MEASUREMENT OF INTERSTITIAL ‘FLUID’ PRESSURE BY MEANS OF A COTTON WICK IN MAN AND ANIMALS: AN ANALYSIS OF THE ORIGIN OF THE PRESSURE

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SUMMARY

1. A method for measuring interstitial ‘fluid’ pressure by using a wick consisting of long-stranded cotton wool at the interface of the tissue is described.

2. A correct measurement of hydrostatic fluid pressure was obtained when the wick, connected to a suitable transducer, was applied to filter paper in which the channels contained fluid at a known subatmospheric pressure.

3. A mean subatmospheric pressure of $-1.6\ \text{cmH}_2\text{O}$ was recorded in the subcutaneous tissue of the normally hydrated frog; pressure fell with dehydration and rose with overhydration.

4. A mean subatmospheric pressure of $-2.8\ \text{cmH}_2\text{O}$ was recorded in the subcutaneous tissues of the abdominal wall and scalp of the normally hydrated rat. Simultaneous measurements made at symmetrical sites showed a high degree of correlation.

5. A comparison of interstitial ‘fluid’ pressure in the subcutaneous tissues of the scalp (measured by a wick) and the abdominal wall (measured by a Guyton capsule), in both anaesthetized and conscious rats showed some degree of correlation. There was, however, a wide scatter of values.

6. The interstitial ‘fluid’ pressure in the rat, measured by both wick and capsule, became more negative when the animals underwent frusemide diuresis; the capsule pressures fell more rapidly for a given degree of fluid loss.

7. The wick method was applied to the subcutaneous tissues of the arm in normal man; a mean atmospheric pressure of $-3.4\ \text{cmH}_2\text{O}$ was recorded in five subjects. There were no untoward sequelae.

8. The forces responsible for the measured pressure have been analysed. The recorded subatmospheric interstitial ‘fluid’ pressure in the rat rose towards atmospheric pressure and sometimes became positive when the wick was removed, soaked in

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increasing concentrations of hyaluronic acid, and reinserted in the tissues. This did not happen in similar experiments in which the wick was soaked in bovine albumin, rat plasma or saline. The large macromolecules of hyaluronic acid therefore exert a force which opposes the forces responsible for the subatmospheric interstitial pressure.

9. It is suggested that the apparent subatmospheric hydrostatic pressure is due to the osmotic forces developed by the hyaluronic acid molecules in the interstitial tissues trapped by their mutual entanglement in a sieve of collagen fibrils acting as a semipermeable membrane.

The measurement of interstitial 'fluid' pressure has been attempted by many workers using a variety of techniques. Meyer & Holland (1932) and Burch & Sodeman (1937) measured the minimum pressure required to force small volumes of normal saline into tissues through a needle. These authors assumed that this pressure was approximately equal to that existing in the tissues before injection. In the subcutaneous tissues of man they found pressures in the range of 0 to +8 cmH₂O. Before injection of fluid, meaningful pressure measurements could not be obtained. McMaster (1946) refined the needle technique so that only minute quantities of saline were injected; he concluded that the method did not measure the true interstitial pressure but rather the breaking point at which the tissues were forced apart by the injected fluid. McMaster (1946) referred to this as 'interstitial resistance'. In oedematous tissues he was able to demonstrate the presence of free fluid, the pressure of which could be measured without prior injection. Wiederhielm (1969) demonstrated the presence of free fluid in the interstitium of the bat wing; by using a micropipette, he recorded a positive pressure from this tissue.

McMaster (1941a, b) showed that if Locke's solution was brought into contact with the interstitial space of the skin through a very fine needle, the fluid was intermittently drawn in. The pressure required to prevent this influx was unfortunately not determined, but this observation raised the possibility that the interstitial pressure might be below atmospheric. This was substantiated when Guyton, Armstrong & Crowell (1960) measured subatmospheric pressures in chronically implanted perforated capsules in the dog. It was claimed that the capsular fluid was in continuity with the surrounding interstitial 'fluid.' Guyton (1963) demonstrated that the capsule pressure varied in accordance with Starling's hypothesis when capillary hydrostatic or plasma oncotic pressures were varied.

Scholander, Hargens & Miller (1968) described a new method for measuring interstitial pressure which also recorded subatmospheric pressures. These authors used a saline-filled plastic catheter with a cotton wick pulled into one end. The wick was inserted subcutaneously to establish contact with the interstitial space. Pressure was transmitted through the catheter–wick assembly to a manometric recording device. By means of a model experiment Scholander et al. (1968) validated the principal of the technique and recorded subatmospheric pressures in the subcutaneous interstitial spaces of several species of animal.

The wick and capsule methods have been thought to measure the hydrostatic pressure of the interstitial space (Guyton, 1963; Scholander et al., 1968; Stremme, Maggert & Scholander, 1969; Ladegaard-Pedersen, 1970). However, it is probable that there is little, if any, free fluid in the interstitial space (McMaster & Parsons, 1939a, b; Guyton, Scheel & Murphree, 1966) and the possibility must be considered that the negative pressure is generated by the osmotic effect of the macromolecules of ground substance, trapped by mutual entangle-
Interstitial ‘fluid’ pressure

ment in a sieve of collagen fibrils acting as a semi-permeable membrane (Fessler, 1960; Laurent, 1970).

We have modified Scholander’s technique and evaluated it with a view to clinical application. Pressures have been measured in the frog and rat in various states of hydration. In a comparative study, simultaneous recordings have been made with the capsule and wick technique in the same animal. A small number of studies have been carried out in man. Experiments have been performed to test the hypothesis that the negative pressure is due to osmotic rather than hydrostatic forces. A preliminary presentation of some of this data has already been made (Snashall, Lucas, Guz & Floyer, 1970).

MATERIALS AND METHODS

Catheter–wick system

Wicks made from a ‘long fibre’ cotton wool (Sea Island) were used throughout this study. The fibres have a mean length of 6 cm and a fairly uniform width (approx. 10 µm). Before making up the wick the material was combed to remove shorter and weaker fibres. The wick was then pulled into the end of the catheter by means of a silk thread (5/0 gauge) which passed through the catheter and was secured at the opposite end (Fig. 1a). The wick protruded 0.5–1.0 cm from the tip of the catheter which was made of polypropylene (i.d. 1.15 mm ‘Intracath’, Bard Davol Ltd). The system was filled with saline and the wick boiled to ensure sterility and to remove air bubbles. Microscopical examination of wicks after boiling confirmed this to be an effective method for removing air bubbles. The wick was inserted beneath the skin through a thin-walled needle (i.d. 1.6 mm).
The catheter–wick assembly was connected to a semi-conductor strain gauge (type SE 3/81, SE Laboratories Ltd) with a volume displacement of 0·01 mm³/100 mmHg. The signal was amplified by using a carrier amplifier (type SE 423/2E) in conjunction with a u.v. recorder (type SE 2005). The drift of this system at atmospheric pressure was less than 0·1 cmH₂O over a period of 30 min. Amplification was such that 1·0 cmH₂O pressure equalled 1·0 cm deflection on the recording paper. Linearity over the range −20 to +20 cmH₂O was within 0·5% of full scale deflection (10 cmH₂O). This system was free from hysteresis and able to discriminate pressure changes of 0·1 cmH₂O. Zero reference point was always taken at the mid-point of the wick.

Validation of wick method

Model experiment. We have tested the validity of the method by means of the model experiment originally described by Scholander et al. (1968). This is illustrated in Fig. 1(b). The wick was firmly applied to a piece of filter paper, wetted with 0·9% saline, by means of a weight (W). The paper was dipped into a beaker of 0·9% saline. If the filter paper acts as a hollow tube the pressure measured by the wick should equal atmospheric pressure less the measured height (H) cmH₂O. Under normal laboratory conditions, the height (H) was initially overestimated by 6–10%. However, when the experiment was repeated in an atmosphere of 100% humidity, H was estimated to an accuracy of ±1%. It was therefore concluded that evaporation from the filter paper had lowered the fluid pressure and that when evaporation was prevented, the wick method measured accurately the hydrostatic pressure of the fluid in the filter paper. Varying the size of the weight from 0·5 to 3·0 kg did not affect the recorded pressure.

Static and dynamic response of the system in vivo. A wick was inserted into an anaesthetized rat and connected to the recording apparatus. Continuous pressure measurements were made while the animal was rapidly raised or lowered a known distance. The response of the system is shown in Fig. 2. The magnitude of the pressure change recorded was that of the change in height of the animal. The time constant for recording a step change in pressure caused by raising or lowering the animal was less than 1 s. This was true even with the wicks pulled into the tubing with far more force than usual. This system was therefore considered adequate for measuring changes of interstitial pressure.

The experiment demonstrated the free flow of fluid between the tissue spaces and the transducer. The manoeuvre was performed during the course of each biological study to ensure the validity of the measurement.

Comparability of wick pressures from symmetrical sites. This was tested by using two wicks simultaneously in ten rats. They were inserted in symmetrical sites on opposite sides of anaesthetized rats. Pressures from the wicks were compared after 20 min equilibration. The two pressures correlated closely (Fig. 3).

Physical properties of the wick. The wick was examined microscopically to determine whether the channels between the fibres were small enough to impede the passage of macromolecules. Several wicks were viewed at a magnification of ×50 at a point where the fibres had been pulled into the tubing and were therefore tightly packed together (Fig. 4a). Observations were made through a small hole cut in the semi-opaque catheter. By using an eyepiece graticule, the distances between individual fibres were measured in wicks that had been pulled into the catheter with varying tightness. Each wick showed a wide range of channel widths and even the tightest wicks contained channels of up to 50 μm in diameter (Fig. 4b). Such
channels appear to rule out the possibility of the wick acting as a semi-permeable membrane. The loosest wicks equilibrate rapidly with the surrounding tissue and were found most satisfactory for pressure recording.

![Graph](image)

**Fig. 2.** Static and dynamic response of the system *in vivo*. Each step involved raising or lowering the animal by 1.6 cm. The time-constant for recording a step change in pressure is less than 1 s. Fluctuations in the wick pressure recording after each step are movement artifacts associated with respiration.

![Graph](image)

**Fig. 3.** Correlation of simultaneously recorded wick pressures from symmetrical sites on opposite sides of the rat.

To determine whether the wick could act as a semi-permeable membrane when in contact with macromolecular solutions, pressures were measured with the wick immersed in saline, in dextran '70' 6%* and in hyaluronic acid 1 g% and 2 g%†. In each case the hydrostatic

* Average mol. wt. 69,000, elliptical molecules approx. 500 Å in length and 17 Å in width (Squire, Bull, Maycock & Ricketts, 1955).
† Mol. wt. 10⁶; each molecule occupying a sphere of diameter up to 1 μm (Ogston, 1970).
pressure measurement was unaffected by the nature of the solutions. This suggests that under these conditions the wick does not act as a semi-permeable membrane.

**Histology of the wick in situ.** To observe any possible inflammation caused by the presence of the wick, biopsies were taken at up to 3 h after implantation. These studies were carried out in the subcutaneous tissues of the rat. The biopsy specimens were frozen in hexane at $-70^\circ$ for Cryostat sections. Four biopsies failed to show any signs of acute inflammation (Figs. 5a and 5b). To establish whether or not cotton fibres were left in situ after removal of the wick, rat subcutaneous tissues were biopsied directly after the wick had been removed. Since cotton is doubly refractile (Fig. 5c) sections were examined by crossed polarizers. No doubly refractile material was found in six biopsy specimens. This is an important consideration if this method is to be applied to man.

**Needle technique**

A saline-filled needle (i.d. 0.147 mm) was introduced into the subcutaneous tissues of the flank of five anaesthetized rats, and connected to the above recording apparatus. An attempt was made to measure interstitial pressure.

In each instance it was found impossible to obtain a steady recording by using an open-ended needle and pressure fluctuated wildly. Raising and lowering the animal caused no constant change of pressure. In five rats 0.1 ml of saline (0.9%) was injected through the needle into the tissues and the recording system was reconnected. Positive pressure (+2.7 to +4.2) cmH$_2$O was now recorded from each rat. The pressure was steady; raising the animal produced a rapid rise in pressure which fell to the base value when the animal was lowered. When the pressure was continuously recorded it fell gradually to a value near atmospheric (+0.4 to −0.6 cmH$_2$O) in 27–42 min after injection. At this time the recording began to fluctuate and a change in pressure could no longer be obtained by raising and lowering the rat.

**Capsule technique**

A polythene cylinder (3.5 cm × 1.5 cm diam.) perforated by 200 1 mm diameter holes was inserted via a dorsal incision into the space between the thigh and abdominal wall in forty rats. After 5 weeks the capsules were surrounded by a fibrous coat and a tissue-lined cavity within the capsule contained free fluid (Floyer, 1966); experiments were performed in rats at least 5 weeks after implantation. Pressure measurements were made with a needle (i.d. 0.325 mm) inserted into the fluid reservoir through one of the perforations and attached to the previously described recording apparatus. Zero reference was always taken at the mid-point of the capsule.

The perforated capsule method has been assessed in the dog (Guyton, 1963), the rat (Floyer, 1966; Green, 1969) and the rabbit (Stromberg & Wiederhielm, 1970).

**Biological experiments**

**Wick pressure in the frog (Rana temporaria) and the effect of altered hydration**

The skin of the frog’s back was anaesthetized with Lignocaine 1%. The wick was introduced via a small incision and was positioned 1–2 cm away from the point of insertion. After 5 min equilibration, pressure was recorded with the animal at rest. The pressure in seventeen frogs
Fig. 4. (a) Photomicrograph of cotton fibres of the wick viewed at a point where the fibres had been pulled into the catheter, and therefore tightly packed together. Magnification \( \times 160 \).
(b) Distribution of channel width in wicks of varying tightness. Even the tightest wicks contain channels of up to 50 \( \mu \text{m} \).

(Facing p. 40)
FIG. 5. (a) Photomicrograph of a section through rat skin stained with Toluidine Blue, magnification ×60. The wick has been in the subcutaneous tissues for 3 h. Its position can be more clearly seen in 5(c) in which the same section is viewed by cross polarizers. (b) Photomicrograph of the same section magnification ×225. There is no evidence of acute inflammation in the tissues in immediate contact with the wick. (c) Photomicrograph of the same section, magnification ×60, viewed through crossed polarizers to demonstrate the doubly refractile cotton fibres of the wick in subcutaneous tissues. Note the doubly refractile material in the epidermis are hair follicles.
was measured in this way and after this their state of hydration was altered. Some frogs were dehydrated in a dry container exposed to room air, whereas others were immersed in 0.45% saline in an attempt to overhydrate them.

**Measurement of wick pressure in the rat**

Female Glaxo Wistar albino rats weighing between 175 and 250 g were used throughout this study. Wicks were inserted subcutaneously under light anaesthesia. At least 20 min was allowed for equilibration of pressure. The scalp and the anterior abdominal wall were used.

Fifty-four measurements in the scalp were made in eighteen prone anaesthetized rats. Fifty-seven recordings in the abdominal wall were made in nineteen supine, anaesthetized rats.

**Comparison of pressures measured by the wick and capsule techniques in the rat**

Simultaneous measurements of wick and capsule pressures were performed in the same animal. Ether anaesthesia has been shown to affect capsule pressures (M. A. Floyer, unpublished work) and it was therefore desirable to record from the conscious as well as the anaesthetized rat. Pressures were studied in two groups of animals. In group 1, thirty-four comparisons were made in eleven prone conscious rats that were at rest in a restraining metabolic cage, under quiet draught-free conditions. The scalp was chosen for recording wick pressure, being the only practical site in the conscious animal. In group 2, fifty-four comparisons were made in ten anaesthetized rats. To maintain comparability, recordings were made from the same site as in the unanaesthetized group.

**The effect of salt and water depletion in the rat, on wick and capsule pressures**

Baseline recordings of wick and capsule pressures were made under ether anaesthesia in ten animals which were then arbitrarily divided into two groups. The test group of five animals received 2 mg of frusemide by intraperitoneal injection while the remaining animals acting as controls received an equal volume (0.2 ml) of water by the same route. Both groups were allowed to regain consciousness. Fluids were withheld from the test group. Subsequent measurement of wick and capsule pressures were made after a 3 h interval. The rats were anaesthetized and weighed before each measurement.

Wick and capsule pressures were also recorded continuously in five conscious rats at rest in a metabolic cage and in which an indwelling catheter had been inserted 1 week previously into a lumbar vein. Frusemide (100–150 mg/kg) was administered through the catheter in divided doses to achieve a urine output amounting to approx. 6% body weight in 2 h.

**Wick pressure in man**

Ten measurements of wick pressure have been attempted in five subjects, aged between 18 and 50 years. Autoclaved wicks were introduced under local anaesthesia into the subcutaneous tissues of either the antecubital fossa or the back of the hand. Pressures were recorded with the subject at rest and supine with the recording site at mid thoracic level.

**Investigation of osmotic factors as a cause of the subatmospheric wick pressures by soaking the wick in osmotically active solutions**

Two saline-soaked wicks A and B were inserted subcutaneously in symmetrical sites on either side of the mid-line of the rat abdomen, 1–3 cm apart. When the pressures were steady
wick A was removed, soaked in a solution of human umbilical hyaluronic acid (Sigma Laboratories, London), and reimplanted in an untraumatized site close to its previous position. After stabilization, the pressure was measured again. Wick B, on the opposite side, was withdrawn at the same time, resoaked in saline and reinserted in the same manner. Wick A was again removed, washed in saline and reinserted. The experiment was repeated by removing wick B, soaking it in hyaluronic acid and reinserting it, while wick A acted as the control. In thirty-five experiments on fourteen rats, twelve different concentrations of hyaluronic acid (0.1–3 g%) were used, and their effects on the wick pressure were related to the concentrations of the solution.

Three different concentrations of bovine albumin (3, 6 and 10 g%) were used to soak the wick and the experiment was repeated as described above; twelve observations were made in four rats.

The wick was also soaked in heparinized plasma previously obtained from a donor rat of the same strain. The above experiment was then repeated eight times in four rats, by using the plasma both undiluted and diluted with 0.9% saline.

The effect of reimplantation alone was studied on twenty-seven occasions in fifteen rats. A saline-soaked wick was allowed to equilibrate in the tissues; it was then removed, washed in 0.9% saline and reinserted in an untraumatized site close to its previous position.

RESULTS

Measurements in the frog

In seventeen normally hydrated frogs the mean baseline wick pressure was $-1.6 \text{ cmH}_2\text{O}$ (SD ± 0.45). As the frogs were dehydrated the wick pressure fell, whereas in two animals in which the weight increased with overhydration, wick pressure rose (Fig. 6).

![Fig. 6. Changes in wick pressure associated with changes in weight caused by altering the degree of hydration in frogs.](image-url)
Measurements in the rat

Fifty-one out of fifty-four wick-pressure scalp measurements and all fifty-seven of the abdominal wall measurements in the anaesthetized rat gave subatmospheric pressures. The mean pressure from the scalp was $-2.85 \pm 2.5$ cmH$_2$O and was almost identical with that in the abdominal wall, $-2.80 \pm 1.4$ cmH$_2$O.

Comparison of wick and capsule pressure in the rat

The comparison in conscious and anaesthetized rats respectively is shown in Figs. 7(a) and 7(b). Both graphs show a wide scatter of points, but for both groups of animals the slope of the regression line differs significantly from zero in the conscious group ($0.001 < P < 0.005$) and in the anaesthetized group ($P < 0.001$). Both graphs show a positive intercept on the wick-pressure axis. The correlation coefficients ($r = +0.53$ conscious rats; $r = +0.40$ anaesthetized rats) are both significant at the 1% level (Snedecor & Cochran, 1967, Table A12).

The effect of salt and water depletion on wick and capsule pressures in the rat

The group of anaesthetized rats that received frusemide all showed a fall of both wick and capsule pressure after 3 h (Fig. 8). One control animal also showed a fall of wick and capsule pressure, but in the rest of the controls, pressures were higher than baseline at 3 h. The mean weight loss in the test animals was 4.25% and in the controls 0.4%.

The results of continuous measurements during a frusemide induced diuresis are shown in Fig. 9, Figs. 10(a), 10(b) and 10(c). Diuresis usually began within 10 min of frusemide administration. Fig. 10 shows the fall in wick and capsule pressure and the fall in weight during the diuresis in one animal over 2.5 h. Fig. 10 (a, b and c) indicates wick and capsule pressures plotted against a fall in body weight. The wick pressure remained roughly constant until 4–5% of body weight had been lost as urine, after which pressure fell rapidly (Figs. 10a and 10c). By contrast, the capsule pressure fell more steadily (Figs. 10b and 10c). In three rats there were sufficient simultaneous wick and capsule measurements to permit the use of a one-tailed Wilcoxon paired test (Siegel, 1956) to the sequence of observations in each experiment. This showed a significant difference between the results of the two methods in each of the three experiments ($P < 0.005$). The same test was applied to the pooled simultaneous data from six rats. A significant difference was again found ($P = 0.00003$).

Wick pressures in man (Table 1)

Five successful recordings of wick pressure in man have been made which gave a mean pressure of $-3.4$ cmH$_2$O. The remaining five attempts, three of which were in one subject, gave pressures above atmospheric; on removal these wicks were markedly blood stained. This is a much higher incidence of bleeding than in the rat in which only approx. 1 in 10 recordings were unsuccessful on account of haemorrhage. Observations of the sites of pressure measurements for 5 months have revealed no evidence of infection or foreign-body reaction.

Osmotic forces as a cause of the subatmospheric wick pressure

The results of a typical experiment are shown in Fig. 11. Soaking a wick in 1% hyaluronic acid raised the pressures to a less negative value; soaking in 2% hyaluronic acid gave a positive pressure. The effect was reversed when the wick was washed in 0.9% saline. Fig. 12 shows the changes in pressure produced by different concentrations of hyaluronic acid. At hyaluronic
Fig. 7. (a) Correlation of simultaneously recorded wick and capsule pressures in the conscious rat showing the regression line (---) and 95% confidence limits (---) of the line. (b) Correlation of simultaneous recorded wick and capsule pressures in the anaesthetized rat showing the regression line (---) and 95% confidence limits (---) of the line.
**Interstitial 'fluid' pressure**

**Fig. 8.** The effect of a frusemide-induced diuresis in the rat. The test group A received 2 mg of frusemide and was compared with the control group B that received an equal volume (0.2 ml) of water. Pressure recordings were made with the rat anaesthetized, but were allowed to recover consciousness between recordings. ——, Wick; ——, capsule.

**Fig. 9.** Frusemide induced diuresis in a conscious (220 g) rat. Wick (— — —) and capsule (— — —) pressures were recorded continuously and urine output (— — —) was measured at 10 min intervals.
Fig. 10. Frusemide-induced diuresis in the conscious rat. Changes in wick pressure (a) and capsule pressure (b) are plotted against urine output (expressed as a percentage of body weight loss). In (c) the mean changes of wick and capsule pressure are plotted against percentage weight loss; --, wick pressure; ---, capsule pressure.
acid concentrations of 0.2% and below there was a small, inconstant effect, but above this concentration the pressure invariably rose. At concentrations of 2% and 3% the pressure usually became positive. By contrast, soaking the wick in albumin solutions gave small in-

<table>
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<tr>
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Fig. 11. The result of a typical experiment on the effect of soaking the wick in 1% and 2% hyaluronic acid. Wicks A (---) and B (----) soaked in saline, were inserted subcutaneously in the rat. At times indicated by the numbered arrows both wicks were removed, soaked in hyaluronic acid or saline, and reinserted close to their previous positions. (1) Wick A soaked in 1% hyaluronic acid, Wick B soaked in saline. (2) Wicks A and B washed in saline. (3) Wick B soaked in 1% hyaluronic acid, Wick A soaked in saline. (4) Wick B soaked in 2% hyaluronic acid, Wick A soaked in saline. (5) Wicks A and B washed in saline.

constant changes, (+0.9 to −0.7 cmH₂O; mean = 0.0 cmH₂O). The plasma experiments gave similar results (+0.7 to −0.5 cmH₂O; mean = +0.04 cmH₂O). These results are of the same order as those of the control saline experiments (+0.9 to −0.5 cmH₂O mean = +0.01 cmH₂O) (Fig. 13).
FIG. 12. Changes in wick pressure after soaking the wick in solutions of hyaluronic acid. Each arrow represents one experiment. The wick, soaked in saline, was inserted into the flank of the anaesthetized rat. After pressure measurement had been made the wick was withdrawn, soaked in hyaluronic acid and reinserted. The tail of each arrow represents the saline reading, the head that of the hyaluronic acid.

FIG. 13. Changes in wick pressure after soaking the wick in saline, bovine albumin solutions or rat plasma. Each arrow represents one experiment. The wick, soaked in saline, was inserted into the flank of an anaesthetized rat. After pressure measurements had been made, the wick was withdrawn, soaked in the test solution and reinserted. The tail of each arrow represents the saline reading, the head that of the test solution.
DISCUSSION

The wick pressures we have measured in the frog are comparable with those reported by Scholander et al. (1968) and Stromme et al. (1969). In the brown rat (Rattus norvegicus) Stromme et al. (1969), using similar techniques, found a mean pressure of \(-6.4 \text{ cmH}_2\text{O}\) which is more negative than the wick pressures in our experiments. We have demonstrated a significant correlation between the wick and capsule pressures. Wick pressure tends to be less negative than the capsule; the reason for this is not apparent.

In the model experiment the wick is pressed into close contact with the filter paper, the channels in the wick becoming continuous with those in the filter paper, so that there is an unbroken column of fluid from the surface of the saline to the pressure transducer. If this transducer it at the same height as the wick–filter paper interface it records a pressure that is subatmospheric by the height of the interface above the surface of the saline. This is the pressure of the fluid in the channels of the filter paper through which the saline flows freely.

In vivo, the purpose of the wick is to enable the saline in the catheter to make adequate contact with the interstitial space. The wick supports the tissues at the end of the catheter creating saline-filled cavities in equilibrium with the surrounding interstitial tissue. Without the wick, tissue blocks the catheter and no meaningful pressure can be recorded. In the same way, a steady pressure cannot be obtained with an open needle until a small volume of saline is injected through the needle to form a cavity in the tissues. However, due to the recoil of the tissues disrupted by the injection a positive pressure is then recorded. The advantage of the wick is that the tissue tension is opposed by the wick fibres and not by the saline in the interstices of the wick. The recording of interstitial ‘fluid’ pressure is thus unaffected by solid forces within the tissues. The ability of the wick to support solid tissue forces while recording interstitial ‘fluid’ pressure was demonstrated in the model experiment in which the wick accurately measured the ‘fluid’ pressure in the filter paper while supporting a wide range of weights.

The wick improves contact between the catheter and the interstitial space allowing fluid to flow between the tissues and the recording system to attain pressure equilibrium. Since all pressure transducers have a finite volume displacement, it is necessary for free flow to occur between the tissues and the transducer to obtain a reading. Such flow was demonstrated by raising the animal and observing a prompt rise in pressure equal to the height to which the animal had been raised. Subsequent lowering of the animal was associated with a fall in the original pressure. We have found that a reliable and repeatable reading cannot be obtained unless there is evidence of free flow.

Light microscopy of the interstitial space reveal areas of P.A.S. positive material (Ham & Leeson, 1969). The issue of whether this space contains free fluid in the absence of oedema has never been satisfactorily settled. Vacuoles which appear to contain fluid have been seen in the ground substance with the light microscope (Chase, 1959; Dennis, 1959) and with the electron microscope (Gersh & Catchpole, 1960), but appear to occupy only a small proportion of the interstitial space. Injected water-soluble dyes do not diffuse evenly in this space (McMaster & Parsons, 1939a, b). The resistance to the flow of fluid down a hydrostatic-pressure gradient in normal subcutaneous tissues is enormous; with oedema the resistance falls several hundred-thousandfold (Guyton et al., 1966).

Wiederhielm (1969) claimed to have demonstrated the presence of free fluid in the interstitial space of the bat wing by observing particles undergoing Brownian movement in spaces of
up to 2 μm across. There are serious objections to regarding the tissues he observed as normal and free from oedema in view of the prolonged immobility to which the wing had been subjected. Further, Wiederhielm (1969) was unable to demonstrate the phenomenon of Brownian movement in the interstitial space of the frog mesentery. The balance of evidence suggests therefore that little, if any, interstitial water exists in the free liquid state.

Biochemical analysis of the interstitial space suggests that it consists of a network of collagen fibres between which there is a gel consisting of about 1% hyaluronic acid and other glycosaminoglycans (Laurent, 1970; Ogston, 1966). Hyaluronic acid is a large molecule (mol. wt $10 \times 10^6$ to $8 \times 10^6$). It consists of polysaccharide chains in the form of an expanded random coil, roughly spherical with a diameter of about 4000 Å. The coil encloses a large amount of water; the hydrodynamic volume of the molecule in a solution occupies about 1000 times the space of the unhydrated polysaccharide chain (Ogston & Stanier, 1951, 1953). There is evidence of entanglement of molecules in solution at concentrations as low as 0.1% (Ogston, 1970). At 1% the molecules show an 80% overlap. Because of this, the osmotic pressure of a solution of hyaluronic acid increases sharply with concentration; a 1% solution has an osmotic pressure of 6.7 cmH₂O and a 2% solution 24.5 cmH₂O (Loewi, 1961).

Laurent and his colleagues (Laurent & Pietruszkieicz, 1961; Laurent, Böjrck, Pietruszkieicz & Persson, 1963) have described the 'sieve' effect; smaller molecules such as serum albumin (70 Å) can pass through solutions of hyaluronic acid with little hindrance, whereas larger molecules (PLP, 3650 Å) (Laurent, 1970, Fig. 9) do so much less readily. This is due to the network formed by the chains of the hyaluronic acid molecule in solution (Laurent, Öbrink, Hellsing & Wasteson, 1969).

If the interstitial space consists of a network of collagen fibres with free fluid between, the wick should measure the hydrostatic pressure of this fluid in the same way as it measures the hydrostatic pressure of the fluid in the interstices of the filter paper in the model experiment. Since, as we have shown, the wick itself does not act as a semi-permeable membrane, the presence of protein or hyaluronic acid in this fluid will not affect pressure readings. However, if the tissues contain no free fluid, the saline introduced by the wick will come into contact with the interstitial gel. If the boundary layer of the gel, consisting of enmeshed molecules of hyaluronic acid supported by collagen fibres, acts as a means of restricting diffusion of these large molecules (i.e. as a semi-permeable membrane) osmotic forces will be set up on account of the concentration of large molecules in the gel. Saline will diffuse into the gel; equilibrium will occur when the hydrostatic pressure of the remaining saline is subatmospheric and equal to the osmotic pressure of the solution of large molecules in the gel. It would be expected that a wick dipped in solutions of hyaluronic acid of similar concentrations to that present in the tissues would record higher pressures in tissue measurements than one dipped in saline, since the osmotic factors would be balanced. Our results suggest that this is so. Since albumin molecules pass relatively freely through the network of hyaluronic acid chains, it would not be expected that soaking the wick in albumin solution or in plasma would affect the measured tissue pressure. Our findings are in keeping with this idea. We suggest that the apparent subatmospheric hydrostatic pressure measured by the wick is due to these osmotic factors.

It is possible that there is a similar explanation for the negative pressure measured by the capsule. Capsules implanted for several weeks become lined with granulation tissue. Osmotic forces might be set up between the interstitial gel in these tissues and the free fluid in the middle...
Interstitial ‘fluid’ pressure

of the capsule, giving rise to a negative hydrostatic pressure in the fluid. The protein content of the fluid will not affect the pressure.

Guyton (1969) calculated that a subatmospheric interstitial pressure, taken with current estimates for mean capillary pressure and for plasma and tissue ‘fluid’ oncotic pressure, satisfied Starling’s hypothesis. Our suggestions do not change the argument; fluid filtered from the capillaries will be at subatmospheric pressure until absorbed into the gel.

Guyton (1965) demonstrated the relationship between interstitial ‘fluid’ pressure (measured by the capsule) and interstitial fluid volume. Loss of relatively small amounts of interstitial fluid led to a marked fall of pressure. Increasing interstitial fluid resulted in a sharp rise in pressure until atmospheric pressure was reached; at this point oedema appeared and large increases of fluid resulted only in small increases in pressure. These findings can be interpreted in terms of osmotic forces. With concentrations of hyaluronic acid over 1%, marked increases of osmotic pressure occur with small changes in concentration (Laurent, 1970). Relatively small changes in interstitial tissue hydration will therefore produce marked changes in the osmotic pressure of the gel. However, with overhydration the gel will become saturated with water and free fluid will appear at or above atmospheric pressure.

Wick pressure measurements during frusemide dehydration are in keeping with this hypothesis. With increasing dehydration the wick pressure at first falls slowly but then much more rapidly. Simultaneous capsule pressure measurements show a more gradual fall; capsules contain tissue in which there are many blood vessels and it is possible that the intracapsular pressure is influenced by changes in intravascular volume. With rapid dehydration the intravascular volume will fall in advance of the interstitial tissue volumes.

The small number of successful measurements on human subjects gave subatmospheric pressure readings comparable with those obtained in animals. There was minimum discomfort and no evidence of unpleasant sequelae. The wick method should therefore prove of use in the study of human interstitial pressure both in normal subjects and in patients with disorders of fluid balance.

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P. D. Snashall et al.


Interstitial 'fluid' pressure


