3.3
120	8.0	112.3	4.7
140	9.4	130.8	6.4
150	10.0	140.0	7.3
160	10.7	149.8	8.4
180	12.0	169.5	10.8
200	13.4	187.0	13.1
220	14.7	205.9	15.9
240	16.0	224.8	18.9
250	16.7	233.8	20.5
260	17.3	242.4	22.0
280	18.7	261.7	25.7
300	20.0	280.0	29.4

Table 6. Theoretical calculation of velocity head loss $(\frac{v2}{2g})$ during calibration of the cannula by flow measurements with free discharge resulting from selected pressures. Cross sectional area of cannula = $\frac{\pi}{4}(0.039)^2$ cm^2 = 1.19×10^{-3} cm^2.

Pressurizing gas	Flow (ml./15 min)	Soln. temp. (°C)	O_2 content (ml./l.)
95% O_2 / 5% CO_2	205	16	13.4
	140	38	10.5
N	205	16	4.5
	140	38	4.2

Table 7. Oxygen content of perfusion solution delivered at the arterial cannula following passage through the apparatus under the experimental conditions. Oxygen content of solution on exposure to atmosphere at 20°C was 5.4 ml./l.

Perfusion (hr)	Perfusate O_2 content (ml./l.)		% reduction in O_2 content	
	Soln. temp. 16°C	Soln. temp. 38°C	Soln. temp. 16°C	Soln. temp. 38°C
1	4.9	2.3	64	79
2	4.6	1.3	66	88
3	6.2	3.1	55	71
4	5.3	3.4	60	68
5	6.8	3.5	50	67

Table 8. Oxygen uptake by isolated ear of the rabbit perfused with solution at temp. of 16° and 38°C having oxygen contents of 13.4 ml./l. and 10.5 ml./l. respectively. Four ears perfused in each case.

Perfusion (hr)	Perfusate O_2 content (ml./l.)		a.-v. O_2 difference (ml./l.)		Inflow vol. (l.)		O_2 uptake (ml.)	
	Soln. temp. 16°C	Soln. temp. 38°C	Soln. temp. 16°C	Soln. temp. 38°C	Soln. temp. 16°C	Soln. temp. 38°C	Soln. temp. 16°C	Soln. temp. 38°C
1	4.9	2.3	8.5	8.2	0.842	0.734	7.2	6.0
2	4.6	1.3	8.8	9.2	0.860	0.578	7.6	5.3
3	6.2	3.1	7.2	7.4	0.855	0.591	6.2	4.4
4	5.3	3.4	8.1	7.1	0.838	0.662	6.8	4.7
5	6.8	3.5	6.6	7.0	0.816	0.711	5.4	5.0

Table 9. Oxygen uptake by isolated ear of the rabbit perfused with solution at temp. of 16° and 38°C having oxygen contents of 13.4 ml./l. and 10.5 ml./l. respectively. Four ears perfused in each case.

t	Solution temp. 16°C		Solution temp. 38°C	
	Observed	Calculated	Observed	Calculated
	1.4	1.6	2.3	1.3
	2.0	2.0	4.7	4.4
	2.5	2.5	7.5	8.3
1	3.0	3.0	11.0	12.2
	3.5	3.5	15.1	16.3
	3.9	3.9	19.7	19.7
	4.4	4.4	23.3	22.8
2	4.7	4.9	26.0	25.5
	5.2	5.4	28.2	27.8
	5.6	5.7	30.1	29.7
	6.1	6.0	31.5	31.2
3	6.3	6.6	32.8	32.5
	6.8	6.9	33.8	33.6
	7.1	7.3	34.7	34.3
	7.5	7.7	35.5	35.1
4	8.0	8.2	36.0	35.6
	8.5	8.4	36.4	36.0
	8.8	8.9	36.9	36.4
	9.3	9.3	37.2	36.6
5	9.7	9.5	37.4	36.8

Table 10. Observed and calculated values for cumulative oedema formation (net outward filtration) in isolated rabbits' ears perfused at a solution temp. of 16° and 38°C. t = time of perfusion in hr. n = no. of ears perfused, (expt. no.). 16°C, n = 2, (24, 47). 38°C, n = 4, (38, 39, 40, 41).

Environmental temp. (°C)	Soln temp. (°C)	Increasing oedema formation
16	16	↓
32		
16	38	
21		

Table 11. The effect of altering the environmental temp. on the relative accumulation of oedema fluid in the perfused isolated ear of the rabbit.

TABLES 12 to 16

CHAPTER 4

t	w	$\frac{dw}{dt}$	$w\frac{dw}{dt}$	w^2
3	2.5	0.50	1.25	6.25
4	3.0	0.50	1.50	9.00
5	3.5	0.45	1.58	12.25
6	3.9	0.45	1.76	15.21
7	4.4	0.40	1.76	19.36
8	4.7	0.40	1.88	22.09
9	5.2	0.47	2.42	27.04
10	5.6	0.45	2.53	31.69
	$\Sigma =$ 32.8	$\Sigma =$ 3.62	$\Sigma =$ 14.68	$\Sigma =$ 142.89

$$\beta = \frac{8 \times 14.68 - 32.8 \times 3.62}{(32.8)^2 - 8 \times 142.89}$$

$$= \frac{-1.30}{-67.28} = 0.02$$ i.e. the extent to which oedema fluid already formed retarded further net outward filtration.

$$\alpha = \frac{3.62 + 32.8 \times 0.019}{8}$$

$= 0.53$ i.e. the ultimate value of gross outward filtration.

Table 12. Calculation of values of β (equation 6) and of α (equation 7) in derivation of capillary gross outward filtration. Example of evaluation for isolated rabbits' ears perfused at a solution temp. of 16°C (expt. no. 24, 47). Compare with fig. 9 & fig. 10.

t	$w_r - \frac{\alpha}{\beta}$	$\left(w_r - \frac{\alpha}{\beta}\right) e^{\beta t_r}$
3	-25.45	-27.05
4	-24.95	-26.42
5	-24.45	-26.90
6	-24.05	-27.00
7	-23.55	-26.85
8	-23.25	-27.22
9	-22.75	-27.06
10	-22.32	-27.02

Mean C = -26.94 i.e. a correction factor calculated by using central values of t. The correction factor allowed for the increase in weight of the ear due to filling of the blood vessels with perfusion solution being unduly predominant, since oedema developed comparatively slowly during perfusion at a temperature of 16°C.

Table 13. Calculation of best value of C (equation 11) in derivation of capillary gross outward filtration. Example of evaluation for isolated rabbits' ears perfused at a solution temp. of 16°C (expt. no. 24, 47).

t	βt	$e^{\beta t}$	$e^{-\beta t}$	$1-e^{-\beta t}$	w eqn. 8	$Ce^{-\beta t}$	w eqn. 12	w obs.
1	0.019	1.02	0.980	0.020	0.58	-26.40	1.6	1.4
2	0.038	1.04	0.962	0.038	1.06	-25.93	2.0	2.0
3	0.057	1.06	0.943	0.057	1.59	-25.42	2.5	2.5
4	0.076	1.08	0.926	0.074	2.06	-24.98	3.0	3.0
5	0.095	1.10	0.909	0.091	2.54	-24.50	3.5	3.5
6	0.114	1.12	0.893	0.107	2.99	-24.08	3.9	3.9
7	0.133	1.14	0.877	0.123	3.43	-23.60	4.4	4.4
8	0.152	1.17	0.855	0.145	4.05	-23.01	4.9	4.7
9	0.171	1.19	0.840	0.160	4.47	-22.60	5.4	5.2
10	0.190	1.21	0.826	0.174	4.87	-22.22	5.7	5.6
11	0.209	1.23	0.813	0.187	5.23	-21.92	6.0	6.1
12	0.228	1.26	0.794	0.206	5.76	-21.38	6.6	6.3
13	0.247	1.28	0.781	0.219	6.13	-21.02	6.9	6.8
14	0.266	1.30	0.769	0.231	6.46	-20.70	7.3	7.1
15	0.285	1.33	0.752	0.248	6.93	-20.25	7.7	7.5
16	0.304	1.36	0.735	0.265	7.40	-19.80	8.2	8.0
17	0.323	1.38	0.725	0.275	7.69	-19.55	8.4	8.5
18	0.342	1.41	0.709	0.291	8.12	-19.08	8.9	8.8
19	0.361	1.44	0.694	0.306	8.53	-18.68	9.3	9.3
20	0.380	1.46	0.685	0.315	8.80	-18.45	9.5	9.7

Table 14. Calculation of values of w (uncorrected) = $\frac{\alpha}{\beta}(1 - e^{-\beta t})$ (equation 8) and of values of w (corrected) = $\frac{\alpha}{\beta} + Ce^{-\beta t}$ (equation 12) in derivation of capillary gross outward filtration. Example of evaluation for isolated rabbits' ears perfused at a solution temp. of 16°C (expt. no. 24, 47). The corrected values of w are an excellent fit for the observed values.

t	w	$\frac{dw}{dt}$	$\log\frac{dw}{dt}$	$t \log\frac{dw}{dt}$	t^2	log t	t log t
3	7.5	3.15	1.147	3.441	9	1.10	3.30
4	11.0	3.80	1.335	5.340	16	1.39	5.56
5	15.1	4.35	1.470	7.350	25	1.61	8.05
6	19.7	4.10	1.411	8.466	36	1.79	10.75
7	23.3	3.15	1.147	8.029	49	1.95	13.65
8	26.0	2.45	0.896	7.168	64	2.08	16.63
9	28.2	2.05	0.718	6.462	81	2.20	19.82
10	30.1	1.65	0.500	5.000	100	2.30	23.00
11	31.5	1.35	0.300	3.300	121	2.40	26.40
12	32.8	1.15	0.140	1.680	144	2.49	29.90
Σ=75	Σ=225.2	Σ=27.20	Σ=9.064	Σ=56.236	Σ=645	Σ=19.31	Σ=157.06

$B = \beta$ = the extent to which oedema fluid already formed retarded further net outward filtration.

α = the ultimate value of gross outward filtration.

$k = \alpha\beta$

$A = \log k = \log\alpha + \log\beta$

$$B = \frac{75 \times 9.064 - 10 \times 56.24 + 10 \times 157.06 - 75 \times 19.31}{10 \times 645 - (75)^2} = \frac{241.64}{824} = 0.29 = \beta$$

$$A = \frac{9.064 - 19.31 + 75 \times 0.293}{10} = \frac{11.73}{10} = 1.173$$

$$\text{Log}\,\alpha = A - \log\beta = 1.173 - \bar{1}.228 = 2.401$$

$$\therefore \alpha = 11.00$$

$$\frac{\alpha}{\beta} = 37.54$$

Table 15. Calculation of values of B, β, A and α (equation 21) in derivation of capillary gross outward filtration. Example of evaluation for isolated rabbits' ears perfused at a solution temp. of 38°C (expt. no. 38, 39, 40, 41) Compare with fig. 9, 10 & 11.

t	βt	$e^{\beta t}$	$e^{-\beta t}$	$1-e^{-\beta t}$	$\frac{\alpha}{\beta}(1-e^{-\beta t})$	$\alpha(1-e^{-\beta t})$	αt	$\alpha t e^{-\beta t}$	w eqn. 22	w obs.
1	0.29	1.34	0.746	0.254	9.54	2.79	11	8.21	1.3	2.3
2	0.59	1.80	0.556	0.444	16.66	4.88	22	12.23	4.4	4.7
3	0.88	2.41	0.415	0.585	21.97	6.43	33	13.70	8.3	7.5
4	1.17	3.22	0.311	0.689	25.87	7.58	44	13.68	12.2	11.0
5	1.47	4.35	0.230	0.770	28.91	8.47	55	12.65	16.3	15.1
6	1.76	5.81	0.172	0.828	31.09	9.14	66	11.35	19.7	19.7
7	2.05	7.77	0.129	0.871	32.70	9.59	77	9.93	22.8	23.3
8	2.34	10.40	0.096	0.904	33.94	9.94	88	8.45	25.5	26.0
9	2.64	14.00	0.071	0.929	34.87	10.23	99	7.03	27.8	28.2
10	2.93	18.70	0.053	0.947	35.55	10.41	110	5.83	29.7	30.1
11	3.22	25.00	0.040	0.960	36.04	10.57	121	4.84	31.2	31.5
12	3.52	33.80	0.030	0.970	36.42	10.68	132	3.96	32.5	32.8
13	3.81	45.20	0.022	0.978	36.71	10.78	143	3.15	33.6	33.8
14	4.10	60.40	0.017	0.983	36.92	10.83	154	2.62	34.3	34.7
15	4.40	81.50	0.012	0.988	37.10	10.88	165	1.98	35.1	35.5
16	4.69	109.25	0.009	0.991	37.20	10.90	176	1.58	35.6	35.0
17	4.98	145.30	0.007	0.993	37.27	10.92	187	1.31	36.0	36.4
18	5.27	195.00	0.005	0.995	37.36	10.95	198	0.99	36.4	36.9
19	5.57	262.90	0.004	0.996	37.40	10.96	209	0.84	36.6	37.2
20	5.86	352.00	0.004	0.997	37.43	10.98	220	0.66	36.8	37.4

Table 16. Calculation of the values of $w = \frac{\alpha}{\beta}(1 - e^{-\beta t}) - \alpha t e^{-\beta t}$ (equation 22) in derivation of capillary gross outward filtration. Example of evaluation for isolated rabbits' ears perfused at a solution temp. of 38°C (expt. no. 38, 39, 40, 41). The calculated uncorrected values of w are a very good fit for the observed values.

TABLES 17 to 19

CHAPTER 5

Internal diameter of A (cm)	For air to enter $p \quad P - 2T/r$ (dynes/cm^2)	For water to leave $p \quad P + 2T/r$ (dynes/cm^2)
0.10	10^6 - 2,920	10^6 + 2,920
0.35	10^6 - 834	10^6 + 834
0.70	10^6 - 417	10^6 + 417

Table 17. Theoretical calculation of order of pressures involved for air to enter different diameter side tubes, A, of constant pressure burettes, and for water to leave through the side tubes. p, outward lateral pressure at A; P, atmospheric pressure; T, surface tension of water (273 dynes/cm^2); r, internal radius of A.

d (cm)	Actual velocity (cm/sec)	Critical velocity (cm/sec)	Ratio of $\frac{\text{Actual velocity}}{\text{Critical velocity}}$
0.2	52.5	1,500	0.035
0.3	23.3	1,000	0.023
0.4	13.1	800	0.016
0.5	8.4	600	0.014

Table 18. Ratio of actual velocity to critical velocity for moist air at 18°C and 760 mm Hg pressure entering the side air tube of an improved constant pressure device. Actual velocity calculated as $6Q/\pi d^2$, assuming Q = 1.1 ml./sec; d: internal diameter of side air tube. Critical velocity calculated as $2000\mu/d\rho$.

Orifice diameter (cm)	Rate at which bubbles blown	Mean bubble diameter (mm)	$\frac{\text{Bubble volume}}{\text{Orifice diam.}}$ (cm^2)
0.3	Slow: cling momentarily	0.55	0.29
0.4	to orifice	0.57	0.24
0.3	Fast: do not cling moment-	0.56	0.31
0.4	arily to orif- ice	0.61	0.30

Table 19. Calibration of bubble diameter at orifice diameters of 3 and 4 mm, together with ratio of volume of bubble to diameter of orifice at slow and fast rates of bubble formation. Each calculated bubble diameter is the mean of 6 observations.

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ADDITIONAL PAPERS

With the assistance of my co-workers I have made publications reporting aspects of the work detailed in the thesis, as follows:-

Chapter 1

1) The influence of ascorbic acid on the healing of heat injuries to the cornea of guinea pigs. (1949). Scott. Soc. exp. Med. 8th October. With Campbell, F. W. & Garry, R. C.

2) The influence of ascorbic acid on the healing of corneal heat injuries in guinea pigs. (1950). Brit. J. Nutrit. 4, 32-42. With Campbell, F. W. & Garry, R. C.

3) The influence of ascorbic acid on vascularisation of the cornea. (1950). Brit. J. Nutrit. 4, vi. With Campbell, F. W.

4) The role of ascorbic acid in corneal vascularisation. (1950). Brit. J. Ophthal. 34, 329-334. With Campbell, F. W.

Chapter 2

1) Responses to temperature in the isolated rabbit ear. (1952). J. Physiol. 118, 59-60P. With Levinson, N.

2) Vascular responses in the isolated ear of the rabbit. (1952). J. Physiol. 119, 14P. With Levinson, N.

Chapter 3

1) Vascular responses to temperature in the denervated isolated rabbit ear. (1953). J. Physiol. 122, 35-36P. With Levinson, N.

Chapter 4

1) A mathematical analysis of net capillary filtration rate in the decentralised perfused rabbit ear. (1953). Abstr. Commun., XIX int. physiol. Congr. 313-314. With Donaldson, W. A., Levinson, N. & Silvey, S. D.

Chapter 5

1) An improved Mariotte constant pressure device. (1952). J. Physiol. 118, 4-5P. With Garry, R. C.

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[*Brit. J. Nutrit.* 1950, 4, 2.]

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Ascorbic Acid and Healing of Heat Injuries in the Guinea-pig Cornea

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(*Received* 29 *October* 1949)

It is accepted that the repair of connective tissue is defective in scurvy. Wounds of the cornea, however, may be superficial, involving only the epithelium, or they may be deep and invade the collagenous substantia propria. Moreover, the power of proliferation of the epidermis is not impaired in scurvy (Wolbach & Howe, 1926); in keeping with this finding, Galloway, Garry & Hitchin (1948–9) found that eye wounds, produced mechanically and confined to the corneal epithelium, healed with undiminished speed in guinea-pigs maintained on a deficient intake of ascorbic acid. The epithelium covering the cornea is said to heal by proliferation and migration of epithelial cells, so that no new formation of collagen is required (Arey & Covode, 1943; Buschke, Friedenwald & Fleischmann, 1943; Mann, 1944). The present investigation was designed to study and compare the rate of healing of both superficial and deep corneal wounds in normal and in subscorbutic guinea-pigs.

Another factor may be of importance; ascorbic acid is known not to be uniformly distributed throughout the body, and it seems to have a very selective distribution within the cornea itself, although its exact location is by no means settled. Schmid & Bürki (1943), using a histochemical method, found the greatest concentration in the superficial epithelial layers of the cornea. The substantia propria had a lower content, although there was a high concentration in the region of Bowman's and Descemet's membranes.

Henkes (1946), on the other hand, who extracted and titrated ascorbic acid from various regions of the cornea, found no ascorbic acid in Bowman's membrane, a little in the corneal epithelium and a high concentration in the substantia propria and in Descemet's membrane. The subepithelial portion of the substantia propria contained the highest concentration of vitamin C. Of importance for our work was the fact that, in experimental scurvy in guinea-pigs, the ascorbic acid disappeared from the cornea in from 2 to 3 weeks, although the glutathione content remained unchanged. Pirie (1946), using microtitration, found the concentration of ascorbic acid to be greatest in the corneal epithelium.

Such findings made it essential that all our experimental guinea-pigs should start with a uniform high content of ascorbic acid. Half the animals had then to receive a diet calculated to produce a subscorbutic state over a period of time necessary to allow the ascorbic acid to disappear from the tissues of the cornea. To maintain complete saturation of the tissues of a guinea-pig with ascorbic acid Kellie & Zilva (1939) believe that 20 mg. by mouth daily is necessary. However, as small a dose as 2 mg. daily

will prevent microscopic and macroscopic signs of scurvy. Kuether, Telford & Roe (1944) believe that 21 days are required to bring a guinea-pig to a steady state of saturation with ascorbic acid.

We had also to try to design an apparatus to test the strength of the cornea after injury, since incised wounds in scorbutic guinea-pigs are structurally weak after apparent healing (Jones, Bartlett, Ryan & Drummey, 1943), and Bourne (1944) claims that there is a direct relationship between the tensile strength of scar tissue and the content of ascorbic acid in healing, incised skin wounds.

EXPERIMENTAL

Animals

Female non-pregnant guinea-pigs were used, with initial weights between 450 and 650 g. The animals were weighed every 2nd day. The guinea-pigs were kept in groups of five or six in wire and metal cages 24 × 18 × 12 in. Cages were sterilized twice weekly. The animals were kept and examined in one room with a temperature between 65 and 75° F.

Diets

Basal diet. The basal diet was crushed rat-cake cubes (Thomson, 1936) well moistened with water. These cubes are free from ascorbic acid. To supplement the diet six drops of cod-liver oil (minimum content 500 i.u. vitamin A and 50 i.u. vitamin D/g.) were added daily to the diet of each guinea-pig. The mash was placed in low-set troughs so that the animals had easy access to the food. Food and water were given without stint.

Ascorbic acid. In order to ensure that the guinea-pigs had a uniform initial level of saturation with ascorbic acid, 20 mg. ascorbic acid (Roche Products Ltd.) were given orally in 2 ml. water once a day. The solution was given to the animals by pipette within a few minutes of its preparation. This was given daily for 21 days to all guinea-pigs to obtain tissue equilibrium (Jones *et al.* 1943).

Controls. Control animals were injured after 21 days of saturation with vitamin C, and the daily intake of 20 mg. was continued thereafter.

Deficient animals. After a preliminary 21 days of saturation with 20 mg. ascorbic acid, the animals were given 0·5 mg. ascorbic acid in 1 ml. water every 2nd day for a further 21 days. Only then were injuries made to the cornea, the dosage of 0·5 mg. ascorbic acid every 2nd day being continued thereafter.

Apparatus

Applicator. Small metal cylinders, having a flat end of 1 sq.mm. area, were heated among lead shot in a hot air oven to 120 or 180°. These applicators were used to produce superficial corneal burns.

Cautery. To produce deeper lesions a cautery was made from a loop of 32 s.w.g. platinum wire. A pre-determined constant voltage was fed to the cautery through a relay, type LF/FS (Londex, Ltd.), connected to the 1 sec. contacts of an a.c. time

clock (C. F. Palmer (London), Ltd.). This circuit allowed the current to flow through the cautery for exactly 1 sec.

Compression balance. The apparatus shown in Fig. 1 was used to find the weight required to rupture the excised guinea-pig eye. This compression balance was used in two ways. First, by attaching a metal bar to the pivoted platform *C* (Fig. 1, *X*) it was possible to estimate the strength of a corneal lesion. Secondly, without the bar, the resistance of the sclera to compression could be found.

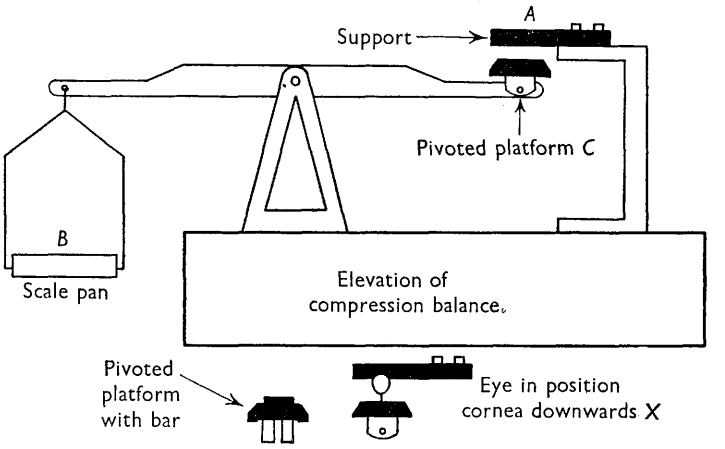

Fig. 1. Compression balance for estimating strength of cornea and of sclera. *X* shows position of bar and eye during estimations of corneal strength. The eye is compressed between a fixed support *A* and a platform *C*. The force is applied by adding weights to the scale pan *B*. Both the support *A* and platform *C*, pivoted on the end of the lever, are hollowed out to hold the excised eye.

Technique

Anaesthesia. The corneas were anaesthetized in all cases by instilling into the conjunctival sac two drops of a 2% amethocaine hydrochloride, B.P., solution. Anaesthesia was complete in 2 min. and infliction of the injuries caused no discomfort since the corneal reflex was never elicited and the animals remained quiet. No signs of distress appeared after injury and in no case did infection occur.

Superficial corneal heat injuries. The applicator was removed with heat-insulated forceps from its bed of lead shot in the oven, and exactly 2 sec. later the sq.mm. surface was applied firmly to the cornea 2 mm. from the corneo-sclerotic junction at '12 o'clock'. Contact was maintained for exactly 5 sec. Histological examinations showed that such injury affected only the epithelium over the cornea.

Deep corneal heat injuries. The cold cautery was pressed firmly and vertically on the cornea 2 mm. from the corneo-sclerotic junction at '12 o'clock'. The current was allowed to flow for exactly 1 sec. and the cautery was removed 1 sec. later. This gave a standard heat injury similar to that used by Campbell & Michaelson (1949). Histological examination showed that the resulting lesion was 1 mm. in diameter, destroying the corneal epithelium and the anterior two-thirds of the substantia propria.

All thermal injuries were carried out by the same operator (F.W.C.) who was un-

aware whether a control or a deficient animal was being injured. The injuries were inflicted alternately on the eyes of deficient and control animals.

Methods of examination. To assess the degree of healing, two drops of 2% aqueous sodium-fluorescein solution were instilled into the conjunctival sac and allowed to act for 1 min. exactly. Excess solution was removed first by mopping with filter-paper and then by instilling 6 ml. Ringer solution: the eye was examined immediately thereafter in darkness under a mercury vapour ultraviolet dark bulb lamp (General Electric Co. London, type M.B.W./V.) 8 in. from the injured eye. The intensity of fluorescence was compared with that of six dry strips of Whatman no. 1 filter-paper, 3 × 1 in., which had been impregnated with different known quantities of sodium fluorescein. Seven degrees of fluorescence intensity were recognized. Their value in terms of sodium fluorescein as μg./sq.cm. filter-paper, is shown in Table 1.

A fresh injury in all cases fluoresced brightly and appeared to have the degree of intensity of strip no. 6. As healing progressed, the intensity fell off gradually and was evaluated against the other strips with smaller fluorescein content.

Table 1. *Arbitrary standards used to estimate intensity of fluorescence*

Values given to intensity of fluorescence	Sodium fluorescein (μg./sq.cm. filter-paper)
6	32
5	16
4	8
3	4
2	2
1	1
0	No fluorescence

This subjective evaluation of the intensity of fluorescence from the damaged area of the cornea was very possibly influenced by the size of the lesion. But we clearly received the impression that the intensity of the fluorescence was affected by the stage of the healing process. However this may be, the intensity of fluorescence thus evaluated gave a reasonable assessment of the progress of healing.

In addition, the degrees of corneal oedema, of vascularization and of opacity were noted under intense focal illumination with the aid of a binocular loupe.

The complete examination was carried out on each animal by one observer (I.D.F.) at intervals of exactly 8 hr. day and night until fluorescence was absent at three consecutive examinations. Thereafter, each eye was examined at 24 hr. intervals.

RESULTS

General

Control guinea-pigs were healthy and gained weight continuously before and after operation.

The deficient animals showed a gain in weight during the initial period of saturation. This gain in weight continued for 10 days on the decreased intake of 0·5 mg. ascorbic

acid every 2nd day. Thereafter, they began to lose weight (Fig. 2), becoming quiet and less active, and the coat had a staring appearance.

After the lesions had ceased to show fluorescence, the guinea-pigs were maintained on their respective diets and were thereafter killed at varying intervals for physical and histological examination. A deficient animal and its corresponding control were examined together.

Post-mortem examinations were carried out on all animals. There were no gross macroscopic signs of disease.

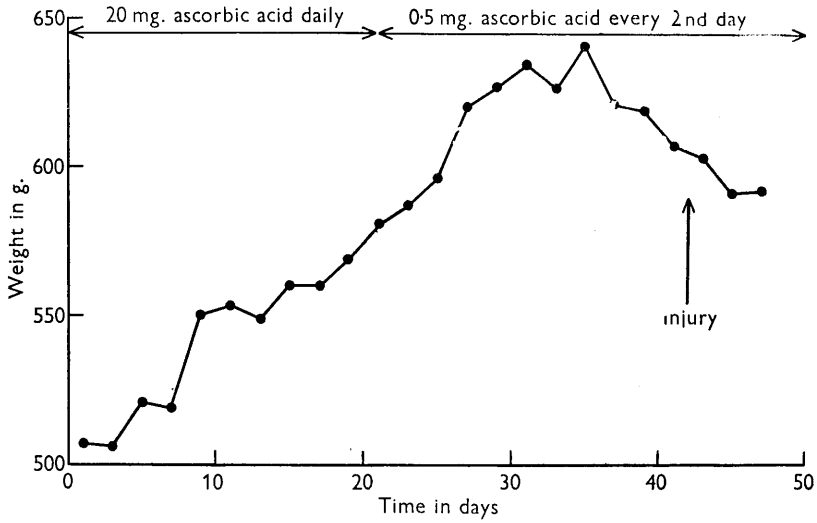

Fig. 2. Curve plotted from mean weights on alternate days of guinea-pigs in deficient group.

Superficial corneal heat injuries

Using the applicator heated to 180°, six control and eight deficient eyes were injured. The time of healing, as indicated by cessation of fluorescence, is shown below. The difference of 1 hr. between deficient and control animals is statistically not significant ($P > 0{\cdot}9$).

No. of wounds	Mean period for healing (hr.)*	Difference between control and deficient animals
6C.	56·0 ± 7·2	Not significant, $P > 0{\cdot}9$
8D.	55·0 ± 5·5	

* Value with its standard error.
C. Controls (20 mg. ascorbic acid daily).
D. Deficient animals (0·5 mg. ascorbic acid on alternate days).

An attempt was made to evaluate the mean fluorescence in both groups at injury and subsequently at 8 hr. intervals. A value 0 was given when fluorescence was absent, a value 6 when fluorescence was most intense. The values attributed to the intensity of fluorescence from each lesion were summed for each group and divided by the number of eyes in the group. There was no difference between the behaviour of the two groups (Fig. 3).

Even after cessation of fluorescence a faint corneal opacity persisted in four out of the fourteen eyes. Wolff (1947) states that a healed epithelial defect does not leave an opacity. Probably, therefore, we had injured to a slight degree Bowman's membrane,

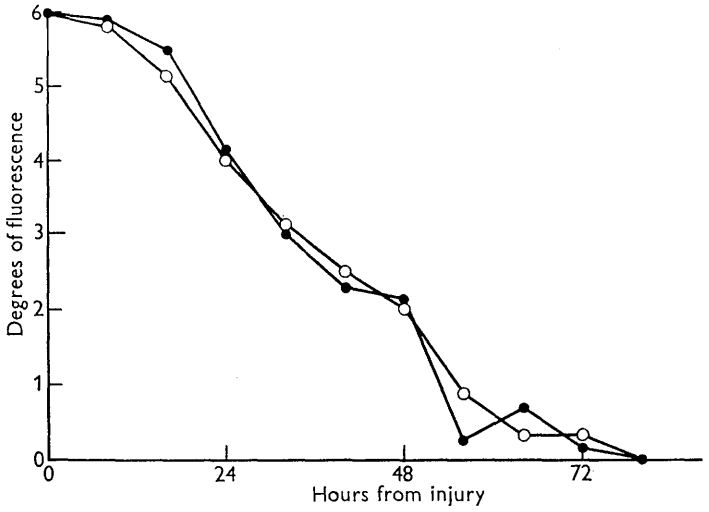

Fig. 3. Graph showing decline of mean intensity of fluorescence from corneal injuries made by applicator at 180° (see p. 34). O—O, control animals; ●—●, deficient animals.

and possibly the substantia propria, when using the applicator at 180°. We repeated the experiment on ten control and eight deficient eyes using the applicator initially heated to 120°. The results were as follows:

No. of wounds	Mean period for healing (hr.)*	Difference between control and deficient animals
10 C.	26·4 ± 1·22	Not significant
8 D.	25·0 ± 1·81	$P > 0{\cdot}5$

* Value with its standard error.
C. Controls (20 mg. ascorbic acid daily).
D. Deficient animals (0·5 mg. ascorbic acid on alternate days).

Once more the difference between the end-points as indicated by the disappearance of fluorescence was not significant ($P > 0{\cdot}5$). In this series all opacities disappeared within 96 hr. from the time of injury.

It is interesting to note that the mean time for healing was markedly affected by the initial temperature of the applicator. The difference was highly significant ($P < 0{\cdot}001$) as the following figures show:

No. of wounds	Mean period for healing (hr.)*
14 at 180°	55·43 ± 4·232
18 at 120°	25·78 ± 1·034

* Value with its standard error.

Deep corneal heat injuries

With this more severe type of injury there was a highly significant difference between the rate of healing in deficient and in control animals as indicated by the disappearance of fluorescence. The results are shown below:

No. of wounds	Mean period for healing (hr.)*	Difference between control and deficient animals
32 C.	94·00 ± 6·02	Highly significant
32 D.	125·75 ± 7·02	$P = 0.001$

* Value with its standard error.
C. Controls (20 mg. ascorbic acid daily).
D. Deficient animals (0·5 mg. ascorbic acid on alternate days).

In guinea-pigs with deep corneal lesions opacities remained at the site of injury in all animals and persisted until they were killed.

During the healing process, assessment of the intensity of fluorescence, in the manner described above, gave the graph shown in Fig. 4. After the first few hours the lesions in the control animals gave, on the average, less intense fluorescence than the lesions in the deficient animals.

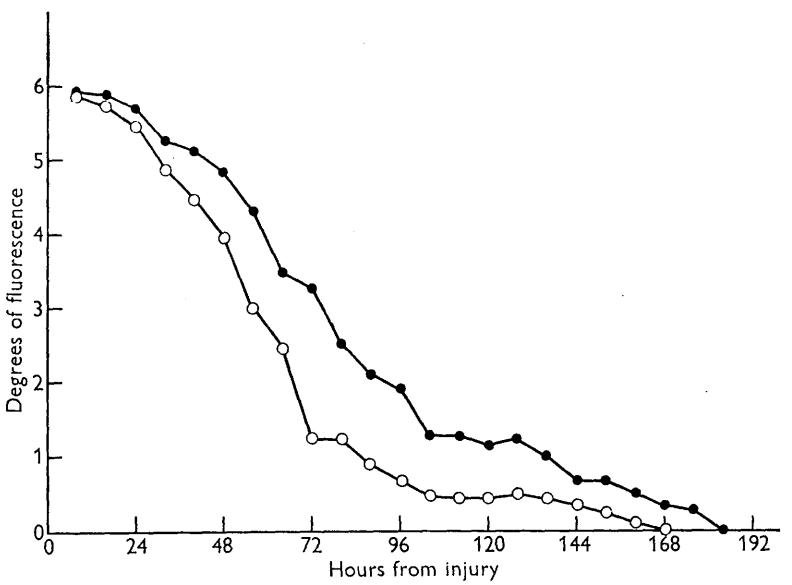

Fig. 4. Intensity of fluorescence following deep injuries to the cornea. O—O, control animals; ●—●, deficient animals.

The strength of the injured cornea

In preliminary experiments, enucleation of eyes, previously injured by cautery, not infrequently led to perforation through the site of the lesion, although healing had so far progressed that all fluorescence had ceased. Obviously disappearance of fluorescence cannot be taken as an index of restoration of strength. Moreover, we received

the impression that such rupture was more frequent among deficient animals than among controls.

We therefore designed the compression balance and subjected to stress twenty-eight control eyes and twenty-six deficient eyes from the experiment with deep corneal lesions.

To estimate the degree of compression necessary to rupture the cornea through the site of the lesion, the excised eye was placed, cornea downwards, with the bar of the pivoted platform along the '3 to 9 o'clock' meridian of the eye (Fig. 1, *X*). In this way considerable pressure was built up in the anterior chamber. If, however, the eye had not perforated by the time 1100 g. had been applied to the scale pan, the eye tended to slip off the bar.

Twenty-one out of twenty-eight control eyes slipped off the bar without perforation. This is indicated by a * sign after the highest value reached before slipping of the eye (Table 2). This value was perforce used for statistical analysis. In the deficient group twenty-one out of twenty-six eyes perforated. The χ^2 test shows that such a result could only occur by chance once in a thousand ($\chi^2 = 14{\cdot}64$).

Table 2. *Weights required to cause perforation of cornea*

Interval after injury (hr.)	Perforation pressure	
	Controls (kg.)	Deficient animals (kg.)
218	0·8	—
218	0·7	—
242	0·8	0·5
242	0·9	0·7
264	1·1*	0·7
264	1·7*	0·7
288	1·4*	0·9
288	1·2*	0·3
288	0·3	0·8
288	0·7	0·6
314	1·4*	0·9
337	0·9	0·8
337	1·3*	0·9
528	1·2*	0·5
528	1·4*	0·9
552	1·2*	0·9
552	1·4*	0·9
631	1·5*	1·4*
631	1·4*	1·2
672	1·6*	1·4
672	1·6*	1·5*
672	1·7*	1·5
672	1·4*	1·6
696	1·7*	1·6*
770	1·3*	1·4
770	1·4*	1·5*
2004	1·4*	1·4
2004	1·6*	1·6*

* Indicates that the eye has slipped from the apparatus before perforation through the lesion had occurred.

Controls: 20 mg. ascorbic acid daily.

Deficient animals: 0·5 mg. ascorbic acid on alternate days.

Inspection of Table 2 shows that, up to 552 hr. following injury, the eyes from deficient animals perforated at a lower pressure than those from controls. Analysis of results up to 552 hr. is shown below. The difference between the two groups is statistically significant.

No. of wounds	Mean weight for perforation (g.)*	Difference between control and deficient animals
17 C.	1082 ± 85·9	Significant
15 D.	733 ± 48·5	$P < 0{\cdot}001$

* Value with its standard error.
C. Controls (20 mg. ascorbic acid daily).
D. Deficient animals (0·5 mg. ascorbic acid on alternate days).

The results from eyes injured more than 552 hr. previously were not included in the analysis, since all eyes from the controls, and many from the deficient animals, slipped off the bar before perforation.

Table 2 shows also that in both groups, as healing progressed, a greater weight was required to cause perforation.

The strength of the sclera

After perforation of the cornea with loss of aqueous humour, the relatively large lens of the guinea-pig eye was displaced forward and prevented escape of vitreous humour. The bar was removed from platform *C* and the eyes were placed, cornea upwards, to be compressed between the fixed support and the platform. Weights were added to the scale pan until the sclera ruptured. In these circumstances the eyes could not escape from the apparatus and the weight necessary for rupture of the sclera was obtained in every case.

The mean weights required for deficient and for control animals are given below. There is no statistically significant difference between the two groups.

No. of eyes	Mean weight for rupture (g.)*	Difference between control and deficient animals
17 C.	1700 ± 71·2	Not significant
32 D.	1681 ± 48·5	$P > 0{\cdot}8$

* Value with its standard error.
C. Controls (20 mg. ascorbic acid daily).
D. Deficient animals (0·5 mg. ascorbic acid on alternate days).

DISCUSSION

The significance of our findings depends to some extent on evaluation of the intensity of fluorescence after application of sodium-fluorescein solution. We accept, as do ophthalmologists, that disappearance of fluorescence indicates the restoration of an epithelial covering at the site of injury. We took care to standardize the strength (2%) of the sodium-fluorescein solution instilled, the time of contact and the interval which elapsed before examination, since we had the suspicion that, in the early stages when the epithelial layers are few, the fluorescein with prolonged contact may penetrate to

the regenerating tissues below and give a faint fluorescence. We found, too, that the presence of extraneous light interfered with the accurate assessment of the intensity of fluorescence.

Superficial heat injuries of the cornea

The repair of heat injuries, probably confined largely to the epithelial cells, is not influenced by ascorbic-acid deficiency, a finding in keeping with the results of Galloway *et al.* (1948–9), who inflicted mechanical wounds on the corneal epithelium. It may be that epithelial cells can divide and proliferate quite independently of a supply of ascorbic acid. On the other hand, corneal epithelium may be able to fill in a defect of moderate size without new formation of cells. If this be so, then there is no reason to expect a deficiency of ascorbic acid to delay healing of pure epithelial lesions.

Deep heat injuries of the cornea

Repair of injuries which penetrate Bowman's membrane and destroy the collagenous tissue of the substantia propria should be influenced by the presence or absence of ascorbic acid.

As judged by the time required for complete epithelialization, the healing of deep corneal wounds in scorbutic guinea-pigs is very definitely retarded. Yet we have just shown above that the process of simple replacement of epithelium is not impaired by a lack of ascorbic acid. We can only conclude that the slower rate of epithelialization in scorbutic guinea-pigs with deep wounds was due to the absence of a suitable substratum of collagenous tissue. This is in keeping with the hypothesis of Hartwell (1929) and with the suggestions of Galloway *et al.* (1948–9).

That there was delayed restoration of collagenous tissue in the deep corneal wounds of deficient guinea-pigs is borne out by the results of the experiments with the compression balance. There was, however, in the deficient guinea-pigs, no general weakening of the connective-tissue coat of the eye since the force required to rupture the sclera was similar in both deficient and control animals.

SUMMARY

1. Standard superficial and deep heat injuries were made on the corneas of guinea-pigs receiving either a wholly adequate (20 mg. daily) or a deficient (0·5 mg. every 2nd day) intake of ascorbic acid.

2. The progress of repair was estimated both by instillation of a standard sodium-fluorescein solution and by subjection of the excised eyeballs to compression in a special balance.

3. The healing of superficial lesions, confined to the corneal epithelium, was not impaired by a deficiency of ascorbic acid.

4. On the other hand, deeper lesions, involving the substantia propria of the cornea, healed more slowly in the deficient guinea-pigs.

5. The healing lesions were structurally weaker in the scorbutic animals.

6. Thus, although restoration of corneal epithelium as such may be independent of an adequate supply of ascorbic acid, yet the rate of epithelialization of a wound of the

cornea involving collagenous tissue does depend on the provision of a suitable fibrous tissue substratum and in turn on an adequate intake of ascorbic acid.

We are indebted to J. B. de V. Weir for help in design of the compression balance and for guidance in statistical analysis. We wish also to express our indebtedness to Roche Products Ltd. for the supply of ascorbic acid, and to the Rankin Medical Research Fund of the University of Glasgow for a grant to cover expenses.

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THE ROLE OF ASCORBIC ACID IN CORNEAL VASCULARIZATION*

BY

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Introduction

ALTHOUGH the cornea normally has no blood vessels, yet, under certain conditions, new vessels freely enter the substance of the cornea from the limbal plexus.

To explain this invasion many theories have been advanced. In riboflavin deficiency there is corneal vascularization. Since riboflavin is part of an oxidation enzyme system, Bessey and Wolbach (1939), and Johnson and Eckardt (1940), believe that anoxia is a stimulus for corneal vascularization. Campbell and Michaelson (1949) brought forward evidence that injury to the cornea close to the limbus sets free a humoral substance which then diffuses to the limbal vessels to stimulate new vessel formation. Julianelle and Lamb (1934) injected an antigen into the cornea of a sensitized animal. This evoked vascularization of the cornea.

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Mayer and Chaffee (1940) and Bacsich and Riddell (1945) look at the problem from a somewhat different angle. They believe that avascular tissues, cornea, cartilage, and Wharton's jelly, normally contain a substance inhibiting vascularization. One could postulate that injury to the cornea destroys or inhibits formation of this hypothetical substance, thus permitting invasion of blood vessels.

Cogan (1949), on the other hand, believes that vascularization is always preceded by oedema of the cornea involving the tissues of the limbus. The resulting reduction in the corneal tissue compactness allows invasion by blood vessels.

We recently studied the influence of lack of ascorbic acid on the rate of healing of heat injuries to the cornea of guinea-pigs (Campbell, Ferguson, and Garry, 1950). Our findings are summarized in Table I. We also found that there was marked persistence of structural weakness in the corneal stroma at the site of the lesion, presumably due to defective formation of collagen.

TABLE I

Time for Epithelial Healing of Deep Corneal Heat Injuries in Guinea-pigs

No. of wounds	Mean time of healing (hours)	Difference between controls and deficient animals (hours)
32 control 32 scorbutic	94·0 (± 6·02)* 125·8 (± 7·02)	31·8 (Highly significant)

't' = 3·43 $P < 0.01$ *Standard Error of the Mean.

During this investigation we took the opportunity to observe the incidence and progress of vascularization in the normal controls and in the scorbutic animals.

Method

Animals.—Female non-pregnant guinea-pigs were used. Their initial weights were between 450 and 650 g.

Diets.—The basal diet was crushed rat-cake cubes (Thomson, 1936) moistened with a little water. These cubes are free from ascorbic acid. To supplement this six drops of cod liver oil were added daily to the diet of each animal.

Ascorbic Acid.—To ensure that the cavies had a uniform initial level of saturation with ascorbic acid, 20 mg. ascorbic acid (Roche) were given orally in 2 ml. water once per day. This intake was given for 21 days in all cavies to obtain tissue equilibrium (Jones, Bartlett, Ryan, and Drummey, 1943).

Control Animals.—Control animals were injured after 21 days of saturation with Vitamin C and the daily intake of 20 mg. was continued thereafter.

Scorbutic Animals.—After a preliminary 21 days of saturation with 20 mg. ascorbic acid per day, the animals were given 0·5 mg. ascorbic acid every second day for a further 21 days. The injuries were then made to the animals, the dosage of 0·5 mg. ascorbic acid every second day being continued thereafter.

Apparatus.—The lesions were produced with a cautery made from a loop of 32 S.W.G. platinum wire. A predetermined constant voltage was fed to the cautery through a relay connected to the one second contacts of a Palmer A.C. time clock. This circuit allowed the current to flow through the cautery for exactly one second when required.

Technique.—The cornea was anaesthetized by instilling into the conjunctival sac two drops of a 2 per cent. pontocaine hydro-chloride solution. The operations did not appear to cause discomfort since the corneal reflex was never elicited and the animals remained quiet. No signs of distress appeared after the operation and in no case did infection occur.

The cold cautery was pressed firmly and vertically on the cornea 2 mm. from the limbus at 12 o'clock. The current was allowed to flow for exactly one second and the cautery was removed one second later. This gave a standard heat injury similar to that used by Campbell and Michaelson (1949). Histological examination showed that the resulting lesion was 1 mm. in diameter, and that the corneal epithelium and the anterior two-thirds of the substantia propria were destroyed.

All thermal injuries were carried out by the one operator, who was unaware whether a control or a scorbutic animal was being injured.

Examination of the injury, including degree of vascularization and of oedema, was made at eight hourly intervals by one observer with the aid of a binocular loupe and focal illumination. Once epithelial healing had occurred observations were continued at 24-hour intervals.

Results

During healing, vascularization of the cornea occurred in nine out of 32 eyes in control guinea-pigs, and in nineteen out of 32 eyes of deficient animals (Table II). The results were analysed by the

TABLE II

Incidence of Vascularization in the Control and Scorbutic Groups

Eyes	No. of vascularized corneae	No. of nonvascularized corneae
Control	9	23
Scorbutic	19	13

$\chi^2 = 5{\cdot}1$ $P < 0{\cdot}05$ (Significant).

χ-squared method ($\chi^2=5{\cdot}1$). Such a result could occur by chance only less than 1 in 40 times, and we may therefore presume that the greater incidence of vascularization in the scorbutic animals is significant.

This higher incidence might be due to the greater time required for healing in the group of deficient animals (Table I) for it is a common clinical observation in man that a corneal ulcer of long duration is more likely to induce corneal vascularization than one of short duration. If this explanation be true, then the eyes with vascularization will be associated with the injuries taking longer to heal. Table III shows the mean time of healing for the vascularized and nonvascularized eyes in the scorbutic group. The

TABLE III

Comparison of the Mean Time of Wound Healing in the Vascular and Non-vascular Cornece in the Scorbutic Group

No. of Eyes	Mean time of healing (hours)	Difference in Means (hours)
19 vascular	127·6 (± 9·3)*	4·5
13 nonvascular ...	123·1 (±11·5)	(Not significant)

't' = 0·79 P > 0·4 *Standard Error of the Mean.

difference of 4·5 hours between the two groups is not significant. A greater difference in the means exists in the control group (Table IV). The difference of 17·7 hours is again, however, not significant. There is thus no conclusive evidence to suggest that, under

TABLE IV

Comparison of the Mean Time of Wound Healing in the Vascular and Non-vascular Corneae in the Control Group

No. of Eyes	Mean time of healing (hours)	Difference in Means (hours)
9 vascular	106·7 (± 13·8)*	17·7
23 nonvascular ...	89·0 (± 6·3)	(Not significant)

't' = 1·33 P > 0·1 *Standard Error of the Mean.

the conditions of this experiment, the greater incidence of vascularization in the scorbutic group is due to the longer time required for epithelial healing.

The onset of vascularization, the time of its maximum extent, and the time of disappearance all tended to be delayed in the scorbutic group compared with the control group (Table V).

TABLE V

Comparison of the Progress of Corneal Vascularization in Control and Scorbutic Groups

Vascularization	Mean Time (hours)				Difference in Means (hours)
	Control (9 eyes)		Scorbutic (19 eyes)		
Onset	33·7	(±8·30)*	52·6	(±8·79)*	18·9
Maximum ...	64·9	(±6·99)	79·6	(±9·31)	14·7
Disappearance	134·2	(±33.44)	146·5	(±21·56)	12·3

*Standard Error of the Mean.

The time of onset and the time of maximum oedema are shown in Table VI. The difference between the means of the times of onset is not significant. However, the scorbutic group did take

TABLE VI

Comparison of the Progress of Corneal Oedema in Control and Scorbutic Groups

Oedema	Mean Time (hours)		Difference in Means (hours)
	Control (32 eyes)	Scorbutic (32 eyes)	
Onset	17·3 (±2.53)*	16·5 (±1·83)*	0·8
Maximum ...	25·8 (±2·78)	42·8 (±6·22)	17·0

*Standard Error of the Mean.

significantly longer to reach the time of maximum oedema. The figures could be not be analysed for the time of disappearance of the oedema as some of the animals were killed for histological examination before the oedema had disappeared.

Discussion

The cornea is known to contain high concentrations of ascorbic acid, although there is some disagreement concerning the partition of ascorbic acid between the various layers of the cornea (Schmid and Bürki, 1943; Henkes, 1946; Pirie, 1946).

In experimental scurvy in guinea-pigs, ascorbic acid is known to disappear from the cornea in from 2 to 3 weeks (Henkes, 1946). Nevertheless, the cornea shows no obvious change even in severe and prolonged scurvy in human beings or in guinea-pigs. On the other hand, ascorbic acid is known to be necessary for formation of collagenous tissue in the repair of wounds in the cornea. Thus, the injuries we inflicted to the cornea possibly unmasked a deficiency not otherwise apparent. Presumably new formation of collagen makes additional metabolic demands which cannot be adequately met in a state of scurvy. Just as ariboflavinosis evokes spontaneous vascularization of the cornea, avitaminosis C may lead to vascularization when there is the extra metabolic demand following on injury.

Presumably failure to satisfy fully a metabolic need, however caused, leads to accumulation of metabolites. These metabolites may act as a direct stimulus to new blood-vessel formation. On the other hand, the metabolites may lead to oedema and opening up of the lamellae of the substantia propria, and thus permit invasion of blood vessels. Is it too much to suggest that the

accumulation of metabolites is the humoral factor postulated by Campbell and Michaelson (1949)?

This hypothesis is, of course, in the highest degree speculative, but our new finding that ascorbic acid deficiency significantly increases the incidence of vascularization of the cornea after injury does seem to warrant an attempt to find a common causal factor to explain the invasion of the avascular cornea by blood vessels in the conditions where such invasion is known to occur.

Summary

(1) A method is described for producing small standard heat injuries to the cornea.

(2) These injuries were inflicted on control and scorbutic guinea-pigs.

(3) New vessel invasion of the cornea following the injury occurred with significantly greater frequency in the scorbutic group than in the control group.

(4) The causal factor in new vessel formation in the cornea is discussed in the light of these findings. It may be that repair of an injury makes additional metabolic demands which cannot be met in a state of ascorbic acid deficiency. As a result, metabolites accumulate and evoke by some means, not yet fully understood, vascular invasion of the corneal substance.

We are indebted to Professor R. C. Garry for helpful criticism and encouragement. We also wish to express our indebtedness to Roche Products Limited for a liberal supply of ascorbic acid, and to the Rankin Medical Research Fund of the University of Glasgow for a grant to cover expenses.

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Journal Physiology, Vol. 118.

Responses to temperature in the isolated rabbit ear. By I. D. FERGUSON and N. LEVINSON. *Institute of Physiology, University of Glasgow*

Perfusion of the ear through the central artery was commenced within 3 min of removal of the ear from the rabbit. Oxygenated Locke's solution at pH 7·3–7·4 containing 0·154 M-NaCl, 0·0054 M-KCl, 0·00225 M-$CaCl_2$, 0·000052 M-$MgCl_2$, 0·00596 M-$NaHCO_3$ and 0·000417 M-NaH_2PO_4 was perfused under constant pressure (Ferguson & Garry, 1952). The temperature of the perfusion fluid (F_t) and of the ear environment (E_t) could be varied at will. The rate of inflow and of weight increase due to oedema were recorded. Forty-six experiments were carried out.

At $F_t = 38°$ C the inflow rate was initially rapid but then fell quickly to reach a minimum at the end of 2 hr. During this period the oedema increased steadily and rapidly. Thereafter the inflow rate rose steadily while the rate of oedema formation decreased. At $F_t = 16°$ C the initial inflow rate was slow, due to an observed spasm of the central artery. Subsequently the inflow rate became rapid and remained significantly greater than the inflow at $F_t = 38°$ C. The oedema, meanwhile, increased steadily but only slowly. These characteristic responses were also obtained in one and the same ear by changing the temperature of the perfusing fluid.

More rapid inflow and greater oedema formation occurred at $F_t = 45°$ than at $F_t = 38°$ C, but vascular damage was indicated by bullae formation.

Perfusion at $F_t = 38°$ C with an E_t artificially lowered from 21 to 16·5° C led to a slightly greater inflow and to a significant reduction in rate of oedema formation. Perfusion at $F_t = 16°$ with an E_t artificially raised from 16 to 23·5° C led to a significant increase in the rate of oedema formation, but the inflow rate was not consistently affected.

These results may be explained by postulating that in the isolated ear the responses to temperature are similar to those in the intact ear (Grant, 1930; Grant, Bland & Camp, 1932; Van Dobben-Broekema & Dirken, 1950).

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Vascular responses in the isolated ear of the rabbit. By I. D. Ferguson and N. Levinson. *Institute of Physiology, University of Glasgow*

During perfusion of the blood vessels of the isolated ear of the rabbit with Tyrode's fluid at constant pressure, a decrease in the temperature of the perfusion fluid from 38 to 16° C was shown to cause a more rapid rate of inflow and a decreased rate of formation of oedema (Ferguson & Levinson, 1952). These results might be attributed to different values of 'interstitial pressure' (McMaster, 1946), to different values of pressure drop across the cannula or to alteration in the vascular pattern.

It was possible that at the lower temperature the lower 'interstitial pressure', due to the decreased rate of oedema formation, was a main factor in the production of the more rapid rate of inflow. However, measurement of the 'interstitial pressure' lent little support to this concept.

It was also considered possible that at the higher temperature the increased downstream pressure, due to the decreased rate of inflow through the cannula, was a main factor in the production of the increased rate of oedema formation. At either temperature, increase in the downstream pressure increased both the rate of inflow and the rate of oedema formation. However, an increase in the temperature of the perfusion fluid from 16 to 38° C at the same downstream pressure still caused a slower rate of inflow and an increased rate of oedema formation.

It is suggested that in the isolated ear of the rabbit, a decrease in the temperature of the perfusion fluid from 38 to 16° C results in an alteration in the vascular pattern. It may be that the fluid is partially diverted from the capillaries through vessels of larger calibre, such as arteriovenous anastomoses.

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Vascular responses to temperature in the denervated isolated rabbit ear. By I. D. FERGUSON and N. LEVINSON. *Institute of Physiology, University of Glasgow*

Unilateral denervation of the blood vessels of the rabbit ear was carried out, under general anaesthesia, by extirpation of the superior cervical ganglion and excision of 1 cm length of each of the ventral and dorsal auricular nerves (Feldberg, 1926). All experiments were carried out 21–24 days later. By the use of methylene blue, it was demonstrated histologically that, while motor end-plates were present in the auricular muscles of both denervated and innervated ears, there was a complete absence of nerves to the blood vessels in denervated preparations.

The technique for perfusion of the isolated rabbit ear was that previously described (Ferguson & Levinson, 1952*a*). In innervated ears the rate of inflow was more rapid when the temperature of the perfusion fluid was 16° C than when it was 38° C (Ferguson & Levinson, 1952*b*). In the denervated ears, with the fluid either at 16 or 38° C, after a fluctuation in inflow during the first 30 min, there was little difference in the rates of inflow at either constant temperature, both being approximately midway between the extremes of inflow characteristic of the innervated preparations. Inflow responses to rapid alterations in fluid temperature from 38 to 16° C or vice versa were more delayed and less marked in the denervated ears.

Oedema developed more rapidly in the denervated ears both at 16 and at 38° C, but the pattern of the development of the oedema at either temperature was not affected by denervation. In addition, alterations in the rate of change in oedema formation in response to rapid alterations in fluid temperature were similar in denervated and innervated preparations.

It is concluded that in the denervated isolated ear there was a diminished response of the larger vessels to differences in temperature of the perfusion fluid. Denervation also resulted in an increased capillary permeability.

We are indebted to the Rankin Research Fund of the University of Glasgow for a grant to cover expenses.

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DONALDSON, W. A., I. D. FERGUSON, N. LEVINSON and S. D. SILVEY. (Glasgow, Scotland). **A Mathematical Analysis of Net Capillary Filtration Rate in the Decentralised Perfused Rabbit Ear.**

Perfusion of the rabbit ear through the central artery was carried out using a technique previously described (1, 2). The increase in weight due to net filtration from the capillaries into the tissues was recorded. The net rate of filtration across the capillary membrane could not be measured experimentally.

The rate of accumulation of the net filtrate may be expressed by: $\frac{dw}{dt} = \alpha\ (1 - e^{-\gamma t}) - \beta w$ where t = time, w = weight of filtrate in g; $\alpha\ (1 - e^{-\gamma t})$ represents the net rate of filtration across the capillary membrane, thus taking into account capillary permeability, area of filtration bed and downstream pressure, α representing the ultimate value of net filtration and γ describing its rate of change. β describes the extent to which oedema fluid already formed retards further net filtration.

In perfusion experiments at a fluid temperature of 38°C, preliminary inspection of the data suggested the simplification $\gamma = \beta$. Values for the constants α and β were determined by the method of least squares to give the best fit for w, where $w = \frac{\alpha}{\beta}\ (1 - e^{-\beta t}) - \alpha\, t e^{-\beta t}$ ($t = 0$ when $w = 0$).

In experiments at 16°C the corresponding simplification is: $-\frac{dw}{dt} = \alpha - \beta w$ whence $w = \frac{\alpha}{\beta}\ (1 - e^{-\beta t})$ ($t = 0$ when $w = 0$). However, at 16°C the initial

increase in weight of the ear due to filling of the vascular bed with perfusion fluid unduly predominates in relation to the decreased rate of net filtration. This is allowed for in the solution $w = \frac{\alpha}{\beta} + c\, e^{-\beta t}$ of the differential equation.

Analysis thus permits the calculation of the net rate of filtration across the capillary membrane by evaluation of $\alpha\ (1 - e^{-\beta t})$ at 38°C and by α at 16°C.

1. Ferguson, I. D. and Levinson, N., *J. Physiol.* **118**, 59, (1952).
2. Ferguson, I. D. and Levinson, N., *J. Physiol.* **119**, 14, (1952).

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An improved Mariotte constant pressure device. By I. D. FERGUSON and R. C. GARRY. *Institute of Physiology, University of Glasgow*

The Mariotte flask is conveniently used to obtain a constant head of pressure. It is possibly not generally realized that the central air inlet tube is not only not necessary but also an actual disadvantage. This is especially so if it is desired to measure accurately the volume of fluid leaving a standard calibrated burette under constant pressure.

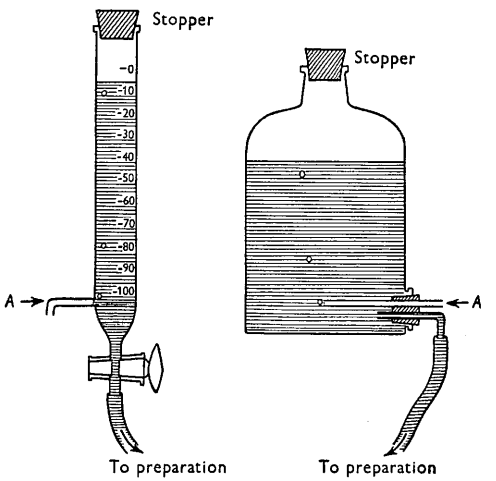

Fig. 1. Constant pressure device applied to a burette and to an aspirator bottle. *A*, air inlet port; level from which constant pressure is measured.

All that is required is a stoppered fluid reservoir (burette or bottle) with two orifices below the level of the surface of the fluid and as near to the bottom as possible. Fluid is delivered from the lower opening and air enters by the upper orifice. The device applied to a burette and to an aspirator bottle is shown in Fig. 1. The pressure at which fluid is delivered remains as constant as in the conventional Mariotte flask, being determined in this case by the height of the air inlet port, *A*, above the preparation. A convenient internal bore for the inlet port is 3–4 mm. Standard burettes for filling from below usually have a side limb of suitable bore. If rapid delivery of fluid is desired from a standard burette it may be necessary to cut off the terminal portion of the tip.

During filling and refilling, the air entry port, *A*, is closed temporarily by the finger or by a clip on a short length of rubber tubing, while the stopper is removed. Once the stopper is replaced the air entry port should be reopened so that a small quantity of fluid may be ejected through the air entry port.

[P.T.O.

The present simple device, then, has the following advantages over the usual type of Mariotte flask: (1) The volume of fluid delivered can be directly measured on a burette with standard calibration if an air entry port is blown below the lowest graduation. (2) One is not humbugged, when filling or refilling, by a pressure initially in excess of the constant head desired for the perfusion or infusion. (3) On refilling there is no variation in pressure due to failure to re-insert a stopper and central tube, as in the Mariotte flask, to exactly the previous level.

RÉSUMÉ

C O N T E N T S

STATEMENT OF ORIGINAL WORK

I carried out the entire work detailed in Chapter 1.

The technique of perfusing the isolated rabbit's ear, which was used in the experiments gaven in Chapters 2 and 3, was devised by me. I also carried out the initial experiments and planned the investigation. Thereafter, initially I supervised, and subsequently I co-operated in the carrying out of the perfusion experiments, in the general analysis of the results, and in the denervation procedures. I applied the differential equation to the experimental results in order to derive the gross outward filtration. Two of the evaluations carried out by me are given in Chapter 4.

The development from Stage 2 onwards of the improved constant pressure burette described in Chapter 5 was made by me. I also investigated the hydrodynamics involved.

STATEMENT OF THE EXTENT TO WHICH I HAVE AVAILED MYSELF OF THE WORK OF OTHERS

The problem from which this thesis stems, that of the influence of ascorbic acid on the healing of corneal heat

injuries in guinea-pigs, was suggested by Professor R. C. Garry. Dr. F. W. Campbell devised the method of production of the standard heat injuries. He also carried out the injuries of the corneae of the guinea-pigs.

Dr. N. Levinson of this Department carried out the greater proportion of the perfusion experiments described in Chapter 2 and assisted in the general analyses of the results. He was a co-worker in the denervation experiments described in Chapter 3.

Mr. W. A. Donaldson and Dr. S. D. Silvey of the Mathematics Department of the University undertook the construction of the differential equation considered in Chapter 4.

The suggestion of Stage 1 in the development of the improved constant pressure burette described in Chapter 5, was made by Professor R. C. Garry.

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