

BEHAVIOUR OF INTERSTITIAL BIOPOLYMERS IN STRETCHED MUSCLE AND IN  
HAEMORRHAGIC SHOCK

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## ABSTRACT

Mechanical properties or colligative properties of the interstitium form the basis for each of the component projects which consist of a clinical problem in which the interstitium plays a role. A first section examines mechanical properties of interstitial biopolymers; a clinical study of irrecoverable deformation in human muscle (anal sphincter) under increasing extension ratios in 64 patients with symptomatic haemorrhoids who underwent a modified anal stretch procedure, using a retractor fitted with a strain gauge. Follow-up assessment and anal force measurements were available in 49 (77%); 26 who considered themselves 97-100% improved (Group I), and 23 whose relief of symptoms ranged 30-96% (Group II). There were significant differences in initial force and stress at lower extension ratios, suggesting that tissues in group II patients were stiffer.

A second section is concerned with interactions between albumin and interstitial biopolymers under varied states of hydration, to test the classical hypothesis that shock is accompanied by transcapillary protein leak, recently challenged by others who found reduced labelled albumin clearance in injured patients who survived resuscitation. Experiments to investigate the rate of loss of labelled albumin ( $^{125}\text{I}$ -SA) from the plasma following haemorrhagic shock, reinfusion, and saline loading, and the extravascular concentration of tracer in various tissues were carried out in anaesthetized rats. Labelled albumin left the plasma less rapidly than in controls, and appeared in increased concentration extravascularly in ear. Contrary to classical teaching, there may be no increase in overall capillary permeability to protein, but entrapment of label within the interstitium.

To F.A. Simeone, M.D., Professor, Health Sciences, Emeritus, Brown University, Providence, Rhode Island; Teacher, Friend, and Chief, who gave the writer his first academic appointment, and an interest in shock research, this work is gratefully dedicated.

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## INTRODUCTION

This work concerns the mechanical and the colligative properties of the interstitium. Mechanical properties of macromolecular systems are dependent on the proportions, distribution, and individual characteristics of the constituents. This is particularly true of biomacromolecules in living mammals; for example, structure-property relationships of connective tissue - the interstitium - vary with age in various tissues (Baer and Torp 1974, Lakatta and Yin 1982) due, in part, to change in modulus of the native collagen and elastin; the relative proportions vary from tissue to tissue. Connective tissue has been characterized by Scott (1983) as, "the natural fibre-reinforced composite material"; systems of insoluble fibres which take the stresses of movement and maintenance of shape, and soluble polymers trapped in the mesh which imbibe water to resist compressive forces. This interstitial matrix also serves as an important link in the transport of proteins and other substances from the plasma to the lymph. A first section is directed toward a better understanding of the mechanical properties of interstitial biopolymers. This section consists of a clinical study of irrecoverable deformation in human muscle (anal sphincter) under increasing extension ratios using a modified rectal surgery retractor fitted with a strain gauge. A second section is concerned with interactions between albumin and interstitial biopolymers; a study of the rate of loss of labelled albumin ( $^{125}\text{I}$ -SA) from the plasma following shock, reinfusion, and saline loading, and the extravascular concentration of tracer in various tissues in anaesthetized rats.

## The Interstitium

A gel made up of fibrous insoluble proteins (principally collagen, 39 nm and glycoproteins 13 nm) and entrapped soluble polymers - chiefly hyaluronate (MW 8,000,000 daltons) and proteoglycans (MW 10,000,000 daltons)- and water, the interstitium is part of the connective tissue of animals, including man, extending as far down the phylogenetic tree as the earthworm and sea cucumber (Scott 1983). Claude Bernard (1859) termed this gel the, "milieu interieur". The final common path for nutrient supply, waste removal, communication, defence, and repair at the postcapillary level, it is essential to a stable cellular environment. The fibrous proteins and the state of hydration of the gel phase influence the colligative and mechanical properties of the tissues (Sandberg 1981, Meyer 1983). Meyer (1983) studied tissue swelling pressure and exclusion of globular proteins in intact, and enzymatically degraded human umbilical cord, and found that the most important contribution was that of the polysaccharides. The proportions of the fibrous proteins vary, and are one important determinant of mechanical properties; the size of the interstitium and its state of hydration are others; the size of the interstitium is about 30-40% of the total in skin, 25-40% in skeletal muscle, and 20-30% in the viscera in mammals (Aukland and Nicolayson, 1981).

The interstitium represents 10-25% of total body weight by various estimates (Parving, H.-H., 1974; Aukland and Nicolaysen 1981). The composition and functional dynamics of the interstitium have been characterized by Zweifach and Silberberg (1979) as a "thick membrane" between blood capillaries and terminal lymphatics made up of a gel, a

flexible sponge of collagen fibers in which are enmeshed hyaluronate and proteoglycans. Measurements of interstitial pressure by several investigators mentioned above have revealed subatmospheric pressures, yet pressure within the lymphatic system has been reported to be positive and close to ambient (Wiederhielm, 1969).

Some inferences about the behaviour of the interstitium in affecting macrosolute transport can be gained from the work of a number of investigators who have used mixtures of biopolymers as an analogue of the gel phase. Ogston and Phelps (1960) and Ogston and Preston (1966) showed that hyaluronic acid (HA) may reduce markedly the partition of diffusible solutes between HA and buffer solutions by exclusion. Gerber and Schubert (1964) used cartilage-derived protein-polysaccharide complex in the dialysis system of Ogston and Phelps containing a Millipore filter as a membrane to measure the effect of the complex on serum albumin exclusion, which was constant, in spite of differences in temperature, salt concentration, and pH. This is surprising in view of the high negative - charge density of such nondiffusible macromolecules, and the amphoteric properties of albumin. At low salt concentration (0.1 - 0.02) and at 5 deg C, albumin behaved as if strongly bound to the protein - polysaccharide complex. Pal and Ghosh (1973) and Ghosh (1975) called attention the effect of lowering pH on polyanion-polycation interactions in vitro. At lower pH values, the basic proteins attained a stronger charge, thereby increasing their binding ability with polyanions such as hyaluronate.

In the case of mechanical properties, when increase in hydration of

the gel occurs in a closed space, as in skeletal muscle encased in a fascial compartment, heart enclosed in the pericardium, or lung restricted by the thorax, a proportional loss of functional capacity ensues. If one of the fibrous elements deteriorates - for example in emphysematous lung (Pierce, Hocott, and Ebert 1961) - then loss of normal pressure-volume relationships follows. If the collagen fibers increase in number in proportion to organ weight or in structure, as in hypertrophied or aged heart muscle (Schaub 1963, Verzar 1969), then mechanical properties and function are altered. Collagen and elastin are the principal determinants of the modulus of relaxed muscle (Banus and Zetlin, 1938). Smooth muscle structures, on the other hand, in general operate under a state of tonic contraction, and when stretched (e.g. a gravid uterus) maintain tension at an extended fibre length. Precisely what has been stretched, and to what degree irrecoverable deformation may be related to unsatisfactory clinical results were the questions which were the basis for the following clinical study.

## I i) VISCOELASTIC BEHAVIOUR IN ANAL MUSCLE-CONNECTIVE TISSUE COMPLEX

### Introduction

Anorectal disease remains a significant cause of disability and need for medical care, primarily in the Western world. Advanced haemorrhoids have in the past eventually required surgical removal. In 1969, Lord introduced stretching the anal sphincter muscles under anaesthesia for the relief of symptoms of hemorrhoids, a procedure first described by Recamier in 1838 for the treatment of fissure. Other surgeons have reported successful clinical trials; Anscombe et al (1974) performed a randomized study of 200 patients in which the anal stretch procedure was compared with hemorrhoidectomy. The two groups did not differ on clinical assessment, but those who had undergone anal stretch left hospital sooner, returned to work earlier, and were more pleased with their result, than those who underwent hemorrhoidectomy. The authors observed differences in the force needed to dilate the anus in certain patients. It was suggested that complications may follow too vigorous dilatation.

Apart from relief of symptoms, anorectal pressure measurements have been used to quantify clinical results. Hancock and Smith (1975) reported measurement of rectal pressure in patients before and after anal stretch. Symptoms were associated with a rectal pressure of 100 mm Hg or greater; postoperative success was associated with a rectal pressure of less than 60 mm Hg. Normal rectal pressure, usually measured hydraulically with a probe surrounded by a balloon, is about 60 mm Hg. Creve and Hubens (1979) measured segmental rectal pressure in patients before anaesthesia and eight days following either

hemorrhoidectomy or anal stretch, and in normal controls. There was no difference in the mean values for pressure between the three groups in the beginning - patients were only slightly higher than controls - but postoperatively, lower pressures were measured in the patients in the stretch group compared with the hemorrhoidectomy group.

In a pilot study (Shami et al, 1982) carried out in eleven patients undergoing anal stretch under anaesthesia to a measured extension (35%), stress relaxation was recorded. Decay of load with time at constant extension seemed to vary from patient to patient. One patient required subsequent hemorrhoidectomy; none was incontinent postoperatively.

Thus, a clinical problem requiring stretching of human muscle and connective tissue for treatment provided an opportunity to make observations in human subjects. The occasional occurrence of incontinence in older patients who have undergone anal stretch suggested irrecoverable deformation, and prompted the present study of anal sphincter force during stretch procedures. Because anal tissue may have viscoelastic properties, damage could be caused to the tissues not only by stretching to an excessive degree, but also by stretching too rapidly. Attempts have therefore been made to measure the degree of viscoelasticity of the tissues. The range of strains to which the anus is subjected during normal defaecation can be inferred from the work of Gibbons et al (1988) who measured anal circumferential force in 16 normal males aged 20 - 76 years (mean=35), using a series of cylindrical plastic probes 1 - 3 cm in diameter. All subjects could accept the 2 cm probe, suggesting that an anal circumference of 6.28 cm may be that present on defaecation in most people, but in some, circumferences of 7.85 cm or 9.43 cm were

tolerated. Enhancing anal "tone" by squeezing with the voluntary external sphincter increased the slope of the straight - line relationship between force and diameter, while reducing anal "tone" by the recto - anal inhibitory reflex (inflating a rectal balloon) decreased it. The authors proposed a mathematical model consisting of two concentric homogeneous isotropic linear elastic cylinders, the inner anal lining, thick-walled, the outer, circular thin-walled muscle. The model agreed with the findings in human subjects.

#### Materials and Method

Sixty-four patients, 47 men and 17 women, aged 22-73 years (mean 45, SD= 15) with symptomatic haemorrhoids who did not improve while receiving a high-fibre diet were admitted as day cases for a standardized anal stretch procedure under general anaesthesia. When rectal bleeding was the main complaint, sigmoidoscopy was carried out if it had not been practicable in the Outpatient Department; rectal cancer was found in two patients who subsequently underwent resection, and are not included in the study group. Barium enema and random rectal mucosal biopsy to exclude inflammatory bowel disease were carried out in selected patients.

The mechanical properties of the anal muscle connective tissue complex were measured during and after surgery using a modified retractor. A spring-loaded, two bladed, self-retaining retractor (Fig 1) was fitted with a strain gauge to monitor the force applied to the blades. The sensor was connected to a Wheatstone bridge and the resulting signal was recorded on a Heath polygraph. The retractor blades could be progressively separated by turning a screw. The minimum separation of the blades was 0.3 cm, and under these conditions, the circumference

Retractor equipped with a strain gauge.

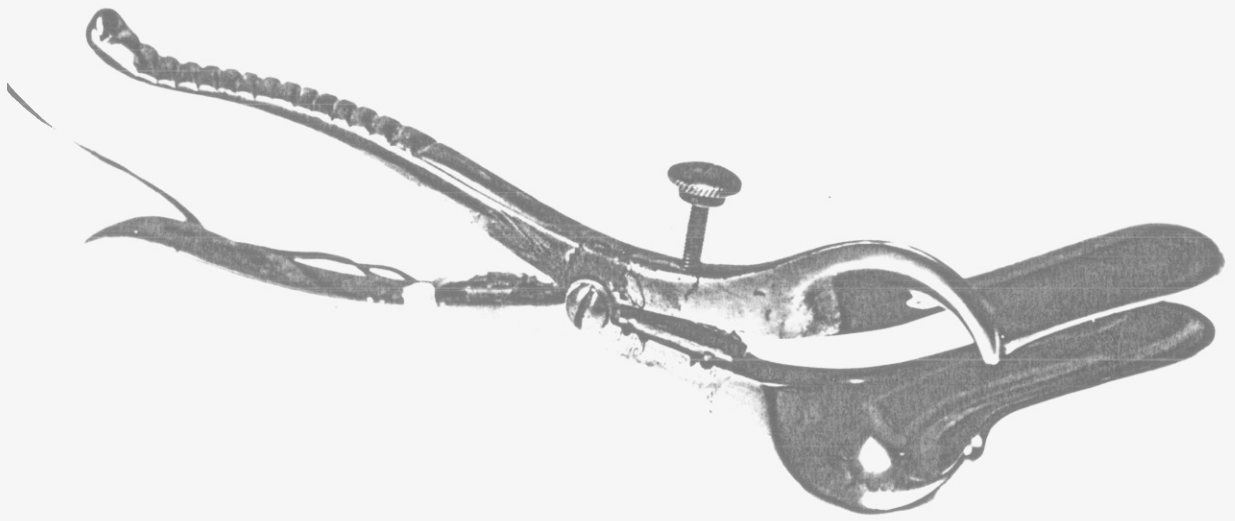


Fig.1

around the outside of the blades was 8.6 cm ( $C_0$ ). Each turn of the screw increased the blade separation by 0.3 cm, thereby increasing the outer circumference of the blades by 0.6 cm. The circumference at each turn of the screw is denoted  $C_n$  and this corresponds to the distance around the mucosal surface of the anal sphincter.

The degree of stretch of the sphincter is denoted by an extension ratio  $E$ , given by

$$E=(C_n-C_0)/C_0$$

The first measurement on each patient was performed after induction of anaesthesia but before the stretch procedure.

After zeroing the sensor and balancing the bridge, a 500 gm weight was suspended from the instrument to provide a standard force (5 N) in order to calibrate the equipment. The instrument was introduced with blades separated 0.3 cm ( $C_0= 8.6$ ), so that the sensor received a force reflecting initial anal sphincter tension; this was taken to be the initial force. The set-screw was turned smoothly five turns over about one minute, the force at the completion of each full turn being recorded continuously. The mucosal length of the sphincter complex was increased from 8.6 to 11.6 cm; for this  $E = 0.35$ . The force resulting from one turn was used to estimate the stress of the muscle-connective tissue complex. This is calculated as the force per unit area of the sphincter complex, estimated to be initially  $1 \text{ cm}^2$  and  $0.74 \text{ cm}^2$  after 5 turns when the circumference had increased by 3 cm, corresponding to an extension ratio for the anal sphincter complex of 0.35. The force was monitored for 15 seconds after attainment of full extension. The decrease in force in this period was considered to represent stress relaxation. Finally, the set-screw was turned

smoothly through five turns to decrease extension while the force was recorded continuously. The extension ratio at which force reached the initial level was taken as an index of length relaxation (creep). A final anal force measurement was recorded when the retractor separation was reduced to 0.3 cm, and the instrument was withdrawn. Further stretching to five fingers (about 20 cm or  $E = 1.33$ ) was then carried out manually with elbows extended and in full pronation (to limit force) over a period of five minutes (the preference of the particular surgeon, M. O.-S.). In addition to age and other relevant clinical details, the type of anaesthesia was recorded. Except where noted below, muscle relaxant drugs were not used; these do not have an effect on anal pressure measurements, which are chiefly the result of the internal (smooth muscle) sphincter. For relief of pain postoperatively, certain patients received a caudal anaesthetic block, most often with 0.5% Marcaine, a long-acting procaine-like anaesthetic. Caudal anaesthesia affects principally the pain and temperature nerve fibres, but may have an effect on motor fibres as well.

The preliminary approval of the Institutional Committee on Research Involving Human Subjects was obtained. As the retractor is a conventional surgical instrument for anorectal procedures, no informed consent other than that for anaesthesia and surgery was obtained.

The clinical outcome of the treatment and follow-up (resting anal force and voluntary sphincter force (obtained by the insertion of the instrument with 0.3 cm blade separation) were determined 1-21 months postoperatively. The data were compared by the student's t-test (Wetherill, 1967) for equal and unequal population variance. Significance was assumed if the value  $p$  was less than 0.05.

## Results

The calibration curve for the retractor strain gauge showed a linear relationship between applied force and output ( $r=0.96$ ).

There were no surgical complications among the anal - stretch patients. One patient who had concomitant anal surgery required reoperation for bleeding, but recovered. The patients were interviewed blindly 1-30 months (mean 10.9 months) postoperatively by the same person (not the surgeon) who asked by what percent the patient considered himself improved and what symptoms remained. Complete protocols were available in 49 patients (77%); 26 (Group I) reported themselves more than 97% improved, although 8 (31%) still reported some symptoms, including: itching (1), minor faecal incontinence 24 hours to 3 weeks postoperatively (4), bleeding (2), protrusion (1); 23 patients (Group II) stated that they were 30-97% improved with: itching (1), temporary incontinence (1), bleeding requiring further investigation (2), and pain (2) suggesting recurrent fissure-in-ano. The remaining 15 Group II patients had noted a return of preoperative complaints to varying degrees. Ten patients who received a nerve block or muscle relaxant were excluded, reducing the numbers for data analysis to 22 in Group I, and 17 in Group II.

There was no significant difference between the two groups as regards age, or sex. Initial anal stress (force per unit cross sectional area of the sphincter complex, assumed to be  $1 \text{ cm}^2$ ) averaged 555 (SD=251)  $\text{g cm}^{-2}$  ( $n=22$ ) in Group I patients, and 879 (SD=805)  $\text{g cm}^{-2}$  ( $n=17$ ) in the less satisfactory Group II ( $p<0.05$ ). The relative stress values of the Group II patients after one, two, and five turns (Extension ratios

0.07, 0.14, and 0.35 respectively) were significantly greater than those observed in the more successful Group I patients. As shown in Fig 2, the relative stress after one turn (Extension ratio 0.07) averaged 1095 (SD=525, n=22)  $\text{g cm}^{-2}$ , or 1.095  $\text{N m}^{-2}$ , in Group I patients vs 1565 (SD=606, n=17)  $\text{g cm}^{-2}$ , or 1.565  $\text{N m}^{-2}$ , ( $p < 0.05$ ). Fourteen patients received a caudal anaesthetic nerve block and one received a muscle relaxant, succinyl choline chloride. Follow-up data are available in ten of these; five in Group I and five in Group II. The differences in stress values seen at two turns (Extension ratio 0.14) in the two entire Groups were not seen in these subsets who had a neuromuscular block at either nerve radical level or at the motor end plate; Group I, 1071 (SD=836, n=5), Group II 992 (SD=609, n=5)  $\text{g cm}^{-2}$ . The small number of patients makes interpretation of these data difficult. As mentioned above, caudal anaesthesia affects principally the pain and temperature fibres, but may have an effect on motor fibres as well.

There was no significant difference between the two groups in the stress relaxation observed at maximum extension ( $E=0.35$ ); Group I, 956 (SD=618)  $\text{g cm}^{-2}$ , n=17 (five records were off scale at  $E=0.35$ ), Group II, 894 (SD=579)  $\text{g cm}^{-2}$ , n=17 nor in length relaxation (creep), the extension ratio at which force fell to the initial, or prestretch, value. The latter values were 0.21 (SD=0.06, n=22) in Group I, and 0.2 (SD=0.07, n=17) in Group II. In both Groups, the value was more than twice the initial extension ratio, suggesting some irrecoverable deformation of the tissues.

The follow-up force (FUF) measurements in unanaesthetized patients were higher than those recorded initially at surgery (IF) in both Groups, and did not differ significantly; the ratio FUF/IF averaged

Graph illustrating difference in relative stress (see text p.16) ordinate in  $\text{g cm}^{-2}$  at increasing strain (abscissa) in unfavourable cases (o) compared to successful ones (closed circles). Bars are Standard Deviation.

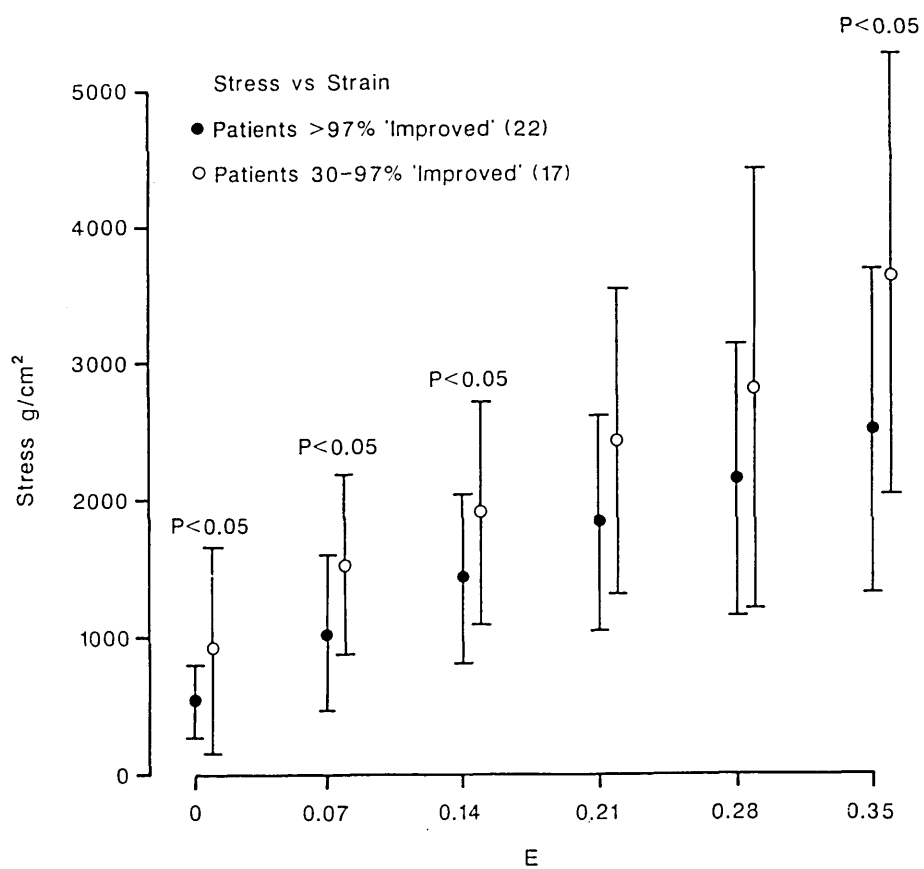


Fig.2

2.12 (SD=1.6, n=22) in Group I vs 1.51 (SD=1.12, n=17) in Group II patients. There was no significant difference in the developed force in excess of the passive stress of the voluntary rectal sphincter between Groups I and II. The values were 329 (SD=274, n=17) g cm<sup>-2</sup> in Group I patients, and 327 (SD=223, n=14) g cm<sup>-2</sup> in the less favourable Group II patients. The period of follow up was greater with the patients in Group II (30-97% improved), perhaps because these patients continued to return to the Outpatient Department.

## Discussion

The data must be interpreted in the light of the clinical exigencies of the situation; ideally one would have preferred to make at least paired observations for each data point, and allowed steady-state forces to develop at each level of extension. In addition, a number of simplifying assumptions were made. The initial stress and length relaxation (creep) were estimated at the initial level of extension with the retractor inserted and not at zero anal force. Initial insertion of the instrument with blades separated 0.3 cm caused some deformation, therefore the "initial force" and creep were not measured on the undeformed tissue.

We cannot be certain that there were no differences in sphincter dimensions between Groups I and II. The effects of six hours fast and a general anaesthetic were ignored in comparing peroperative measurements with those in the Outpatient Department. However, while our own force measurements and the hoop stress measurements made by Gibbons et al (1988) are different, if our measurements of initial force are converted to Nm<sup>-1</sup> and compared, the mean initial force falls

on their line for mean resting circumferential force in healthy subjects without anaesthesia. The significant differences observed in tissue mechanics, particularly at lower extension ratios may imply that the tissues being stretched in Group II patients were inherently stiffer, and may therefore be more likely to undergo irrecoverable deformation, when subjected to the considerable further extension by the surgeon.

The decay of force at maximum extension ratio 0.35 which was similar in both Groups implies viscoelastic behaviour of the anal tissue. Whether the viscoelasticity is associated with the connective tissue or muscle cannot be determined by this study. The observations in the pilot study (Shami et al, 1982) showed greater stress relaxation in older patients. This is interesting in the light of Haas's (1977, 1980) reports of deterioration in elastin in the anal connective tissue-muscle complex in older patients. However, in the present study, there was no significant difference in age between the two Groups. Neill et al (1981) found post-stretch incontinence, if present, was usually in older patients. The index of creep was relatively great in both Groups (but not significantly different between them). Some caution is necessary, however, because the time course of viscoelastic recovery of the tissue may be greater than the period of observation available in these patients. The greater stress at extension ratio 0.14, seen in Group II compared with Group I was not seen in the small subsets of patients who received a nerve block. Although the number of subjects in the subsets are too small to draw firm conclusions, this observation could suggest that muscle was affected rather than connective tissue. Frenckner and von Euler (1975) found that pudendal block obliterated electromyographic activity

and voluntary contraction in the external sphincter, and concluded that the internal sphincter contributed most of the pressure in the anal canal, but only 40% after rectal distension, which relaxes the internal sphincter.

Clinicians who carry out this procedure are familiar with the sense of sudden yielding of the tissues when carrying out anal stretch; whether due to yielding of the internal sphincter or rupture of fibrous bands as suggested by Lord (1969) is not certain. In the present study, histological examination was available in only one patient who required removal of the rectum for cancer following anal stretch, when a high initial anal force measurement was observed; review of histology revealed invasion of the muscle-connective tissue complex by tumour, but no evidence of possible mechanical injury to muscle. High-amplitude anal pressure waves were found in one patient during mechanical stretching. Terramoto et al (1981) and Hancock (1975) suggested that such waves are an etiologic factor in hemorrhoids.

The differences in both Groups between peroperative force (IF) under anaesthesia and follow-up force (FUF) without anaesthesia are probably the effect of general anaesthesia on the one hand, and anxiety on the other. That the ratio of follow-up force to peroperative force (FUF/IF) was lower in Group II patients is due primarily to the higher initial force in these patients, and suggests increased muscle tone rather than rigidity due to connective tissue components in determining the relative stresses which were observed.

## Conclusions

1. The results of a study of 64 patients who underwent a modified anal

stretch procedure for symptomatic hemorrhoids unrelieved by taking a high-fibre diet have been reported.

2. Follow-up assessment and anal force measurements were available in 49 (77%); 26 who considered themselves >97% improved, and 23 whose relief of symptoms ranged 30-97%. Excluding 10 patients equally divided between the two groups, who received a caudal nerve block or muscle relaxant peroperatively, there were no significant differences between the two groups as regards age, sex, length relaxation, decay of force at maximum extension, follow-up force, or voluntary sphincter force. Significant differences in initial force suggest that the tissues in Group II patients were stiffer.

3. Anal stretch procedures should probably be done in a manner which takes account of the tissue stress, and not simply to a constant level of extension. Slower extension may be appropriate in those patients who exhibit elevated stress at low strain ratios. A larger clinical series would be necessary to prove this point.

## II. MOVEMENT OF SERUM ALBUMIN THROUGH INTERSTITIAL MACROMOLECULES IN SHOCK

### Introduction

Haemorrhage sets in motion a number of haemodynamic and neuroendocrine responses, roughly proportional to severity, which act to preserve tissue perfusion in vital organs such as brain and heart. Following a severe bleed, when added blood, fluid, and serum albumin have been given, if the subject survives, a stage of post-resuscitation overhydration of the interstitium follows. The classical explanation of these changes has been leakage of plasma proteins through damaged capillaries, with shift of the normal plasma-to-lymph osmotic pressure relationship (Starling, 1896). Resuscitation after haemorrhagic shock leads to expansion of both the interstitial fluid, and intracellular fluid spaces (Blalock, 1930; Wiggers, 1942). The commonly accepted explanation of capillary leak as a cause has recently been challenged by Lucas et al (1982), who have suggested entrapment of serum albumin in the interstitial fluid space (IFS) as an alternative explanation. The present study seeks to examine this hypothesis in shocked rats. Changes in the distribution of extravascular serum albumin induced by hemorrhagic shock in rats will be examined. A better understanding of these factors may improve therapy in shock.

Although the clinical picture of shock due to wounding and blood loss has been recognized for a long time (Drucker, Chadwick, Gann, 1981),

an understanding of the basic mechanisms in shock has been hampered by the diversity of methods and uncertainties in the interpretation of the results of experimental studies. Haemorrhage in mammals sets in motion a number of regulatory mechanisms which act to preserve tissue perfusion for those organs with the highest oxygen requirement such as brain and heart. Blood vessels are constricted and cardiac output and urine flow are reduced, but the mean arterial pressure is maintained until refill from the IFS restores the plasma volume deficit. So effective is this restoration that blood loss of 1200 ml in healthy human subjects may produce little change in haemodynamics (Moore and Ball, 1964).

In contrast, when bleeding is severe, after it is finally controlled and fluid and red cell deficits in the plasma are restored by autologous blood, there remains a disturbance in tissue oxygenation and metabolism. It has been suggested that this might affect the behaviour of the macromolecules in the IFS either by exclusion (Carrico 1981), regional reduction in hydration (Shires et al, 1960; Larsson et al, 1981), increased hydration in specific organs (e.g. lung; Demling, 1980; Modig, 1983), impaired solute mixing by reduced cardiac output (Small and Homer 1979), or binding of albumin to polyanions at reduced pH (Ghosh 1975). After resuscitation, trauma patients (and elective surgical patients) enter a stage of recovery - Phase II (Lucas et al, 1982) - in which there is fluid retention both in the IFS and intracellular space.

If the subject survives Phase II, hypertension, mobilization of fluid and diuresis begin and the interstitial fluid space shrinks - Phase III- (Ledgerwood and Lucas, 1974). The classical explanation of these phenomena has been that there has been a leak of serum protein from

damaged capillaries (Phase II) which ceases on recovery (Phase III) followed by a return of normal plasma-IFS volumes and oncotic relationships.

The mechanism by which these changes occur has been challenged by Lucas(1980,1982), who reported that, in a series of 138 severely injured patients, the plasma albumin transfer rate (inferred from the slope of the radioiodinated serum albumin disappearance curves) to the IFS was lower than normal in phase two but was elevated in phase three. The observed increase of albumin in the IFS in phase two must therefore be due to an even greater reduction in albumin re-entry into lymphatics. Thus movement of albumin appears to depend on the state of hydration of the IFS which may lead to albumin entrapment or exclusion. These data may explain the reported failure of supplementary albumin in the treatment of severely shocked patients (Lucas et al 1980).

If the sequestration of fluid in the IFS occurs in an organ in a confined space, such as brain, heart, or lung, there is a proportional decrease in function. This has been amply demonstrated by Snashall (1980) who reported the importance of the osmotic constituents of the interstitium to pulmonary interstitial compliance in mammals.

Not all investigators agree that the IFS volume decreases in shock proportionally to the fluid transfer to the intravascular compartment. Shires et al (1960) determined the simultaneous radiosulfate,  $^{131}\text{I}$  albumin, and  $^{51}\text{Cr}$  red blood cell spaces in dogs bled to shock levels, and while the functional extracellular fluid was reduced 21% over that due to shed plasma, concluded that this was due to a translocation of fluid in the body during haemorrhagic shock - possibly into cells -

rather than a shift of fluid from extravascular to intravascular extracellular fluid. Larsson et al (1981) have suggested that methods of estimation of this space have been the problem. They proposed a double-isotope technique for studying simultaneously the plasma water and extracellular fluid volumes; rats bled 40% of total blood volume to 40 mm Hg and then allowed to recover after 10-90 minutes showed 50% restoration of plasma water within ten minutes. The total extracellular fluid volume did not change significantly, but was reduced regionally in liver, spleen, and subcutaneous fat during the same period. Reviewers were critical of the minor degree of shock produced, and the time allowed for equilibration following injection of isotopes.

Of major importance, as it influences the treatment, is the lack of understanding of the dynamics of the interstitial fluid space (Small, 1979; Demling, 1980). The IFS is a multicomponent biopolymer system, the ease of diffusion through which may be influenced by pH (Pal, 1973), and degree of hydration (Comper, 1978), both of which might be altered in shock. The next section describes experiments designed to study movement of fluid and labelled albumin and EDTA in shocked rats.

## II.i) REDISTRIBUTION OF $^{125}\text{I}$ SERUM ALBUMIN AND FLUID IN RATS IN HAEMORRHAGIC SHOCK

### Introduction

This section seeks details of movement of fluid and labelled albumin in shock of the same severity as that in Lucas' trauma victims, in animals, where the restraints imposed by the clinical situation do not apply.

Albumin is the principal macromolecule in plasma and is a major determinant of colloid osmotic pressure of  $3 \times 10^3 \text{ N m}^{-2}$  (30 cm  $\text{H}_2\text{O}$ ) (Caro et al, 1978). The normal concentration range is 3.5-4 g/dl, slightly less in lymph. Filtration across capillary walls depends on hydrostatic pressure difference, osmotic pressure, and lymph flow; the pressure difference depends on capillary pressure and interstitial pressure; the latter can be affected by lymph flow (Starling, 1896). Alteration of any these factors may change Interstitial Fluid Volume (Aukland and Nicolaysen, 1981). Landis (1927) obtained an average capillary pressure in vivo. Pappenheimer and Soto-Rivera (1948) used the term, "isogravimetric capillary pressure, the total pressure opposing net filtration of fluid from the capillaries". Plasma protein concentration in the Interstitium averages 1.6-2.0 g/dl (Wiederhielm et al, 1976), which exerts a colloid osmotic pressure substantially higher than that predicted by Landis, or Pappenheimer and Soto-Rivera. The osmotic pressure of mixtures of plasma proteins and hyaluronate are greater than the algebraic sum of the two components (Ogston and Phelps, 1960; Ogston and Preston, 1966); according to Wiederhielm et

al, 1976) they must be considered a two phase system in osmotic equilibrium, half free, the remainder in the gel phase. In hemorrhage, capillary pressure falls to as low as 8-10 mm Hg and reabsorption leads to reduction in circulating plasma protein concentration by 30-50% (Mellander and Lewis, 1963). Lymph flow is drastically reduced, then is resumed with regional differences; greater from the splanchnic bed than the limbs (Rusznay et al, 1967). Others have explained the redistribution of protein and fluids in the body by changes in cardiac output ( Small and Homer, 1979); albumin resuscitation in shocked dogs led to improved cardiac output as measured by thermodilution.

The initial objectives of this project are to confirm the loss of protein, small molecules, and fluid from the plasma into the IFS of various tissues in a Wiggers (1942) preparation modified for the rat. The rat as a subject for shock study is well established (Shires 1960, 1964, Larsson, 1981), and the properties of its connective tissue elements have been studied in depth (Baer and Torp 1974).

## Materials and Method

Sixty-five Sprague-Dawley rats of both sexes 150-200 gm body weight were fed Rat and Mouse Maintenance Diet ( Special Diet Services Limited, Witham, Essex, U.K.) until the evening before the experiment, and allowed water ad libitum. Animals were anaesthetized with either an ethyl ether-room air mixture and intramuscular fentanyl citrate (Hypnorm, Crown Chemical Company, Ltd, Lamberhurst, Kent TN3 3DJ, England) 0.1-0.16 mg/kg, or with the latter at a dose 64 microg/kg plus intraperitoneal sodium pentobarbital 26 mg/kg, repeating 7 mg/kg hourly. As shown in Fig.3, the animal was positioned supine on a heating pad, and rectal temperature was monitored. The left carotid artery was cannulated with a 10 mm x 0.63 mm i.d. Portex tube and heparin 3 mgm/kg was injected (expts 013-030). A 10 cm x 1 mm Portex central venous catheter was inserted via the right external jugular vein. The trachea was intubated, and the animal was allowed to breathe room air spontaneously. Arterial pressure was recorded using a Statham P23Gb electromanometer (Hato Rey, Puerto Rico) and a uv recorder. After baseline measurements and a blood sample, the animal was bled into a sterile (.05% Chlorhexidine Gluconate, Hibitane, ICI, U.K. for 24 hours) heparin-saline filled siliconed reservoir set at 54.4 cm (40 mm Hg) at an overall average rate of 0.66 (SD=0.4) ml/kg/min for an average of 40 (SD=26) minutes. The rate of bleeding was limited to 1 ml/kg/min for ten minutes by a resistance in the line, then opened to the reservoir. In spite of these measures, there was variation in the duration of bleeding (20-60 minutes) to produce shock, defined as the time at which 10% of shed volume was taken back from the reservoir, at

Wiggers shock model modified for the rat

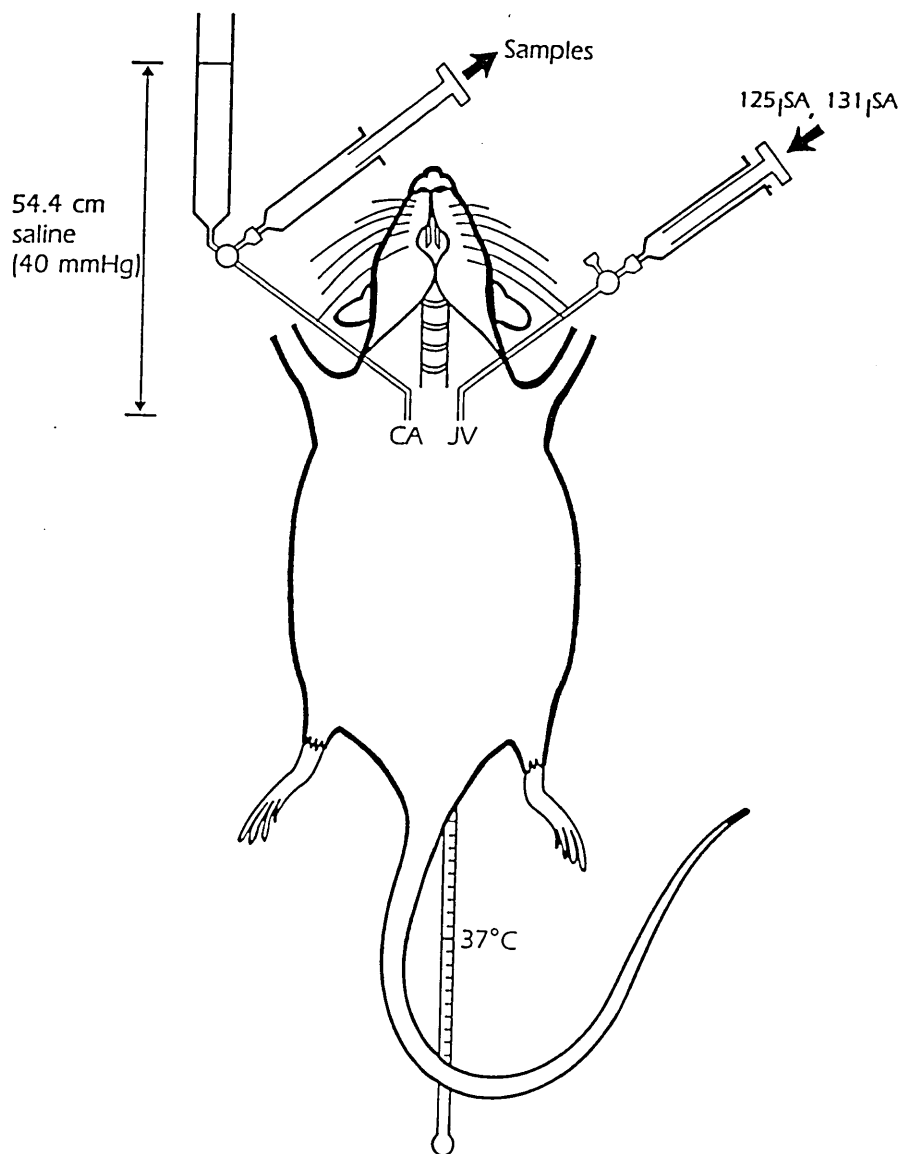


Fig.3

which time the reservoir was elevated and shed blood and an equal volume of saline were infused. Controls which were neither bled nor reinfused were studied by the same protocol. Following reinfusion, 100 microCi  $^{125}\text{I}$  serum albumin (0.5 mgm) dissolved in 0.5 ml 0.95 % saline solution was injected and washed through with a equal volume of 0.95 % saline solution (included in calculation of infused volume). In experiments 6-10, Evans Blue Dye and  $^{51}\text{Cr}$  ethylenediamine tetraacetate (EDTA) were used as plasma volume and IFS probes respectively, but results obtained with the dye were discarded as it was not reliably assayed in digested tissue specimens in comparison to a dye-free animal because of turbidity, which gave misleading spectrophotometric results. The data for the one hour  $^{51}\text{Cr}$  EDTA Distribution Space proved useful, and are presented under RESULTS

Arterial blood samples (0.2 ml) were taken at 5, 25, 60, ( and 120 minutes in the last 20 experiments); the last sample was taken 5 minutes after  $^{131}\text{I}$  serum albumin 100 mc in 0.5 ml (+0.5 ml wash volume) was injected. A rigorous sampling protocol was used to ensure accurate plasma samples for counting; the dead-space of the system was measured (0.7ml), and before each sample, an amount in excess of this volume, and the contents of the hub of the stopcock, were aspirated before sampling, and the aspirated volume mixed with heparinized saline was reinjected; it may be noted below, that there was an insignificant ( $t=1.2$ ) decline in Hct values in both groups, probably the result of sample removal and mechanical red blood cell trauma. All radioisotopes were obtained from Amersham International P.L.C., Amersham, U.K.; Bovine Serum Albumin (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) was radioiodinated using the chloramine-T method (Bolton, 1977) and dialyzed for 24 hours to minimize free

Iodine present. Paired determinations were made of : hematocrit, total plasma protein (spectrophotometric method), plasma volume, and tracer concentrations. In 13 two-hour experiments, blood sugar (orthotoluidine method, Hultman, 1959) and flux of  $^{125}\text{I}$  SA into 1 cm  $0.0004\text{ gm}$  linen subcutaneous abdominal wicks (Aukland and Fadnes, 1973) were also measured.

The anaesthetized animals were frozen in liquid nitrogen to minimize further fluid shifts, sectioned transversely to obtain tissue samples of ear (pinna), brain, heart (but not blood in chambers), lung, liver, kidney, bladder urine, hairless skin of palms and soles, and sacrospinalis muscle for estimation of tracer and water content in tared vessels.

#### Analyses:

Cecil spectrophotometer model 292; 20 microliter samples diluted to 1.0 ml, total plasma protein estimated at an optical density of 280 nm using a Deuterium source, Evans Blue Dye at 580 nm, and plasma glucose as aldosesaccharide at 625 nm, using a tungsten source.

Packard Autogamma counter, half hour per sample, subtracting background; paired, tared isotope samples.

Hematocrit: micro method using Benjamin capillary tubes, flame-sealed, 3000 rpm for 10 min. The tubes were fractured through the buffy coat, and the plasma reserved for counting and total protein analysis.

#### Calculations:

Total plasma volume was determined by indicator dilution technique (Gibson and Evans, 1937), injecting 0.5 ml containing 100 microCi of the isotope intravenously, followed by 0.5 ml 0.95 % saline, allowing

five minutes for mixing, and removing 0.2 ml samples from the arterial cannula. Paired samples of injected solutions and plasma, 0.020 ml were placed in tared counting tubes for gamma counting for 30 minutes, subtracting background. The appropriate corrections for atomic spectral overlap were made in the calculations of tracer concentration/ml plasma, and estimation of plasma volume made by the expression:

$$\text{Plasma Volume} = (C_i \times V_i \times V_p) / C_p$$

Where:  $C_i$  = concentration (cpm/ml) of isotope solution injected

$C_p$  = quantity of isotope (counts) in plasma sample

$V_i$  = Volume of isotope solution injected

$V_p$  = Volume of plasma sample

Extravascular tissue water, ml/kg was calculated from paired tissue samples dried at 94 degrees C to constant weight, and subtracting plasma water estimated from  $^{131}\text{I}$  counts.

$V_p$  ml/kg, the calculated fluid loss from the circulation between the time of reinfusion and the end of the experiment; reinfusion plasma volume less final plasma volume (taking account of volume of label and saline added in latter determination) divided by body weight in kilograms.

Tissue Blood Volume (TBV) = Tissue Plasma Volume Fraction  $\times$   $1/(1-\text{Hct})$

Differences between shocked and control animals were examined by

t-test analysis of data, for equal and unequal population variance (Weatherill, 1967), using computer programs written for this purpose. Results were also evaluated by a commercial statistical program, MINITAB (Ryan, 1982). Data were considered significant if the value, p was less than 0.05.

## RESULTS

A pilot experiment was conducted to establish the amount of unbound radioiodine. 100 microCi <sup>125</sup>I serum albumin was purified by dialysis in two changes of 500 ml saline over 48 hrs in Visking membrane 12,000 MW pore. An aliquot was then injected into a rat, and a blood sample taken at 60 minutes, centrifuged, and the plasma was placed in a Sepharose CL6B column. It was found that 0.13% unbound <sup>125</sup>I was present; this was deemed insignificant for this study.

The data have been divided into two groups; an initial "Development Experiments" group, occurring in a period during which anaesthetic, surgical, and technical problems were identified, and a "Experimental Results" group of 31 animals.

### Development Experiments

Of 28 animals, 22 received diethyl ether induction followed by intraperitoneal Fentanyl Citrate 0.2 mgm; of these 17 died, many of apparent respiratory causes. With the addition of preliminary tracheotomy and tracheal intubation, and Fentanyl Citrate alone for induction of anaesthesia, followed by hourly intraperitoneal Sodium

Pentobarbital, the results improved. Complete data are available in 8 animals.  $^{51}\text{Cr}$  EDTA was injected immediately after reinfusion, and  $^{125}\text{I}$  BSA one hour later; the animals were killed after a further five minutes. The  $^{125}\text{I}$  BSA content of tissue samples was assumed to represent the plasma concentration; this was then used to estimate the extravascular  $^{51}\text{Cr}$  content from the total tissue content. The one hour  $^{51}\text{Cr}$  EDTA Tissue Distribution Space was calculated in these experiments by dividing  $^{51}\text{Cr}$  tissue counts/gm corrected for plasma-derived  $^{125}\text{I}$  counts by  $^{51}\text{Cr}$  counts/ml in the one hour post-injection plasma sample; these data for various tissues are presented below.

#### Experimental Results

Fifteen of 24 shocked animals completed the protocol (LD 38); data are based on ten one hour experiments (four shocked, six controls) and 21 two-hour experiments (eleven shocked and ten controls), a total of 31.

#### Cardiovascular Response to Haemorrhage:

Loss of 26.4 ml/kg (SD=0.4) of blood was accompanied by obvious peripheral vasoconstriction in the feet, bradycardia (mean reduction in heart rate 24%, SD=0.11%), and hypotension (Fig.4), the result of experimental design. The heart-rate pressure product (RPP) was 23396 (SD=10712) in bled animals as compared to controls (RPP=57615 (SD=11771), as shown in Fig.5. Reinfusion was usually accompanied by a hyperdynamic circulation, with a wide pulse pressure, and an elevated mean arterial pressure, 139 (SD=38) mm Hg in comparison to controls, 106 (SD=20) ( $p < 0.05$ ).

Arterial pressure, all experiments; hypotension (40 mm Hg) in bleeding animals (o), the result of experimental design, followed by post-reinfusion hypertension in comparison with controls (closed circles); bars are Standard Deviation.

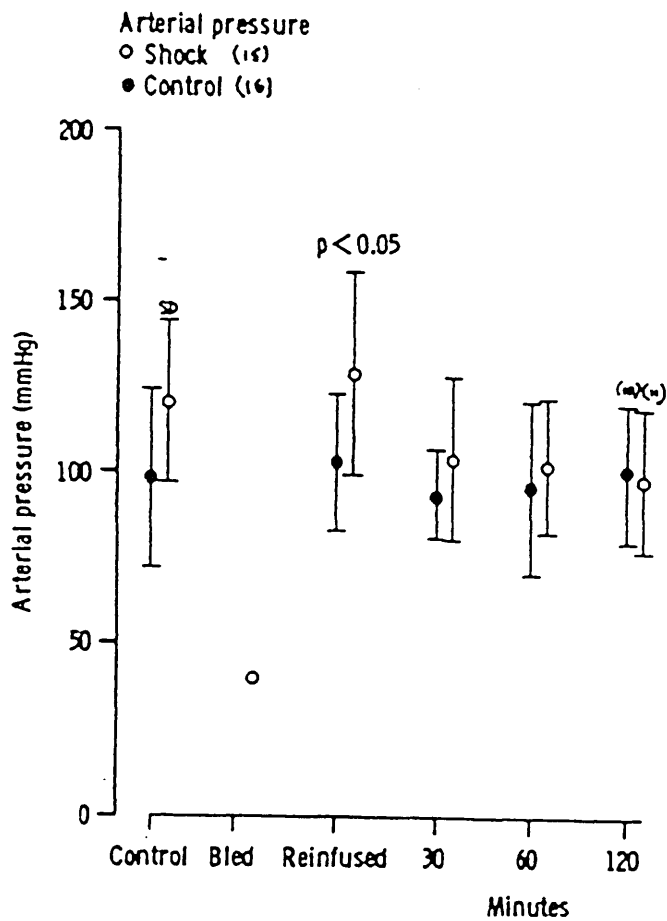


Fig.4

Heart Rate-Mean Arterial Pressure Product (RPP); bleeding is accompanied by bradycardia in shocked animals; bars are Standard Deviation.

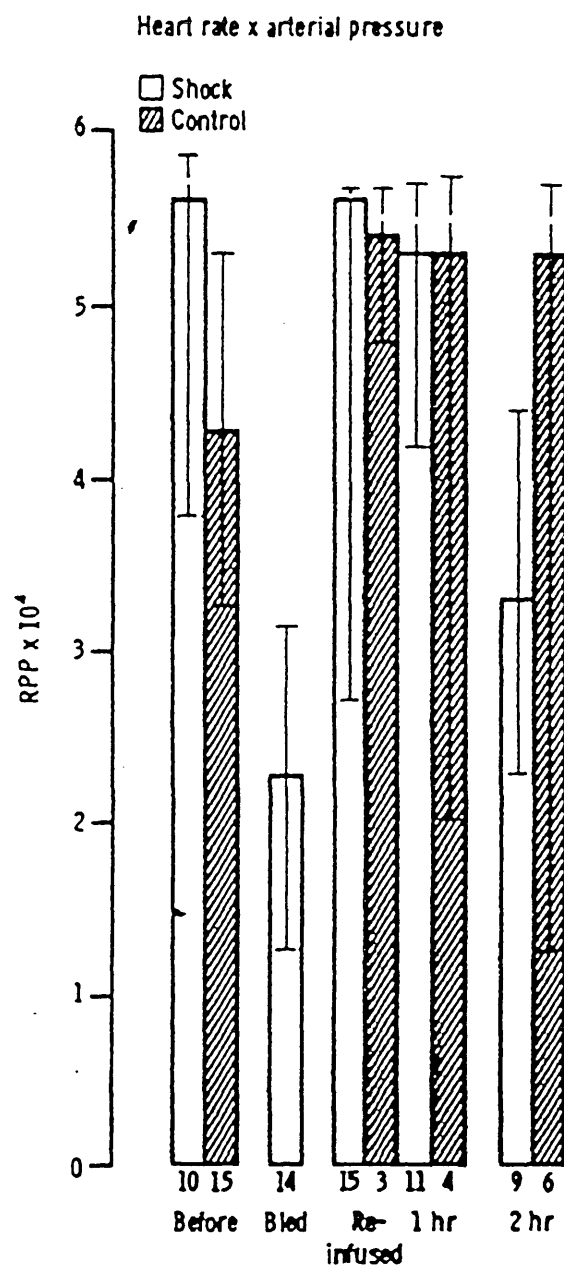


Fig.5

#### Hematocrit (n=31)

As shown in Fig.6, values for the hematocrit in animals observed 1-2 hours following shock and reinfusion (Hct) showed no significant differences between control and shocked animals, and did not change significantly with time. The limitations of using the central vessel hematocrit while studying changes in tissues instead of the tissue hematocrit, which would be less, was recognized and accepted. A small reduction in Hct is expected because of blood sampling. These values are consonant with those published (Altman and Dittmer, 1974; Everett et al, 1956), but differ in the shocked animals from the published results of Larsson et al (1981) who found a significant difference in Hct in bled rats after 90 minutes (34.5, SD=2.6 in controls vs 42.9, SD=2.2 in bled animals;  $p < 0.001$ ), but the animals were not reinfused.

The calculated red blood cell mass (RBCM), measured at the end of the experiment, was reduced in shocked animals ; mean=35.3 ml/kg (SD=6.3) compared to control animals (mean 39.3; SD=6.5, not significant).

#### Total protein (n=31)

The value for serum total protein, normalized to reinfusion period, was unchanged in shocked animals after one to two hours. As shown in Fig.7, values were 8(SD=1.0, n=15) g/dl in bled animals vs 7.9(SD=1.0, n=16)g/dl in controls.

#### Mass Balance:

#### Plasma Volume (n=31)

As shown in Fig.8, a histogram of Mass Balances in shocked and control

Hematocrit, normalized to time of reinfusion; there is no significant difference between shocked, reinfused animals, and controls; bars are Standard Deviation.

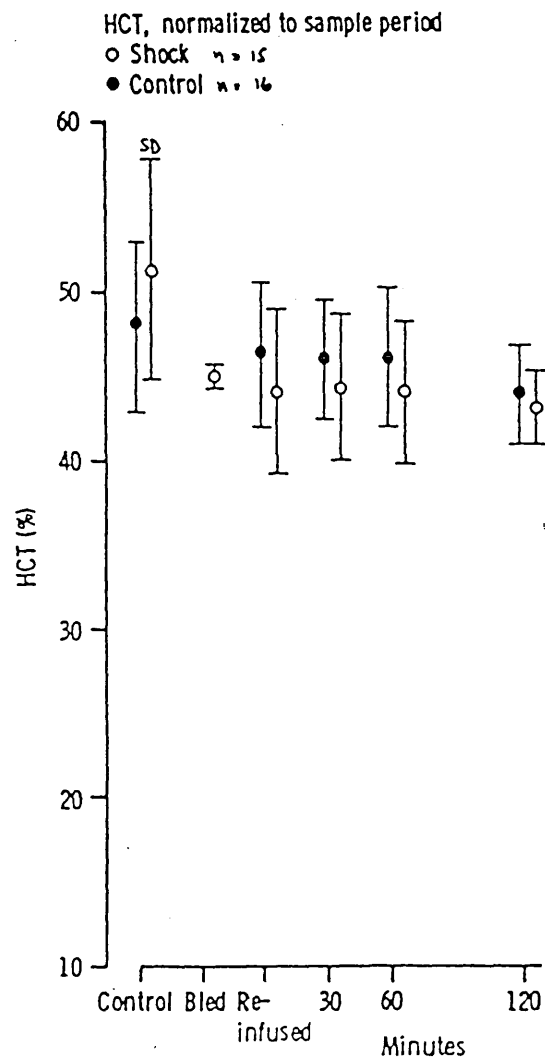


Fig.6

Total Protein, all experiments normalized to reinfusion period, was reduced in bled animals following reinfusion (probably dilution), but not after after two hours; bars are Standard Deviation.

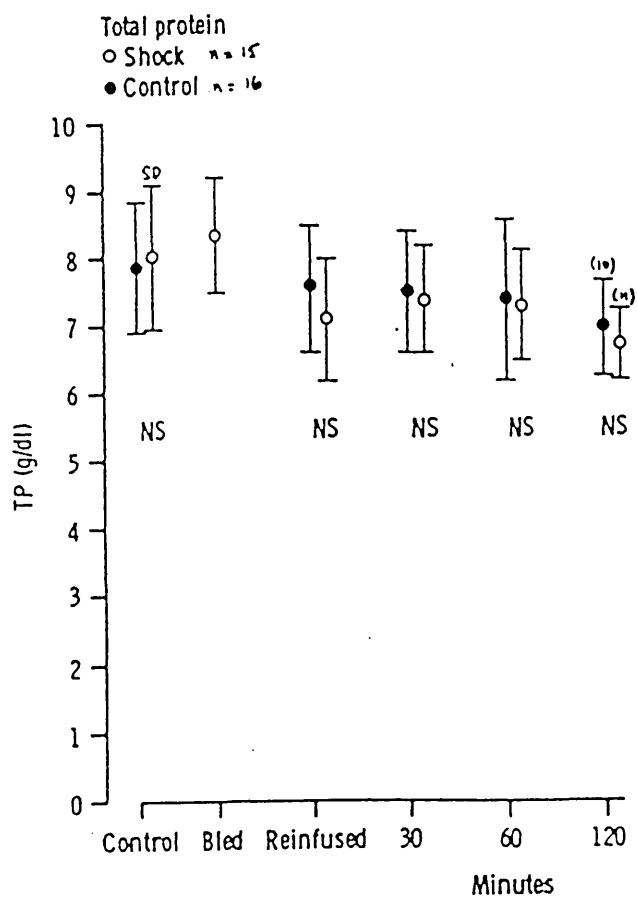


Fig.7

# Mass Balance

post-reinfusion plasma volume (PVR) is increased over that for controls two hours after shock, reinfusion, and saline load; Final Plasma Volume (PVP) is not significantly different between the two groups; calculated fluid lost from the circulation (VP) is increased in shocked animals, in comparison to controls; bars are Standard Deviation.

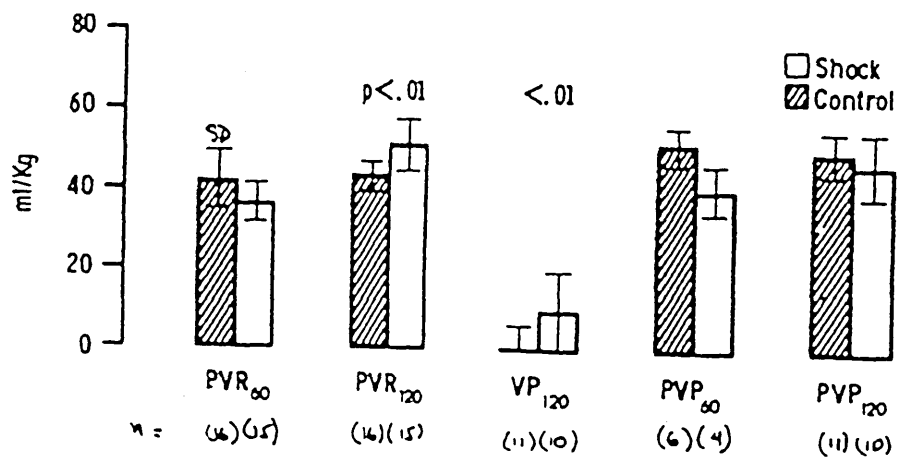


Fig.8

animals, the values for PVR, the post-reinfusion plasma volume, averaged 50 (SD=6.9) ml/kg for all 15 shocked animals, greater than 16 controls because of added fluid infusion, 39 (SD=3.7) ml/kg ( $p<0.01$ ). The value for the final plasma volume (PVP) in 14 shocked animals studied one to two hours was 44.8 (SD= $\pm 8.5$ ) ml/kg as compared with 16 controls, 50.6 (SD= $\pm 4.7$ ) ml/kg, not significant. The insignificant increase in plasma volume in controls is probably experimental error for the determination in small animals. These values are consistent with published data for the rat (Altman and Dittmer, 1974), and their difference suggest that the added saline given at the time of reinfusion left the circulation. In rats studied for two hours, the calculated fluid lost from the circulation,  $V_p$  ml/kg, given by PVR-PVP averaged 9.2 ml/kg (SE=3.6) in comparison to -1 ml/kg in controls ( $p<0.01$ ).

#### Plasma Iodinated Serum Albumin Washout (n=31)

The slope of the decay of  $^{125}$  radioiodine activity in the plasma during the post-reinfusion period is the result of two principal factors, renal losses (estimated by counts/ml bladder urine removed as a frozen pellet and weighed), and redistribution of labelled albumin between plasma and tissue (and perhaps reticuloendothelial system as well). Fig.9 shows clearance of  $^{125}$  I cts/ml normalized to initial counts/ml 5 minutes following injection. After reinfusion,  $^{125}$  I SA appeared to leave the circulation less rapidly in shocked animals than in controls; the ratio, counts/ml after 1 hour to initial counts/ml was 0.89 (SE=0.02, n=14), and 0.85 (SE=0.02, n=16) in controls ( $p<0.05$ ). The difference was less striking in animals observed two hours. This slower plasma clearance may be due in part to loss of

After shock and reinfusion, labeled radioiodine left the circulation less rapidly in shocked, reinfused animals than in control animals. Ordinate is plasma counts/ml; abscissa is time,  $t$  normalized to initial plasma concentration; bars are Standard Error.

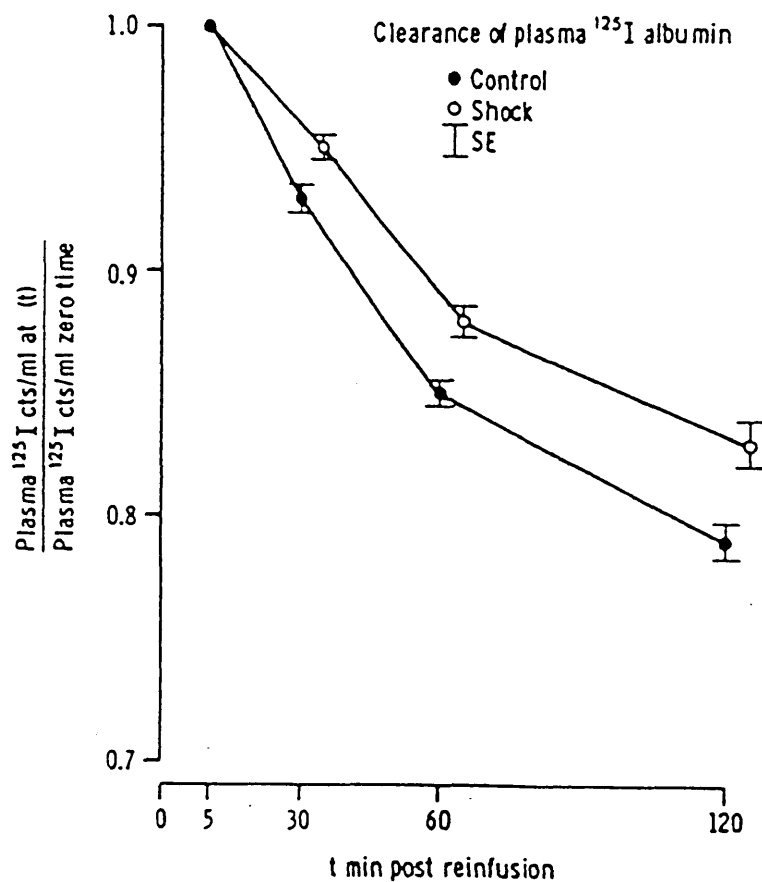


Fig.9

water from plasma of the shocked animals, thereby increasing concentration of tracer. As mentioned above, an average of 9 ml/kg of plasma appeared to be lost during the experimental period. Urinary loss of  $^{125}\text{I}$  was less in shocked animals; no micturition was observed, but there appeared to be six-fold less in the urine of shocked animals compared with controls.

#### Tissue, Fluid and Radioiodinated Albumin Flux:

##### Tissue Blood Volume (n=30)

In 10 shocked, reinfused animals studied for two hours, the average calculated TBV is significantly reduced in kidney, 0.3 (SD=0.14) g/g vs 0.43 (SD=0.16) g/g in controls ( $p < 0.02$ ). (Fig.10). These differences were not evident in animals studied for one hour after reinfusion. The values for control animals are similar to those published for the rat (Everett, 1956; Studer and Potchen, 1971).

##### Extravascular Tissue Water (n=30)

Average extravascular tissue water (EVTW) values, expressed as percent of dry weight are shown in Fig.11. In 10 two hour animals, EVTW was elevated after shock and reinfusion in kidney, 285 (SD=39.6) vs 210.1 (SD=65.5) in controls ( $p < 0.01$ ); this difference was not significant in one-hour experiments. The values in controls are similar to those published for the rat (Altman and Dittmer, 1974; Spector, 1957).

##### Tissue EDTA Content (n=8)

In Development Experiments,  $^{51}\text{Cr}$ -Ethylenediaminetetracetate (EDTA)

Tissue Blood Volume in various tissues of animals studied for two hours show reduction in kidney, in shocked animals in comparison to control animals; bars are Standard Deviation.

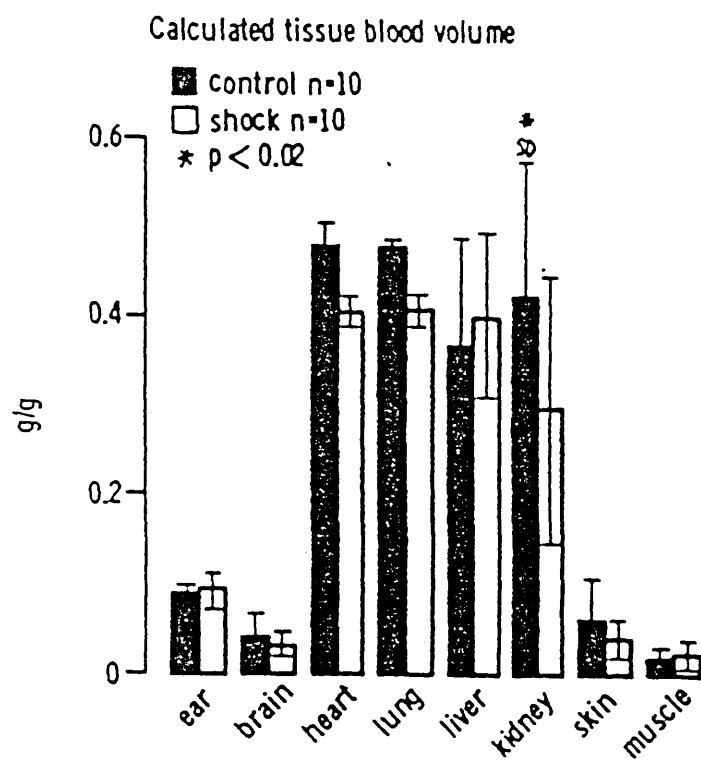


Fig.10

Extravascular Tissue Water was increased in kidney ( $p < .01$ ), in shocked, reinfused animals studied for two hours, compared to controls; bars are Standard Deviation.

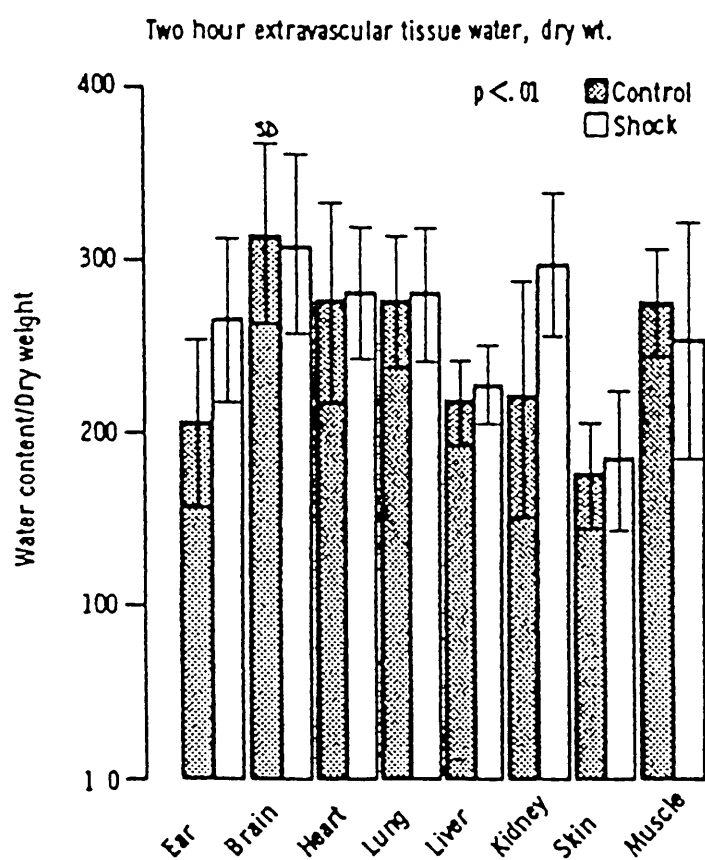


Fig.11

was used as the small-molecule probe, while Evans Blue Dye was used for estimation of plasma volume following reinfusion. As shown in Fig.12, there was increased EDTA (and water) in skin in shocked animals, 0.7 (SD=0.11)g/ml as compared to controls, 0.5 (SD=0.18)g/ml ( $p<0.07$ , not significant). The values in control animals are similar to those published for the rat (Larrison et al, 1981; Reed and Wiig, 1984).

#### Extravascular $^{125}$ Radioiodine Content (EVRC) (n=30)

The results are shown in Fig.13. The EVRC was elevated after shock and reinfusion in ear, 0.064 (SD=0.05) vs 0.009 (SD=0.007) ( $p<0.001$ ), in comparison with control animals studied for two hours. In ten one-hour experiments, elevation of EVRC in ear after shock and reinfusion seen in two hour experiments was not seen. The values for control animals are similar to those reported for the rat (Studer and Potchen, 1971; Dewey, 1959), and the rabbit (Kwong and Egan, 1985; Owen, 1976), although none happen to have recorded values for ear.

#### Appearance of $^{125}$ I in Subcutaneous Linen Wicks (n=13)

Wicks of linen surgical thread were placed in the abdominal subcutaneous tissue after preliminary surgery, but before bleeding, in such a way that 1 cm (.004 gm) was subcutaneous. They were removed weighed and counted 30, 60, 120 minutes after reinfusion. The counts per gm of wick were normalized for plasma  $^{125}$  I cts/ml, and corrected for absorbed plasma by multiplying by the fraction, wick  $^{131}$  I cts/gm divided by final plasma  $^{131}$  I cts/ml. The resulting value averaged 0.08 (SD=0.06) in shocked animals, and 0.09 (SD=0.05) in controls, not

Tissue EDTA Content in one hour Development Experiments; the differences are not significant; bars are Standard Deviation.

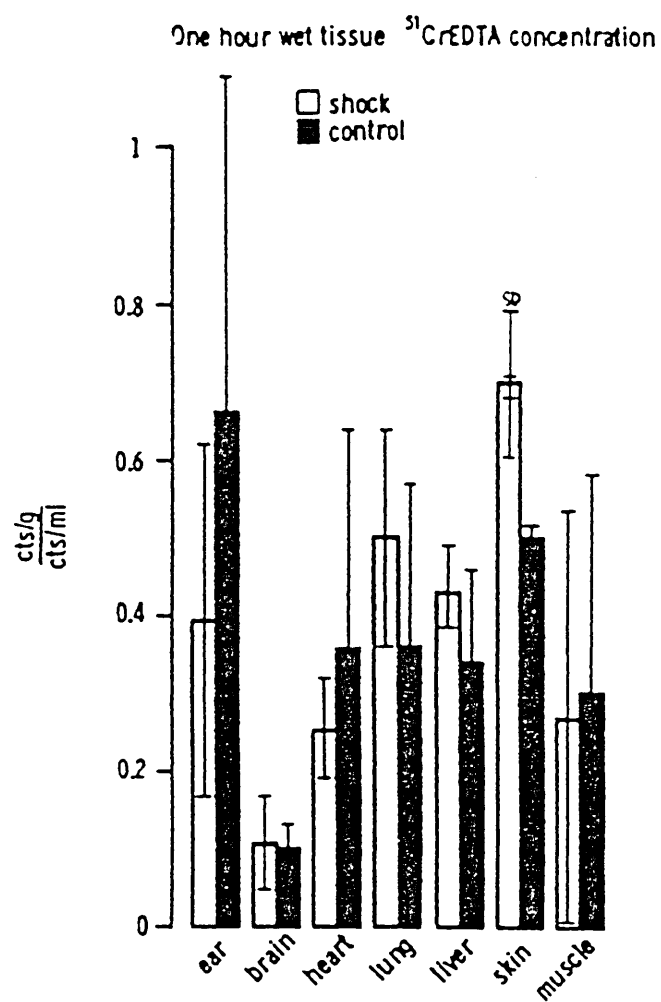


Fig.12

Extravascular  $^{125}\text{I}$  Albumin Concentration in various tissues; elevated after shock in ear, in comparison to control animals studied for two hours; bars are Standard Deviation.

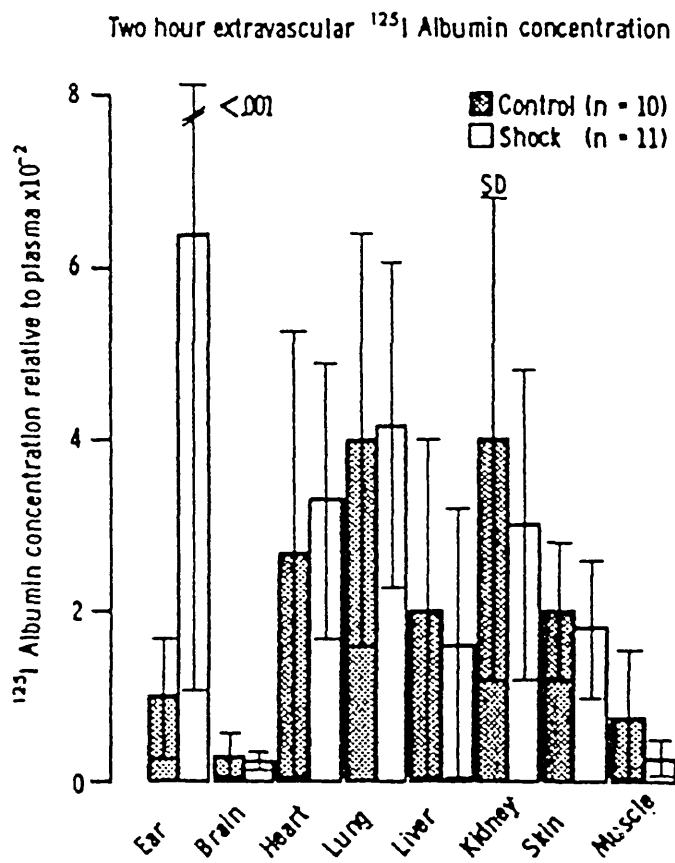


Fig.13

significant.

#### Blood Glucose Concentration (n=13):

The blood glucose, corrected for the sampling period (since duration of bleeding varied), was consistently lower in shocked animals following reinfusion; at two hours following shock and reinfusion, glucose concentration was 25.5 gm/dl plasma (SD=12) vs .63 (SD=14) ( $p<0.01$ ) in controls (Fig.14).

#### Discussion

Once technical details were refined during the development phase of this project, shocked animals behaved in a consistent way in comparison to controls. The familiar clinical picture of oligemic hypotension was seen; vasoconstriction, particularly in the ears and forefeet, and led to death of 38% during the procedure, most often near the end of the experiment.

#### Hemodynamic Response to Haemorrhage and Resuscitation:

During bleeding, the most obvious immediate observation in the present study was bradycardia (average reduction from pre-shock value was 24%,SD=0.11) at the end of the haemorrhage and before reinfusion. Others have reported this; Castenfors & Sjostrand ( 1972) have described reduction of this response by atropine. Pendleton et al (1980) have reported that vagotomy and compounds which suppress phenylethanolamine N-methyltransferase would also inhibit this response through the central nervous system. The response is not seen

The blood glucose, corrected for sampling period, as duration of bleeding varied, was significantly lower in shocked animals following reinfusion, in comparison to controls; bars are Standard Deviation.

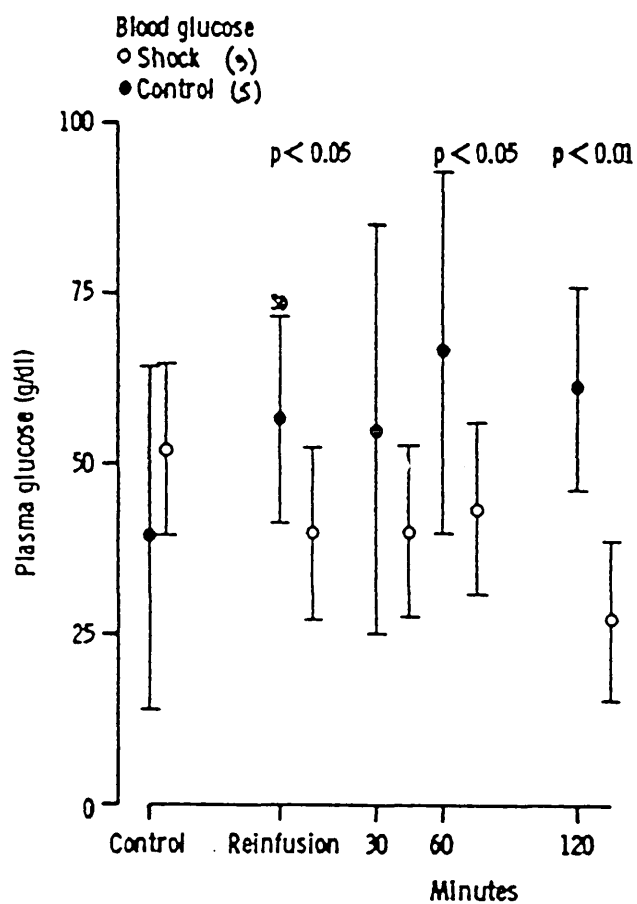


Fig.14

in all murine breeds, but is observed in the Sprague-Dawley rat used in the present study. McCafferty et al (1981) conducted further studies in which rate and volume of blood loss in rats were varied. If arterial pressure remained above 100 mm Hg, heart rate increased as blood loss increased, below this level, bradycardia proportional to reduction in systemic arterial pressure resulted, suggesting a baroreceptor mechanism. These experiments were conducted under chloralose anaesthesia;

barbiturates are known to obtund the baroreceptor reflex in rabbits (Normann, 1971). In the present study, Fentanyl citrate, which preserves cardiovascular reflexes (Shaw, 1983) was the primary anaesthetic agent, supplemented by an initial, and hourly small doses of sodium pentobarbital; the centrally-mediated bradycardia was not abolished. Secher et al (1984) have reported bradycardia with severe blood loss in four patients, tachycardia returned with resuscitation; two patients subsequently died.

The observed hyperdynamic circulation in the post-reinfusion period in shocked animals remains unexplained, but suggests the presence of vasoactive substances in the circulation in shock, a subject which has dominated the field of shock research for many years; (Wiggers (1942), Frank et al (1945), and Shorr et al (1945). Catecholamine release is the most likely explanation (Pirkle and Gann, 1975).

While not in the same species, Pardy and Dudley (1979) have carried out sequential acute haemorrhages in barbiturate-anaesthetized greyhounds, and described six phases of haemodynamic changes following acute haemorrhage, depending on whether bleeding stops, or recurs. The

initial increase in total peripheral resistance to maintain systemic arterial pressure in the face of hypovolemia is ineffective with sequential haemorrhages. This has a familiar clinical parallel; a hypovolemic patient with a narrow pulse pressure, and a normal systolic arterial pressure may collapse when given a general anaesthetic if not "topped up" with added blood or other volume replacement first.

In the present study, the Heart Rate-Pressure Product (RPP) has been used as an overall rough estimate of the state of the circulation. The RPP is taken to be the product of heart rate and mean arterial pressure; central venous pressure or left atrial pressure are usually subtracted from mean arterial pressure, but the former were not recorded in the present study. Wilkinson et al (1980) studied RPP in acute experiments in dogs under Halothane-Morphine anaesthesia and found RPP to be the best correlate of myocardial oxygen consumption over a wide range of ventricular afterload states. Of particular value clinically as a non-invasive measurement of cardiovascular performance, it has been shown by Gobel et al (1978) to correlate well with myocardial oxygen consumption (nitrous oxide method) in normotensive men with ischemic heart disease.

That 38% of shocked animals died is in keeping with studies reported by others in small mammals (Lazarus, 1984). The immediate cause of death in irreversible shock has been a matter of controversy for 40 years, and has led many to consider a biochemical abnormality (Shorr, 1945; Frank, 1945). The classical view of Wiggers (1942), Crowell (1961, 1962), and Goodyear (1967) has been the heart as a major factor

in irreversible haemorrhagic shock. Under barbiturate anaesthesia, Ohnishi et al (1984) reported improved survival, reversal of hypotension and oliguria, but no improvement in distribution of renal blood flow between medulla and cortex in shocked rabbits given a synthetic inotropic agent, M6434. Similar animals treated with Dopamine or Dobutamine died within 24 hours. The central role of endogenous vasoactive amines in cardiovascular adaptation to haemorrhage is well accepted, but the mechanisms continue to be the source of controversy. Bond and Johnson (1984), in barbiturate-anaesthetized rats with and without adrenalectomy, demonstrated that beta adrenergic receptors do not play a major role in compensation to shock, but that alpha 2 adrenoreceptors, and to a lesser degree, alpha 1 adrenoreceptors play the major role in the response to haemorrhage. In the present study, shocked animals were hypertensive following reinfusion and saline loading (mean arterial pressure 139(SD=38) vs 106(20), in comparison with controls. Ledgerwood and Lucas (1974) observed this in 20 of 33 severely injured patients following successful resuscitation, more pronounced in those given albumin. The cause was thought to be an acute hypervolemic state following mobilization of sequestered fluid, and can be treated by fluid and colloid restriction, or urgently, by vasodilators and diuretics. The amount of albumin given the rats during injection of labels in the present study is similar to that given patients, 2 mgm/kg.

Faden and Holaday (1979), and Albert et al (1982) reported that the opiate antagonist, naloxone, can increase survival in shocked animals by blocking endorphins released by the anterior pituitary during stress. While the response is apparently centrally mediated (Pendleton

et al, 1980. Feverstein et al, 1984), the mechanism is unknown.

Apart from effects of different anaesthetic agents, if any, the pre-shock state of the animal is apparently important. In the present study, in spite of being housed in a supervised vivarium, occasional animals had crusted nares and conjunctivae, and invariably died when anaesthetized, and are not included in this report. Diet is apparently of importance.

Lazarus (1984) has demonstrated a protective effect of a low-protein diet on rats subjected to severe shock, with evidence of preservation of Desoxyribonucleic Acid (DNA). Of animals on a standard diet, 33% died; all had evidence of breakage of DNA into small component molecular species. Of animals fed a low-protein diet, 83% survived, none had evidence of DNA degradation. In the present study, animals received standard pelleted feed. Spontaneously hypertensive rats (mean arterial pressure  $160 \pm 4.9$  mm Hg) under barbiturate anaesthesia have a reduced capacity for venoconstriction as a compensation for hypovolemia in comparison to controls (Burke, et al, 1984); the authors inferred a greater contribution of transcapillary fluid flux to compensation in the hypertensive rats. In the present study, only 2 of 65 animals had an initial systolic arterial pressure of 160 mm Hg, and both survived the 2 hour post-reinfusion protocol; in these animals, values for  $V_p$ , calculated fluid lost from the circulation, and 125 I plasma disappearance indices were within standard deviations of the values for the 2 hour shock group.

Whatever the cause of shock - anaphylactic, cardiogenic, haemorrhagic,

or septic - the final result is inadequate tissue perfusion. The calculated tissue blood volume, yielded values similar to data published by others in control animals, and were significantly reduced in kidney in shocked animals reinfused and observed for two hours.

The possibility of bacterial infection superimposed on haemorrhagic shock as a cause for some of the changes observed must be considered. Cultures from blood drawn by heart puncture in two successive Development Experiments showed no growth after two weeks.

The metabolic consequences of shock include a shift from aerobic to anaerobic metabolism resulting in metabolic acidosis with a compensatory decrease in serum bicarbonate, not documented in the present study. A high serum potassium concentration is expected, the result of shift of potassium from the intracellular to the extracellular compartment (Shires et al, 1981) because of a failure of cell membrane function - the sodium-potassium "pump" (Illner et al, 1972).

In special tissues, such as kidney and liver, these universal consequences have specific effects, roughly in proportion to reduction in local tissue perfusion: decrease in renal cortical blood flow, glomerular filtration, tubular sodium and water reabsorption, and eventually acute renal failure; depression of hepatic and skeletal muscle cellular membrane potential difference and abnormal levels of high energy phosphates (Gallagher and Shires, 1977; Peizman et al, 1981). The only measure of the "sick cell" in the present study was

serial estimation of blood glucose, which revealed serious hypoglycemia in shocked rats compared to controls. The findings were similar to those reported by Rhodes (1980) who demonstrated failure of hepatic oxidative phosphorylation in shocked rats; depletion of glycogen stores led to hypoglycemia. Hepatic cellular injury (degradation of ATP) can be prevented in shocked dogs by pretreatment with a xanthine oxidase inhibitor, Allopurinol (Cunnigham et al, 1978) with increased survival. The effect is thought to be due to stabilization of lysosomal membranes by maintaining adequate levels of ATP.

#### Water Loss From The Circulation

Plasma volume is regulated through humoral and renal mechanisms, the interstitial fluid volume (IFV) by local, and perhaps neural mechanisms (Reed, 1984). Protein concentration, chiefly albumin, in both compartments is an important determinant of osmotic pressure. In non-oedematous peripheral tissues, albumin has been calculated to distribute in 50-70% of the total interstitial volume at lymph concentrations (Bell et al 1980). The state of hydration of the interstitium is of importance in movement of plasma protein (Rothschild et al 1955). Mullins and Bell (1981) have shown 41% increase in extravascular albumin mass in muscle following a 10% saline load of the circulation in unshocked rabbits anaesthetized with pentobarbital.

Measurement of IFV pressure and constituent concentrations by various techniques such as subcutaneous capsules (Guyton, 1963), wicks, or

micropipettes (Wiig et al 1981, 1983) have yielded varied results, probably because of the inherent invasiveness of the techniques. In the development stage of present study, an attempt was made to use volume change in the rat tail immersed in a graduated cylinder containing liquid paraffin; the magnitude of any change was too small to be detected. Aukland and Fadnes (1973) and Garetto and Hargens (1976), using wick methods similar to that in the present study, found an interstitial fluid protein concentration of 3.5 gm/dl, equivalent to a colloid osmotic pressure of 11 mm Hg. The tissue surface of incised wounds has yielded fluid containing a higher potassium concentration than in plasma (Haljamae and Freden, 1970). Lucas and coworkers (1982) found labelled albumin left the circulation at the rate of 7%/hr in presumably normal individuals; to reach the lymphatics, protein must traverse the IFV. Much less is known about regulation of the IFV. At least three possibilities exist: the simplest; that the IFV is a passive overflow system without autoregulation (Reed and Wiig, 1984); alternately, controlled by receptors in tissues (Zweifach and Silberberg, 1979). The third possibility is that regulation is mediated through automatic local adjustments of forces into, and out of, the interstitium (Guyton et al 1975; Aukland and Nicolayson, 1981). Skin and muscle studies by the latter support this proposal. In a study to prove the existence of interstitial volume receptors, Reed and Wiig (1983) measured interstitial fluid volume (IFV) by the 2 hour distribution volume of 51 Cr EDTA in control rats (IFV= 0.22 ml/g body weight, skin IFV= 0.06 ml/g body weight), and those given a diuretic (Lasix) to produce acute or chronic dehydration and those given a saline load. Only their control values are comparable to the present study in shocked,

reinfused, and possibly overhydrated rats. In the present study, converted to their units, skin IFV in control rats averaged 0.07 ml/g body weight.

Decrease in fluid movement to the extravascular space in lung in shocked animal was reported by Demling (1980) who noted reduced lymph collection from a previously-created lymph fistula in shocked unanaesthetized sheep. Following saline resuscitation, there was increased lung lymph flow without evidence of increased plasma protein permeability (protein lymph/plasma ratio). When resuscitation in the same model was carried out with saline load of 2.5x shed volume, there was increase in extravascular lung water.

Movement of fluid (and protein) were the chief evidences of a change in the interstitium of shocked rats in the studies reported here; the kidney appeared to show a relatively large shift of water to the interstitium, since tissue blood volume (TBV) was reduced and water content was enhanced. In clinical terms, this describes the pale, swollen kidney of acute renal failure. The control values for kidney TBV in the present study compare favorably with those reported by Everett (1956) and Studer and Potchen (1971). Proteinuria (trichloroacetic acid precipitation) was present in half of the animals in each group and remains unexplained.

The kidney is well recognized as a target organ in shock. Hopkins et al (1963) reported impaired renal function in shocked trauma victims who underwent resuscitation and surgery; glomerular filtration rate and estimated renal blood flow fell. Oliguria following trauma and

surgery may also be the result of vasopressin release (Dudley et al, 1954). Because of assumed renal autoregulation, cardiac output is not an important determinate of urine output and solute excretion so long as a minimum mean arterial pressure is maintained in patients with intraoperative blood loss (Kennedy et al, 1964). The role of atrial stretch receptors in canine renal autoregulation was described by Henry and Pierce in 1956. More recently, Busija (1984) has demonstrated that in awake rabbits, previously anaesthetized with a short-acting barbiturate, the renal vascular response to hypotension is dependent upon arterial oxygen partial pressure. Increase in renal vascular resistance (mean arterial pressure/renal blood flow, radioactive microsphere technique) was greater in severely hypotensive, hypoxic rabbits than those hypotensive with a normal arterial oxygen partial pressure.

#### Radioiodinated Albumin Clearance:

That the kinetics of plasma protein movement can be estimated from plasma clearance curves has been the subject of controversy; clinical studies by Lucas et al (1982), and Fleck et al, (1985) have used the one hour disappearance of of radio-iodinated serum albumin from plasma (normally about 7% per hour), but Lassen and Parving (1985) have emphasized that while Transcapillary Escape Rate (TER) of labelled albumin in the steady state (control animals in the present study) is not affected by minor changes in plasma volume, in a non-steady state such as shock, these changes may be important because of possible plasma protein losses to the extravascular space, and radioactivity in plasma samples must be corrected for changes in hematocrit by

multiplying by  $(1-Hct)/Hct$ , because of the error otherwise introduced by changes in plasma water. These authors further claim that TER is a measure of unidirectional flux of albumin, assuming back-flux is negligible. They suggest that filtrative flux (solvent drag) is a major determinant of TER because an increase in central venous pressure increases the albumin extravascular mass, quickly counteracted if lymph flow increases normally (lymph flow ceases in shock)(Parving et al, 1974; Lassen et al, 1974). The shocked animals in the present study had decreased clearance of  $^{125}I$  SA from the circulation, averaging 8.5%/hr vs 10.5%/hr in controls ( $p < 0.05$ ). The rats were in the early (2 hr) post-shock and resuscitation state, unlike the patients studied by Lucas et al (1982) who were studied 12-36 hours after resuscitation; in these, decreased clearance of labeled albumin, 6%/hr vs 7%/hr for volunteers was observed. As mentioned above, average values for Hct in the present study did not vary significantly between shock and control animals. If the correction proposed by Lassen and Parving in  $counts/ml \times (1-Hct)/Hct$  are made, the rate of plasma clearance of  $^{125}I$  albumin from the plasma was still slower in shocked animals one hour after reinfusion; 0.95 (SE=0.06, n=14) vs 0.88 (n=16,  $t = 1.13$ , not significant). On the other hand, Paaske (1983) has reported both mathematical and experimental evidence to support his claim that TER is an arbitrary rate constant which cannot be attributed to any one physiological process. The rate of renal clearance and water clearance on plasma albumin concentration would be other important factors, but were not measured in Paaske's study.

In the present study, radioiodine counts in subcutaneous linen wicks

did not differ in the two groups. What changes are indicated by this technique is uncertain; plasma concentration, lymph flow, or plasma leakage from trauma (Aukland and Fadnes, 1973). In shocked, reinfused rats in the present study, labelled albumin left the circulation more slowly than controls, hematocrit did not differ significantly, yet labelled albumin in the wicks, normalized to contemporaneous plasma did not differ significantly; averaging  $0.08(\text{SD}=0.06)\text{g/ml}$  vs  $0.09(\text{SD}=0.05)\text{g/ml}$  in controls. Protein concentration in wick fluid was not measured. If lymph flow was reduced in shocked animals, then a greater wick concentration of labelled albumin would be expected in comparison to controls; that the wick concentrations of labelled albumin did not differ, while rate of loss of labelled albumin was lower in shocked animals, may suggest interstitial entrapment. Lucas (1986) claims that skin lymphatic protein concentration fairly accurately reflects the interstitial protein concentration; Bell and coworkers (1980) assert that albumin distributes in about 70% of the total interstitial volume at lymph concentration in non-oedematous peripheral tissue. The introduction of six 2-0 linen threads into the abdominal subcutaneous tissue produces local trauma, and the observations must be interpreted with this in mind. Wicks in the subcutaneous tissues as a sample of interstitial albumin give varying results; Aukland and Fadnes (1973) found subcutaneous wicks in rats contained albumin concentrations 50% that in plasma, unchanged over 2 hours, with a greater albumin/globulin ratio than plasma (as in lymph).

The possibility of antigenicity of bovine albumin to the rat concerned Dewey (1959) who used labelled rat and rabbit plasma proteins to study

vascular-extravascular exchange between the two; there were no overall differences, except in liver and spleen, presumably due to catabolism. An uncontrolled variable in the present study is whether injected heterologous (bovine) albumin is metabolized more rapidly than normal turnover of endogenous albumin, a question also raised by Lucas (1984) in clinical studies. Sterling (1951) reported that human albumin labelled with 2-3 iodine atoms per molecule remained tagged after intravenous injection into rabbits and behaved like native protein by immunochemical tests. Warner (1976) reported the presence of dimer species in samples of bovine serum albumin. Studer (1971) also excluded the possibility of rapid breakdown of heterologous plasma in the short term on the basis that immune reactions to foreign protein are not seen in the rabbit until 8 days post-injection (McFarlane, 1964).

In the present study, the high extravascular albumin concentration in liver and the small EVRS in brain are consistent with those reported by (Studer and Potchen, 1971). The latter was thought by Studer to represent the contribution of the choroid plexus. Extravascular  $^{125}$  I was elevated after shock in ear, in comparison with control animals. This could be due to local changes in permeability, or an altered interstitium; the interstitium in the ear of shocked animals was not grossly overhydrated, as the water and EDTA content were lower in shocked animals studied for one hour. These events may be time-dependent as in ten one-hour experiments, elevation of EVRC in ear after shock and reinfusion seen in two hour experiments was not seen. The values for control animals are similar to those reported for the rat (Studer and Potchen, 1971; Dewey, 1959), and the rabbit (Kwong and Egan, 1985; Owen and Triffitt, 1976), although none happen to have

recorded values for ear.

## Conclusions

1. The results of a study whose objective was to demonstrate redistribution of serum albumin and water in the interstitium in 31 anaesthetized rats, 15 shocked by controlled hemorrhage, has been reported.

2. After shock and reinfusion, 125 I serum albumin left the circulation more slowly than in controls.

3. Extravascular tissue water was increased in kidney, compared to controls.

4. Extravascular 125 I was elevated after shock in ear, in comparison with control animals. This could be due to local changes in permeability, or an altered interstitium; the interstitium in the ear of shocked animals was not grossly overhydrated, as the water and EDTA content were lower in shocked animals studied for one hour.

5. The significance of these data to those of other investigators has been discussed.

## GENERAL DISCUSSION

These seemingly disparate projects can be studied together, as each involves the mechanical and colligative properties of an array of macromolecules, the proportions, concentrations (hydration), and distributions of which influence their behaviour, just as in the case in an interpenetrating polymer network (IPN). An IPN is a mixture of polymers, of varied proportions, with varied mechanical properties, often with greater strength than either constituent on its own (Sperling, 1981). Like the IFS, polymerization of one (or both) monomer(s) occurs in the presence of the other, to distinguish from mechanical blends obtained by mechanically mixing previously formed polymers (Kim et al, 1976). These principles will be applied to two tissues which have been examined in the present study.

## SKIN

Mammalian skin is the largest organ (and IFS) in the body; 16% of adult weight, 1.5-2 m<sup>2</sup> adult surface area. Structurally, it is a fibrous composite of collagen in a planar array and elastin fibers embedded in an amorphous matrix (an IPN); of its dry weight, 80% is collagen, 4% is elastin (Lanir, 1979), the latter degenerates with age (of interest, in the light of age-related changes in anal elastin noted by Haas (1980). Considered biomechanically, it is highly

non-linear and anisotropic under time-dependent stress-strain-time relationships. Hyaluronic acid occupies the space between the fibers. In its extracted form it is viscous at physiological pH, but may become viscoelastic under low pH (Gibbs et al, 1968). In the present study, the mechanical properties of skin have not been examined alone, but only as part of the muscle-connective tissue complex of the anus. The tissue biomechanics of skin have been extensively studied by others. Ferguson and Barbenel (1981) studied extension - load characteristics of the forearm skin of human volunteers. All displayed the classical non-linear response of soft connective tissue; an initial compliant phase where low load produced large extension, followed by increasing stiffening, varying with direction of stretch, and varying greatly in other body sites, proportional to the depth of skin surface wrinkles. Brody, Peng, and Landel (1981) studied volar forearm skin during flexing and extending the elbow, and noted time-dependent hysteresis - thought not to be an artifact - which they attributed to viscous flow of amorphous phase between fibrous components, with prompt recovery. This was not seen in burn scar, or in lymphoedematous skin, both of which showed progressive softening with cycling - suggesting movement of water out of the matrix and into the lymph - an example of the interplay between mechanical and colligative properties. In the present study, the anal muscle-connective tissue complex did not behave in the classical way; this may be because of the myogenic response of smooth muscle, or from the fact that the baseline measurement of Initial Force was, in fact, not measured at zero extension. The fact that caudal nerve block abolished the differences between the later favourable and unfavourable patients suggests that the unusual behaviour is the

result of changes in muscle tone. Lanir (1979) suggested that viscoelasticity in skin originates primarily from the collagen fibers, at variance with the mechanical behaviour of lymphoedema or burn scar in humans cited above. One of the major in vivo features of skin is the pre-existing stress and strain fields (strain history, like "memory" in synthetic polymers). Dupuytren (1834) and Langer (1861) observed that circular skin punctures resulted in elliptical holes; Langer, and later, Cox (1942) mapped cadavers with trajectories connecting the major axes - now recognized as the lines along which incised wounds gape (Langer's lines) - of importance in clinical surgery.

Rat skin mechanical properties change in relation to maturation; advancing age decreased stress and moduli at low strain ratios, but increased them at high strain ratios (Vogel, 1981). The biochemical bases for these observations were provided by Flandin et al (1981) who studied saline extractability and chemical characterization of rat skin collagen from birth to 16 weeks. Collagen content increased from 10% in newborns to 60% at six weeks, when collagen solubility is greatest, displacing other proteins. Infantile-type collagen had disappeared by this time. With uniaxial stretching, the elastin and collagen fibers gradually straighten and align in the axis of loading (Craik et al, 1965). Daly (1969) showed that elastase-treated skin showed an increase of stress-free length on uniaxial stretch, loss of elastic recovery, but the same strength, stiffness, and viscoelastic properties at high strain as controls - the latter the result of collagen fibers. The contribution of the

matrix to skin biomechanics is the subject of controversy. Lanir (1979) and others have suggested that ground substance flows between the fibers with stretching, perhaps reducing friction between fibers. On the other hand, Harkness and Harkness (1959) found little difference in rate of creep of newborn rat skin (which differs from mature murine skin) treated with hyaluronidase in comparison to controls.

Movement of fluid and macromolecules in rat skin did not differ strikingly in shocked, reinfused animals in the present study in comparison with controls; extravascular tissue water and EDTA were increased in the palmar skin of shocked animals studied for one hour, compared to controls, but not significantly so ( $p < .07$ ). This difference would probably have been detected mechanically, but this was not done. Extravascular radioiodinated albumin was significantly increased in ear - largely skin and cartilage - in comparison to controls. In dogs, Bell et al (1981) demonstrated exclusion of fibrinogen from toe pad, and albumin from skin from part of the sucrose space, using constant infusion of sucrose, and bolus injection of labelled albumin and fibrinogen. In the rats studied in the present report, the small size of tissue samples may have been a disadvantage.

## MUSCLE

The tissue mechanics of muscle has been of interest to physiologists; the authoritative work of Hill (1970) has been referred to above, and is still used as a benchmark for biomechanics studies of muscle tissue. In the clinical study reported here, patients with stiffer anal muscle-connective tissue complex peroperatively had a poorer result than those with more pliant tissues; whether due to a stiffer spring, or a more sluggish dashpot (Hill,1970) is not clear. From the fact that the 20% of patients who had a caudal nerve block as part of surgical anaesthesia did not show this difference suggests that muscle, rather than connective tissue, is involved.

Age is well established as a factor in studies of muscle mechanics in animals. Mohan and Radha (1980) noted that collagen content in all classes of muscle - slow, fast, and cardiac - increased 30-50% between 5-25 months, and became less degradable. In the rats in the present study, the state of hydration and extravascular labelled albumin concentration in sacrospinalis muscle did not differ significantly in bled, reinfused animals, compared to controls. No mechanical properties of muscle were investigated.

The IFS, by comparison is about 15% collagen, having a Young's modulus of  $10E9 \text{ N/m}^2$  with failure at 8-10% extension, a variable amount of elastin, and the rest proteoglycans, largely hyaluronate, holding about 20% of body weight in water, but varying from one tissue to another. Unlike the IPN, the constituents are heterogeneous, polydisperse, and discontinuous. In some human tissues, the moduli

vary with site in the same tissue, for example, autopsy-derived thoracic aorta has the lowest strength at the origin of the left subclavian artery (Hyashi and Kennedy, 1983), and this is the most frequent site of rupture in accident victims. In the IFS, water content of the amorphous phase is of great importance in determining mechanical properties; as seen in cartilage, increased water yields a high modulus, underhydration leads to tissue which behaves more like an elastomer.

The orientation of the high-modulus component is of importance to the mechanical properties of both IPN and IFS. As pointed out by Wainwright (1976), crossed rigid reinforcing fibres in an amorphous polymer sheet reach a final small fibre angle at which the load is borne largely by the fibres, with portional shortening at 90 degrees to the load; at large fibre angles, the initial load is borne by the amorphous component. Crossed fibrillar and crossed helical array form the basis of most cylindrical animals and some vertebrates. Arrangement of fibres in a parallel orientation as in tendon, the deformation is purely tensile. If the fibres are arranged discontinuously and randomly as in human skin and anus (Haas, 1983), a more isotropic material results; flexible, moderately extensible. The amorphous phase is also important; the force required to move the fibres through the amorphous phase - viscoelastic, in the case of the proteoglycans - means the behavior is therefor frequency-dependent.

The diffusivity of aqueous macromolecules through IPN would be the result of the individual solubility coefficients of the substituents and their proportions, assuming no interaction yielding a third

polymer and no voids are present. The diffusivity of macromolecules through the interstitium, from the available data, is variable; in shocked rats,  $^{125}\text{I}$  SA left the circulation more slowly, and was present in extravascular tissue in increased amounts in ear, in comparison to controls.

Irrecoverable deformation, creep, is a property of both pliant composites, IPN, IFS; it has been demonstrated in the anal muscle-connective tissue complex in patients undergoing a therapeutic stretch procedure in the present study. It cannot be proven that this effect was limited to the interstitium, the evidences suggests muscle was principally affected.

#### CONCLUSIONS

1. The results of two projects, each considering interstitial biopolymers as pliant composite materials with unique mechanical and colligative properties has been presented.
2. Mechanical properties, including irrecoverable deformation, have been considered in a clinical study of 64 patients undergoing anal force measurements at the time of therapeutic anal stretching to an extension ratio of 1.33. Significant differences between those who considered themselves "cured" and unsatisfactory patients were noted in initial force and at lower extension ratios.
3. Colligative properties, restricted mobility of interstitial labelled serum albumin in comparison with a labelled small molecule

(51 CR EDTA) has been demonstrated in shocked rats, in which disappearance of intravascular labelled serum albumin was less rapid and was recovered in increased amounts extravascularly in certain tissue (ear), in comparison to controls.

4. The distribution, composition, and hydration of the interstitial biopolymers have important effects on the mechanical and colligative properties of the containing tissues.

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