the bronchus. The lungs and bronchi from most of the sheep have subsequently been examined by Dr Brian Heard of the Department of Pathology.

Results

(1) Preliminary analysis at the time of writing suggests that there is a certain variability in the results from sheep to sheep but that the readings are little influenced by the degree of negative pressure to which the lung is subjected or by the time since resection (the experiments are always performed within 2 hr of death). In five out of six sheep only slight contribution to elastance seemed to be made by the surrounding lung. The elastance of the bronchi (in the lung) varied from 0.9 to 3.5 cmH₂O/ μ l.

(2) Observations have also been made on several patients. Most of these were undergoing routine diagnostic bronchoscopy because of haemoptysis or suspected carcinoma. They did not have an important degree of wheeze or airways obstruction. The results have not yet been fully analysed but the figures for bronchial elastance are so far within a range similar to that in sheep.

It should be possible by this technique to measure bronchial elastance both on inspiration and on expiration and in this way not only to calculate static tone and its variation in disease, but also to measure the effect of antispasmodics and to confirm or refute our previous finding of increase in dynamic tone with expiration in some wheezy patients.

The formation of collaterals

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OBSTRUCTION of an artery supplying part of a vital organ like the heart or brain may lead to infarction of the tissue which it supplies but sometimes vascular obstruction is silent. Symptomless occlusion of one internal carotid artery or a major division of the coronary tree is by no means uncommon.

Large collateral vessels by-passing obstructed arteries are a common finding. Everyone is familiar with the skeins of tortuous vessels seen on an arteriogram of a patient with obstruction of the superficial femoral artery in the leg. The processes by which collaterals form are obviously of importance and yet very little is known about them.

Recently experimental work on vascular obstruction in the retinal circulation of various animal species including pigs and monkeys has given a new insight into the mechanisms of formation of collaterals.

Method of study

The retinal blood vessels are uniquely accessible to repeated study. The evolution of vascular changes at the same site can be studied over a period varying from hours to months and at the end of the experiment the precise area can be located for histological examination.

Colour photographs of the retina give only limited information about circulatory changes. However, use of colour filters and injections of sodium fluorescein dye through a catheter positioned in the carotid artery make it possible to visualize all orders of vessel size down to and including the capillaries. When this technique is combined with ciné photography it is possible to measure the velocity of flow in retinal arterioles.

Vascular obstruction can be produced by several means but the most convenient is the intra-carotid injection (Ashton & Henkind, 1965) of glass microspheres which produce a sudden and localized occlusion of a branch retinal arteriole.

Circulatory changes following acute vascular obstruction

The retinal circulation has no arcades or bridges between one arteriole and another. The vessels are end-arterioles and it might be predicted that obstruction of such a vessel would lead to complete cessation of flow within its vascular territory. Surprisingly, this is not so. Immediately following arteriolar obstruction by glass emboli the blood column in the obstructed artery becomes darker in colour, but flow con-

tinues although in the reverse direction and at a velocity only 1–5% of the normal. The source of the flow is the periphery of the territory of the obstructed vessel where there is an interface between its capillary circulation and that of surrounding patent arterioles.

FIG. 1. The diagram illustrates the changes in the direction of flow in the capillaries following obstruction of an arteriole. Before obstruction flow in both C_1 and C_2 proceeds forward into C_3 and thence into the vein. After the arteriole feeding C_2 is blocked flow reverses at the junction of C_1 and C_2 and proceeds back into the obstructed arteriole.

Fluorescence angiograms show conclusively that this collateral flow takes place through capillaries (Fig. 1). As the red cells entering the main stem of the blocked vessel have already passed through capillaries in an area of reduced flow, the oxygen saturation is low.

The genesis of capillary collateral flow

Capillary collateral flow depends upon the anatomy of the capillary bed and the pressure relations at the junctions (Fig. 2). If each arteriole was linked to its companion vein by a unique capillary bed no collateral flow could take place. However, in the retina and many other organs the capillary bed is better described as a branching network which is not limited to the territory of a single vessel and spreads through the organ fed by many arterioles and drained by many veins (Wiedman, 1963). Anatomical divisions may limit the number of capillary junctions, for example in the retina relatively few capillaries cross from one side to the other of a major arteriole.

When an arteriole is blocked the pressure in its branches begins to fall towards the tissue pressure. When the pressure in a capillary fed

by the blocked arteriole (C_2 , Fig. 1) falls below that at the junction (X, Fig. 1) between capillaries C_1 and C_2 flow reverses in C_2 and proceeds in the reverse direction to normal. The factors that limit the effectiveness of collateral flow are the number and length of the capillary links C_1 and C_2 and the pressure gradient. Such flow is also of relatively poor quality as the blood has already passed through a partially ischaemic capillary bed before entering the arteriole which it will leave to supply capillaries in the central part of the ischaemic territory which have no direct communications with surrounding patent arterioles through capillary bridges. In an extended network the number of capillary anastomoses will be related to the surface area of the territory of the vessel and, if the organ is normally uniformly perfused, the demand for blood flow will be equivalent to its volume. It can readily be seen that the effectiveness of capillary collateral flow will fall off inversely with the size of the vessel involved.

Experimental studies in animal retinae show that when arterioles only 15 or 20 μ in diameter are blocked then the tissue downstream often survives but if the vessels are larger, focal necrosis of the retina takes place with formation of a cotton-wool spot consisting of swollen nerve fibres and other cellular elements (Ashton *et al.*, 1966).

FIG. 2. Capillary phase fluorescence angiogram 35 min after arteriolar obstruction. Fine branches of the blocked arteriole are filling from the surrounding capillaries (arrows). Reproduced by kind permission of the *British Journal of Ophthalmology*.

Development of larger collaterals

Flow through capillary collaterals is slow and their chief importance is that they provide the framework for development of larger collaterals. Larger channels take at least 24 hr to form and cannot usually be detected less than 3 days after the vessel is blocked. After several weeks they become so large that flow into the area is as great as it was before the feeding arteriole was blocked. Initially, these larger channels are thin-walled but after about 6–8 weeks they develop a cellular wall in which smooth muscle cells appear.

Eventually one or more of the collaterals, usually the one with the shortest path between the blocked vessel and an adjacent patent one (Fig. 3) take on all the characteristics of an arteriole.

FIG. 3. A diagram of the same area as Fig. 1, 4–6 weeks later. Both C_1 and C_2 have dilated and formed smooth muscle in their walls. They are now indistinguishable from surrounding small arterioles and the result is that the blocked large arteriole is connected to the nearby patent one by an arteriolar bridge.

Why do larger collaterals develop?

As capillary collaterals do not begin to dilate appreciably until at least 24 hr after vascular obstruction it is probable that this change depends upon remodelling of structure rather than vasomotion. However, capillary dilatation is not general. For example, it does not occur in the centre of the ischaemic area. Both ischaemia and a substantial distending pressure appear to be necessary to allow capillaries to dilate, and this only occurs in capillaries connected to surrounding patent vessels.

Once a capillary bridge begins to dilate the

tension in its wall will rise rapidly. Laplace's Law states that:

$$\text{'Tension (T) = Transmural Pressure (P}_{TM}) \times \text{radius (r)'}.$$

Increase in diameter of a capillary connected to a patent arteriole will also increase P_{TM} towards the arterial pressure and further increase r . Muscular elements which appear in the wall of the dilating vessel probably develop locally from cells of multi-functional mesenchyme (Wissler, 1967).

Why are there no arteriovenous shunts?

Collapsible vessels with tone exhibit pressure-flow characteristics that have been termed 'a vascular waterfall' (Permutt & Riley, 1963). In such a system the driving pressure is not the difference between the arterial and venous pressures but the arterial minus the critical closing pressure.

$$\text{Critical closing pressure (P}_c) = \frac{\text{Active tension (T}_A)}{\text{Resting radius (r}_0)}$$

Changes in the capillary circulation would then have no effect on the inflow of blood from arterioles unless the capillary pressure exceeded P_c . This may explain why capillaries in normal circulations play a passive role. In an ischaemic area T_A probably falls so that the vascular waterfall no longer holds. Changes in the capillary circulation can then alter flow and permit larger collaterals to function. However, in this situation there seems no good reason why the same processes should not lead to formation of larger channels between arteries and veins in the ischaemic area. Further work will be needed to clarify this problem.

The importance of capillary collateral flow

The network of capillary collaterals and their ability to form the scaffolding through which larger channels form provides great flexibility in the terminal vascular bed. Slow occlusion of an arteriole in any circulation could be compensated by formation of collaterals so long as there are capillary links with surrounding patent vessels. Clearly the mechanism cannot operate if there are no such links as will be the case if a single vessel feeding the whole of an encapsulated organ such as the eye or kidney is occluded.

The capillary collateral mechanism may explain why implantation of an internal mammary artery into the myocardium can provide a functional anastomosis with the coronary circulation.

If the end of the implanted artery forms a communication with capillaries of granulation tissue from the myocardium these will dilate and later take on the morphological and functional characteristics of arterioles joining the two arteries.

The capacity to form larger collaterals may be limited in diseased or older vessels and they form too slowly to prevent tissue necrosis if larger arterioles are occluded. However, there is evidence that collaterals form in the same way in the human retina (Dollery *et al.*, 1967).

Further work will be needed to establish the relative importance of various factors that may facilitate or retard the formation of collaterals.

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Studies on the metabolism of angiotensin

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THE pressor octapeptide angiotensin is formed by the action of the enzyme renin on a plasma substrate. The amino-acid sequence of angiotensin II was determined by Skeggs *et al.* (1956), and synthesis of biologically active peptides was subsequently achieved by Bumpus, Schwarz & Page (1957) and by Schwyzler *et al.* (1958). The level of angiotensin found in arterial blood in healthy individuals is usually less than 20 ng/100 ml (Kahn *et al.*, 1952; Massani *et al.*, 1966). Raised levels of angiotensin have been found in the arterial blood of patients with oedema or sodium depletion and in some patients with hypertension (Kahn *et al.*, 1952; Boucher *et al.*, 1964; Massani *et al.*, 1966).

The significance of raised plasma levels can only be assessed if knowledge is available as to the speed with which angiotensin liberated into the circulation is removed from it. Such evidence as is available suggests that angiotensin is rapidly removed from the circulation, but there is little knowledge of the mechanism involved. Moreover, little is known of the relationship between the biological effects and plasma levels, or of the relationship between the destruction of angiotensin by plasma enzymes and the limitation of biological activity.

Methods in this field are difficult to apply to these problems. Hitherto, all assays of angio-

tensin in biological fluids have depended on biological assay. Previous attempts to devise labelling techniques with ^{131}I have led to almost complete loss of biological activity (Cruz-Coke, 1946; Barbour & Bartter, 1963), while randomly tritiated material is unstable (Barbour & Bartter, 1963) and has the disadvantage that radioactive fragments of various types may make isolation and identification of metabolic products difficult.

Some of these difficulties have been overcome by the use of [^{35}S]phenylisothiocyanate to form [^{35}S]phenylthiocarbamyl (PTC) angiotensin (Osborn, Louis & Doyle, 1966). By this technique angiotensin II can be specifically labelled with [^{35}S]phenylisothiocyanate, and the derivative so formed can be readily separated from angiotensin II and retains biological activity. PTC angiotensin exhibits about 25–30% of the pressor effect of the unlabelled octapeptide, and has a parallel dose-response curve (Fig. 1). Moreover, during tachyphylaxis to unlabelled angiotensin, pressor responses to PTC angiotensin also disappear (Fig. 2). These observations suggested that the biological site of action and the metabolism of the labelled derivative might be similar to that of the original octapeptide, and that [^{35}S]PTC angiotensin might be of value in elucidating the site of action and metabolic fate of angiotensin.

The suggestion has been made that variations in plasma angiotensinase activity might be of

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