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EFFECT OF WALL SHEAR STRESS AND TRANSMURAL PRESSURE
ON THE UPTAKE OF MACROMOLECULES BY THE ^{AORTA}~~AORTIC WALL~~

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by

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ABSTRACT

The aim of this study was to examine the short-term effect of steady wall shear stress and transmural pressure on the uptake of macromolecules by the rabbit thoracic aorta. An in situ perfused artery preparation was used in order to ensure that the wall was intact and achieve good control of experimental parameters. Great care was taken to purify radioactive tracers employed. The wall concentration profile of tracer was determined by measuring the radioactivity in sequential frozen sections cut parallel to the luminal surface. It was found that wall shear stress in the range 0 - 75 dyne cm^{-2} did not damage the endothelial surface, the major barrier to macromolecule uptake, and had no immediate effect on the uptake of ^{131}I -albumin. These results do not contradict theories which correlate hemodynamic factors and the distribution of atheromatic plaques in the arterial tree, but seem to show that if the wall shear stress has any effect upon macromolecules transport (other than as a result of damage) the effect would not be discernible except on a long-time scale. Using the same experimental preparation a very distinct immediate increase was observed in the uptake of ^{131}I -albumin and ^3H -dextran when the transmural pressure was raised from 30 to 180 mm Hg. This augmentation was immediately reversed by reducing the pressure and hence apparently not due to damage to the endothelial surface. The wall concentration profiles also demonstrated a sharp increase in the uptake from the lumen around 130 mm Hg. The cause of this increase and its potential significance in vascular pathology is considered.

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I. INTRODUCTION

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1.1 General consideration

The main objective of this thesis was to examine experimentally the possible influence of fluid mechanical factors on the uptake of macromolecules by the arterial wall. An in situ perfusion technique was used in the present work. This permits good control of mechanical and fluid parameters and the artery appeared to be undamaged. The influence of steady transmural pressure and of hydrodynamic wall shear stress applied for short periods was studied. These experiments may be relevant to arterial pathology; atherosclerosis is found more often in patients with hypertension (see, for example, Framingham, 1971) and atherosclerotic lesions tend to occur at certain sites in the arterial tree: in large central arteries rather than in peripheral ones and particularly in the region of bends, branches and stenoses. (McGill et al., 1963). This distribution of atherosclerotic lesions led to the suggestion that local hemodynamic forces may be of importance.

1.2 Main theories of hemodynamic factors in atherogenesis

A number of theories of atherogenesis have taken into account hemodynamic factors. Shear stress, blood pressure, blood turbulence and boundary layer separation have been considered as possible causes. These theories will be described only briefly because they have been reviewed in detail (Glagov, 1973; Cessner, 1973; Walton, 1975; Bergel et al., 1976) and all do not have direct relevance to the present work.

1.2.1. Theories based on wall shear stress

Caro et al (1971) advanced a theory which attempts to relate shear-dependent mass transfer to atherogenesis. The analysis of post-mortem data led these workers to suggest that early atheroma in man occurs preferentially in regions in which wall shear stress is expected to be relatively low. In contrast, regions of the wall which experience high shear stress are relatively spared from the development of atheromatous lesions. To account for these observations these authors hypothesized that the rate of mass transfer between the blood and the arterial wall is higher in regions of high wall shear and material synthesized in the wall tends to accumulate in regions of low wall shear because efflux via the intima is inhibited.

Another theory (Fry, 1969a) suggested that high shear stress, as for example on the flow divider at bifurcations, damages the endothelium and leads to increased "wall permeability" thus promoting the development of atherosclerosis.

1.2.2 Theories based on pressure

Texon (1957, 1963) and Texon et al. (1965) suggested that over a portion of the cardiac cycle the luminal hydrostatic pressure could be lower than the subendothelial pressure and that this could lead to damage to the endothelium and eventually to the development of atheromatic lesions.

Recently Kenyon (1979) argued theoretically that by virtue of the compressibility of the arterial wall (Harrison and Massaro, 1976; Yamartino et al., 1974) fluctuation of

blood pressure can produce a force tending to lift off the endothelial cells.

1.2.3 Theories based on turbulence of blood flow and fluctuations of blood velocity

Mustard et al. (1963) and Mitchell and Schwartz (1965) suggested that fluctuations of blood velocity cause platelets and other particulate matter in the bloodstream to coalesce and eventually to form mural thrombi or elevated plaques on the intimal surface.

Wesolowski et al. (1965) suggested that atherosclerotic lesions can develop in regions of turbulence as a result of injury to the arterial wall.

1.2.4 Theory based on flow separation

Fox and Hugh (1966) argued that regions of local flow separation at bifurcations and along curved segments cause the formation of stagnant zones. They suggested that the bloodstream stagnates locally thus allowing platelets and fibrin to form a mesh at the intimal surface which traps lipids and leads to their eventual coalescence to form atheromatous plaques.

1.2.5 The filtration theory

This is based on the view that atherogenesis is due to a tissue reaction to substances filtered through the arterial wall as the result of the transmural pressure and accumulated in the intima (Page, 1954). The filtration hypothesis, that plasma components enter the arterial from plasma (as suggested by Virchow in 1856) and are transported

across it (as formally proposed by Anitschkow in 1933), has been extended (Wilens, 1951; Adams et al., 1967; Walton, 1975; Stein and Stein, 1973; Schwartz and Benditt, 1972; Bjorkerud and Bondjers, 1973; Ross and Glomset, 1976; Smith, 1974). Such deposition could be facilitated by high blood pressure, high blood lipid concentration or by an increase of intimal permeability. Consequently endothelial injury, which can increase uptake of macromolecules, could play a key role in the development of atherosclerosis.

1.3. Previous experimental results

1.3.1 Non-uniformity of macromolecule uptake in vivo

Non-uniformity of macromolecule uptake by the arterial wall in vivo was demonstrated using Evans Blue dye (EBD) (Okuneff, 1926; Duff, 1931). This diazo-dye bonds strongly to serum albumin, a property which led to its use as an optical marker for albumin. The EBD-albumin complex has been reported to be stable in vivo (Gregersen and Rawson, 1943). Following injection there is non-uniform accumulation of the dye along the arterial tree, forming a characteristic pattern of blue and white areas as shown in Fig. 1 (Bell et al., 1972). This pattern has been demonstrated in different species: dog (McGill et al., 1957; Caro et al, 1971; Fry, 1973), pig (Packham et al., 1967; Somer and Schwartz, 1971; Fry, 1973; Bell et al., 1974a, 1974b) and rabbit (Friedman and Byers, 1963). In view of the recent discussion by Fry et al (1977) on EBD accumulation in vivo, it appears that the areas of blue staining are commonly taken to represent regions of increased wall uptake not only of albumin but also of other macromolecules. This has been

demonstrated in the pig aorta for lipoproteins labelled with ^3H -cholesterol (Somer and Schwartz, 1971) for ^{131}I -fibrinogen (Bell et al., 1974a) and ^{131}I -albumin (Packham et al., 1967; Bell et al., 1974b). Attempts have been made to determine the cause of this increase in wall uptake. Duff (1931) suggested that areas of high uptake correspond with regions in which the aortic wall is most plentifully supplied by vasa vasorum. Bell et al. (1974a) examined the transmural distribution of ^{131}I -albumin in blue and white areas of the pig aorta and interpreted focal differences in ^{131}I -albumin uptake as indicating focal spontaneous areas of "increased endothelium permeability". Gerrity et al. (1977) found that these blue areas of the pig aorta consistently showed a variety of endothelial and subendothelial features not observed in areas with lower dye accumulation. They suggested that these structural differences may account for the observed blue-white differences in uptake of proteins found by Bell et al. (1974a, 1974b). It has also been reported that endothelial cell turnover is increased in areas of the pig aorta which have a high rate of EBD uptake in vivo (Caplan and Schwartz, 1973). Other studies described regional and focal variations in arterial macromolecular transport. Duncan et al. (1962) found that labelled albumin enters the inner layer of canine aortic wall fastest in proximal regions and progressively slower in distal regions. Electron microscopic studies of Huttner et al. (1973) point to regional differences in permeability to electron-dense protein tracers in arterial endothelium of the normotensive rat: thoracic aorta was found to be more permeable than other arterial regions examined. A non-uniformity of uptake of macromolecules along the arterial tree thus appears to be established. It

has been suggested, but never demonstrated, that this may be due to regional variations in intimal permeability and structure. Hemodynamic factors have also been considered in this connection because one may expect regional variations of wall stress, and because it has been suggested (Fry, 1969b; Caro et al., 1971) that the effect of hemodynamic factors on the uptake of macromolecules is mediated by the endothelial cells. Again, this has not been conclusively shown. The main reason is that the lack of detailed knowledge of the temporal and spatial variation of hemodynamic parameters prevent a quantitative demonstration of correlations between the non-uniformity of macromolecular uptake and wall stress.

1.3.2 Transendothelial transport of macromolecules

Transport of molecules across the capillary wall and the arterial endothelium have been reviewed in detail (Michel, 1972; Chien, 1978). Ultrastructural studies of various endothelia suggest two possible pathways for transport of macromolecules through the arterial endothelium layer. Large molecules including ferritin (Florey and Sheppard, 1970; Palade and Bruns, 1968) and lactoperoxidase (Stein and Stein, 1973) are considered to be transported exclusively via the endothelial vesicles. Small molecules, such as horseradish peroxidase (Karnovsky, 1967) seem to enter both via the vesicles and through the intercellular clefts.

Fry (1972) made a quantitative comparison of the uptake of the EBD-albumin complex by the thoracic aorta in specimens with intact endothelium and those from which the endothelial cell layer had been removed by gentle stroking with a camel

hair brush. The injured samples showed a marked increase in the rate of dye uptake. Similar increases following endothelial injury have been found for ^{125}I -albumin (Siflinger et al, 1975) and lipoproteins (Ghosh et al., 1976). All these studies indicate that the endothelium is an important barrier in the exchange of macromolecules between the blood and the wall. This implies that arterial macromolecular transport strongly depends on the integrity of the endothelium.

1.3.3 Influence of fluid mechanical factors on arterial endothelial cells and mass transport

Endothelial cells in the dog aorta are oriented parallel to the axis of the vessel and when their orientation or the flow pattern is altered the cells realign in the direction of flow within a few weeks (Flaherty, 1972). Reidy (1978) found that there is a sudden change in orientation of cells around branches of the rabbit aorta and aortic-iliac bifurcation. These findings strongly suggest that hemodynamic factors may influence endothelial cell orientation.

Fry (1968) quantified the acute changes in endothelium histology associated with an induced increase in blood velocity. This was done by implanting an intra-aortic device designed to produce a rapid convergence of the blood stream into a narrow channel along the ventral part of the dog thoracic aorta. It was found that, for periods as short as one hour:

- i. endothelial cell degeneration can be produced acutely either by turbulence or by increased shear stress on the cell surface exposed to flowing blood;

- ii. a time-averaged shear stress in excess of 379 dyne cm⁻² causes rapid deterioration of the endothelial surface.

Fry pointed out, however, that it is not known whether a shear stress of this magnitude can occur in the normally functioning vascular bed.

Fry (1969b) studied the interaction between mechanical factors and EBD uptake in vivo. Using the same preparation, as that described in his 1968 paper, he found that an increased shear stress at the blood wall interface was associated with architectural changes in the interface and with an increased flux of EBD. He also offered evidence to show that the flux of EBD increases to a significant extent with exposure to stresses lower than the acute critical shear stress.

There have been other attempts to understand possible effects of mechanical factors on macromolecular uptake in vivo. Somer et al. (1972) experimentally induced coarctation in the pig aorta. They found an increase in EBD uptake proximal to the coarctation and a decrease distal to the coarctation. Other studies (Newman et al. 1977; Stehbens, 1978) reported modifications in the EBD pattern in vivo with alterations of hemodynamic factors. In these in vivo experiments, hemodynamic changes are complex and poorly understood in terms of fluid dynamics. It is therefore impossible to identify any single component as being alone responsible for the change observed. Also, possible contribution of endothelial damage (by the surgical procedure or by the flow itself) is unknown.

In vitro techniques have generally been used because they allow good control of the experimental conditions. Caro and Nerem (1973) and Caro (1974) found for dog common carotid arteries, perfused for 30-50 min. with serum containing ^{14}C -4-cholesterol linked to lipoproteins, that the uptake of the label was dependent on the level of steady shear stress at the wall. A shear dependent transport of ^{131}I -albumin between blood and the arterial wall of the dog carotid artery was also found in vitro (Nerem et al., 1976). The latter study suggests furthermore that variation in pressure and flow at frequencies of 1-4 Hz increases the uptake of ^{131}I -albumin.

The effect of blood pressure on arterial macromolecular transport has been relatively little studied. Duncan et al. (1962) in an in vivo study found that elevation of blood pressure produced a marked increase in the rate at which labelled albumin entered the inner layer of the canine ascending aorta, a small increase in the upper descending aorta and no change in the most distal parts of the vessel. Fry (1969b) found that clamping the abdominal aorta in vivo increases the intimal concentration of EBD above the clamp and correlated this with the increase in hydrostatic pressure. Hüttner et al. (1973) found that, in the early stages of experimental hypertension, peroxidase and ferritin from plasma may enter the intima of the rat aorta in increased amounts. They stress that these results do not necessarily indicate permeability changes in the endothelium but may be related, at least partly, to an increased hydrostatic fluid filtration. Indeed, there is evidence to suggest that the transport of material between the blood and the arterial wall is dependent both on pressure-filtration and on molecular diffusion (Wilens,

1951; Yamartino et al., 1974; Harrison and Massaro, 1976; Caro, 1978). Duncan (1965) and Fry (1973) tried to estimate separately the effects of transmural pressure and stretching in vitro on the uptake of albumin by the aortic wall. The large effect of stretching and the absence of a demonstrable effect of transmural pressure suggested that the possible effect of pressure is small relative to that of stretching. This study showed that it is important to take into account the change in dimensions of the artery with pressure. However, since this work was performed in vitro, it is possible that the endothelium layer was damaged. Moreover, the mechanics of the wall may have been altered (Caro, 1978; Vargas et al., 1979). More recently preliminary experiments performed by Chien et al. (1978) indicated that, in an in situ experiment, elevation of transmural pressure from 0 to 100 mm Hg caused an 83% increase in ^{125}I -albumin uptake and a similar increase (54%) in the apparent luminal area was obtained. However, from 0 to 200 mm Hg, the increase in uptake (303%) was much greater than the increase in the apparent luminal area (128%). They explained their result as being due to either a non-linear relationship between effective transport area and apparent luminal area or by an increase in permeability characteristics of the endothelium at this high pressure.

1.4 In vivo and in vitro techniques

In vivo arterial macromolecular uptake studies are difficult to interpret mainly because many of the experimental parameters, including the fluid mechanics and tracer concentration, are difficult to control. On the other hand, in vitro

techniques can introduce artefacts. The problems inherent in these techniques have been reviewed by Caro (1978) who concluded that arterial transport could be seriously disturbed by the following three factors:

1. the excision of an artery, particularly if accompanied by its collapse, can damage its intima and possibly its inner media (Baldwin, 1979);
2. the wall stresses are abnormal when the vessel is restored to in vivo length and normal transmural pressure, because of disruption of adventitial connections. Also, wall stresses are grossly abnormal if the vessel is prevented from expanding by an external restraint;
3. a change in flow in the vasa vasorum may disturb the fields of pressure and thus convection and diffusion in the wall.

In view of these disadvantages, with both in vivo and in vitro techniques, an *in situ* procedure has been used in this work. Moreover, considerable effort was made to ensure that the portions of the artery in which macromolecular uptake was investigated were undamaged.

1.5 Conclusion

It appears from the literature that wall shear stress and transmural pressure can, without damaging the endothelium, influence macromolecular uptake, but that the time scale of these processes is unknown. To study these problems I cannulated and perfused an artery in situ in such a way that a physiological pressure was maintained, the artery was

prevented from shortening, and the adventitial connections remained intact. Because of the non-uniformity of macromolecular uptake in vivo I chose a segment of artery in which the endothelial cells and macromolecular uptake are thought to be uniform. The in situ technique made it possible to study only short-term effects. In order to study the effect of a well-defined steady wall shear stress, a fully developed flow of a fluid containing ^{131}I -albumin was induced in the lumen and a constant mid-artery transmural pressure was maintained. In investigating the effect of steady transmural pressure, the flow rate (and wall shear stress) was maintained as low as possible. In these experiments there was entry of tracer into the arterial wall both via the lumen and the vasa vasorum which were perfused. To study wall uptake it was therefore necessary to determine the wall tracer concentration profiles which were determined by measuring the radioactivity in sequential frozen sections cut parallel to the luminal surface.

2. CHAPTER I: THE EFFECT OF WALL SHEAR STRESS

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2.4 Discussion

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2.4.2 Comparison of results with those reported in the literature

2.4.2.1 Uptake in damaged arteries

2.4.2.2 Wall shear stress dependent uptake

2.4.3 Conclusion

2.1 Preliminary experiments on the carotid artery

During the initial stages of the present study, a technique was developed for cannulating and perfusing the rabbit common carotid artery in situ in such a way as to prevent disturbance to the wall and to its adventitial connections. Transmission electron micrographs and EBD uptake studies confirmed that the artery was intact. During the perfusion studies the luminal hydrostatic pressure and the rate of flow of the perfusate (Tyrode's solution containing 5% dextran) were controlled. The flow was steady and laminar as shown by a linear relationship between the flow rate and the pressure drop across the perfused segment, up to a rate of $1.4 \text{ cm}^3 \text{ s}^{-1}$. Artery diameters were measured with a travelling microscope and the length of the perfused segment was measured with a ruler. Luminal pressure was maintained at about 74 mm Hg. Since macromolecular uptake depends quite strongly on temperature (Siflinger et al., 1975) the temperature was held at $40 \pm 0.5^\circ \text{C}$ by a combination of warming the perfusate and radiant heating.

In a first group of experiments the time of perfusion with the perfusate described above containing ^3H dextran of average molecular weight 70,000 was varied from 0 to 80 min. The flow rate was maintained constant at an average value of $0.4 \text{ cm}^3 \text{ s}^{-1}$. Wall uptake (defined as radioactivity in the wall tissue per unit luminal surface area in the stressed state divided by radioactivity per unit volume of perfusate) was found to increase almost linearly up to 80 min. The average wall uptake rate (defined as uptake divided by time of perfusion with the labelled perfusate) was $4.9 \times 10^{-7} \text{ cm s}^{-1}$ (S.D. = $3.6 \times 10^{-7} \text{ cm s}^{-1}$; 6 arteries). In a second group

of experiments arteries were perfused for 30 min. As shown by results from the first group of experiments, this time is short enough to ensure that steady wall tracer values have not been reached. The flow rate was varied from 0 - 1.5 cm³ s⁻¹, corresponding to a wall shear stress up to 80 dyne cm⁻² which is above the average estimated in vivo shear stress assuming Poiseuille flow. These experiments did not show a dependence of wall uptake on wall shear stress. The average wall uptake was 9.9×10^{-4} cm (S.D. = 4.7×10^{-4} cm, 30 arteries).

It has been assumed, in view of results in the literature that the wall uptake would depend on wall shear stress and the absence of a dependence was thought to result from the masking effect of the large variation of uptake between individual animals. This variation was attributed partly to variation in the arterial wall smooth muscle tone causing changes in artery diameters. Variations in artery diameters would introduce uncertainty into the calculation of wall shear stress and might alter wall uptake. Another possible explanation was the difference, between individual animals, in the contribution of the vasa vasorum to wall mass transport.

In the face of these difficulties, the decision was taken to work with a more elastic artery. The rabbit thoracic aorta was selected because it is relatively straight and because its endothelial cells and macromolecular uptake are thought to be uniform (Reidy and Bowyer, 1977; Bratzler et al., 1977a). Furthermore, experience had been gained in this laboratory in cannulating this artery in situ (Davis and Winlove, 1978). In view of some uncertainty concerning the stability of the dextran tracer used in preliminary experiments on the carotid artery, a study of its stability

was undertaken (see section 3.1.2). In experiments on the aorta ^{131}I -albumin purified by ultrafiltration was used in preference. In order to distinguish the luminal from the adventitial contribution to wall uptake it was necessary to construct concentration profiles for the tracer across the wall. This was done by rapidly freezing artery segments to inhibit diffusion of tracer, cutting sections parallel to the luminal surface on a freezing microtome and counting the activity in these sections (see section 2.2.1.5.)

The methods employed in this work will be detailed in the next section. They were established after many tedious attempts. Confidence in these methods was gained through preliminary experiments. The results of these are given in sections 2.3.1. and 2.3.2.

2.2 Methods

2.2.1 Measurement of wall shear stress dependent uptake

2.2.1.1 Surgical procedures

Male New Zealand rabbits (2 - 2.5 kg) were anaesthetized with 40 mg/kg sodium pentobarbital (Sagatal, May and Baker) injected through the marginal ear vein. A mid-line incision was made through the skin overlying the abdomen, thorax and neck. The trachea was intubated and the lungs were artificially ventilated. The sternum was cut lengthwise and the ribs were displaced laterally with forceps. 1000 I.U. Heparin (Evans Ltd., Speke) were then administered via the ear vein. To avoid excessive loss of label during the perfusion, ligatures were carefully tied around the third to the eighth intercostal arteries about 0.5 cm from their

origins. Great care was taken to avoid damage to the aortic adventitia during the ligation. The surface of the aorta was kept moist with saline. During cannulation, the artery was prevented from collapsing or shortening. With this preparation the infusion of EBD into the vessel in situ showed that the circulation within the vasa vasorum was maintained.

A ligature was tied around the vessel just above the diaphragm and a clamp was placed just below the most distally tied pair of intercostal arteries. The transmural pressure within the thoracic aorta was kept at levels above 50 mm Hg by the action of the heart as judged by the apparent diameter of the aorta. At this stage in the experiment values for the mean arterial blood pressure were measured in three animals with a manometer connected to a catheter placed in a carotid artery and were in the range 55-90 mm Hg. A downstream cannula connected to a reservoir of unlabelled perfusate, warmed to 40°C and held at 100 cm above the rabbit, was inserted through an incision in the ventral surface of the aorta between the clamp and the ligature. The clamp was then removed and the cannula was pushed towards the heart to a point 0.2 - 0.5 cm beyond where the clamp had been positioned and secured with a second ligature. A tap between the cannula and the reservoir was then opened and the aorta was immediately tied just distal to the aortic arch. The rabbit was then sacrificed by stopping the respirator. An upstream cannula clamped at its far end was then inserted into the aorta in the same manner and the artery was gently flushed with the perfusate. The time that elapsed between the beginning of anaesthesia and of

flushing varied from 30-60 min.

2.2.1.2 Perfusion apparatus

Cannulae

Fig. 2 shows a schematic longitudinal section of the cannulae. These were designed to favour the development of Poiseuille flow within the arterial segment:

1. the luminal surfaces of the cannulae were polished and their internal diameter was made 0.4 cm, equal to the estimated mean internal diameter of the thoracic aortic segment, in rabbits of the weight range used, when subjected to a transmural pressure of 100 cm H₂O (73.5 mm Hg);
2. the upstream cannulae was 25 cm long. The length of the tube required for full development of steady laminar pipe flow is (Goldstein, 1938):

$$x = 0.03 R_e d \quad (1)$$

where d is the diameter of the pipe and R_e is the Reynolds number. For all experiments this number was less than 2400 (see Table 1).

The maximal required length of the upstream cannula was therefore 72 times the diameter.

The upstream tubing of the artery consisted of the cannula (62 diameters long) and a straight plastic tube (0.4 cm in diameter, 18 diameters long) aligned with it;

3. the downstream cannulae was 11 cm long. It could be rigidly positioned with respect to the upstream cannula by an adjustable clamp

so as to ensure linear alignment of the cannula with the artery and to maintain the artery at its in vivo length;

4. the outer wall of the cannulae was tapered 1.5 cm from the point where it joined the artery so that there was a step of ~ 0.01 cm (very small compared to the diameter of the cannulae) at the point of junction with the artery. As a result the parabolic velocity profile in fluid entering the artery was not expected to be significantly disturbed over a distance exceeding one tube diameter downstream of the junction (Caro and Nerem, 1973).

Poiseuille flow could therefore be expected within the arterial segment for Reynolds numbers at least up to 2400. Side arms, 0.05 cm in diameter, were attached to the cannulae 1.5 cm away from their tips and these could be connected to manometers.

Perfusion system

The perfusion system is shown in Fig. 3. It was designed to maintain a steady fully developed flow with perfusate in the cannulated segment of the artery and therefore to apply a known steady wall shear stress. Both the flow rate and the transmural pressure could be held constant during exposure of the artery to solutions containing radioactive tracers. Flow was induced by sustaining a pressure difference between two large air reservoirs, each of which had a recess in its base to hold the perfusate. Perfusate levels and flow rates were kept constant with the aid of a servo device (Fig. 4.7). This device was triggered by

contact of the perfusate with the fixed electric contact A in the downstream reservoir which activated a pump between the two reservoirs. The time, t , taken for the fluid level to change from that of contact A to that of contact B (approximately 1 cm), when the pump was switched off, could be measured automatically by the servo device. The volume, V ($\sim 7 \text{ cm}^3$) between the two contacts was measured and the flow rate, Q , could therefore be calculated. Volume flow rates of up to $10 \text{ cm}^3 \text{ s}^{-1}$ were obtained with a roller pump (Watson, Marlow, type 22) and flow rates of $10\text{--}20 \text{ cm}^3 \text{ s}^{-1}$ with a second pump (Watson, Marlow, type H.R.) used in parallel.

The upstream and downstream side arms of the cannulae were connected to vertical manometric tubes (0.2 cm in diameter). The mid-artery transmural pressure, P_o , (see Fig. 3) was estimated as the mean of the heights of the perfusate in these tubes. This pressure was kept constant at $73.5 \pm 0.4 \text{ mm Hg}$ by adjusting the absolute pressure level in each reservoir. On leaving the upstream reservoir, the perfusate flowed through a heating coil before entering the upstream cannula. The temperature of the perfusate, leaving the downstream cannula, was monitored with a thermocouple inserted through a side arm. The temperature was maintained at $40 \pm 0.5^\circ\text{C}$ by adjusting the temperature of the water bath between 41 and 44°C depending on the flow rate. The perfusate could also be circulated through a bypass (see Fig. 3). Three-way taps were used to connect the cannulated artery to the unlabelled perfusate reservoir, the downstream and upstream reservoirs, a reservoir containing Tyrode's solution and a draining reservoir.

2.2.1.3 Fluid dynamics of the system

The fluid dynamics of the system were investigated in two experiments in which the flow rate of perfusate through the artery was varied in steps. For each flow rate the pressure difference between the two side arms was measured with a differential pressure transducer (S. E. Laboratories, type 1150). If Poiseuille flow prevails, the predicted pressure difference is

$$\Delta P = \frac{128\eta LQ}{\pi d^4} \quad (2)$$

where η is the perfusate viscosity (which was measured in an Ostwald viscometer at 40°C), L is the distance between the two side arms and d is the diameter of the cannulae and the artery (0.4 cm).

During these experiments the pressure levels in the reservoirs required to attain a desired flow rate, with a mid-artery transmural pressure of 73.5 mm Hg, were determined.

The roller pumps and surface tension phenomenon at contact A introduced slight variations in the levels of liquid in the reservoirs. The design of the reservoirs was such as to minimize these oscillations. These variations, x , were in the order of 0.1 cm H₂O and could cause oscillations in wall shear stress and transmural pressure. The resulting variation in wall shear stress, τ_w , can be estimated for each reservoir using the Boyle-Mariotte Law assuming a proportional relationship between the pressure difference of the two air reservoirs and the flow rate, and between the flow rate and

and the wall shear stress. We find:

$$\left(\frac{\Delta \tau_w}{\tau_w} \right)_1 = \frac{x S_1}{V_1} \frac{P_1}{P_1 - P_2} + \frac{x}{P_1 - P_2} \quad (3)$$

for the upstream reservoir and

$$\left(\frac{\Delta \tau_w}{\tau_w} \right)_2 = \frac{x S_2}{V_2} \frac{P_2}{P_1 - P_2} + \frac{x}{P_1 - P_2} \quad (4)$$

for the downstream reservoir. The subscripts 1 and 2 refer to the upstream and to the downstream reservoir respectively, P is the pressure in the reservoir, S the surface area of the recess and V the volume of the reservoirs. Experimentally it was found $P_1 + P_2 = 2P_0$ which showed that the hydraulic resistances of the tubes between the side arms and the air reservoirs were equal. After substitution of P_2 by $2P_0 - P_1$ in eqn. 3 and 4, the maximum wall shear stress oscillations can be calculated. We find

$$\left(\frac{\Delta \tau_w}{\tau_w} \right)_{1_{\max}} = \frac{x S_1}{2 V_1} \frac{P_1'}{(P_1' - P_0)} + \frac{x}{2(P_1' - P_0)} \quad (5)$$

and

$$\left(\frac{\Delta \tau_w}{\tau_w} \right)_{2_{\max}} = \frac{x S_2}{V_2} \frac{2 P_0 - P_1'}{P_1' - P_0} + \frac{x}{2(P_1' - P_0)} \quad (6)$$

where P_1' is the minimum pressure used (1114.2 mm H₂O or 81.9 mm Hg). Using this value one obtains

$$\left(\frac{\Delta \tau_w}{\tau_w} \right)_{2_{\max}} = 0.009 \quad \text{and} \quad \left(\frac{\Delta \tau}{\tau} \right)_{1_{\max}} = 0.006$$

this implies that, assuming quasi steady flow, the maximum

oscillation in wall shear stress is $0.9\% + 0.6\% = 1.5\%$ of the steady value. The fundamental frequencies of these oscillations were in the range of $0.5 - 2\text{Hz}$. This gives a value for the frequency parameter ($d\sqrt{\frac{\omega}{\eta}}$) in the range of 4-9. In this range it is very difficult to calculate the exact value of instantaneous wall shear stress oscillations.

The oscillations in the mid-artery pressure can be estimated from the oscillations in pressure measured at one side arm. Measurements, using a pressure transducer (Bell and Howell, type 4-422), showed that these oscillations were less than 0.01% of the mid-artery pressure.

2.2.1.4. Studies of tracer uptake

In these studies the labelled solution was introduced into the perfusion system a few minutes before the start of an experiment and was then rapidly brought up to 40°C by circulation through the bypass. The desired flow rate was achieved by adjusting the pressure difference in the two reservoirs to appropriate levels. The labelled solution was then passed through the cannulated artery. The time that elapsed between isolating the artery segment from the blood supply of the heart and beginning the perfusion with labelled perfusate was 5-15 min. Within the first minute after perfusion was begun, minor adjustments were made to the pressure levels in the air reservoir in order to attain a mid-artery transmural pressure of 73.5 mm Hg.

The flow rate was measured, at least three times during the experiment, using the servo device. It ranged in different experiments between 0.1 and $19\text{ cm}^3\text{ s}^{-1}$ and the solution viscosities, measured at the conclusion of each experiment, were between 0.4 and 3.7 cp .

The difference in the heights of the level of perfusate in the vertical manometric tubes connected to the side arms was measured. The Reynolds number was calculated from the formula

$$R_e = \frac{4 Q \rho}{\pi \eta d} \quad (7)$$

where the fluid density, ρ , was assumed to be 1 g cm^{-3} .

Assuming Poiseuille flow the wall shear rate was calculated from

$$S_w = \frac{32 Q}{\pi d^3} \quad (8)$$

and the wall shear stress from

$$\tau_w = \frac{32 Q \eta}{\pi d^3} \quad (9)$$

2.2.1.5 Handling of tissue

Precautions were taken to minimise further diffusion of the tracer within the wall at the end of the 10 min. perfusion period. The arterial segment was connected via two taps to the draining reservoir and to the Tyrode's reservoir 100 cm above the segment. In this manner the labelled perfusate was flushed from the lumen at a flow rate of about $1 \text{ cm}^3 \text{ s}^{-1}$ without a change occurring in transmural pressure. After the initial flushing period (5s) the loose connective tissue was dissected away from the artery. After a further 30-40 s the ventral portion of the aorta was cut away leaving the dorsal portion with the intercostal roots in situ, and rinsed for 5 s in each of two changes of Tyrode's solution at 4°C . It was then placed, luminal surface downward, on a lightly greased microscope slide, frozen

using a dichlorodifluoromethane aerosol (Bright Instrument) and placed in a cryostat at -30°C . Freezing was always completed within 3 min from the start of flushing. The flattened frozen sheet of tissue was then trimmed along both lateral margins. Portions which had been within 0.5cm of the tips of the cannulae were discarded, in case they had been damaged during cannulation or subjected to local flow disturbances.

Tissue mounting medium (Tryco-M-bed) was frozen onto the cutting block of a rotary retracting microtome (Bright Instrument, model 5030), and cut to give a flat surface with an area of about 2 cm^2 parallel to the cutting plane of the knife. A segment of the artery was thawed and rapidly transferred, luminal surface down, to a second greased slide. A drop of mounting medium was placed on the adventitial surface and the tissue was brought into contact with the flat surface. The slide and therefore the intimal surface was kept parallel to the cut surface by means of a $400\text{ }\mu\text{m}$ thick wire ring which surrounded the tissue. As the artery froze to the surface, the slide was gently eased away. The edges of the tissue were then trimmed to remove any overhanging material and after measuring the projected surface area, serial sections $20\text{ }\mu\text{m}$ thick were cut through the whole thickness of the wall. The knife was cleaned after each cut to prevent the transfer of radioactive tracer from one section to the next. The boundary between the media and the adventitia was noted by a change in the appearance of the section (Bratzler et al., 1977a). Each section was placed on a vinyl strip to thaw and dry, and was then weighed to check the accuracy of sectioning and to allow estimation of the thickness of the

first luminal section.

The volume of each tissue section was calculated from its thickness and projected surface area. The activity of each section was measured in a gamma counter (LKB, type 8000). The typical range of counts for artery sections was 16-1000 counts per min. Slices were counted for 10 min. The activity of the perfusate was determined from 0.04 cm^3 aliquots taken during the perfusion. The adventitial slices were usually not counted. Background rates ranged from 16 to 18 counts per min and the counting rates for aliquots of the perfusate were approximately 10,000 counts per min. The relative tissue radioactivity concentration (RTRC) of each section was calculated as counts per unit time per cm^3 of tissue divided by counts per unit time per cm^3 of perfusate.

2.2.1.6 Perfusing solution

The perfusate was Tyrode's solution with a pH adjusted to 7.4 containing 2% bovine serum albumin (Sigma laboratories) and 2% dextran of molecular weight 70,000 (Pharmacia) to produce a normal colloidal osmotic pressure. Dextran of molecular weight 500,000 was also added to the perfusing solution in some experiments to increase its viscosity. Concentrations of up to 4% were used to produce viscosities up to 3.7 cp.

The label used in these experiments was ^{131}I -human serum albumin (^{131}I -HSA) (Radiochemical Centre, Amersham) at a concentration in the perfusing solution of about $10 \mu\text{Ci cm}^{-3}$. Iodinated albumin is invariably contaminated

with free labelled iodide which is much more readily taken up by the arterial wall than albumin (Winlove et al, 1978) and it was always purified by an ultrafiltration technique: isotope dissolved in 1.5 cm^3 of saline was diluted to 11.5 cm^3 with saline and filtered through a membrane with a nominal cut off at molecular weight 50,000 (Chemical Laboratories Instrument, Model A50T) until the volume was reduced to 1.5 cm^3 . Dilution and filtration were performed five times in a pressure cell 2.54 cm in diameter, equipped with a stirrer. To minimise the further dissociation of the iodinated albumin the tracer was diluted with perfusing solution immediately after filtration and the solution was kept at 4°C except during the actual period of the experiment.

To assess the purity of the tracer, samples of the solution were fractionated - by passing through a gel filtration column - immediately after filtration, at the end of a 10 min in situ perfusion experiment and after an in vitro incubation time of 40 min at 40°C . The chromatographic column used was 100 cm long and 2.6 cm in diameter. It was packed with Sepharose CL6B (Pharmacia) and run at room temperature at a pressure of 200 cm H_2O . The eluant was 0.9% NaCl at pH 7.4. Liquid samples introduced into the column had a constant volume of 2 cm^3 . Eluant collection was by means of a timer-driven turntable adjusted to provide 3.5 cm^3 aliquots. Three hours were necessary for run. The void volume (59 cm^3) was measured with blue dextran (Pharmacia) and the total volume (178 cm^3) with ^3HHO . The column was calibrated by measuring the elution volume of various molecules (see Table 2).

2.2.2. Complementary experiments

2.2.2.1 Microscopic studies

In order to investigate the histological effects of shear stress two arteries were cannulated as described in section 2.2.1.1. One was exposed to a high shear stress (42 dyne cm^{-2}) and the other to a very low shear stress (0.5 dyne cm^{-2}). After 10 min of perfusion, 5% glutaraldehyde - 4% formaldehyde in a pH 7.4 cacodylate buffer was gradually added to the perfusate without disturbing pressure or flow in the artery. After all the perfusate had been substituted, fixation was continued for 100 min at a constant mid-artery transmural pressure of 73.5 mm Hg. The arteries were then post-fixed in osmium tetroxide, dehydrated in absolute ethanol and finally embedded in Epon. Sections ($500 \text{ \AA}$ thick) were cut with a microtome, mounted on coated grids, stained with lead oxide and uranyl acetate and observed in an electron microscope (Jeol, type 100 B) at 80 kV.

The structure of intentionally damaged arteries (see 2.2.2.3) was investigated. Two were rinsed (while still in situ at a transmural pressure of 73.5 mm Hg) with a solution of 0.3% EBD in saline for about 1 min and finally flushed with saline. They were then excised and the luminal surface was examined under a light microscope. Another damaged artery was fixed in situ under the same pressure, embedded in Epon, sectioned (about $1 \mu\text{m}$ thick) and stained with Toluidine Blue for microscopic examination.

2.2.2.2 Time course of uptake

The time course of ^{131}I -HSA uptake was determined, at very low flow rates, using a slightly different method from that described in section 2.2.1. Fig. 8 shows the perfusing system. The artery was perfused with needles (Butterfly, type 16) instead of cannulae. The upstream needle was connected to a reservoir, 100 cm above the aorta, through a heating coil at 41°C and was inserted just below the aortic arch, pointing distally. After insertion, the aorta was tied above the needle and, immediately afterwards, just above the diaphragm. The flow rate was controlled by a variable tap connected to the downstream needle. The flow rate of the labelled solution was kept at about $0.08 \text{ cm}^3 \text{ s}^{-1}$. At this flow rate the pressure drop between the artery and upstream reservoir was sufficiently small to ensure a constant transmural pressure of 100 cm of water in the perfused segment. In these experiments the Reynolds number (given by eqn. 7) was 16. The entrance length required by eqn. 1 must thus be about 2 mm. After the two first millimeters the flow within the artery was therefore fully developed and wall shear rates and wall shear stresses could be calculated using eqn. 8 and 9. These were 13 s^{-1} and 0.2 dyne cm^{-2} respectively. The arteries were perfused for 5 min (2 arteries), 10 min (5 arteries) and 40 min (4 arteries).

2.2.2.3 Experiments with damaged arteries

The method used was the same as in the time-course study (see 2.2.1). The artery was intentionally damaged by twice passing a 0.4 cm diameter silicone rubber tube with a rounded end along the lumen, before the downstream needle was inserted.

During the operation the transmural pressure was decreased to about 20 mm Hg so that the tube fitted tightly the lumen of the artery. The downstream needle was then inserted in the normal way and the transmural pressure was raised to 73.5 mm Hg. The arteries were perfused with the labelled solution for 10 min (3 arteries) 20 min (2 arteries), 40 min (2 arteries) and 60 min (2 arteries).

2.2.2.4 Measurement of uptake in vivo

To compare the uptake of ^{131}I -HSA by the aortic wall in situ and in vivo, two anaesthetized rabbits were injected, through the marginal ear vein, with 600 μCi of purified label in saline. Mean arterial blood pressures were monitored by a manometer connected to a catheter placed in a carotid artery and were 65 and 70 mm Hg respectively. A blood sample was taken after 10 min. to estimate the concentration of label. A needle (Butterfly, type 16) connected to a saline reservoir was inserted into the previously exposed abdominal aorta and the blood was rapidly flushed out of the thoracic aorta through a tap attached to the carotid cannula. Rinsing was continued as the thorax was opened. The ventral portion of the aorta was excised and sectioned as before (see 2.2.1.5).

To investigate the stability of ^{131}I -HSA in vivo, another rabbit was injected with purified tracer and blood samples were drawn after 2 min and 20 min. Plasma from the last sample was fractionated on the gel filtration column (see 2.2.1.6).

2.3 Results

2.3.1 Stability and purity of tracers

No free iodide peak was discernible from the background of the elution curve of the 2 samples of perfusing solution immediately after the ^{131}I -HSA had been purified. The amount of free iodide present was estimated to be less than 0.01% of the total activity corresponding to the iodinated albumin peak. After in situ perfusions at 40°C for 10 min the average concentration of free iodide relative to total activity was 0.03% and rose 0.08% after in vitro incubations at 40°C for 40 min.

It has been found in in situ perfusion studies (Davis and Winlove, 1978) that the concentration of labelled iodide attains an equilibrium value in the thoracic aorta within 10 min and that the wall concentration approaches that of the perfusing solution. This implies that in in situ experiments of the present study, the activity in the wall attributable to uptake of free iodide from the perfusate was about 0.01% of the total activity of the perfusate at 10 min and 0.08% at 40 min. These values are very small compared with those measured (see fig 11, 16, 18, 19). Thus, the free iodide does not interfere with the determination of uptake of iodinated albumin by the arterial wall.

Following the intravenous injection of purified ^{131}I -HSA the activity of the plasma was found to decrease significantly with time. But this decrease was found to be only 4% between samples drawn at 2 min and at 20 min after intravenous injection. It was therefore assumed that the concentration of label during 10 min for in vivo experiments was constant. No free iodide could be detected in the plasma sample drawn 20 min after

injection and the radioactivity was eluted with a single peak like that observed for the purified label, indicating no transfer of label to other proteins (Winlove et al., 1978). These results indicate that ^{131}I -HSA could be considered stable in the 10 min in vivo experiments.

2.3.2. Fluid dynamics of the perfusion apparatus

Fig. 9 shows the relationship between flow rate in the two arteries studied and the pressure difference between the side arms. The data are given in Table 3. The slopes of least mean square regression lines were different from the values predicted from eqn. 2 using $d = 0.4$ cm. The differences between the theoretical and experimental slopes were small, amounting to 0.06% ($P \sim 0.02$) and 4.8% ($P \sim 0.04$) for arteries No.1 and No. 2 respectively. For the highest flow rates the Reynolds numbers were 2370 and 2005 respectively. This means that for Re lower than 2400 the pressure drop per unit length of artery, $\frac{\Delta P}{L}$ can be fairly accurately estimated from the flow rate and viscosity using eqn. 2 assuming $d = 0.4$ cm. This gives support to the determination of the wall shear rate and the wall shear stress from the same measurements using eqn. 8 and 9 which can be written in alternative forms as

$$S_w = \frac{\Delta P}{L} \times \frac{d}{4\eta}$$

and

$$\tau_w = \frac{\Delta P}{L} \times \frac{d}{4}$$

In contrast to the results obtained for the two arteries, the pressure difference measured by the difference of the heights of the perfusate in the vertical manometric tubes for vessels perfused with label was on average 19% higher than that calculated using eqn. 2. Since the pressure difference varied as d^{-4} , this systematic difference may result from the fact that the arteries may not always have had the same internal diameter as the cannulae.

2.3.3. Shear dependent uptake

Medial thickness varied from segment to segment and from rabbit to rabbit. For each segment the distance from the luminal surface to the outer border of the media was normalised to its average value of $170\text{ }\mu\text{m}$ (S.D. $12\text{ }\mu\text{m}$). The RTRC of each section of the arterial wall was plotted as a function of the normalised distances of the middle of the section from the luminal surface. The profiles obtained from three segments of a single artery are shown in Fig. 10. They are typical of those found after 10 min exposure to labelled albumin.

Table 1 gives the values of the hydrodynamic parameters for all arteries studied. The arteries were allocated to one of three groups with shear stresses in the range $1 - 17\text{ dyne cm}^{-2}$ (8 arteries), $17 - 48\text{ dyne cm}^{-2}$ (7 arteries) and $48 - 75\text{ dyne cm}^{-2}$ (7 arteries). The latter value is below the average estimated in vivo shear stress, assuming Poiseuille flow. An average RTRC profile was calculated for each group using the RTRC values interpolated for each artery segment at distances of $10, 20\text{ }\mu\text{m}$ and then at further

20 μm intervals from the luminal surface. The results are given in Table 4 and the three average profiles are shown in Fig. 11. At each location within the media where a group average was obtained, the mean value of the RTRC was compared with the corresponding value in the other groups by Student's t test. No statistically significant differences were found ($p > 0.05$). Also, there was not any significant difference observed between these values and the mean RTRC values found for the five arteries cannulated with needles (see section 2.2.2.2 and Fig. 16) and exposed to a shear stress of about 0.2 dyne cm^{-2} for 10 min. Fig. 13 shows the average uptake profile calculated using data from all 27 arteries. The absence of any significant difference implies that it was not possible to detect an effect of a steady wall shear stress in the range $0.4 - 75 \text{ dyne cm}^{-2}$, under a constant transmural pressure of 73.5 mm Hg on the 10 min concentration profiles of ^{131}I -HSA by the rabbit thoracic aorta perfused in situ.

Since varying shear stress in these experiments is likely to affect only luminal uptake (defined as the ratio of total radioactivity coming through the luminal surface in wall tissue per unit luminal surface area in the stress state to the radioactivity per unit volume of perfusate), the radioactivity of the wall between the luminal surface and the minimum point on the uptake profile was calculated for each artery segment and expressed as "luminal uptake" (see section 2.3.4). On average the projected surface area of the relaxed artery when sectioned was 1.8 times lower than that calculated for the artery when stressed under the conditions of the experiments. The average luminal uptake for each artery is given in Table 1. The luminal uptake for

the five arteries perfused at low flow rates for 10 min with needles were 4.5, 1.9, 4.3, 1.2 and 2.6×10^{-5} cm respectively.

Fig. 14 - 16 show the luminal uptake for the 27 arteries as a function of the shear rate, the shear stress and Reynolds number. The slopes of the least mean square regression lines through these data were found not to be significantly different from zero ($P > 0.50$). Their absolute value and confidence intervals (0.05) are given in each figure. The average uptake was calculated and found to be 2.8×10^{-5} cm (S.D. = 1.1×10^{-5} cm).

Fig. 15 gives the luminal uptake for these 27 arteries as a function of the viscosity of the perfusing fluid. The slope of the least mean square regression line is slightly different from zero ($P \sim 0.03$). Increasing the viscosity of the perfusate tends to decrease the luminal uptake.

Using bivariate regression analysis, partial regression coefficients were calculated for wall shear stress, β_τ , and for viscosity β_η . Again, β_τ was found to be not significantly different from zero ($P \sim 0.4$) and β_η to be slightly different from zero ($P \sim 0.02$).

2.3.4 Time course of uptake

The average-profile obtained in the 5 min, 10 min and 40 min experiments are given in Fig. 16. The 5 min profile shows that practically no label entered the middle of the media and that, as expected, the label can enter the media both through the intimal and the adventitial surfaces. The average concentrations obtained in the 40 min experiments are significantly greater than for 10 min, indicating that

a steady state level was not reached in 10 min. This result justifies the choice of 10 min for the duration of subsequent shear stress experiments. Since equilibrium concentrations had not been reached in the arterial wall, possible variation in the arterial monomolecular transport could be demonstrated. Secondly, because the activity in the central part of the media is very low, it is possible to characterise the luminal uptake by the radioactivity localised between the luminal surface and the minimum concentration point within the media.

2.3.5 Uptake in vivo

Fig. 13 shows the average profile obtained from the two in vivo experiments. Concentrations are somewhat lower than those obtained in situ for the same perfusion times. The difference is particularly important in the outer media close to the adventitia. The luminal uptakes were calculated for these two arteries. The values obtained were 1.6 and 1.9×10^{-5} cm. The average (1.75×10^{-5} cm) is not significantly different from the average value found in situ for the same perfusion time (see section 2.3.3.).

2.3.6 Uptake in damaged arteries

E.B.D. staining experiments on arteries which were perfused and not intentionally damaged, showed that no dye was taken up by cell nuclei except where the cannulae or needles had been in contact with the wall and sometimes in small areas where the intercostal arteries arose from the aorta (regions not included in the uptake studies). This indicates that the endothelial cells were not damaged in the experimental segment by the cannulation and perfusion

techniques (Bjorkerud and Bondjers, 1972). By contrast the two arteries which had tubes passed through the lumen exhibited stained nuclei over 30 and 60% of their luminal surfaces, indicative of intimal damage. Sections from the damaged arteries showed that the internal elastic lamellae were intact except in one small region close to the upstream end of the needle.

Profiles obtained for damaged arteries perfused during 10, 20, 40 and 60 min (see section 2.2.2.3) are shown in Fig. 18. It is seen that:

1. there is a large variation in the wall concentration which does not correlate with the time of perfusion. This variation is probably due to differences in the degree of surface damage between individual arteries;
2. compared with undamaged arteries, the concentrations obtained are much higher when perfused for the same time periods and at the same transmural pressure. Fig. 19 shows the average RTRC profile for 4 damaged arteries perfused for 10 min and for all undamaged arteries. The difference between the concentrations is considerable and greater for the adventitial than for the intimal side. This confirms that the intima is a major barrier for macromolecular penetration into the arterial wall. The difference in concentration on the adventitial side is probably not due to a change in permeability on this side caused by adventitial damage. It is more likely to be a result of the increase in luminal uptake

with tracer coming from the intimal side. Fig. 19 also shows that the total uptake is increased by an average factor of about 4 but that the luminal uptake is increased by a factor of 13;

3. the concentration profiles were relatively flat. A minimum point was apparent only for arteries perfused during 10 min and for one artery perfused for 20 min. The other profiles were very flat for almost all the media and the average RTRC was $8.2 \pm 2.5\%$.

2.3.7 Electron microscopic studies

Transmission electron micrographs of the intimal region of the two arteries fixed during exposure to high and low shear stresses were examined by a colleague closely acquainted with the ultrastructure of the rabbit artery wall. Longitudinal sections of about 10 cells at about four different locations along each artery were observed. No difference between the two arteries could be detected. Fig. 20 shows a transmission electron micrograph of an endothelial cell from an artery which had been exposed to a wall shear stress of 42 dyne cm^{-2} .

2.4 Discussion

2.4.1 The methods employed

The intima of arteries perfused in situ for the study of the effect of wall shear stress were undamaged as verified by light and electron microscopic studies, by dye exclusion and by the fact that uptake was far lower than in the experimentally

damaged arteries (see section 2.3.6). Furthermore, there was no significant difference between uptake measured in situ and in vivo for identical times and under similar average transmural pressures (see section 2.3.5).

The difference in tracer concentration on the adventitial side of the media between the in situ and in vivo experiments could be due to either modification of the perfusion of the vasa vasorum or to an altered lymphatic circulation in the in situ experiments.

2.4.2 Comparison of results with those reported in the literature

2.4.2.1 Uptake in damaged arteries

It is generally considered that the intima is an important barrier for the penetration of macromolecules into the arterial wall (see 1.3.2). Siflinger et al. (1975) found that intimal damage increases the uptake of ^{125}I -albumin by the isolated dog common carotid artery on average by a factor of about 2 for 10 - 15 min studies. I found a slightly larger increase; however, the results may not be comparable because the experiments of Siflinger et al were performed in vitro at very low transmural pressure.

With a transmural pressure greater than 30 mm Hg there is no morphological difference in the ultrastructure of the intimal, central and adventitial part of the media - with exception of the transition zone media-adventitia (Baldwin, 1979). One can thus assume that the accessible volume for macromolecules is likely to be the same for the different parts of the media at a given pressure larger than 30 mm Hg. If one supposes that the media is a single compartment in

which the concentration of tracer in the accessible volume tends to equilibrate with that of the perfusate from the lumen and the vasa vasorum, one would expect a flat concentration profile at equilibrium. If it is assumed that equilibration can occur after intimal damage, the ratio between the tracer concentration in the media and in the perfusate is a measure of the accessible volume for the tracer. Results obtained with arteries with damaged intimas indicate that this ratio is $8.2 \pm 2.5\%$ for the ^{131}I -HSA in the rabbit thoracic aorta.

2.4.2.2. Wall shear stress dependent uptake

Several studies have been made of the effect of wall shear stress on transendothelial transport of macromolecules in arteries. The results differ from those of the present study.

Fry (1969b) implanted a device in the aorta of the dog which markedly altered the blood flow and found that the permeability of the endothelial cell surface to EBD-albumin complex increased with an increase in the wall shear stress in a range in which the endothelial cell appeared histologically normal. The flow patterns obtained in this way would be expected to be significantly different from those in the present study. I ensured that the flows through the perfused segment were not only not turbulent but also steady. Flow oscillations have been implicated in affecting transport (Weinbaum and Caro, 1976). However, the similarity between the tracer concentration profiles obtained in situ in my steady flow experiments and those obtained in vivo by myself and others (Bratzler et al, 1977a) for iodinated albumin uptake, where aortic flow would be disturbed (Seed, 1972; Parker, 1977) suggest that these unsteady effects are

small. This does not exclude a possibly important effect of turbulence upon arterial mass transport when the average wall shear stress is larger than physiological. In the experiment reported by Fry (1969b) the average wall shear stress was relatively well determined but only in the channel interior where it varied between 1300 and 300 dyne cm^{-2} . However, Fry found histologically that the critical wall shear stress (below which cells retain a normal appearance when observed with a light microscope) was 379 ± 85 dyne cm^{-2} . Thus, Fry did not observe an effect of wall shear stress without apparent intimal damage, except when it approached the critical wall shear stress. In my experiments, wall shear stress was always well below the critical value. It is possible that in Fry's experiments, at wall shear stress between 300 and 379 dyne cm^{-2} , only a very small proportion of endothelial cells were damaged. He was unable to demonstrate histologically the presence of this small proportion of damaged cells, but these damaged cells could have been sufficient to increase the permeability of the endothelial cell surface. Only a study of macromolecular uptake after exposing the endothelial surface to a steady wall shear stress close to the critical value would enable one to establish whether wall shear stress can have a short-term effect without irreversibly altering the endothelium.

Caro and Nerem (1973), Caro (1974) and Nerem et al (1976) used an in vitro perfusion technique to study shear dependent uptake in the dog common carotid artery. Some of these experiments used ^{14}C -cholesterol as tracer (Caro and Nerem, 1973; Caro, 1974). It is possible that mechanisms involved in the uptake of cholesterol are different from those of

^{131}I -HSA (Dayton and Hashimoto, 1970; Webb and Cohn, 1971; Adams et al, 1975; Bratzler et al, 1977b) and that the former may exhibit shear dependence. More importantly, all these workers used excised arteries. The endothelial layer has been shown to be easily damaged on excision of arteries (Baldwin, 1979) and, as confirmed by the present study, this can markedly increase macromolecular uptake. It is also possible that such damage was exacerbated on application of a moderate wall shear stress.

The tracer used in Nerem's experiment was not purified and the transmural pressure was not constant. This pressure was 100 mm Hg while the wall shear stress was varied between 5 to 50 dyne cm^{-2} , but it was only 10 mm Hg for stresses in excess of 100 dyne cm^{-2} . Caro (1974) worked at a very low pressure (10 cm H_2O). The morphology of the whole artery changes markedly with pressure and so it is possible that at very low pressures arterial wall mass transport properties are different to those at physiological pressure.

2.4.3. Conclusion

The experiments presented in this thesis were of 10 min duration only. The in vivo and in vitro results, with which my findings are compared, were of a slightly longer duration (1 - 3h). It remains possible therefore that wall shear stress influences the transport of material between the blood and vessel walls (Fry, 1969a; Caro et al, 1971). This effect would not be significant except on a longer time scale. Several experimental facts argue in favour of such a long-term effect of hemodynamic factors upon the arterial wall and upon transport phenomenon (see section 1.3.1 and 1.3.3.). Structural modification could lead to change in transport

phenomenon. At short time periods the wall shear stress above the critical wall value affects transport phenomenon by damaging the intimal surface. One could also ask whether there may not be a limiting value of wall shear stress below which at long time the modification in the transport phenomenon would not affect the arterial wall, but above which metabolic disorders such as atheroma could appear. In order to progress in this field one would need to correlate quantitatively in vivo values of wall shear stress with variations in histological and metabolic factors in the arterial tree. Unfortunately, the in vivo experimental measurement of shear stress poses considerable technical problems (see, for example, Seed and Wood, 1971 or Seed, 1972), while theoretical calculations of wall shear stress involve complex mathematical problems and are still inapplicable to the flow in arteries (see, for example, Smith, 1975 or Parker, 1977).

3. CHAPTER II: THE EFFECT OF HYDROSTATIC PRESSURE

3.1 Methods

3.1.1 Experimental procedures

3.1.2 Tracers

3.1.3 Tracer uptake studies

3.1.4 Microscopic studies

3.2 Results

3.2.1 Uptake results

3.2.2 Microscopic studies

3.3 Discussion

3.1. Methods

3.1.1. Experimental procedures

The same experimental procedure was used as for the time course uptake studies (see section 2.2.2.2). Arteries were initially perfused with inactive solution under a transmural pressure of 73.5 mm Hg. The pressure was then slowly changed to the desired value during about 1 min by adjusting the air pressure in a 10 litre reservoir connected to the reservoirs containing the solutions. Fig. 21 shows a schematic diagram of the apparatus. Transmural pressures up to 180 mm Hg were measured with a mercury manometer attached close to the entrance of the perfused arterial segment. After 2 - 3 min the solution containing the radioactive tracer was introduced with the aid of a three-way tap, without changing the transmural pressure. After perfusion for 10 min the artery was rinsed for 5 - 10 s with the inactive solution reintroduced through the same tap. If the transmural pressure of perfused arteries had exceeded 73.5 mm Hg it was reduced to that value during the rinsing procedure. For transmural pressures lower than 73.5 mm Hg the rinsing liquid was introduced under the same pressure as the pressure during the actual perfusion.

3.1.2 Tracers

^{131}I -HSA and tritium labelled dextran of a molecular weight of about 70,000 (^3H -D70) - Radiochemical Centre, Amersham - were used. The same safeguards against degradation of ^{131}I -HSA were taken as described in section 2.2.1.6. Fig. 22 shows a typical activity profile of an unpurified ^3H -D70 solution after column chromatography-gel

filtration was performed as described in section 2.2.1.6. Radioactivity was eluted in one main peak corresponding to an elution volume identical to that of inactive dextran of molecular weight of about 70,000 (D70) but about 15% of the radioactivity was eluted between this peak and the total volume. The majority of radioactivity eluted at the total volume was most likely tritiated water since around 85% of the activity could be removed by freeze drying.

Prior to each experiment, the commercial ^3H -D70 used was purified by ultrafiltration as described in section 2.2.1.6. After purification the radioactivity was found exclusively in a single peak with an elution volume identical to that of inactive D70.

After incubation of a purified solution for 12 hours at 40°C and at the same concentration as that used in perfusion experiments (about $10\ \mu\text{Ci cm}^{-3}$), the main elution peak was unchanged but a small amount (about 0.4%) of the radioactivity was eluted in a single peak at the total volume. This radioactivity could be completely removed by freeze-drying. After incubation of a purified solution for 12 hours at 90°C , the elution radioactive chromatogram was similar to that for commercial ^3H -D70. These findings indicate that commercial ^3H -D70 is not homogeneous and contains tritiated dextran of lower molecular weight and tritiated water. These impurities can be removed by ultrafiltration. The small proportion of ^3H -HO which may appear during a 10 min perfusion at 40°C does not interfere with the uptake experiments since each artery section was already in the dry state before counting (see section 2.2.1.5).

The ^{131}I -HSA in each sample was determined as described in section 2.2.1.5. The radioactivity of ^3H -D70 samples was determined on a liquid scintillation counter (LKB). Prior to counting the liquid samples (50 to 250 μl) were made up to 250 μl with water and then mixed with 2.5 cm^3 of scintillant (Unisolve 1, BDH). Each artery section (see section 2.2.1.5) was moistened with 10 μl of water and then digested, at room temperature during approximately 12 hours, with 120 μl of a quaternary amine compound (NCS; Radiochemical Centre, Amersham). After digestion each sample was neutralised with 120 μl of a dilute solution of acetic acid (3.2×10^{-2} V/V) and then counted after adding 2.5 cm^3 of scintillant. Each sample was counted for a long enough period (up to 40 min) to obtain a precision better than 5%. No correction for quenching was necessary since the efficiency of counting was the same ($\pm 1\%$) both for tissue and perfusate samples. The background count was around 10 cpm.

3.1.3. Tracer uptake studies

The uptake of ^{131}I -HSA at 10 min was studied under transmural pressures of 30, 70, 100, 130, 150 and 180 mmHg for 3, 4, 3, 3 and 4 arteries respectively. The uptake of ^3H -D70 within 10 min was studied under 70, 130 and 180 mm Hg for 4, 2, and 2 arteries respectively. The RTRC profiles were obtained as described in section 2.3.3. The wall uptake was calculated using the data of Baldwin (1979) to determine the luminal surface area in the stressed state from the known unstressed area. Uptake of the inner half of the media was calculated for each pressure and each tracer.

3.1.4. Microscopic studies

Studies were made with two arteries to find out whether exposure to a transmural pressure of 180 mm Hg caused damage. One artery was perfused for 10 min with an inactive solution and then, while still in situ at the same transmural pressure was perfused with a solution of 0.3% EBD in saline for about 1 min. The other artery was perfused while in situ at a transmural pressure of 73.5 mm Hg with the same EBD solution after having been perfused for 10 min. The two arteries were then rinsed with saline, excised and their luminal surfaces were examined under a light microscope.

3.2 Results

3.2.1 Tracer studies

Fig. 23 shows the profiles obtained as a function of pressure. They reveal a very distinct increase in the relative concentration of tracer in the media with transmural pressure. Profiles obtained for 70 and 100 mm Hg were indistinguishable. The profiles obtained for 30 and 70 or 100 mm Hg were very similar in the inner half of the media but very different in the outer half. Comparing the 70 or 100 and 130 mm Hg profiles, one sees almost no change in the profiles on the outer side of the media but a very distinct change on the inner side. A comparison of the 130 and 150 mm Hg profiles reveals no difference in the outer half of the media. Concentrations in the inner half were much higher at the higher pressure but both fell continuously. Comparison of the 150 and 180 mm Hg profiles shows a clear-cut decrease on the outer side. Concentration in the immediately sub-intimal region was unchanged, but despite the reduction in the

outer side, it was higher at the lower pressure in the inner half of the media. The initial fall in concentration in the average profile for 180 mm Hg was much smaller than that for the 150 or 130 mm Hg profiles. For the 12 segments of 4 arteries perfused at 180 mm Hg, 7 gave a profile of type A, i.e. decreased monotonically in the first half of the media as in all profiles at pressures below 180 mm Hg. 5 gave a profile of type B, i.e. practically flat in the first half of the media. Sometimes both types of profile were obtained in the same artery. Fig. 24 shows two examples of profiles of type A and type B. The initial plateau for type B has an average value of 4.9% (S.D. = 1.9 %) and extends through a relative average distance from the artery lumen of 0.38 (S.D. = 0.07).

Fig. 25 shows the profiles obtained for $^3\text{H-D70}$ as a function of pressure. As with $^{131}\text{I-HSA}$, these profiles show a very definite relative increase in the concentration in the media with transmural pressure. A comparison of 70 and 130 mmHg profiles shows that at the higher pressure the concentration increased sharply for the entire media. Comparing the 130 and 180 mm Hg profiles, the concentration was observed to increase in the inner half but to decrease in the outer half. At 180 mm Hg (6 segments, 2 arteries), the profiles were always of type A.

Fig. 26 gives the result for both $^3\text{H-D70}$ and $^{131}\text{I-HSA}$. At each pressure, the concentration of $^3\text{H-D70}$ was between twice and three times that of $^{131}\text{I-HSA}$ throughout the inner half of the media.

Fig. 27 shows the variation with pressure of the average total medial uptake and the least mean square

regression line for both tracers. The slopes of these lines were found to be significantly ($p < 0.05$) different from zero. Increasing the transmural pressure tends to increase the uptake of ^{131}I -HSA and ^3H -D70 by the aortic media for 10 min periods.

3.2.2. Microscopic studies

EBD staining experiments with arteries perfused at 180 mm Hg showed that no dye was taken up by cell nuclei, indicating that the endothelial cells were not damaged either by the cannulation or by the high perfusion pressure (see 2.3.6). The artery stained under a pressure of 180 mm Hg was a darker colour than the one stained under a pressure of 73.5 mm Hg after prior exposure to 180 mm Hg.

3.3. Discussion

Experiments show that in general the total medial uptake and the local concentration of label within the media increases with pressure. This result is in agreement with earlier studies (see section 1.3.3.). However, the interpretation of the different profiles is not easy since the transport phenomena within and into the arterial wall are complex and their variation with transmural pressure is little understood. I feel that my results can only be properly interpreted with a mathematical model which would have to take into account the various parameters such as the structure of the arterial wall and transport phenomena in the intima, the media and the adventitia.

Bratzler et al (1977c) proposed a theoretical model for the in vivo transport of low density lipoproteins in the wall

of the rabbit thoracic aorta. This model ought to be generalised so as to include the influence of transmural pressure and effects of the type of the macromolecular tracer employed. Some information can be extracted from my results even without recourse to such a model which would be highly artificial (as very little information about the various parameters involved is available).

The total wall uptake increased with transmural pressure (see Fig. 27). It is possible that this increase is the result either of a change in the mechanisms of the transport of macromolecules with increasing pressure or of a dependence on the pressure of the characteristics of fixed transport phenomena.

The considerable increase in the concentration within the inner half of the media at 180 mm Hg as compared with a 70 or a 100 mm Hg pressure looks like that obtained after inflicting deliberate intimal damage (see Fig. 19). Were the arteries perfused in situ damaged by arterial pressures above 70 or 100 mm Hg? The answer is 'no'. Firstly, histological studies exclude any possibility of extensive intimal damage. Secondly, and more importantly, profiles obtained for two arteries perfused with ^{131}I -HSA for 10 min at 70 mm Hg after perfusion for 10 min at 180 mm Hg with an inactive solution were indistinguishable from those obtained when arteries were directly perfused at 70 mm Hg with the same solution.

Transport of macromolecules within the arterial wall can be diffusive or convectional (see section 1.3.3.). Bratzler et al (1977a) indicated that for albumin at normal physiological blood pressure the role of convection is negligible. These workers pointed out that an approximate estimate of the

diffusive permeability of the intimal endothelium at normal physiological blood pressure is given by the luminal uptake divided by the time of perfusion when relatively little solute has entered the aortic wall. With my experimental results this calculation yields a diffusive permeability for ^{131}I -HSA at 70 - 100 mm Hg of $0.47 \times 10^{-7} \text{ cm s}^{-1}$ (S.D. = $0.18 \times 10^{-7} \text{ cm s}^{-1}$). The shape of the type B profile (see Fig. 24) obtained at high pressure is consistent with convection being dominant at raised pressures. Diffusional phenomena would only round off the abrupt variation in the tracer concentration at the level of the penetration front. The height of the plateau obtained for B type profiles could then be a measure either of the accessible volume within the media, which would thus be 4.9% at 180 mm Hg - or of the value for transendothelial tracer transport at 180 mm Hg - which would be equivalent to $3.1 \times 10^{-3} \mu\text{l}$ of perfusate transported through the intima per cm^2 of intimal surface under stress per second.

It has been reported (Vargas et al, 1979) that hydraulic conductivity increases with intimal damage. One should thus expect to be able to observe an important effect upon convection at pressures below 180 mm Hg but only in arteries with a damaged intima. This prediction is borne out by the fact that profiles obtained in damaged arteries perfused with ^{131}I -HSA solutions during 10 min (see Fig. 19) were of type B.

If one considers that in the case of the type B profile the tracer moves within the accessible volume at a constant velocity, one could estimate this velocity - per unit of hydrostatic pressure - using the distance of the concentration front from the lumen of the artery. With my experimental

results for undamaged arteries at 180 mm Hg and damaged arteries at 73.5 mm Hg this calculation yields tracer velocities of 3.5 and $6.8 \times 10^{-8} \text{ cm s}^{-1}/\text{mm Hg}$ respectively. Using a manometric method Yamartino et al (1974) found a value for the hydraulic conductivity in the rabbit aorta in situ which leads to an average water velocity of $7 \times 10^{-8} \text{ cm s}^{-1}/\text{mmHg}$ assuming a relative water space of 0.58 within the media (Bratzler et al. 1977a). This value is in the same order of magnitude to that found in this study for the tracer velocity within undamaged arteries. It is also interesting to note that in the present work an increase in the tracer velocity - per unit of hydrostatic pressure - within the media with intimal damage was found to be 94%. This increase is very similar to the increase of 90% reported for the hydraulic conductivity of rabbit aorta with intimal damage (Vargas et al, 1979). These results give further support to the view that convection phenomena could be important either at high pressures or when the intima is damaged.

It is very likely that the perfusion of the vasa vasorum is pressure dependent and may be low when transmural pressure was 30 mm Hg. This behaviour would explain the very low concentration of tracer found at low pressure (30 mm Hg) in the outer half of the media (see Fig.23). At 180 mm Hg a constant decrease in concentration in comparison with 150 mm Hg was found. This decrease was very pronounced in type B profiles. If convection favours tracer penetration within the media, from the intima, it would tend to oppose penetration from the vasa vasorum. This effect would become more pronounced as convection becomes more important.

Fig. 28 gives the uptake of ^{131}I -HSA within the entire inner half of the media as a function of transmural pressure. It does not necessarily represent the amount of tracer within the media originating from the lumen. However, because the concentrations on the external side of the media are similar at different pressures - for instance at 70, 100, 130 and 150 mm Hg - the increase in the uptake within the inner half of the media probably represents an increase in luminal uptake with the transmural pressure. The curve shows an abrupt rise in luminal uptake around 130 mm Hg. My results indicate that for ^{131}I -HSA this represents the pressure at which convective transport dominates the transport by diffusion within the media of the rabbit thoracic aorta. One could ask if the influence of convective transport within the media could explain, at least partly, the increase in luminal uptake caused by hydrostatic pressure and by intimal damage. Indeed, convection within the media can be increased either by increasing hydrostatic pressure or by damaging the intimal surface. In this hypothesis the intima acts as a barrier to macromolecular uptake under a normal physiological pressure by limiting the hydraulic conductivity of the arterial wall.

It has been frequently indicated that circulating lipids associated with lipoproteins play an important role in atherogenesis (see, for example, Stamler, 1973). The various lipoproteins have different structures and metabolic characteristics (see, for example, Jackson et al, 1976) and their influence upon the initiation of atheromatic plaques are very variable (see, for example, Miller et al, 1977).

It is probable that transmural pressure or intimal damage which modifies the penetration of albumin into the arterial wall, can modify lipoprotein penetration in the same way. This effect would vary according to the lipoprotein involved. By interfering with lipid metabolism of the arterial wall, this could influence atherogenesis.

It is clear that the sharp increase in the intimal permeability found for ^{131}I -HSA is subject to a pressure threshold value. It is interesting to note, in this context, that a systolic pressure of 160 mm Hg or a diastolic pressure of 100 mmHg are considered by the World Health Organisation to be lower limits of arterial hypertension. This condition and hyperlipidemia are major risk factors in atherosclerosis. The present study suggests that arterial transport of macromolecules could well be the link between these risk factors.

SUMMARY

There are reasons to believe that mechanical factors play an important role in atherogenesis. It appears that these factors may be able to modify arterial molecular transport and thus the metabolic equilibrium of the arterial wall. The aim of this study was to examine the short-term effect of steady wall shear stress and transmural pressure on the uptake of macromolecules by the arterial wall.

An in situ preparation was developed for the perfusion of the descending part of the rabbit thoracic aorta under well-defined and steady mechanical stresses. The technique made it possible to maintain the artery in a state very close to the physiological. Histological tests showed that the intima, which is known to be fragile, was not damaged. Arteries were perfused at 40°C with Tyrode's Solution (adjusted to normal colloidal osmotic pressure) to which was added a radioactive macromolecular tracer.

Macromolecules can enter the medial part of the artery from the lumen and from the vasa vasorum. The effect of mechanical factors upon the uptake of macromolecules from the lumen was in particular studied. To establish their effect, it was necessary to determine the concentration profiles of the tracer within the media for each artery. After perfusion under stress, the arteries were immediately excised, frozen and then cut into sections (20 µm thick) parallel to the intimal surface. The concentration profiles were determined from the concentration of the tracer within each section.

Human serum albumin labelled with iodine-131 (^{131}I -HSA)

and tritiated dextran of molecular weight around 70,000 ($^3\text{H-D70}$) were used as tracers. They were carefully purified by ultrafiltration in order to eliminate radioactive species of low molecular weight. Study of $^3\text{H-D70}$ in solution showed that it could breakdown to produce tritiated water and tritiated dextran of lower molecular weight. It was also shown that the decomposition of $^3\text{H-D70}$ and $^{131}\text{I-HSA}$ which occurred, had almost no influence on the concentration profiles.

Profiles of the activity as a function of time for $^{131}\text{I-HSA}$ showed that the media had not reached an equilibrium after perfusion under a transmural pressure of 73.5 mm Hg for a 10 min duration. Furthermore, luminal uptake was calculated from these profiles.

Intimal damage increased the uptake of $^{131}\text{I-HSA}$ from the lumen under a transmural pressure of 73.5 mm Hg by a factor of about 13. This confirmed that the intima is a major barrier to the penetration of macromolecules into the media. Examination of the profiles obtained as a function of time using arteries in which the intima was intentionally damaged showed that the immediate accessible volume to $^{131}\text{I-HSA}$ within the media of those arteries was approximately 9%.

A special apparatus was designed in order to study the effect of steady wall shear stress. The design allowed perfusion of arteries with a variable flow rate under a constant transmural pressure. Particular attention was given to the cannulae used for perfusion of arteries to ensure Poiseuille flow within the arterial segment. Steady wall shear stress was calculated from the diameter of the artery, the flow rate and the viscosity of the perfusion liquid. Under a transmural pressure of 73.5 mm Hg the wall shear stress was

varied between 0 and 75 dyne cm^{-2} . The latter value was not so great as to cause damage to the intima. Within this range wall shear stress did not significantly effect the luminal uptake to ^{131}I -HSA, the mean value being $2.8 \times 10^{-5} \text{ cm}$ (S.D. = $1.1 \times 10^{-5} \text{ cm}$; 27 arteries). It was also shown that intimal ultrastructure of an artery fixed under a wall shear stress of 42 dyne cm^{-2} and a transmural pressure of 73.5 mm Hg were indistinguishable from that of others fixed under zero shear stress and at the same pressure.

Luminal uptake to ^{131}I -HSA measured in vivo was not significantly different from that in situ. This confirms that the intima of arteries perfused in situ is not damaged. It also shows that the effect of oscillations due to the action of the heart were negligible.

Problems arising from the use of in vitro technique do not permit comparisons of my results with those obtained with this type of technique. Nevertheless, the present work does not invalidate theories which try to explain the distribution of atheromatic plaques in the arterial tree by focal variation in wall shear stress. It shows however that if wall shear stress has an effect upon arterial transport of macromolecules (other than from intimal damage), this effect would not be detectable except at long-term. Furthermore, the possibility of an effect of wall shear stress on arterial transport of molecules such as cholesterol associated with lipoproteins is not excluded.

The effect of hydrostatic pressure in the range 30 - 180 mm Hg on the uptake of ^{131}I -HSA and ^3H -D70 in 10 min experiments was studied with an apparatus similar to that used in the study of the effect of wall shear stress.

The results showed that hydrostatic pressure increases markedly the medial uptake of ^{131}I -HSA and ^3H -D70. However, between 70 and 100 mm Hg this increase was insignificant. The increase of uptake with pressure is readily reversible which shows that it is not caused by intimal damage. Profiles measured under various transmural pressures provided new information. At 180 mm Hg the form of the profiles obtained for ^{131}I -HSA changed in comparison with profiles at lower pressures. The uptake from the lumen was found to increase sharply at pressures around 130 mm Hg. These results are interpreted as being due to transport by convection dominating transport by diffusion at high pressures.

The study of the effect of transmural pressure supports the hypothesis that arterial transport of macromolecules plays an important role in atherogenesis. Transport phenomena could well be the link between two main risk factors of atherogenesis: hypertension and hyperlipidemia.

Flow rate [$\text{cm}^3 \text{ s}^{-1}$]	Viscosity [cp]	Reynolds No.	Wall shear rate [s^{-1}]	Wall shear stress [dyne cm^{-2}]	Luminal uptake [cm] ($\times 10^5$)
0.47	1.9	79	75	1.4	2.5
0.81	2.0	129	129	2.6	2.2
0.69	3.7	59	110	4.1	0.8
0.95	3.1	98	151	4.7	1.6
2.0	2.7	236	3.8	8.6	5.0
3.4	1.9	570	541	10.3	2.7
4.3	1.9	720	484	13.0	2.9
5.3	2.0	844	844	16.9	4.4
6.5	1.9	1089	1035	19.7	4.4
7.1	1.9	1189	1180	21.5	4.0
8.1	1.8	1432	1280	23.2	1.9
7.8	1.9	1307	1241	23.6	3.2
7.4	2.0	1178	1178	23.6	3.7
6.9	2.7	813	1098	29.7	2.6
8.4	3.1	863	1337	41.4	2.5
8.2	3.7	705	1305	48.3	0.8
11.6	2.7	1368	1846	49.8	1.6
12.6	2.8	1432	2005	56.1	2.9
14.1	2.8	1603	2244	62.8	1.8
15.4	2.7	1816	2451	66.2	2.6
17.6	2.5	2241	2801	70.0	3.1
18.8	2.5	2394	2992	74.8	3.5

Table 1

Values of the hydrodynamic parameters and of corresponding luminal uptake for all arteries used to study shear dependent uptake of ^{131}I -HSA

Species	Elution volume [cm ³]
Blue dextran	59 (Void volume)
Dextran 500,000	85
Rabbit albumin	115
Dextran 70,000	120
Cytochrome C	163
³ HHO	178)
¹²⁵ I ⁻	178) (Total volume)
Tryptophane	178)

Table 2

Elution volume of various species used to calibrate
the chromatographic column

	Artery No.1	Artery No.2
Distance between side arms [cm]	6.25	6.50
Viscosity [cp]	2.70	2.70
$\frac{\Delta P}{Q}$ [dyne cm ⁻³] given by eqn. 2	268.00	279.00
Slope of the regression line [dyne cm ⁻³ s]	270.00	293.00
t-test : t	2.52	2.22
p	~0.02	~0.04
Correlation coefficient	0.995	0.994

Table 3

Data obtained with the two arteries used to study the relationship between the flow rate and the pressure difference between the side arms

Distance [μm] from the luminal surface	Average concentration value			Standard deviation
	Range of shear stress [dyne cm^{-2}]			
	0-17	20-41	48-75	
10	1.4	1.8	1.6	0.5
20	1.1	1.5	1.3	0.3
40	0.75	1.0	0.8	0.2
60	0.50	0.80	0.6	0.15
80	0.45	0.55	0.65	0.17
100	0.85	0.75	1.0	0.4
120	2.20	1.80	2.05	0.75
140	4.6	3.7	4.0	1.0
160	7.7	6.1	7.0	2.5

Table 4

Average concentrations interpolated from each artery segment at distance 10, 20 μm and then at 20 μm intervals from the luminal surface function of wall shear stress

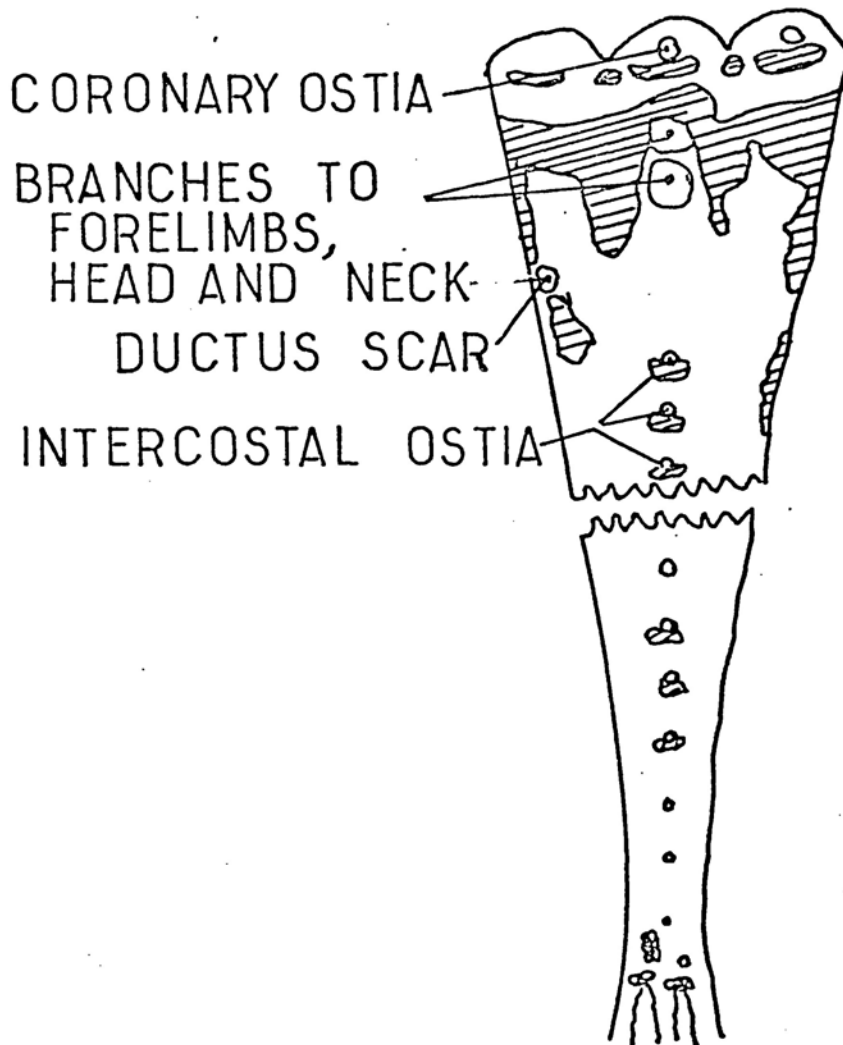


Fig.1 Patterns of Evans Blue uptake in the aorta of the pig.
(from Bell et al, 1972)

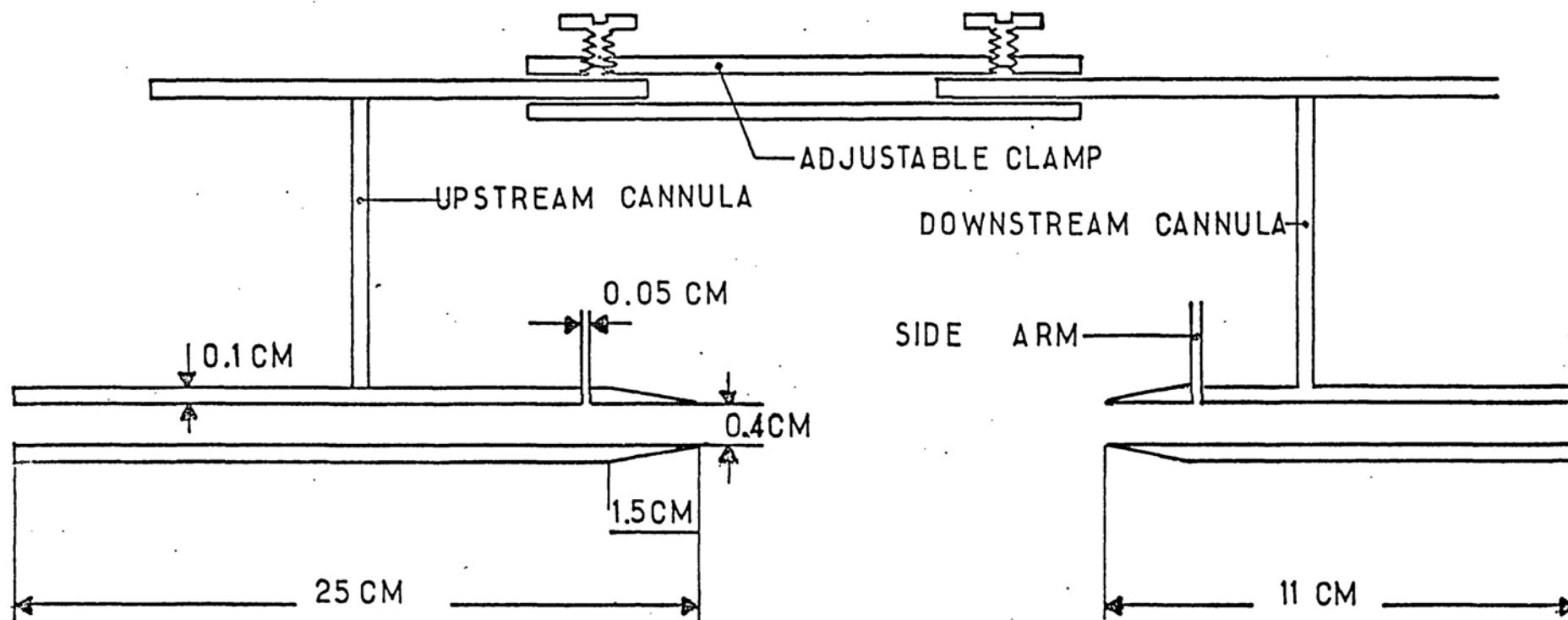


Fig.2 Schematic longitudinal section of the cannulae.

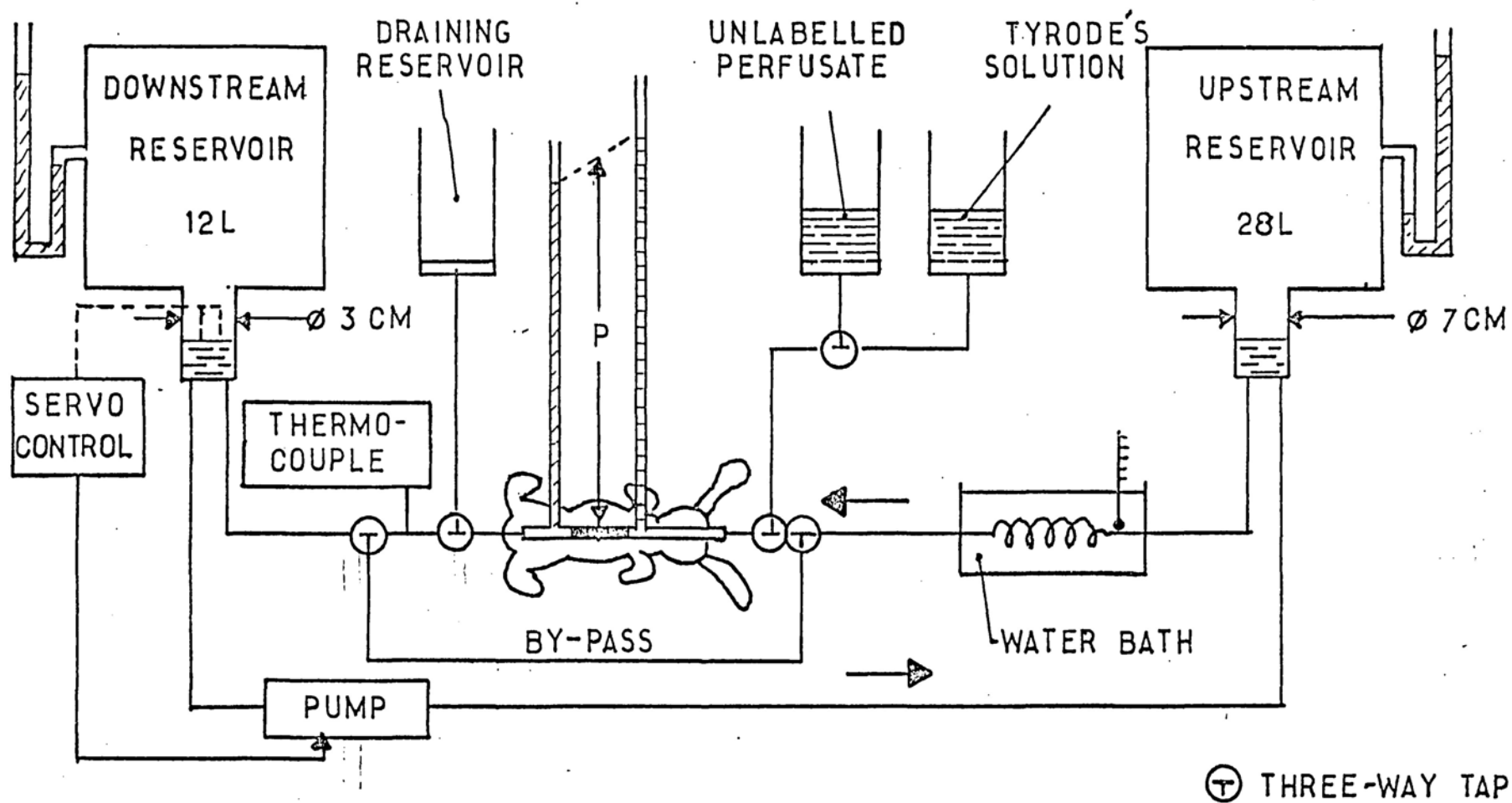


Fig 3 Perfusion system used for the measurement of wall shear stress dependent uptake.

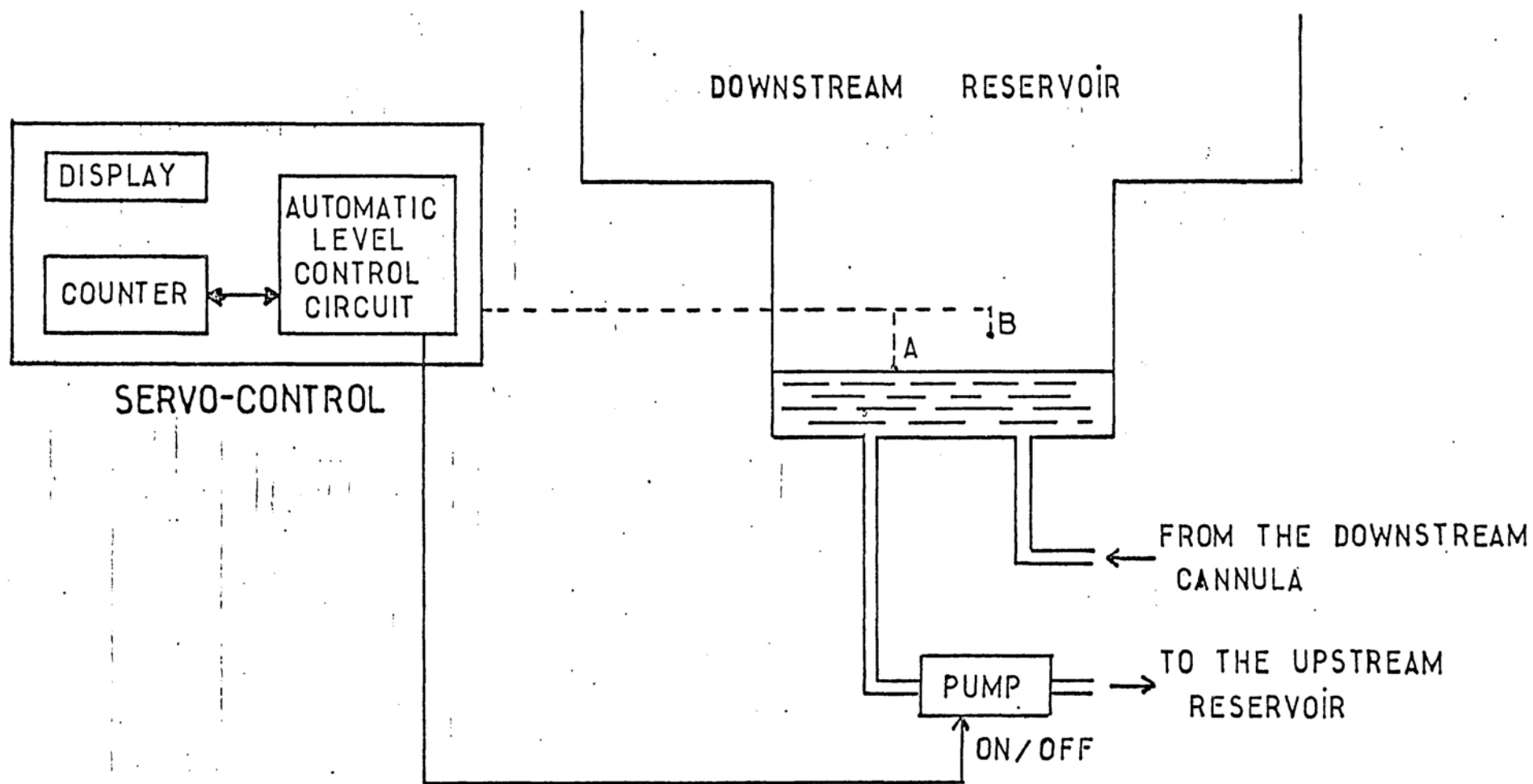


Fig.4 The servo device used to keep constant the flow rate through the perfused segment of artery.

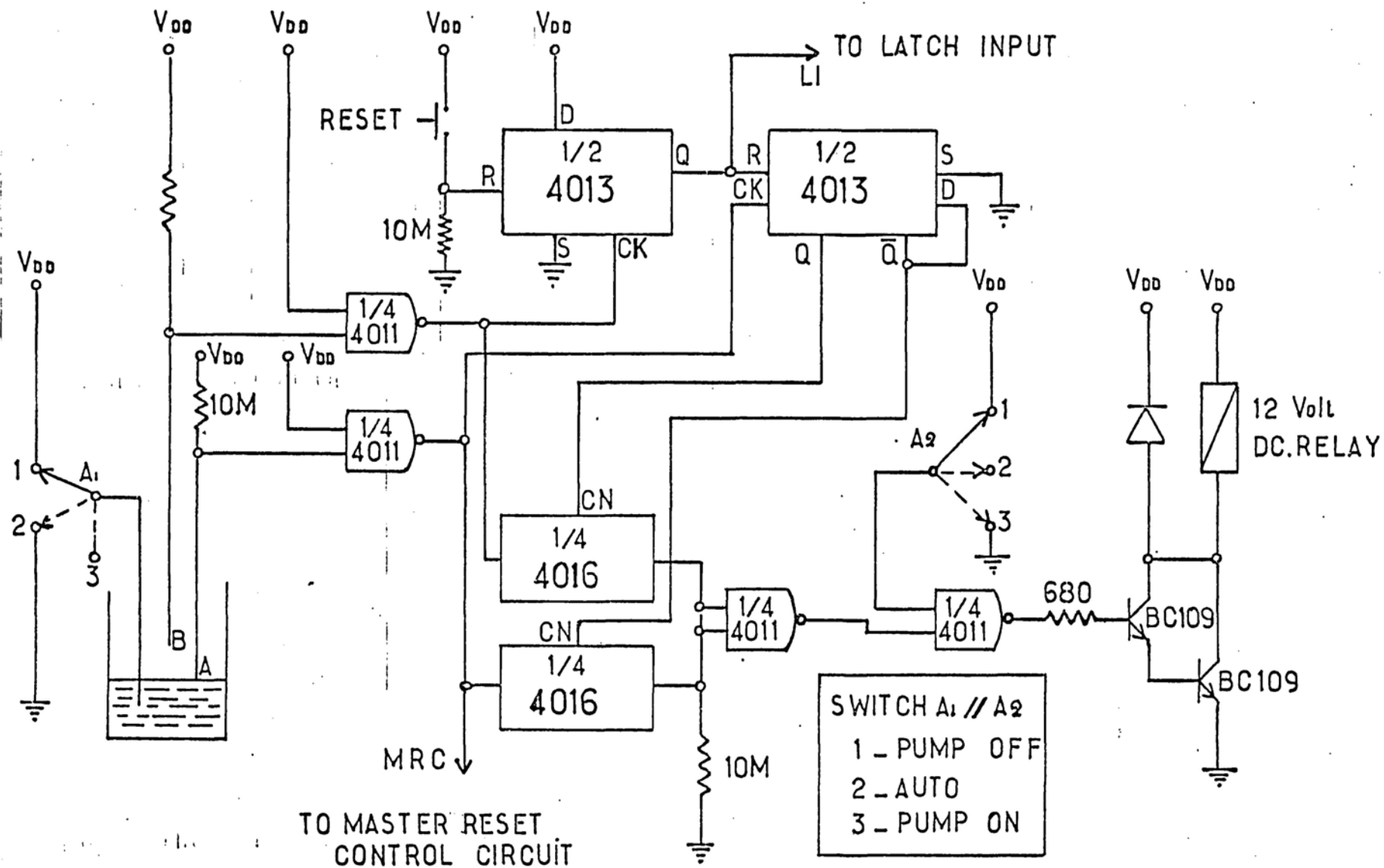


Fig.5 The automatic level control circuit of the servo control.

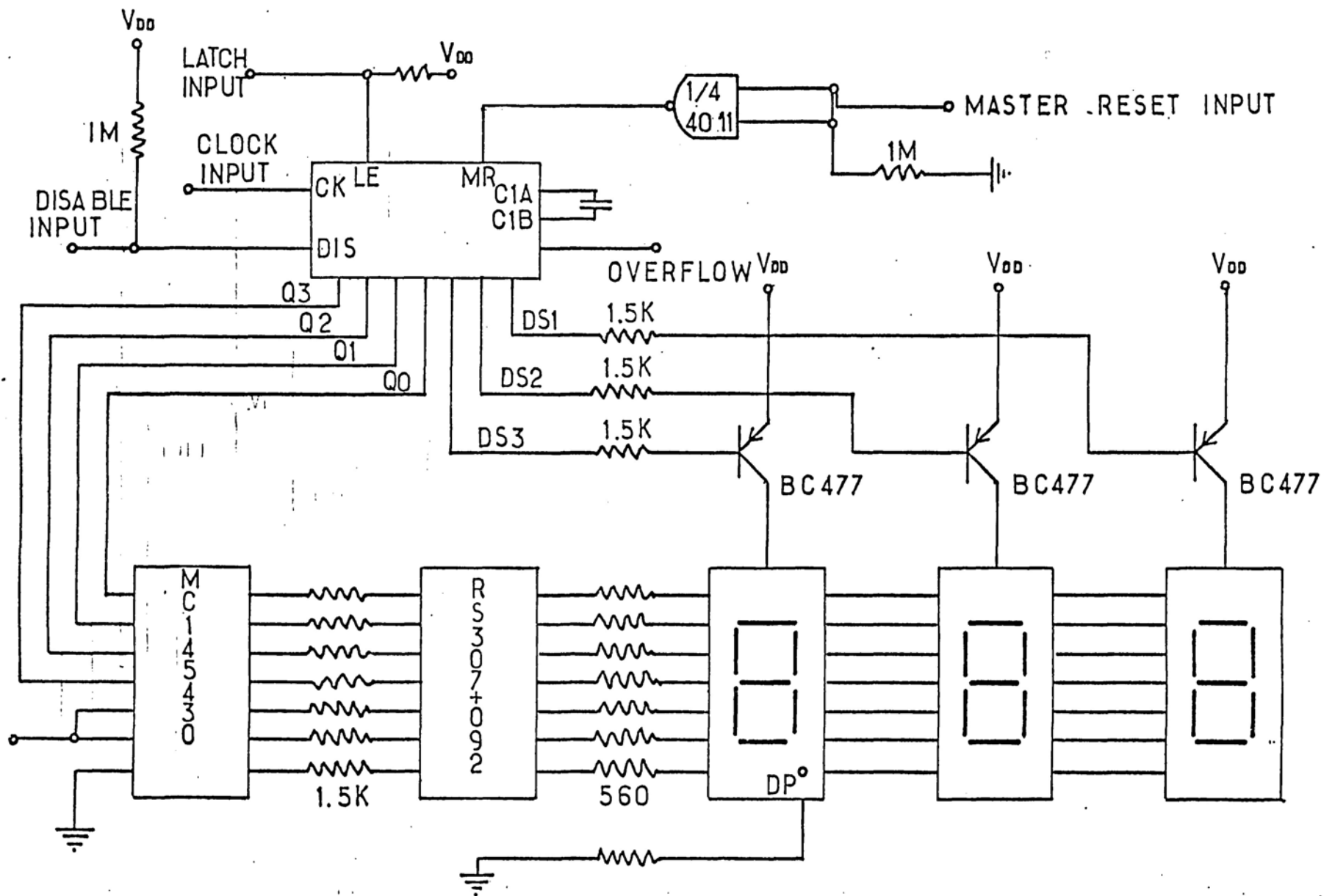


Fig.6 The counter circuit and the display of the servo control

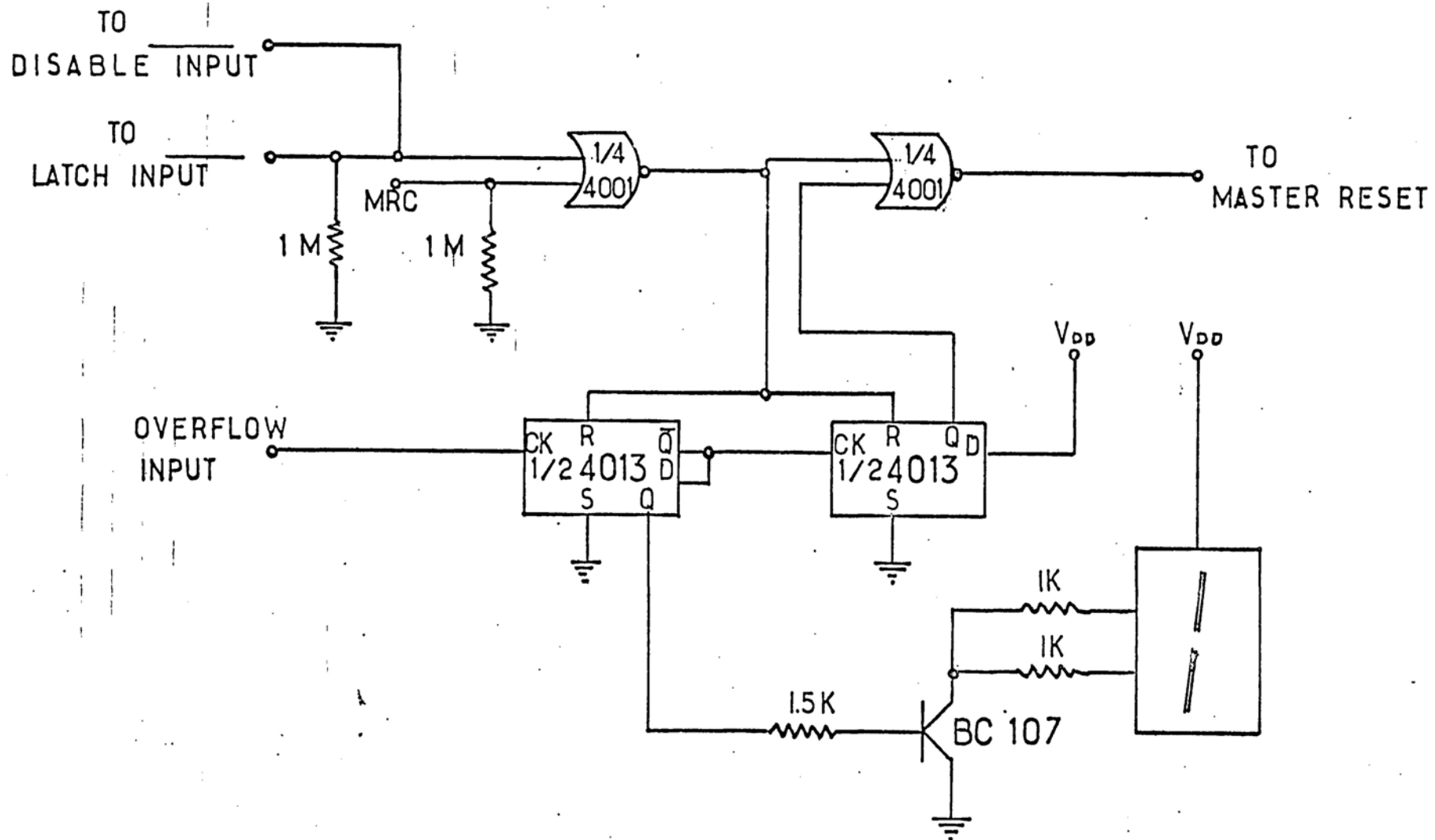


Fig.7 The master reset control circuit and the 1/2 digit logic drive of the servo control

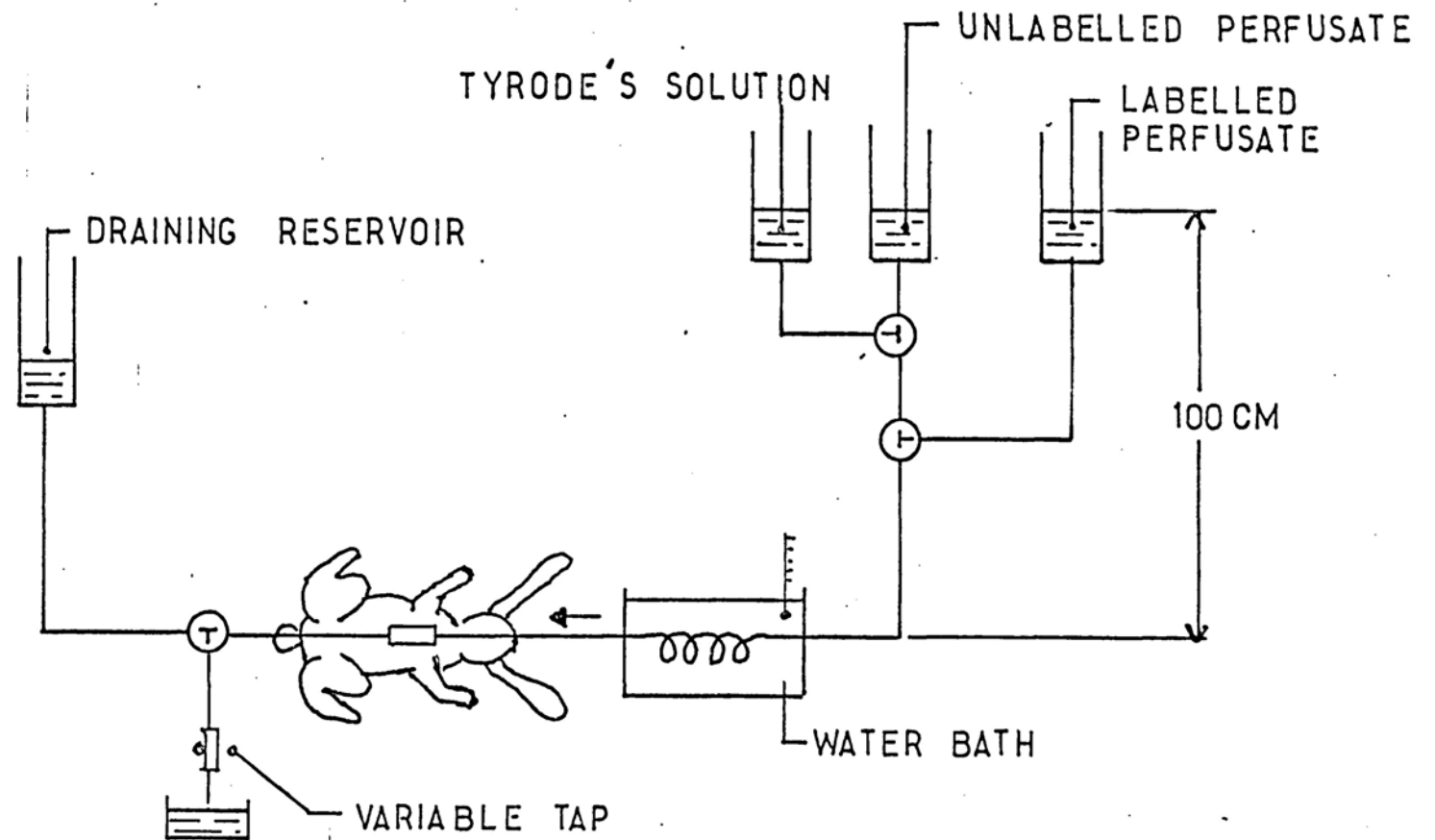


Fig.8 Perfusion system used for the measurement of time course dependence of uptake

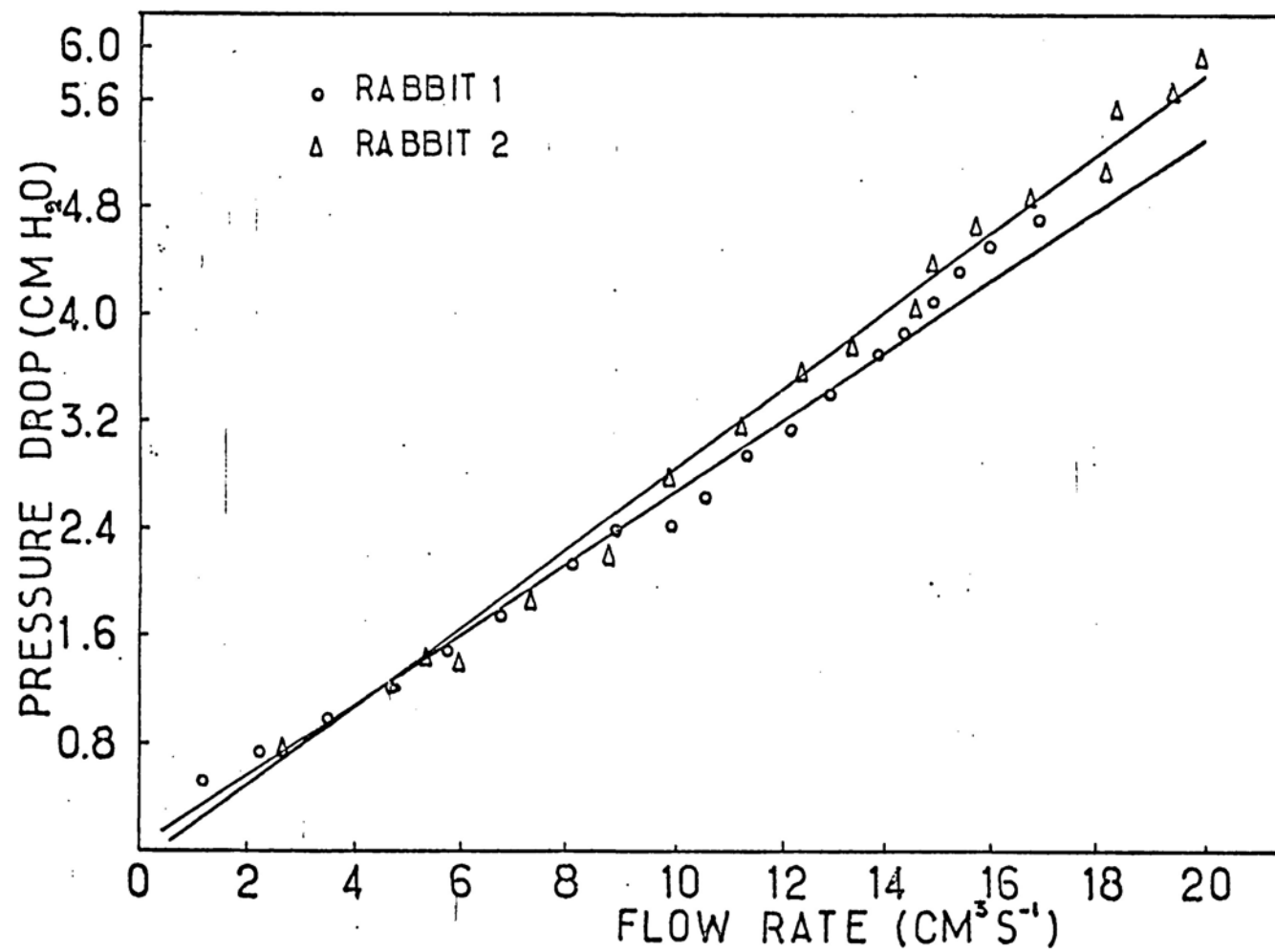


Fig.9 Relation ship between the flow rate and pressure difference between the side arms

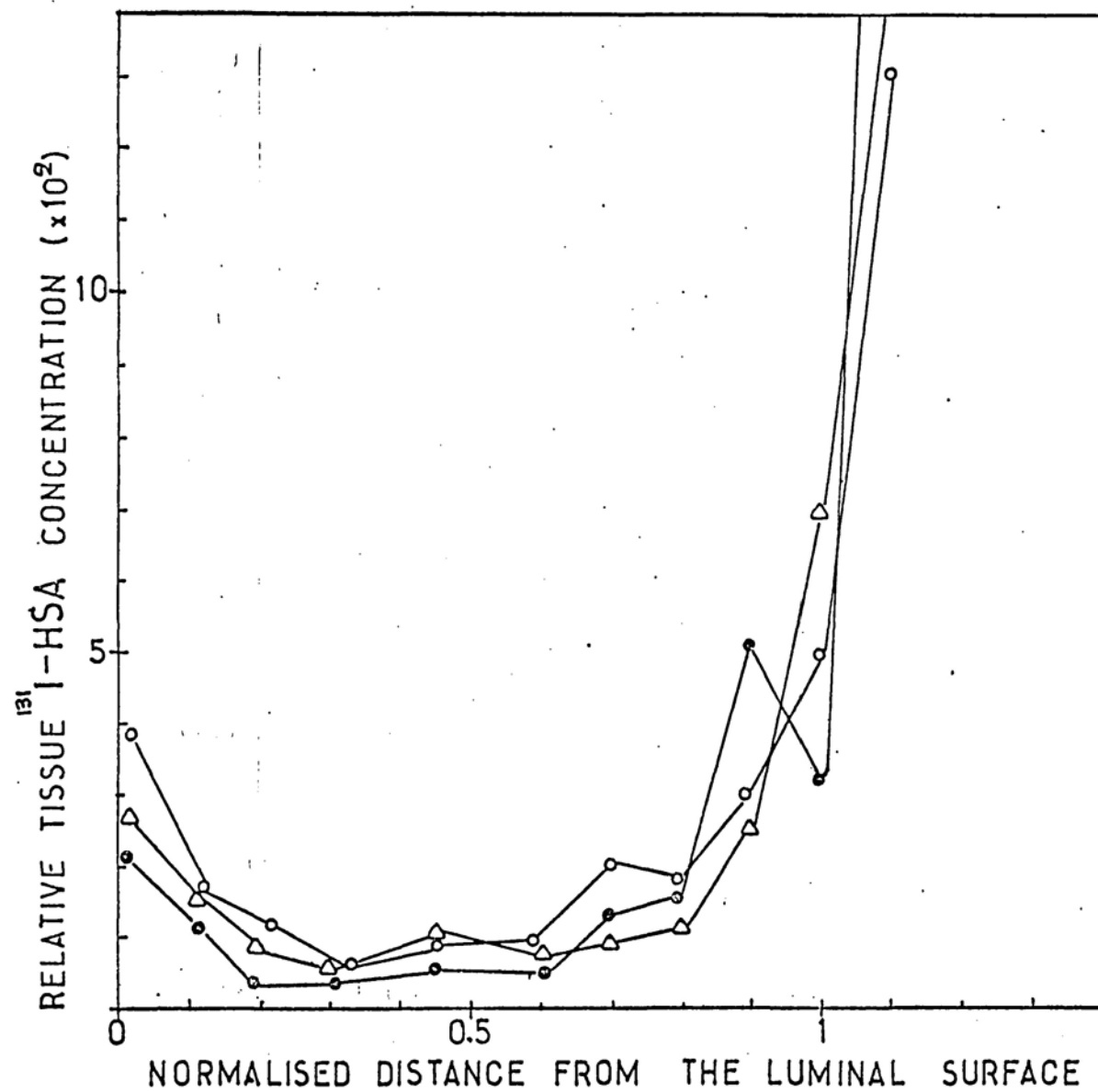


Fig.10 A typical concentration profile for labelled albumin after 10 min. perfusion in three segments cut from a single artery.

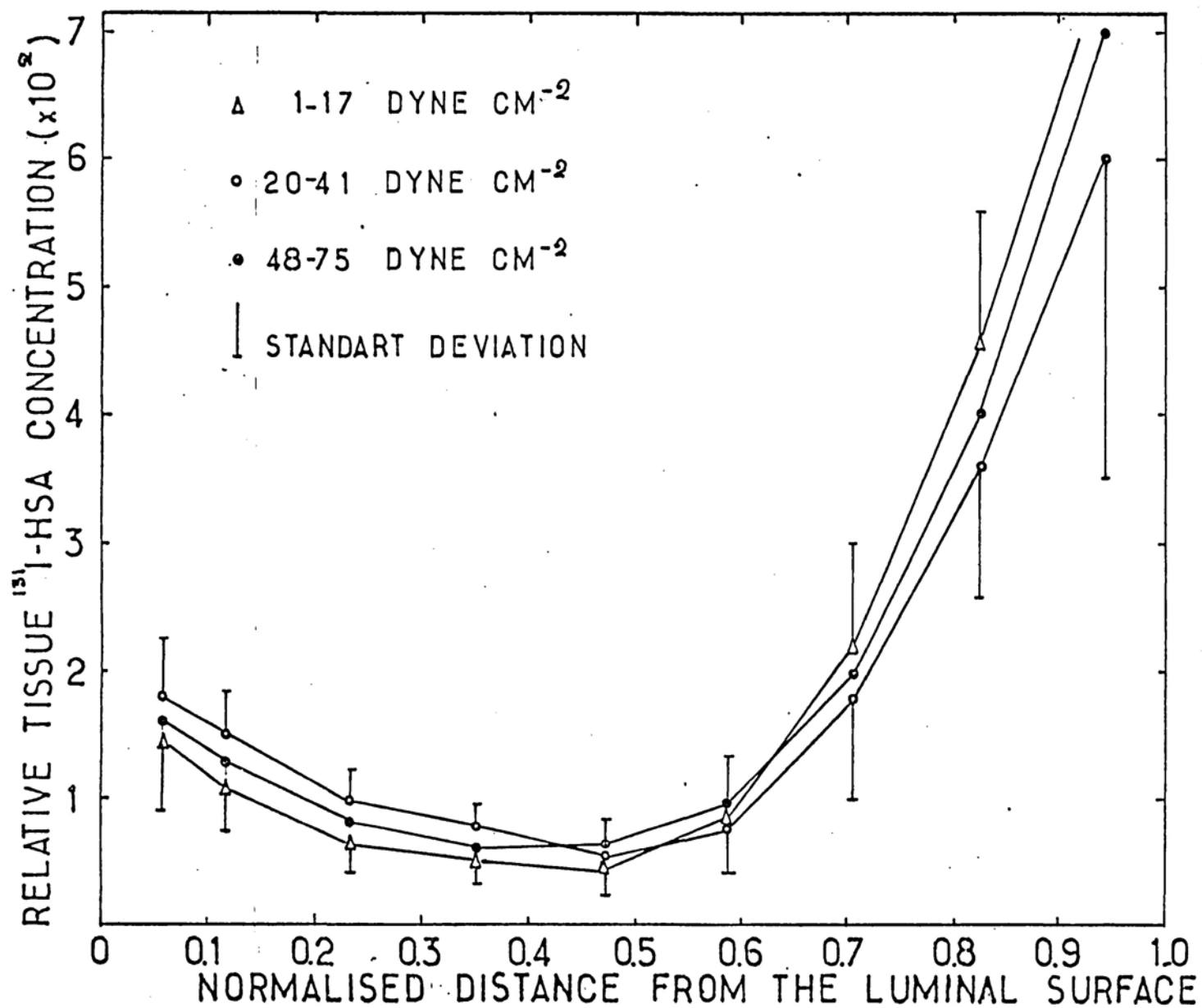


Fig.11 ¹³¹I-HSA concentration profiles vs wall shear stress.

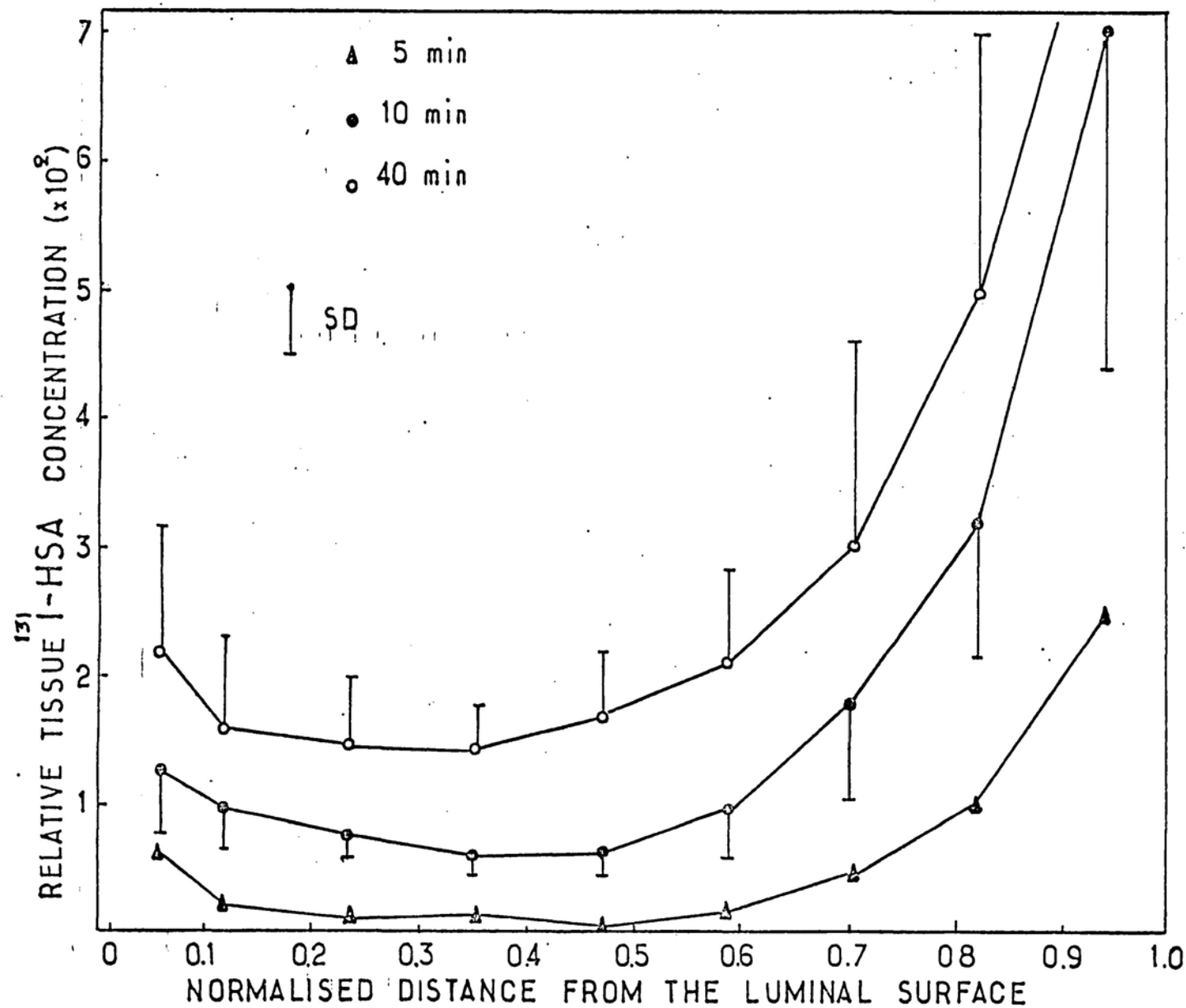


Fig.12 ^{131}I -HSA concentration profiles vs. the time of perfusion.

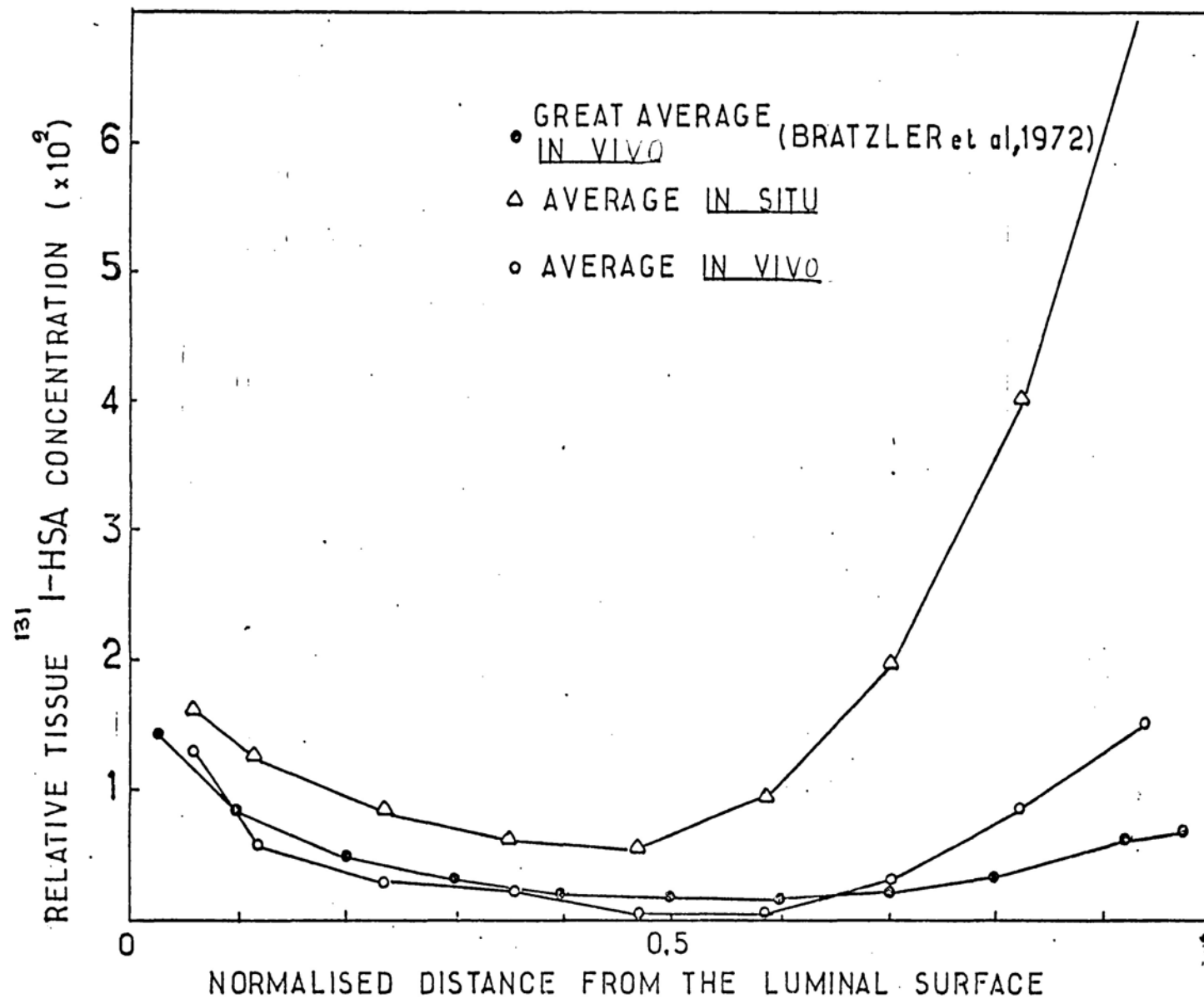
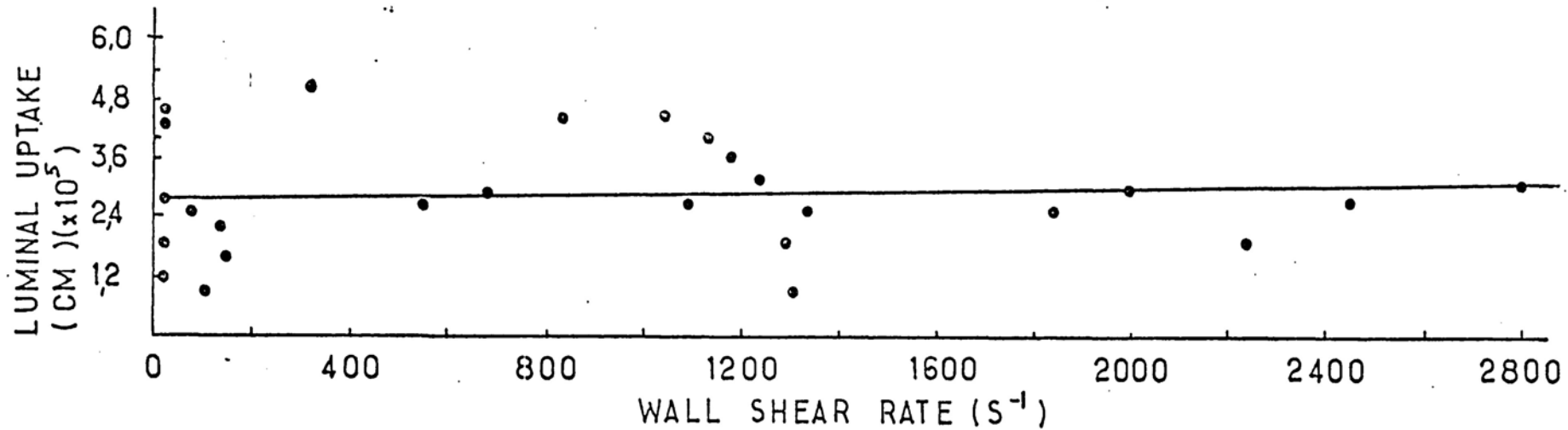


Fig.13 ^{131}I -HSA concentration profiles in vivo and in situ.

SLOPE: 5.3×10^{-3} CMS
 CONFIDENCE INTERVAL OF THE SLOPE (0.05):
 -46×10^{-2} , $+56 \times 10^{-3}$ CM S



SLOPE $-2.5 \times 10^{-1} \text{ CM}^3 \text{ DYNE}^{-1}$

CONFIDENCE INTERVAL OF THE SLOPE (0.05): $-22, +1.6 \text{ CM}^3 \text{ DYNE}^{-1}$

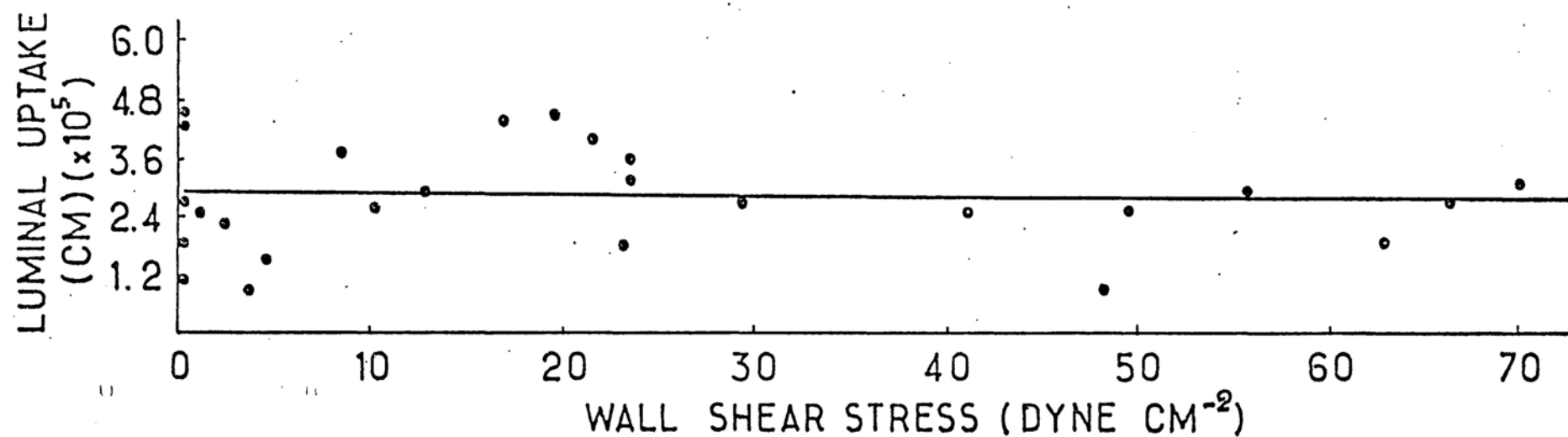


Fig. 15 Luminal uptake vs. wall shear stress.

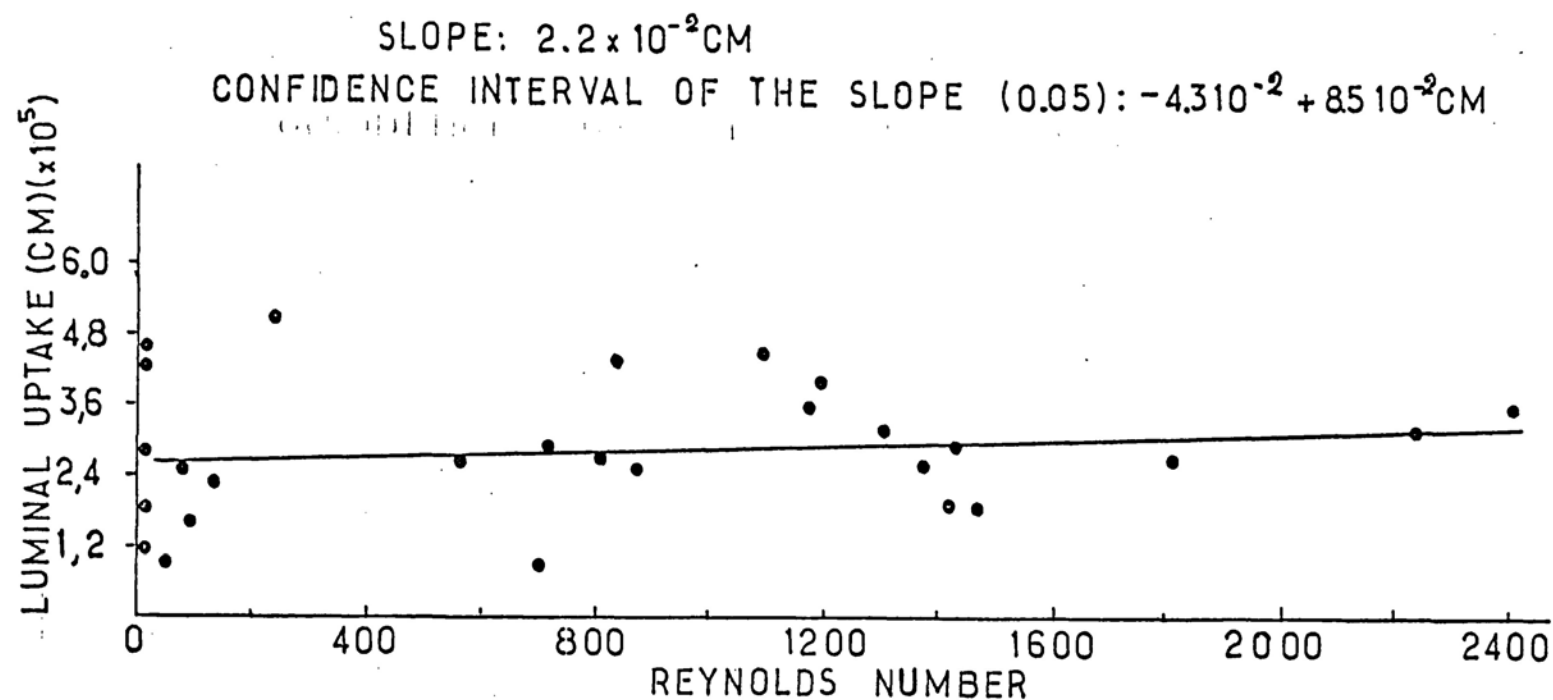


Fig.16 Luminal uptake vs.the Reynolds number of the perfusing solution.

SLOPE -84 CM cP^{-1}
 CONFIDENCE INTERVAL OF SLOPE (0.05): $-150 +12 \text{ CM cP}^{-1}$

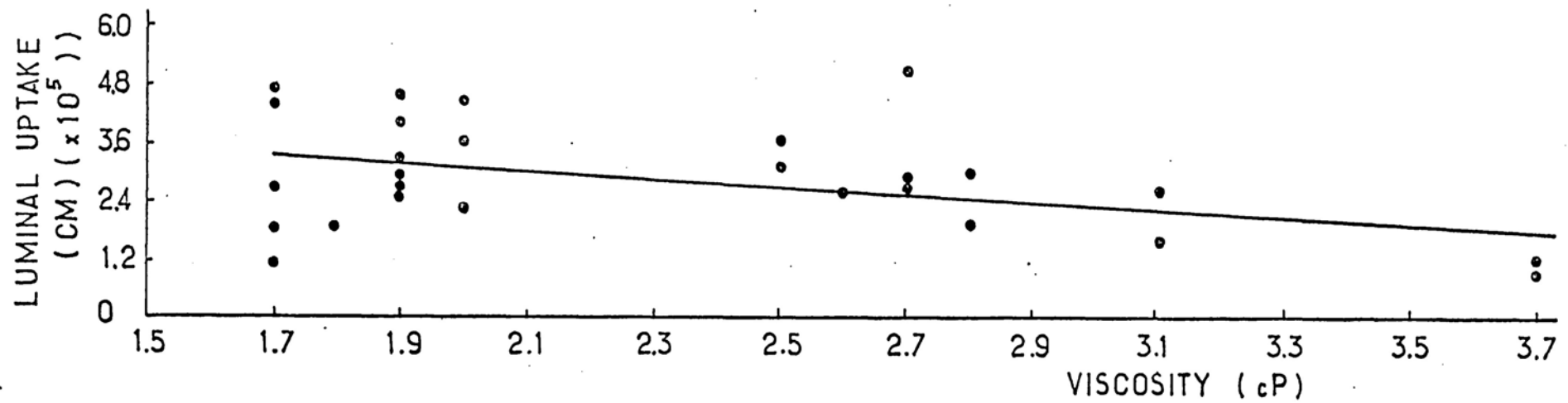


Fig.17 Luminal uptake vs. the viscosity of the perfusing solution.

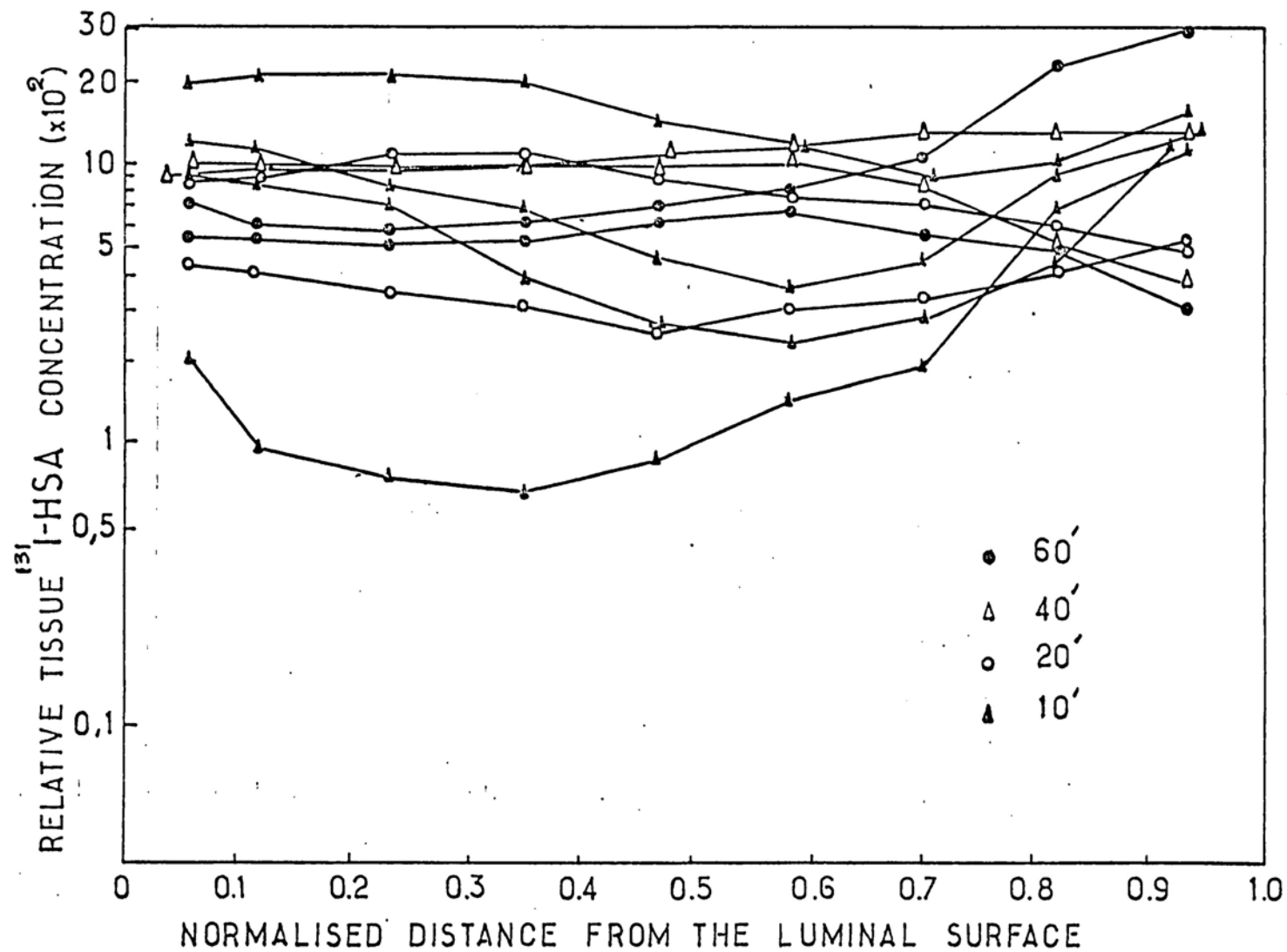


Fig.18: ^{131}I -HSA
concentration profiles
for damaged arteries
vs.time of perfusion.

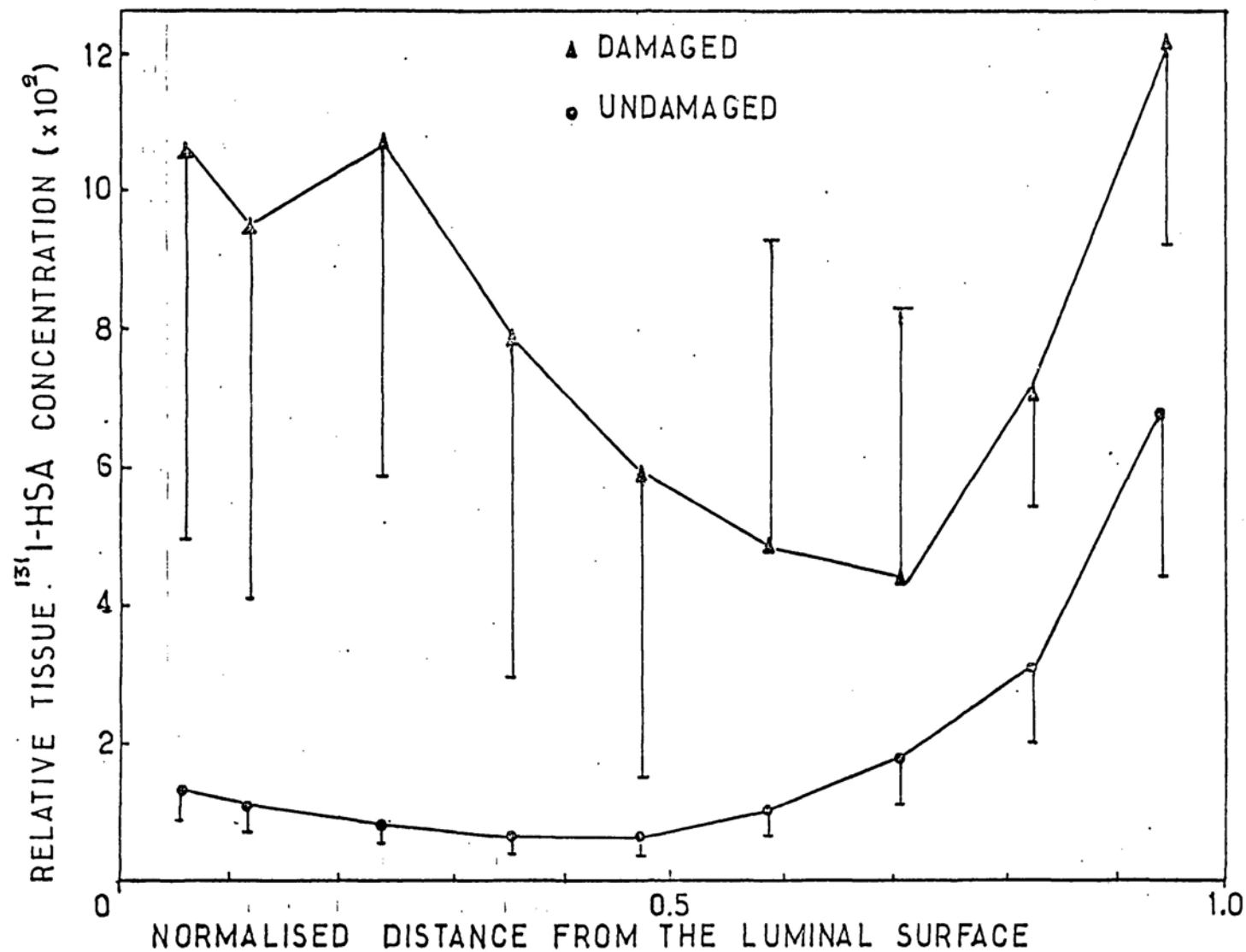


Fig.19 ^{131}I -HSA concentration profiles for damaged and undamaged arteries.



Eig_20 Transmission electron micrograph of the intimal region of an arterie fixed during exposure to high shear stress.

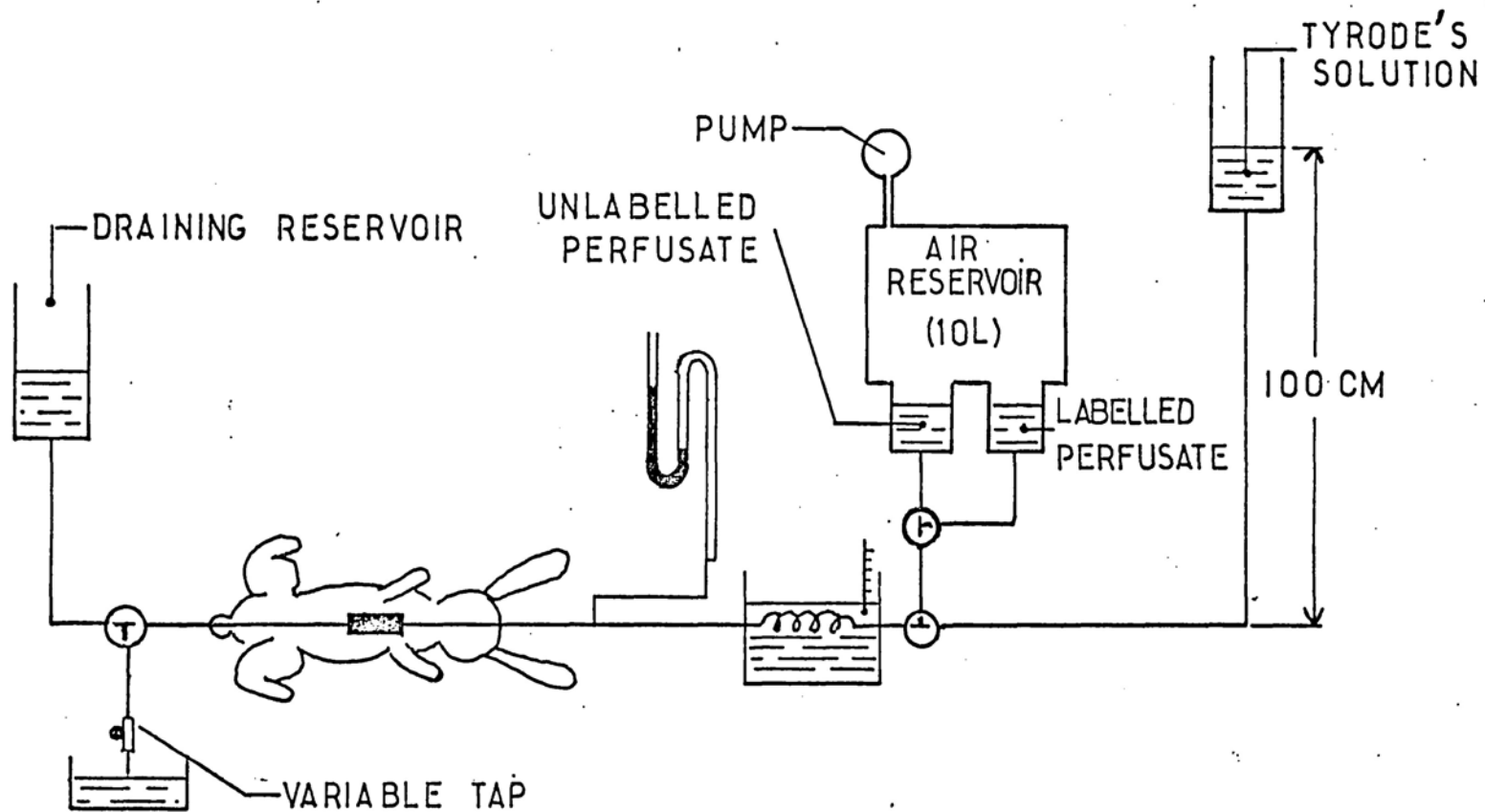


Fig.21 Perfusion system used for the measurement of transmembrane pressure dependent of uptake.

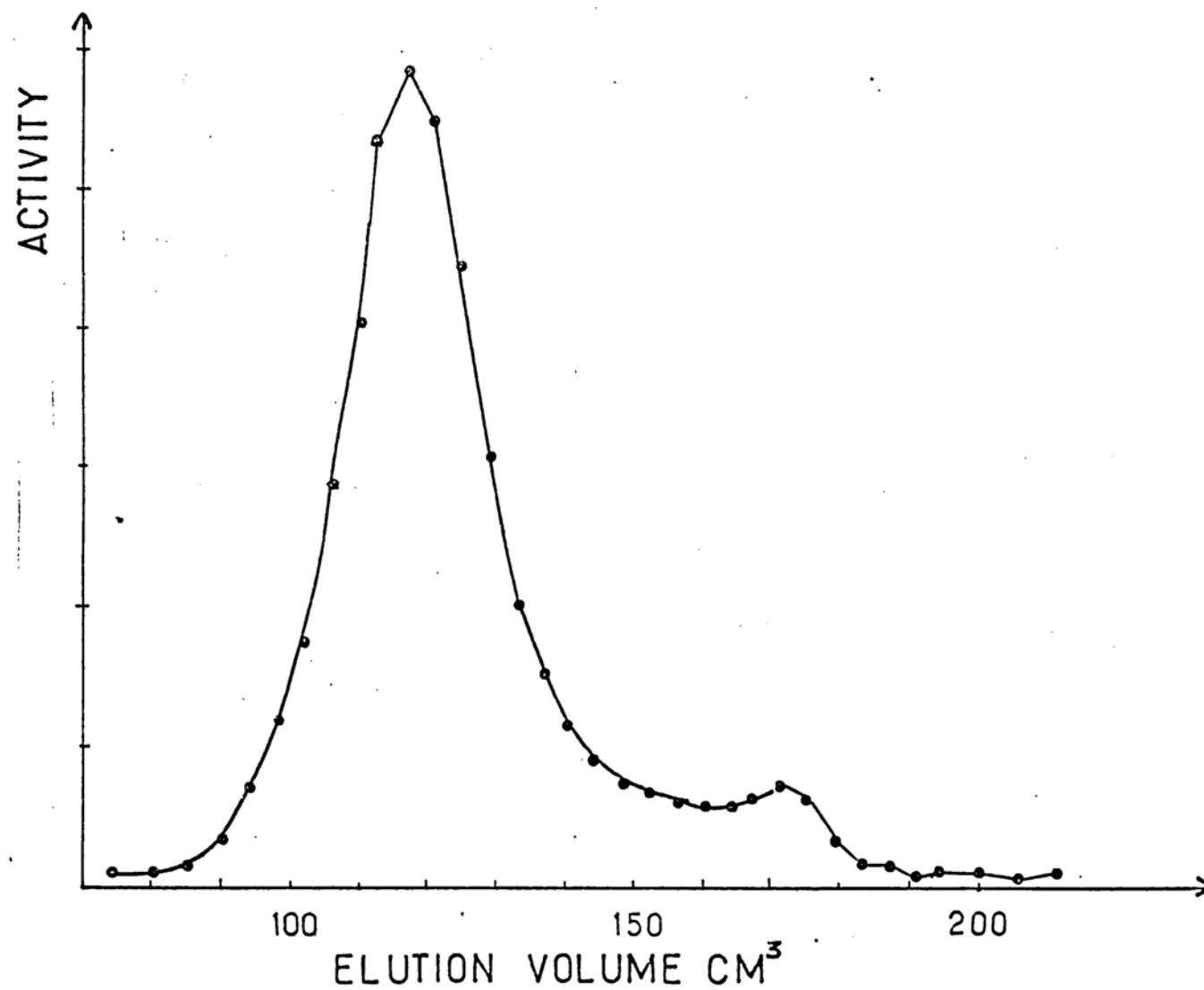


Fig.22 Typical activity profile of a ^3H -D70 solution after column chromatography.

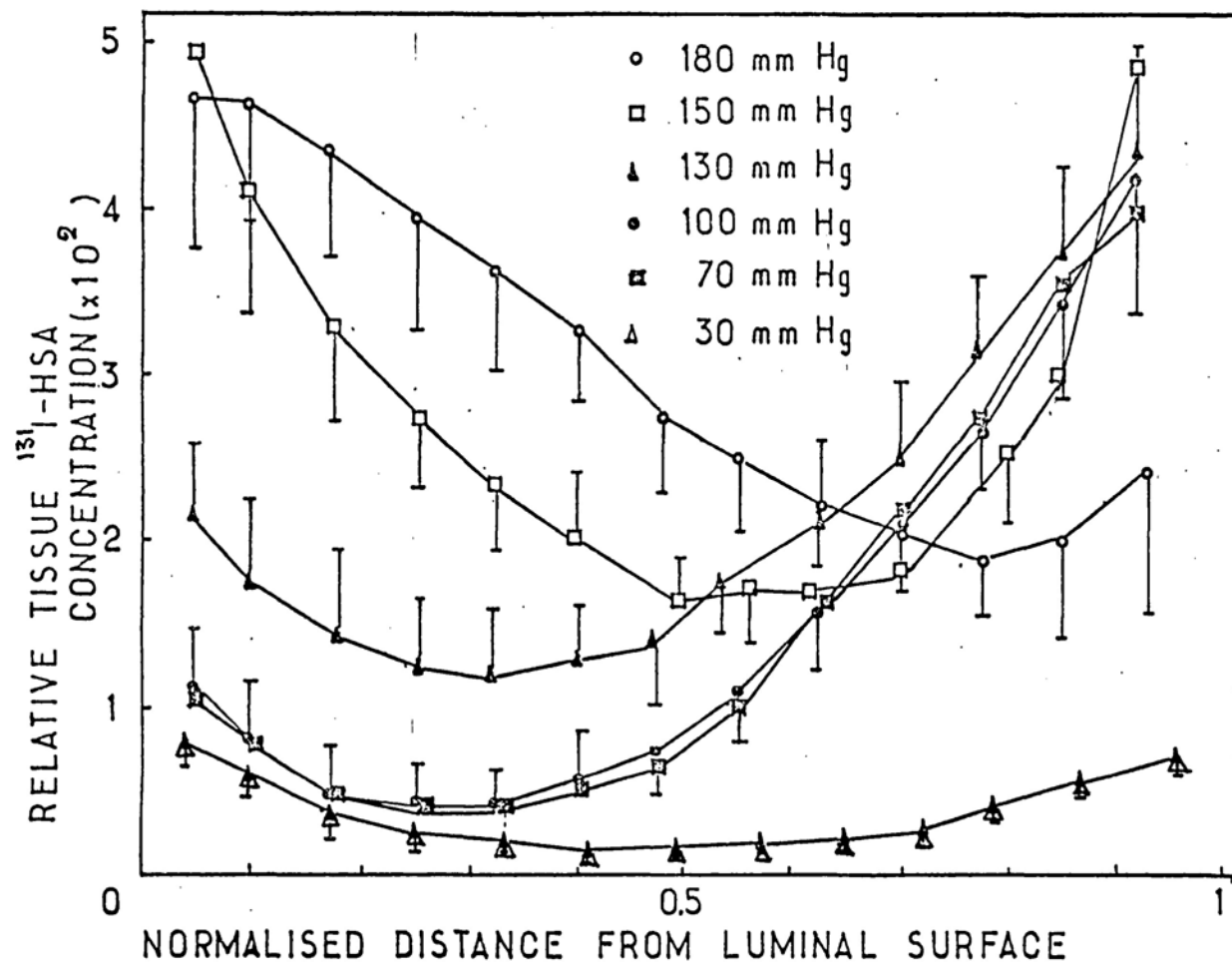


Fig.23 ^{131}I -HSA concentration profiles vs. transmural pressure.

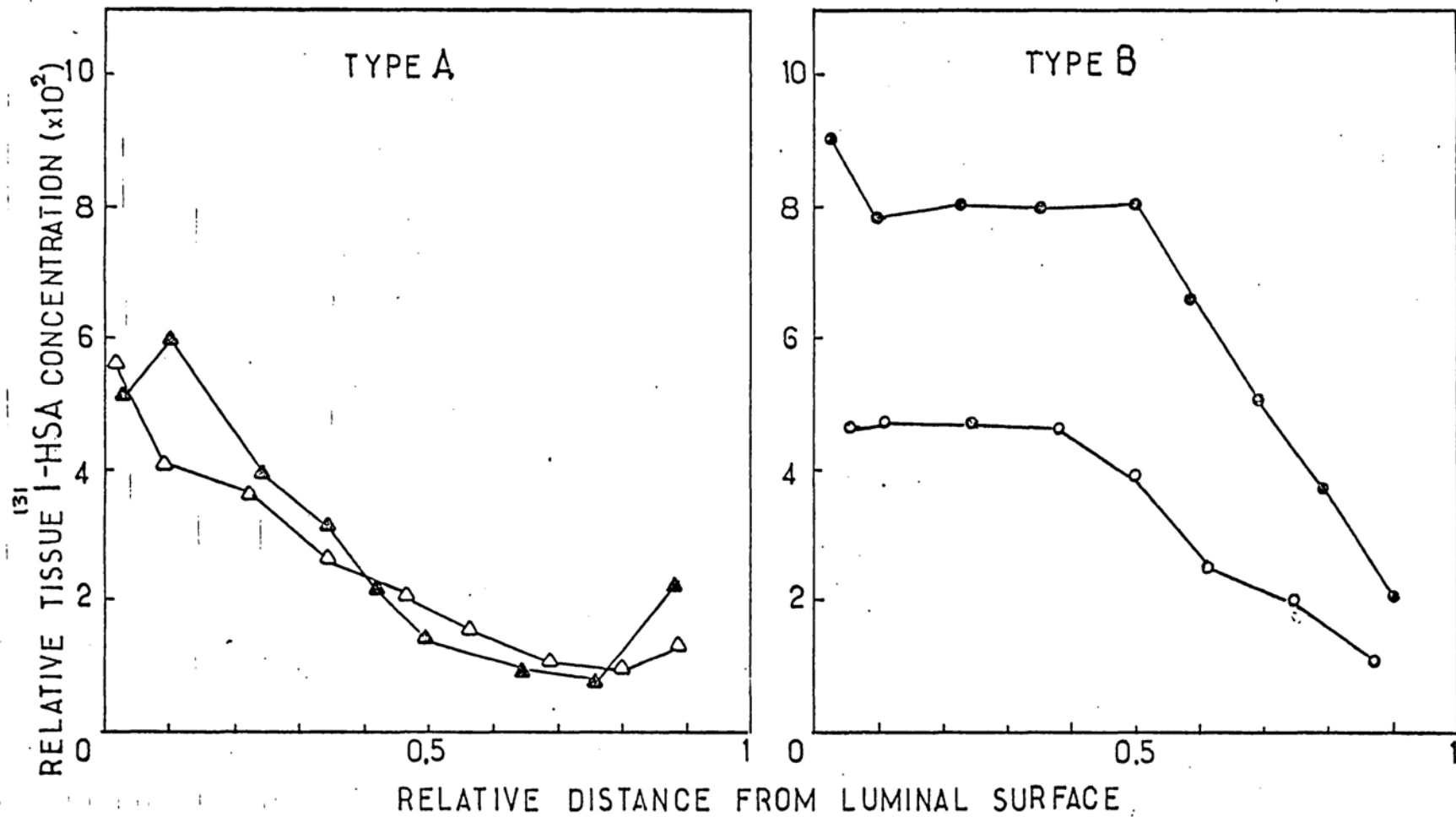


Fig. 24 Examples of A-type and B-type of ^{131}I -HSA concentration profiles for 180 mm Hg transmural pressure.

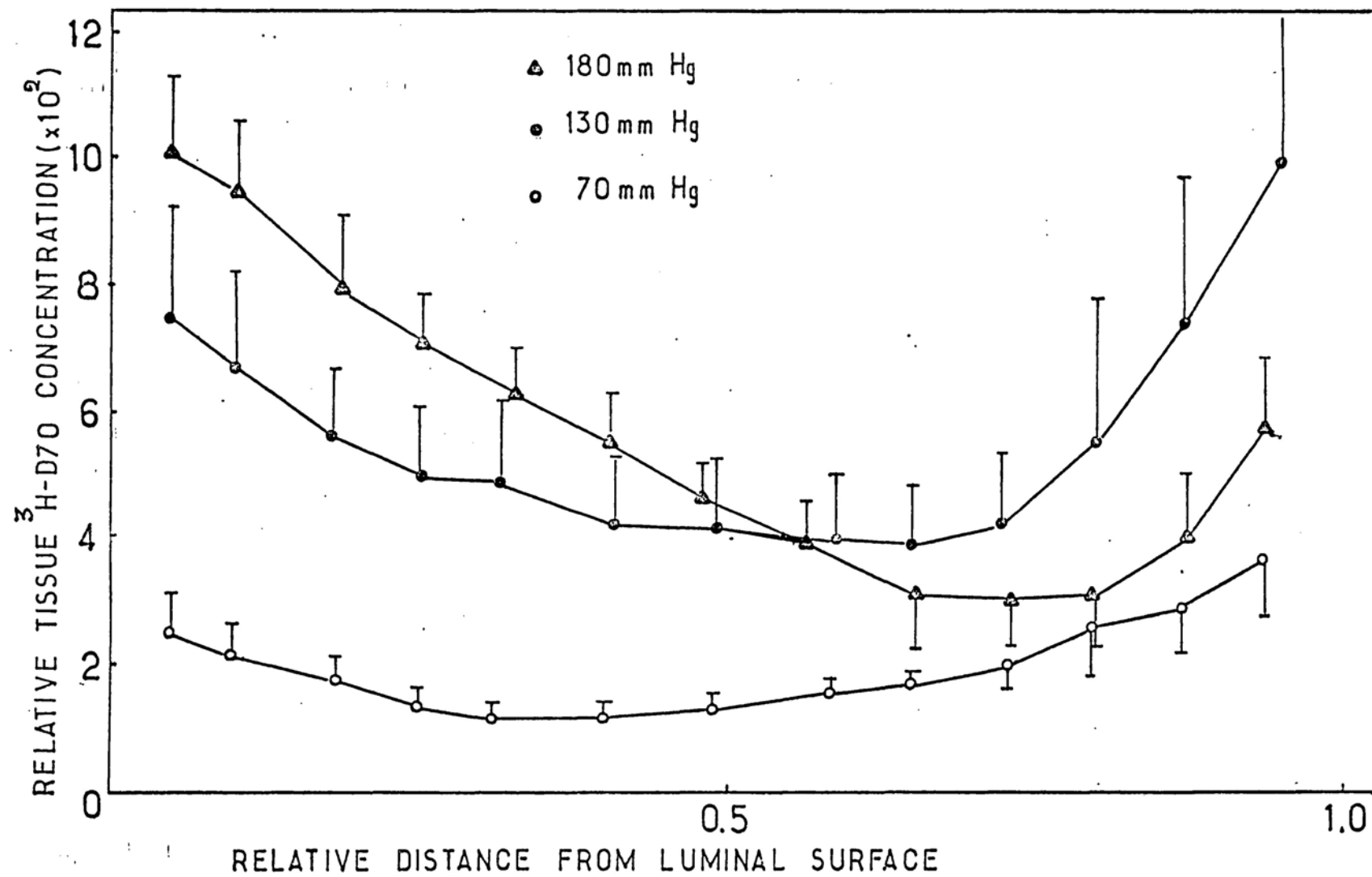


Fig. 25 ^3H -D70 concentration profiles vs. transmural pressure.

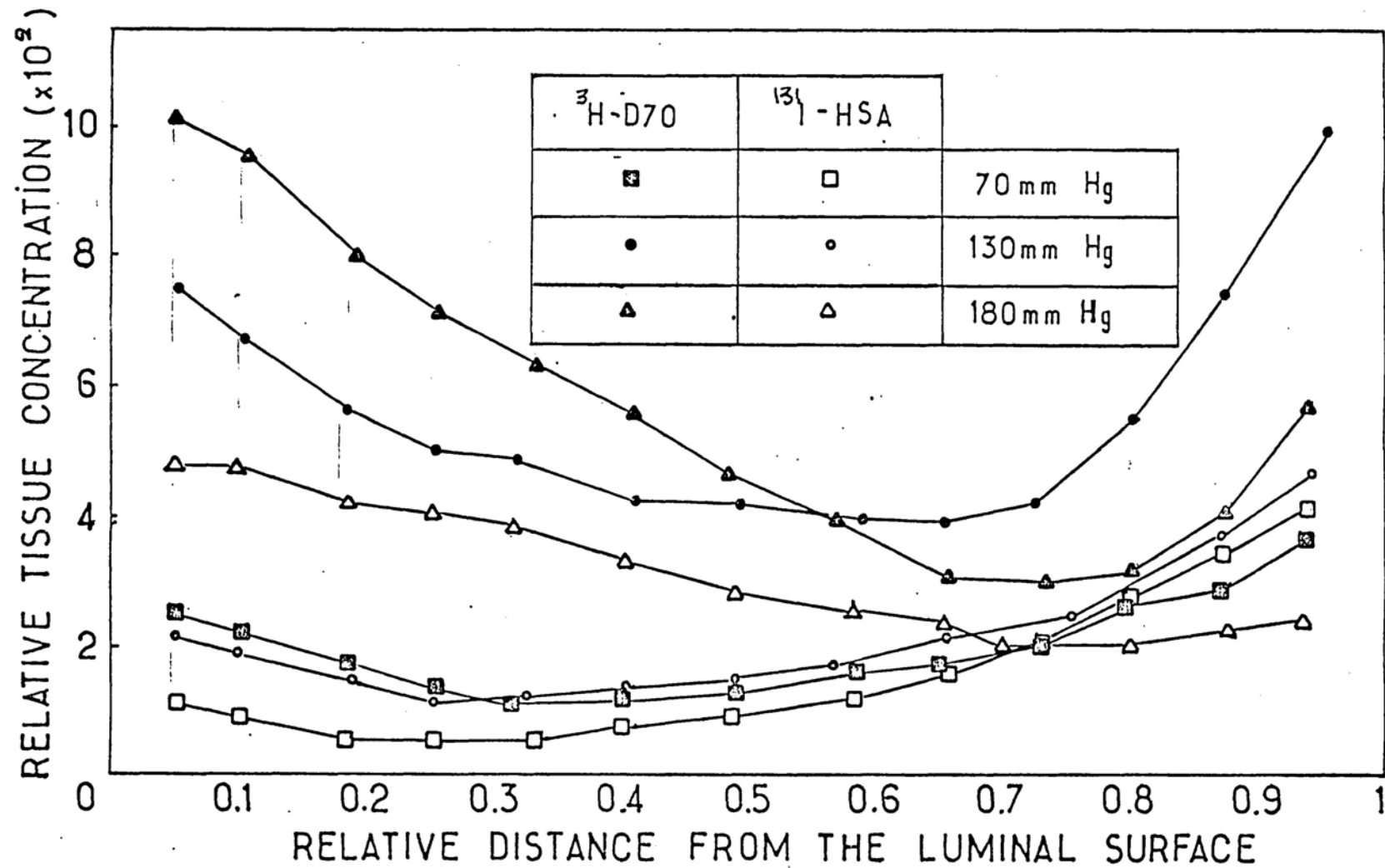


Fig. 26 ³H-D70 and ¹³¹I-HSA concentration profiles vs. transmemural pressure.

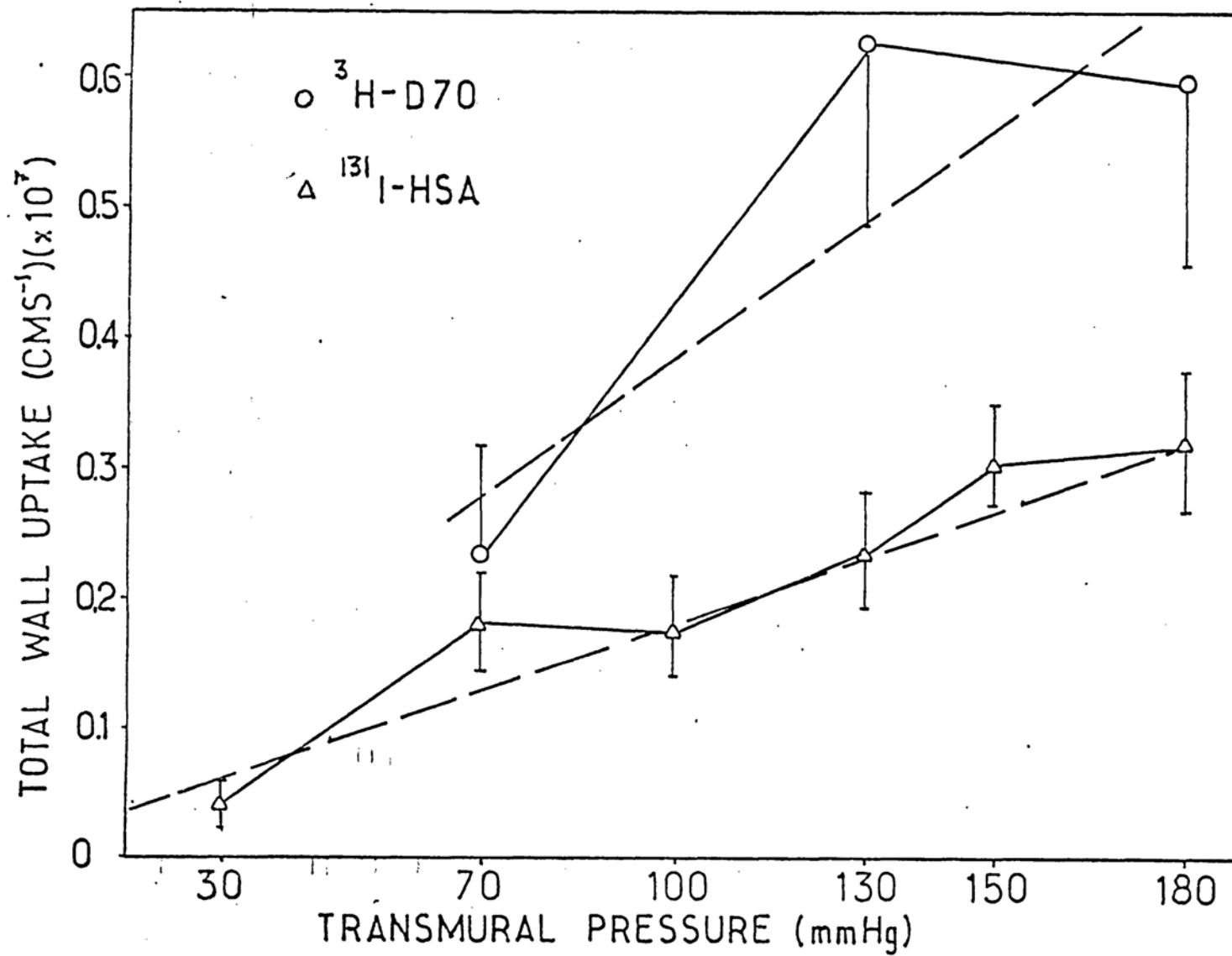


Fig 27 Total wall uptake for ³H-D70 and ¹³¹I-HSA vs hydrostatic pressure.

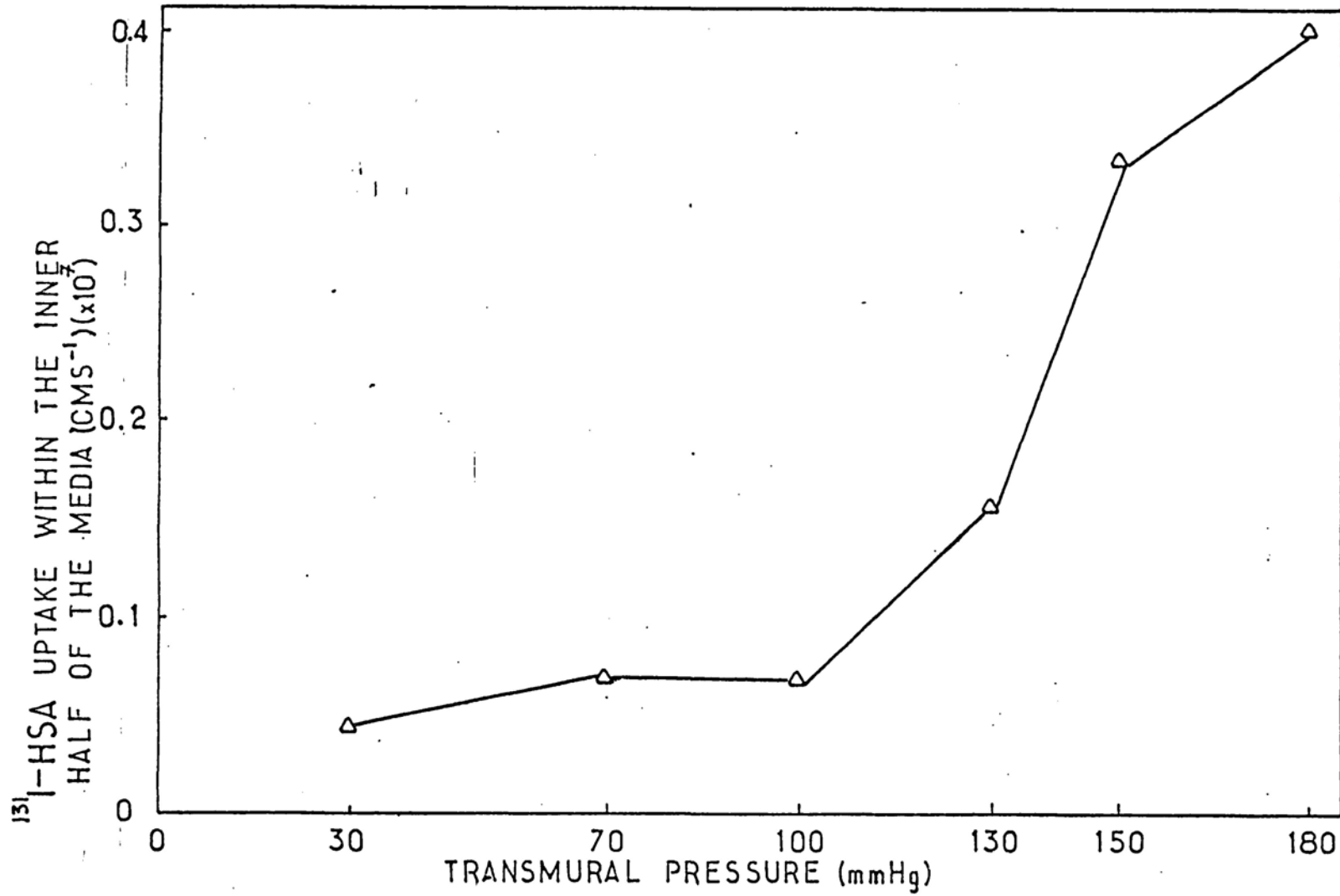


Fig.28 ^{125}I -HSA uptake within the inner half of the media vs.transmural pressure

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