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LOCALIZATION OF THE BLOOD-BRAIN BARRIER OF AN INSECT: ELECTRICAL MODEL AND ANALYSIS

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SUMMARY

The perineurium was found to form the principal barrier to diffusion across the blood-brain barrier system of the cockroach, *Periplaneta americana*, since the resistance across this layer was much greater than that across the underlying neuroglia. An equivalent electrical circuit of the perineurium was then used to analyse recordings made in apparent perineurial cells and the interstitial system. Trans-perineurial resistance was at least 900 Ωcm^2, while the ratio between basolateral and apical membrane resistances was 11:1, indicating that the apical membrane had an area much greater than that of the basolateral membrane. Raising the potassium concentration in the saline produced changes in potential difference (p.d.) and resistance that were interpreted as due to the effect of potassium upon the basolateral membrane. Analysis indicated that the resting electromotive force (e.m.f.) generated by the basolateral membrane was less than that generated by the apical, although the K level in the saline was near that considered to be in the interstitial system. The analysis also yielded a value of 9 for the ratio of shunt resistance to apical resistance. Most changes in recorded values following the K elevation could be simulated by use of the estimated parameters, and an estimation of a change in interstitial K level. From these results, the shunt can be calculated to be an important contributor to the resistance across the perineurium, having a resistance about 0·9 times that of the transcellular resistance.

INTRODUCTION

Among the neuroglia that form the basis of the blood-brain barrier system of the insect, there must be a degree of restriction to the intercellular diffusion of water-soluble substances. In the intercellular spaces of the peripheral neuroglia (the perineurium), this restriction was indicated in the preceding paper to be sufficient to form an electrical resistance (Schofield, Swales & Treherne, 1984*a*). This was shown by finding that the interstitial p.d. that is associated with the integrity of the barrier system is generated across the perineurium. In the present report, it is shown that the perineurium forms the region of highest resistance between the neurones and the blood, and is therefore the chief barrier to diffusion. An electrical model is then used

Key words: Electrophysiology, electrical model, glia, blood-brain barrier, insect, *Periplaneta americana*.

to analyse the effects of high K upon recordings of p.d. and resistance in and across the perineurium, to quantify the degree of intercellular restriction.

METHODS

The abdominal nerve cord from adult male cockroaches, *Periplaneta americana* L, cultured in the laboratory, was mounted in a Perspex chamber (e.g. Treherne, Schofield & Lane, 1973). Microelectrode recordings were then made from the penultimate connectives, as described in the preceding paper (Schofield *et al.* 1984*a*). The composition of the saline flowing over these connectives could be rapidly varied. Axons were electrically stimulated near the terminal ganglion. Experiments were made at room temperature (27–30 °C for the first Results section, 21–28 °C for the others).

Saline was based on that of Treherne, Schofield & Lane (1982) with a lower K concentration and different buffer. The lower K concentration was chosen to be similar to that in the interstitial fluid (Thomas & Treherne, 1975) in the hope that variation between preparations in the permeability of the barrier would then lead to less variation in interstitial K concentration. Composition of the saline was: 127 mM-Na, 3 mM-K, 2 mM-Ca, 2 mM-Mg, 50 mM-mannitol, 5 mM-trehalose, 135 mM-Cl, 3 mM-OH, 8·6 mM-HEPES (pH 7·2). High K saline (130 mM) was made by substitution of K for Na. Salines were filtered (0·45 μm Millipore) immediately before use to remove particles that could adhere to electrodes.

Glass microelectrodes were pulled from thin-walled glass (Clark Electromedical), and filled with 3 M-KCl and 3 mM-HEPES/KOH buffer (5–10 MΩ, pH 7·2). Connectives were penetrated according to the procedure used in the preceding paper (Schofield *et al.* 1984*a*).

Resistances were measured by injecting current pulses through an electrode of low resistance (1–9 MΩ), with the tip in an interstitial position. Pulses lasted 1–10 s, and had an amplitude of around −500 nA. The resulting deflections in the p.d.s recorded by other electrodes were then measured. Values obtained with 1 s pulses sometimes did not quite reach steady-state, and therefore gave underestimates of resistance (by no more than 5 %). Use of longer pulses could introduce inaccuracies in p.d. readings. Correction for the p.d. induced in the agar bridge was made arithmetically at first, and then automatically by voltage clamp in later experiments.

Current-induced deflections were measured within about 50–200 μm from the injection electrode, since preliminary experiments indicated that the deflections declined by about 20 % at a distance of 500 μm from the injection site (the diameter of the connective was about 200 μm), indicating a length constant of about 2·2 mm. Electrodes for recording resistances that were to be compared were at a similar distance from the injection electrode. When obtaining the ratio between deflections in apparent perineurial cells and the interstitial space, the intracellular electrode was driven into the interstitial space at the end of the experiment, whenever possible, to obtain any correction factor that might be required. This factor was always close to unity, indicating that little error was involved in the technique.

Probabilities of differences between values recorded simultaneously were calculated by Wilcoxon matched-pairs signed-rank test. All tests were two-tailed.

MODEL

The perineurium, and the recordings made in and across it (Fig. 1A), can be described in terms of the equivalent electrical circuit shown in Fig. 1B. A paracellular shunt is assigned a resistance (R_s) and source of e.m.f. (E_s). The cell is represented by the basolateral membrane – resistance (R_b) and e.m.f. generated (E_b) – and the apical membrane – resistance (R_a) and e.m.f. (E_a). The characteristics of an individual perineurial cell may reflect characteristics of adjacent perineurial and glial cells, because of the possibility of some electrical coupling through gap junctions (Fig. 1A). No representation is made of intracellular resistance, because of the extremely short distance between the membranes (<0·2 μm: Schofield *et al.* 1984*a*), or of the neural lamella, since the much thicker stroma of the rabbit corneal endothelium is indicated to have little resistance and no e.m.f. (Lim & Fischbarg, 1981).

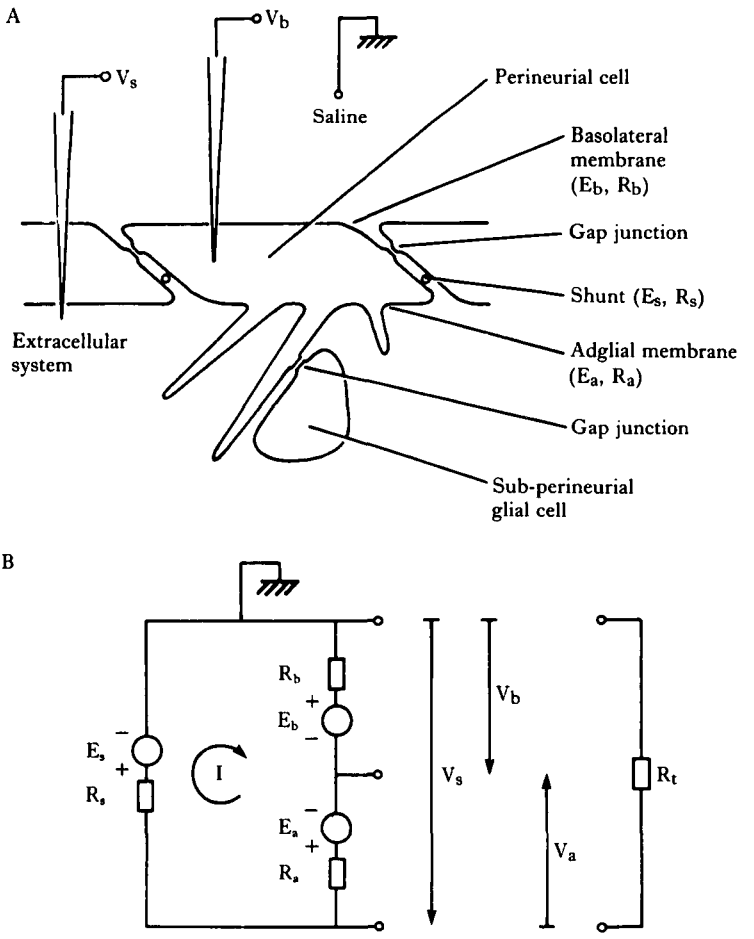

Fig. 1. (A) Schematic transverse section through the perineurium, showing two electrodes recording p.d. across the basolateral membrane (V_b) and in the interstitial system (V_s) relative to the bath at earth potential. (B) Electrical model of the perineurial elements shown in (A) (see text).

Since the circuit is closed, a current (I) can flow through each component of the circuit. With negative values assigned to the e.m.f.s generated by the membranes, I is given by:

$$I = \frac{-E_a + E_b - E_s}{R_a + R_b + R_s}. \quad (1)$$

If $-E_a + E_b - E_s$ is greater than zero, then current flow will be in the direction indicated in Fig. 1B.

The p.d.s recorded in the interstitial system (V_s) and in the perineurium, across the basolateral membrane (V_b), are recorded relative to the saline at earth potential (Fig. 1). The difference between these p.d.s gives the p.d. across the apical membrane (V_a) relative to the interstitial system, from the relationship:

$$V_a - V_b + V_s = 0. \quad (2)$$

Each p.d. is generated by one source of e.m.f., plus the current flowing through the associated resistance:

$$V_a = E_a + IR_a, \quad (3)$$

$$V_b = E_b - IR_b, \quad (4)$$

and

$$V_s = E_s + IR_s. \quad (5)$$

The trans-perineurial resistance (R_t) is given by:

$$R_t = \frac{R_s (R_a + R_b)}{R_a + R_b + R_s}. \quad (6)$$

RESULTS

Resistance across neuroglia

To determine the resistance across the perineurium compared to that across the underlying neuroglia, recordings were made at two different depths in the interstitial system of six preparations, one just under the perineurium and one outside an axon, in similar fashion to that in the preceding paper (Fig. 1A, Schofield *et al.* 1984*a*), while current was pulsed through an electrode with the tip in an interstitial channel near the central longitudinal axis of the connective, at a depth of 112 μm (S.E. 8·4). In the shallower position, at a depth of 18 μm (S.E. 2·2), the resistance was calculated from the deflections in p.d. to be 25 kΩ (S.E. 2·0). At the much greater depth (pair $P = 0{\cdot}031$) of 57 μm (S.E. 5·8), the resistance was 28 kΩ (S.E. 2·0), about 12 % greater (pair $P = 0{\cdot}031$). Since the current density was probably higher at the deeper site, the difference between resistances may have been less.

The bulk of the resistance across the neuroglia is thus provided by the perineurium.

Recordings from perineurium, interstitial system and axons

In 17 preparations, recordings were made simultaneously from an apparent perineurial cell, the interstitial system, and an axon (e.g. Fig. 2), while current was

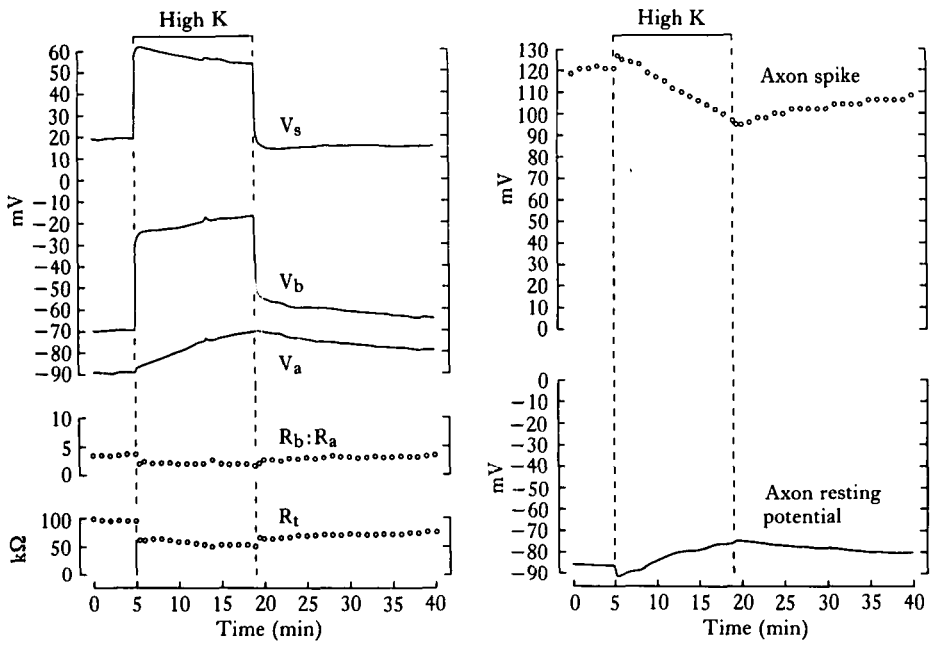

Fig. 2. Effect of high K upon simultaneous measurements of p.d. and resistance associated with the perineurium, and the resting and action potentials of an axon. Deflections in p.d. recordings that were produced by current injection are not shown, for clarity.

pulsed through another electrode nearby in the interstitial system. The apparent perineurial cell had physiological characteristics like those of identified cells (Schofield *et al.* 1984*a*).

The p.d. recorded across the basolateral membrane (V_b) had a value of −60 mV (S.E. 1·3) when the p.d. across the perineurium (V_s) was 15 mV (S.E. 1·4), indicating (equation 2) a p.d. of −75 mV (S.E. 1·9) across the apical membrane (V_a). The p.d. recorded in the axon, −60 mV (S.E. 2·3), relative to the value of V_s, gave the axonal resting potential as −75 mV (S.E. 2·3). An apparent trans-perineurial resistance (R_t) of 72 kΩ (S.E. 3·0) was indicated by the deflections in V_s produced by current injection. Deflections produced in V_b and V_a yielded a value for the ratio between the resistances of basolateral and apical membranes ($R_b : R_a$) of 11 (S.E. 1·3).

The value of R_t will be an underestimate (see Methods) but indicates a resistance of at least 900 Ωcm^2 for an area of connective in the experimental compartment of about 0·0126 cm^2. This will also lead to an underestimate of absolute values of resistance given below, but since the perineurial cells are much interleaved, and since the greatest extent of a cell (including underlying processes) is about 400 μm (see Schofield *et al.* 1984*a*), much less than the length constant of current spread of 2·2 mm (see Methods), it was considered that a sufficient proportion of the cell lay within a sufficiently homogeneous zone of current density to make relatively accurate measurement of resistance ratios. Correction for possible difference in current density was also made wherever possible (see Methods).

Upon raising the K concentration in the saline from 3 to 130 mM, there was

depolarization of V_b by 30 mV (S.E. 2·1), to −30 mV (S.E. 2·0), and also a depolarization of V_a by 4·6 mV (S.E. 0·8), to −71 mV (S.E. 2·1). V_s became more positive by 26 mV (S.E. 1·9). The axon never depolarized by as much as the apical membrane, and often hyperpolarized (Fig. 2); the average change was a hyperpolarization of −0·2 mV (S.E. 0·5). There was a fall in apparent R_t to 55 kΩ (S.E. 2·1) accompanied by a fall in apparent $R_b : R_a$, by 27%, to 8 (S.E. 1·1).

During exposure to high K, there was a depolarization of both V_b and V_a, accompanied by depolarization of the axon (Fig. 2). When exposure was sustained ($N = 15$), a change in axon resting potential by 5 mV (after any initial hyperpolarization) required 168 s (S.E. 32·8). This figure gives some measure of leakiness for comparison in future study, as in the subsequent paper (Schofield *et al.* 1984*b*).

Analysis

The initial changes induced by high K can be interpreted as due to effects principally upon the basolateral membrane (Schofield *et al.* 1984*a*), to reduce the generated e.m.f. (E_b) and resistance (Hodgkin & Katz, 1949). An attempt was made to calculate resistance values from the changes in R_t and $R_b : R_a$, in the manner of Reuss & Finn (1974). Such analysis was too sensitive to small errors in measurement, and did not give consistent results, possibly because the value of $R_b : R_a$ was never close to unity.

$R_s : R_a$, the ratio of shunt resistance to apical resistance, was calculated from the changes in p.d. By combining equations (3) and (5) we find that:

$$V_s = \frac{R_s}{R_a}(V_a - E_a) + E_s. \tag{7}$$

If the e.m.f. generated by the apical membrane (E_a) and any e.m.f. produced by the shunt (E_s) are unaffected by the K elevation, we may deduce that:

$$\frac{dV_s}{dV_a} = \frac{R_s}{R_a}, \tag{8}$$

giving a value for $R_s : R_a$ of 9 (S.E. 1·8).

R_a, R_b and R_s were calculated from equation (6), using $R_b : R_a$ and $R_s : R_a$. The values, which will be underestimated due to an underestimate of R_t (see Methods), were 21 kΩ (S.E. 2·9) for R_a, 192 kΩ (S.E. 21) for R_b, and 146 kΩ (S.E. 29) for R_s.

$R_s : R_a + R_b$, the ratio of shunt to trans-cellular resistance, was found to be 0·9 (S.E. 0·28).

E_a and E_b may be calculated for a given value of E_s. From equation (7) we obtain a value for E_a of −78 mV (S.E. 1·8) if E_s were 0 mV. The value would be −76 mV (S.E. 2·1) if E_s were 10 mV. From equations (4) and (5) we obtain:

$$E_b = V_b + \frac{R_b}{R_s}(V_s - E_s). \tag{9}$$

If E_s were 0 mV, this would yield a value for E_b of −31 mV (S.E. 6·7), rising to +25 mV (S.E. 13) in high K. This is a change of 56 mV (S.E. 7·1). If the relationship with log K concentration were linear, it would have a slope of 34 mV (S.E. 4·3) per decade of K concentration. If E_s were 10 mV, the value of E_b would rise from −50 mV (S.E. 4·5) to +11 mV (S.E. 10·4).

Simulation

Just as the abrupt initial depolarization of V_a may be interpreted as due to coupling to a rapid depolarization of the basolateral membrane, so the slow depolarization of V_b can be suggested to be due to electrical coupling to a gradual depolarization of the apical membrane, as K leaks into the sub-perineurial interstitial system (Schofield *et al.* 1984*a*). To examine whether the coupling would be of the correct magnitude for such an effect, simulation was made of the effects of a rise in interstitial K level, obeying simple diffusion. Starting K concentration was chosen to be 3 mM (Thomas & Treherne, 1975), ending at the level indicated by the slow phase of axonal depolarization, using a previously determined slope (Fig. 2 of Schofield & Treherne, 1978). Relationship between K level and R_a was assumed to be linear (Hodgkin & Katz, 1949), in the same proportion as indicated by effects upon R_b. Depolarization of the apical membrane was assumed to follow the same slope as for the depolarization of the axon, to which it may be similar (see Discussion). The parameters derived from the recordings shown in Fig. 2 were first used to simulate the effect of high K upon E_b and R_b, to generate a change in p.d. and resistance (Fig. 3). The estimated effect of the gradual rise in interstitial K upon E_a and R_a gave a gradual depolarization of V_a, similar in magnitude to the depolarization of E_a (Fig. 3) and thus similar to that to be found in an axon, as observed (Fig. 2). The simulation produced less change in V_b than in V_a, and hence there was a concomitant negative shift in V_s (Fig. 3). These effects were more pronounced than in the recording (Fig. 2), possibly because of a

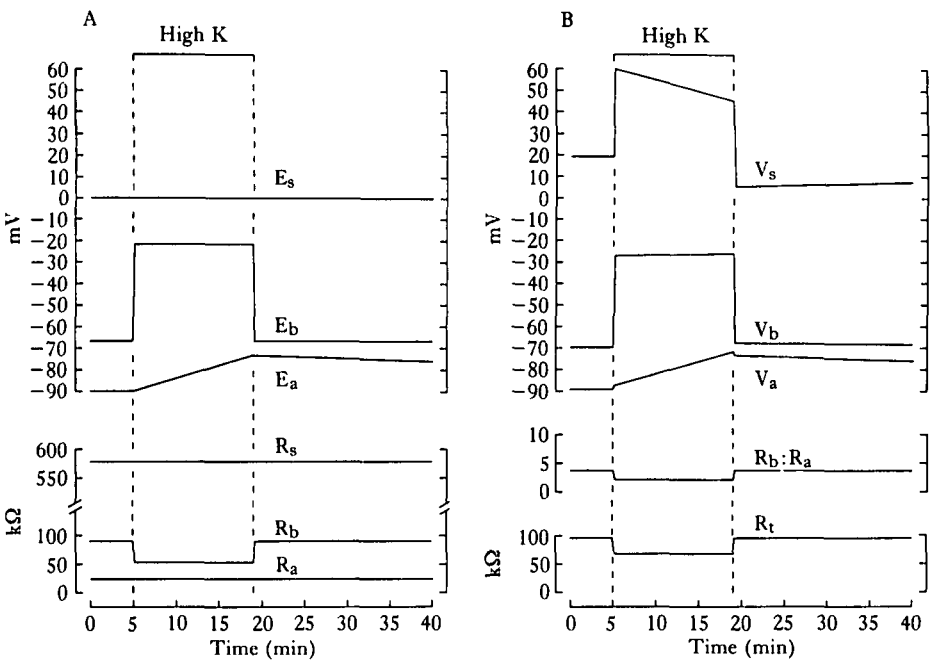

Fig. 3. (A) Estimated effect of high K upon e.m.f.s and resistances in the perineurium, using starting values and a change in interstitial K derived from the recording shown in Fig. 2. (B) E.m.f. and resistance parameters calculated from the values shown in (A).

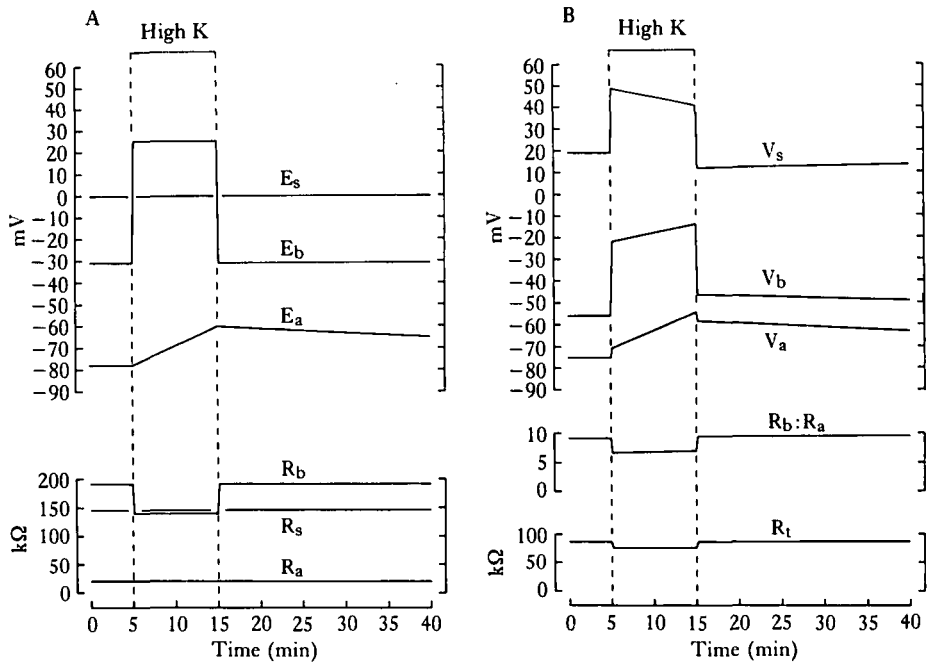

Fig. 4. (A) Estimated effect of high K upon average values of e.m.f.s and resistances in the perineurium, using an average change in interstitial K. (B) E.m.f. and resistance parameters calculated from the values shown in (A).

change in R_b, as might be indicated by the recorded values of $R_b : R_a$ (Fig. 2). Since interstitial K was simulated to rise by only a few mM, there would be little change in R_a, and hence no obvious change in $R_b : R_a$ or R_t (Fig. 3).

Such effects could be simulated over a wide range of parameter values. Thus, the values for E_b, R_s and R_b that were employed for Fig. 3 are far from the typical figures; if average values for all parameters are employed then a reasonable approximation to a typical recording is obtained (Fig. 4), showing, for example, an average value for the initial depolarization of V_a.

DISCUSSION

The principal barrier to diffusion across the insect blood-brain barrier system may be identified as the perineurium from the observation that most of the resistance across the neuroglia lies among the superficial cells. Among the glia just below the perineurium, there are no obvious distinctions in cell-type, intercellular junctions, or interstitial matrix, that could account for such a resistance. Previous observations of a lack of penetration of lanthanum or dyes among the sub-perineurial glia (Lane, Leslie & Swales, 1975; Shaw, 1983*a*,*b*) might mean that insufficient tracer entered the interstitial system in those experiments. Alternatively, it might indicate that these substances can bind to the interstitial anion matrix, as previously observed for lanthanum in desheathed cockroach connectives (Treherne *et al.* 1982), and have the effect of occluding the interstitial system. We see no reason why such results should

be interpreted as evidence that the barrier is 'an extensive property of the CNS tissue' (Shaw, 1983*a*), or lies in some sub-perineurial zone (Shaw, 1983*b*). A localization of the blood-brain barrier in the perineurium is in good agreement with ultrastructural and physiological observations (see Introduction of the preceding paper: Schofield *et al.* 1984*a*). Because the perineurium presents an electrical resistance, it must restrict the diffusion of even the smallest of water-soluble substances, including the ions involved in neuronal signalling (Pichon, 1974; Callec, 1974). It must form a relatively tight barrier, for the average resistance is greater than 900 Ωcm^2, approaching the resistance of the tighter epithelia, 2000 and 3530 Ωcm^2, and certainly higher than in leaky epithelia, 70–300 Ωcm^2 (Table 2 of Erlij & Martinez-Palomo, 1978). In frog, a trans-epithelial resistance of 3000 Ωcm^2 has been recorded for retinal barrier endothelium (Miller & Steinberg, 1977) and 1870 Ωcm^2 for brain capillaries (Crone & Oleson, 1982). Frog choroid plexus has a resistance of only 26 Ωcm^2 (Zeuthen & Wright, 1981).

Several features of the perineurium were assessed from the recordings made in this study, by consideration of an equivalent electrical circuit (Fig. 1). A similar method has been used to determine the parameters of frog retinal endothelium (Miller & Steinberg, 1977), and also of many other epithelia, such as kidney (see Boulpaep, 1971, 1979), small intestine (Okada, Tsuchiya, Iramajiri & Inouye, 1977), salivary duct (Augustus, Bijman & van Os, 1978), urinary bladder (see Finn, 1978), gastric mucosa (see Machen & Forte, 1979) and gallbladder (see Reuss, 1979). In the present study, the model was used to analyse the initial changes in p.d. and resistance measurements that were induced by raising the potassium level in the external medium. These changes may be interpreted as due to the effect of potassium upon the basolateral membrane (Schofield *et al.* 1984*a*).

An asymmetry of the perineurial cells was indicated by both resistance and p.d. measurements. Resistance of the basolateral membrane (R_b) appeared to be eleven times that of the apical membrane (R_a). This difference is unlikely to result from electrical coupling of the apical membrane to the underlying neuroglia, since such coupling appears to be weak (Schofield *et al.* 1984*a*). Instead, if the membranes have similar thickness and specific resistance, it indicates that the basolateral membrane has one-eleventh the area of the apical face, which is known to be thrown into many projections (Maddrell & Treherne, 1967; Schofield *et al.* 1984*a*). For non-mammalian gallbladder there is agreement between the degree of folding and the resistance per apparent area (Henin *et al.* 1977). Because of the difference in membrane resistance, current flow (I) across the cell (Fig. 1) would induce more p.d. in the basolateral membrane than in the apical. At a steady current, the p.d. induced across one membrane would be of opposite sign to the p.d. induced across the other (equations 4, 5). For any given value of e.m.f. generated by the paracellular shunt (E_s), analysis of the recordings can evaluate how much of the p.d. across each membrane would be generated by current flow, and hence how much would come from sources of e.m.f. within the membrane. The shunt pathway is unlikely to contribute much to the p.d. difference since selectivity should be low (see Erlij & Martinez-Palomo, 1978), and gradients of ionic concentration between saline and interstitial fluid are likely to be small (Thomas & Treherne, 1975). If we assign a value of 0 mV to the shunt, then, in preparations bathed in 3 mM-K saline, the analysis indicates that the basolateral membrane generates an e.m.f. (E_b) of −31 mV, which is supplemented

by current flow to produce the resting p.d. (V_b) of -60 mV. The p.d. of -75 mV across the apical membrane (V_a) would be produced by current flow countering a source (E_a) of -78 mV.

Upon raising the potassium level in the bathing medium, there would be a depolarization of the e.m.f. generated by the basolateral membrane, and R_b would decrease, resulting in an increase in the current (equation 1). At an external K level of 130 mM, when V_b was -30 mV, the membrane is calculated to generate $+25$ mV. Thus a given change in K concentration would produce less change in p.d. across the membrane than in generated e.m.f. The change produced by a decade change in K had a slope greater than 34 mV per decade. This is much less than for leech glia (58 mV: Nicholls & Kuffler, 1964), but not greatly different than for some vertebrate glia (e.g. 42 mV, Dennis & Gerschenfeld, 1969). Because the current would couple the basolateral membrane to the apical membrane, the rise in current produced by depolarization of the basolateral membrane would be responsible for the positive shift in V_a of 4·6 mV.

Subsequent depolarization of both V_b and V_a, as K leaked into the preparation, can also be explained in terms of the model. A small increase in K at the apical surface would reduce E_a and R_a. Since there would be relatively little change in R_a, there would be a decrease in current (equation 1). The reduction in E_a would be thus slightly countered by a fall in current, to produce a gradual depolarization of the apical membrane. The gradual depolarization of the basolateral membrane would be produced by the decreasing flow of current through the relatively high resistance of the basolateral membrane.

The difference between the derived values for E_b and E_a could indicate that the K concentration in the interstitial channels is lower than that at the surface of the basolateral membrane. The K level in the interstitial channels is considered to be around 3 mM (Thomas & Treherne, 1975), the same as in the saline, but it could be that the level of free K outside the basolateral membrane is higher than that in the saline, perhaps a result of an unstirred layer effect, or attraction to a charged zone since the K/Cl ratio is higher than in the saline (Treherne *et al.* 1982). Another possibility is that the K gradient is not the only source of e.m.f. in at least one of the membranes. An additional source could be an electrogenic pump, the presence of which may be indicated by the effects of cooling and ethacrynic acid (Pichon & Treherne, 1974). The magnitude of the difference between E_b and E_a will depend upon the value of E_s. As shown in the Results, setting the value of E_s at $+10$ mV decreases the difference, principally by an alteration in the value for E_b.

The interstitial p.d. will be the result of current flow through the shunt resistance, plus whatever e.m.f. is generated by the shunt itself (equation 5). It can thus be seen how this p.d., and the K-induced changes, can indicate the integrity of the barrier, as suggested by earlier recordings at greater depth (Pichon & Boistel, 1967; Treherne, Lane, Moreton & Pichon, 1970) or with the sucrose-gap (Pichon & Treherne, 1970; Treherne *et al.* 1973). Correlation of the size of the K-induced change with the speed of the fast fraction of Na efflux may thus result from the degree of restriction to Na afforded by the shunt, as has been suggested (Tucker & Pichon, 1972). Since the current reflects the properties of the membranes, the initial change in interstitial p.d. will reflect the effect of high K upon the basolateral membrane, just as does the change

in p.d. across the apical membrane. The ratio of the changes thus yields the ratio of shunt resistance to apical resistance, at any constant value of E_s. We can then calculate that the shunt is almost as important in determining the trans-perineurial resistance as is the cell, by a factor of 0·9. This value is about half that obtained in the tight epithelia, 1·6 and 1·7, and four to one-hundred times higher than in leaky ones, 0·009–0·15 (Table 2 of Erlij & Martinez-Palomo, 1978). A gradual increase in V_s during the K exposure would be produced by the reduction in current.

Simulation of the effects of high K, assuming that the changes during the K exposure would be due to effects upon the apical membrane, parallel to the effects upon the axon, show a good approximation of the depolarization of the apical membrane (Figs 3, 4). Where the simulated depolarization of the basolateral face was not as great as the observed change, this might be explained by continuing reduction in R_b (Figs 2, 3). But since a reduction in V_s was often slow or absent (e.g. Fig. 4 of Schofield *et al.* 1984*a*) this might indicate an increase in R_s during the K exposure. A similar discrepancy is found for urea-treated preparations in the subsequent paper and is discussed there (Schofield *et al.* 1984*b*).

Previous models of the production of the p.d. across a blood-brain barrier have not incorporated the effect of current flow across the cell and back through the intercellular resistance, and this may explain some of the discrepancies between observed and predicted values. Thus, the present model gave a closer fit to magnitude and time course of the K-induced change in p.d. across the blood-brain barrier of the cockroach than has a previous model (Fig. 4 of Pichon, Moreton & Treherne, 1971). Time course, especially after the K pulse, is also more closely fitted than in a previous model for crayfish (Abbott, Moreton & Pichon, 1975).

From this analysis, we can see that fluctuations in e.m.f. generated by the basolateral membrane, such as would be caused by fluctuations in the K level in the blood (Lettau, Foster, Harker & Treherne, 1977) will cause fluctuations of smaller magnitude in the p.d. that appears across this membrane, slightly smaller fluctuations across the perineurium, and much smaller fluctuations across the apical membrane. Sensitivity of the apical membrane to interstitial K is also suppressed by current flow. Processes of the apical membrane descend into the connective, and keeping the changes across this membrane to a low level may help keep the interstitial environment steady. It can also be seen that the change in p.d. across the apical membrane produced by a rise in interstitial K is mirrored by a change across the basolateral face. This tracking of p.d., which is accompanied by a steady interstitial p.d. (equation 2), may limit the loss of K into the surrounding medium which might otherwise occur by 'spatial buffering' (see Gardner-Medwin, 1981).

The high resistance of the shunt shows that the intercellular pathway will be nearly as limiting as the cell in controlling the passage of many substances across the insect blood-brain barrier. The extent to which a blood-brain barrier is formed by a restriction to diffusion between the cells has not been determined in previous study, and would be difficult to assess with alternative techniques. Tracers for electron-micrography are larger and more highly charged than the monovalent cations (see Lewis & Knight, 1977), the perineurial cells are too small for autoradiography (Schofield *et al.* 1984*a*) and it is difficult to identify the necessary compartments in radio-isotope studies (Treherne *et al.* 1982). The insect blood-brain barrier can be

made leaky by brief exposure to hypertonic urea (Treherne *et al.* 1973; Schofield & Treherne, 1978; Treherne *et al.* 1982), and whether this is achieved by damage to the shunt is assessed by use of the present technique in the subsequent paper (Schofield *et al.* 1984*b*). Further study will also be attempted of the perineurium, which, although in many places only 0·1 μm in thickness (Schofield *et al.* 1984*a*), must play important roles in metabolism (see Wigglesworth, 1972) and ionic homeostasis (see Treherne & Schofield, 1979, 1981) in the insect nervous system.

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