THE LOCATION OF PENETRATION BARRIERS IN THE GANGLIA OF THE AMERICAN COCKROACH, PERIPLANETA AMERICANA (L.)

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In the period between 1950 and 1960 there were a number of reports of the relative insensitivity of insect nerves and ganglia to various electrolytes, drugs and toxicants which affected vertebrate nervous systems. Examples include: potassium acting on the crural nerve of the locust, Locusta migratoria (Hoyle, 1952, 1953); potassium and acetylcholine acting on ganglia of the American cockroach Periplaneta americana (Twarog & Roeder, 1956, 1957); ionic organophosphate penetration into the nerve cord of the American cockroach (O'Brien, 1959). These findings, coupled with the finding that ionized drugs and toxicants had unexpectedly little effect on insects (O'Brien & Fisher, 1958; Kolbezen, Metcalf & Fukuto, 1954), led to the widely accepted view that insect nerves, and especially insect ganglia, were protected from cations, perhaps by the sheath or sheaths which invest most insect ganglia and nerves. Since insect ganglia are comparable to the brain and spinal cord of vertebrates, such protection would be like that offered by the so-called 'blood–brain barrier' of vertebrates.

The existence of an ion-barrier was challenged when it was shown with radioactive materials that K+, Na+, Ca²⁺ and acetylcholine enter readily the nerve cord of the American cockroach and the stick insect, Carausius morosus (Treherne, 1961a-d, 1962, 1965; Treherne & Smith, 1965). Subsequent studies from this laboratory have explored the penetration of fatty acids, quaternary ammoniums and alcohols into ganglia of the American cockroach (Eldefrawi & O'Brien, 1966, 1967a, b) and willow aphid (Toppozada & O'Brien, 1967) and have shown that the penetration rate of these molecules is reduced by large size and by polarity. Charged molecules do indeed penetrate more slowly than uncharged molecules of comparable size, but the difference is relatively small, in the order of 5- to 15-fold. The 'barrier' is therefore relative rather than absolute. Furthermore, it is the polarity of charged molecules, rather than charge per se, which slows their penetration.

In all of the above studies the location of the barrier (or 'barrier system' as Lajtha (1962) calls it in the analogous vertebrate situation) is uncertain. In principle, the external sheath might be involved, as suggested by Twarog & Roeder (1957); or there might be some more central barrier layer; or the 'barrier system' might be a non-

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localized function of the whole ganglion. A component of this problem is the possibility that the influxes and effluxes studied by Treherne and ourselves might involve only a portion of each ganglion—for instance, the cortex—so that the crucial central neuropile, containing the pharmacologically sensitive synapses, might enjoy a greater protection than was apparent. The present study is intended to clarify these problems by examining the effects of sheath disruption upon efflux and upon the pH-dependence of influx, by providing additional data on the sensitivity of influx to 2,4-dinitrophenol (DNP); and by observing the penetration of methylene blue into intact as compared with sliced ganglia.

MATERIALS AND METHODS

Four analogous compounds were used: butanol-1-14C, sodium butyrate-1-14C, butylamine-1-14C and 3H-butyltrimethylammonium iodide. The first three were purchased from Nuclear Chicago Co. and the fourth was synthesized in this laboratory (Eldefrawi & O'Brien, 1967a). The nerve-cord preparation for influx and efflux studies, described by Eldefrawi & O'Brien (1966), was used; it involves bathing the nerve cord in situ in a physiological solution of the test compound. When influx was studied, the nerve cord was removed, digested (Eldefrawi, 1966) and radioactivity counted. When efflux was studied, the nerve cord was ligated, removed, and transferred to successive washing solutions, each of which was counted. The concentration of the compounds in the physiological solutions bathing the nerve cord was in the range of 1-2 × 10⁻⁶M/l. In the sheath-disruption experiments it was possible to break the sheath surrounding the ganglia using fine needles, without much damage to the underlying tissue; but this procedure failed with the connectives and they were left intact. To study the effect of dissociation on influx of inonizable molecules, four solutions were made for each of the four compounds, with pH values of 6.3, 7.3, 8.3 and 9.3, and influx into the nerve cord was measured in the different solutions.

To study the effect of metabolism on the influx of a metabolizable molecule, the nerve cord was exposed to sodium butyrate for 1 hr., alone and in the presence of DNP (2,4-dinitrophenol), then homogenized in acetone in a Biolab glass micro-homogenizer. After centrifugation the supernatant was chromatographed in butanol-ammonia; then the developed strip was cut into 1 in. sections, and each was put into 20 ml. counting vials with 10 ml. dioxane liquid phosphor (Bray, 1960) and counted in a Tri-carb liquid scintillation counter.

Histology

The semi-intact preparation of the abdominal nerve cord of the American cockroach, described by Eldefrawi & O'Brien (1966), was used. The abdominal nerve cord was bathed in a 10⁻⁸M/l. methylene blue in Ringer at pH 7 for 1.5 hr., before it was removed and cut into five pieces. Each piece was placed upright in a no. 4 gelatin capsule containing Lab-Tek O.C.T. (−30° to −60° C.)* as an embedding material, and the capsule was placed immediately into a test-tube containing isopentane cooled with liquid nitrogen. Sections 5 μ thick were then made using glass knives in an International Equipment Company cryostat, cooled to −40° C. by pouring liquid nitrogen into the refrigeration cabinet. The frozen sections were transferred to glass slides,
Penetration barriers in cockroach ganglia

dried for 10–15 min. in cold air, and fixed in formaldehyde vapours for 10 min. at room temperature. The tissue was kept frozen (below –40° C.) from the time it was put in isopentane immediately after treatment until after the sections had been cut. Thus the chance of translocation of the dye or any distortion in the original pattern of distribution was kept at a minimum.

RESULTS

pH-dependence of Influx

Our earlier studies led to the conclusion that a regulatory system exists in cockroach ganglia, which discriminates against large, polar, charged molecules; and that metabolism of a compound in nerve tissue greatly enhances its influx. However, comparisons of compounds of different properties are sometimes difficult to interpret because of the impossibility of changing one property of a molecule alone, e.g. of altering polarity without affecting size or shape. Perhaps the closest approximation to be

Text-fig. 1. Effect of pH on the influx of butanol (A), butyltrimethylammonium iodide (B), sodium butyrate (C), and butylamine hydrochloride (D), in intact (●) and de-sheathed (×) abdominal nerve cords. The points represent mean values of triplicate determinations.
reached is when one can compare a compound in a protonated and unprotonated form, for there is then a large charge difference, and little difference in size or shape. Such a comparison can be made by studying the pH-dependence of the behaviour of acids and bases, and comparing the dependence with that of pH-insensitive compounds, as reported in the present study.

Let us first consider the effect of pH upon influx into intact ganglia. Text-fig. 1 (points only; crosses will be discussed below) shows the influx, calculated as the molar ratio of radioactivity in the nerve to that in the bathing solution, plotted on a logarithmic scale against pH. The non-ionizable butanol and the completely ionized butyltrimethylammonium showed no pH-dependence. Butanol established a molar ratio of 1 in the nerve cord within 30 min. exposure, and thus its influx was 6.1 times faster than that of butyltrimethylammonium. Influx of the two ionizable compounds butyrate and butylamine was pH-dependent, being higher the larger the percentage of the undissociated species in solution. When 99.9% of either compound was ionized (the \( pK_a \) for butyrate is 4.87 and for butylamine is 10.6), then their influxes were similar to that of the cation butyltrimethylammonium.

If the rate constant of penetration of the protonated form of a compound is \( k \), and if the unprotonated form penetrates \( N \) times faster, then in unit time the amount \( A \) (which can be expressed in any convenient units, including quantities or concentrations) which penetrates is given:

\[
A = F_p k M + F_u N k M,
\]

where \( F_p \) is the fraction protonated at the particular pH, \( F_u \) is the fraction unprotonated, and \( M \) is the molar concentration. Since \( F_u + F_p = 1 \) and \( pH = pK_a + \log(F_u/F_p) \), it follows that

\[
\frac{A}{kM} = \frac{1 - N}{\text{antilog} (pH - pK_a) + 1} + N.
\]

One can therefore plot a family of theoretical curves (which are sigmoidal) showing \( \log A/kM \) as a function of \( \text{pH} - pK_a \) for various values of \( N \) (Text-fig. 2). In a non-specific ion-barrier system, \( N \) would be greater than one for bases, whose protonated form is charged and therefore is the slow-penetrating species, and less than 1 for acids, whose protonated form is uncharged and therefore is the fast-penetrating species. Text-fig. 1 shows essentially the experimental counterpart of Text-fig. 2, with 'molar ratio' in place of \( A/kM \), and with pH in place of \( \text{pH} - pK \); these changes merely shift the curves without changing their slopes.

From the data of Text-fig. 2 one can plot the slope at the inflexion point (as estimated by the slope of a line drawn through the apparently flat middle portion of each curve) as a function of \( N \). Using such a plot, the experimentally observed slopes of Text-fig. 1 can be used to find the corresponding value of \( N \). For butyrate, \( N \) was found to be 3.5; for butylamine it was 6.5. In the case of butanol and butyltrimethylammonium, no pH-dependence of penetration was observed, in harmony with the hypothesis that all the variation observed in the other compounds was a reflexion of changes in the penetrating compounds, and not of changes in the permeability of the cord.

The findings confirm that the presence of a positive or a negative charge restricts the influx of the molecule into the nerve cord. It is believed that the barrier system
restricts the entry of charged molecules not because of the presence of the charge per se, but because of the high polarity the charge imparts to the molecule; since a cation with a relatively high octanol/water partition coefficient (0.69) such as decyltrimethyl ammonium has a higher influx rate \( k = 0.02 \text{ min}^{-1} \) than methanol \( k = 0.03 \text{ min}^{-1} \), which has a higher polarity (partition coefficient 0.15) (Eldefrawi & O'Brien, 1967a).

Some of the early literature discussed ‘the ion barrier’ as if ‘it’ excluded completely all ionized compounds. The above results show that ionization slows penetration, perhaps to the extent of 3- to 7-fold over an un-ionized compound of similar size.

Text-fig. 1 also shows that the pH-dependence is extraordinarily similar in intact ganglia and in ganglia with disrupted sheaths. The only effect of disruption is to increase the influx of butyltrimethylammonium, about 2-fold, independent of pH; and to increase influx of butylamine about 2-fold under acid conditions, when most butylamine would be protonated, and not at all under alkaline conditions, when most would be unprotonated. These findings suggest that a cation-specific barrier effect is caused by the sheath (i.e. the neural lamella or the perineurium, or both) and that this effect accounts for a part only of the total barrier effect of the ganglion.

Effects of sheath disruption on efflux

The standard preparation, whose sheath is intact, will be referred to as condition A. Three kinds of disruption experiments were performed. In one kind (condition B) the cord was disrupted prior to exposure to the radioactive material, then efflux was studied in the normal manner. In the second kind (condition C) disruption was performed after exposure, but before efflux. In the third (condition D) uptake and early efflux were studied in intact preparations, then the sheath was rapidly broken, and the efflux studies continued.
Experiments of type A show biphasic efflux, which has been shown to be analysable into a fast and slow efflux; for the fast and slow components one can calculate pool sizes (i.e. the percentage of the total effluxed material which escapes in the fast and slow phases respectively) and the rate constants $k_f$ and $k_s$ (Eldefrawi & O'Brien, 1966,

![Graphs](image)

**Text-fig. 3.** Effect of de-sheathing before exposure to butanol (●), butyrate (×) and butyltrimethylammonium (○) on their efflux. The points represent mean values of triplicate determinations.

**Text-fig. 4.** Effect of de-sheathing after exposure of an intact nerve cord to butanol (●), butyrate (×), and butyltrimethylammonium (○) on their efflux. The points represent mean values of triplicate determinations.

**Table 1.** Effect of de-sheathing on efflux of butanol, butyrate and butyltrimethylammonium from the abdominal nerve cord

<table>
<thead>
<tr>
<th>Compound</th>
<th>Treatment</th>
<th>Fast Pool</th>
<th>Slow Pool</th>
<th>Total efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanol</td>
<td>A</td>
<td>$t_{o-1}$</td>
<td>$k_f$</td>
<td>$t_{o-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(min.)</td>
<td>(min. $^{-1}$)</td>
<td>(min.)</td>
</tr>
<tr>
<td>Butanol</td>
<td>A</td>
<td>$2.5$</td>
<td>$0.28$</td>
<td>$22$</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>$2.0$</td>
<td>$0.35$</td>
<td>$33$</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>$2.0$</td>
<td>$0.35$</td>
<td>$55$</td>
</tr>
<tr>
<td>Butyrate</td>
<td>A</td>
<td>$3.3$</td>
<td>$0.21$</td>
<td>$16$</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>$3.3$</td>
<td>$0.21$</td>
<td>$33$</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>$3.0$</td>
<td>$0.23$</td>
<td>$30$</td>
</tr>
<tr>
<td>Butyltrimethylammonium iodide</td>
<td>A</td>
<td>$2.1$</td>
<td>$0.30$</td>
<td>$35$</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>$2.1$</td>
<td>$0.33$</td>
<td>$64$</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>$2.0$</td>
<td>$0.35$</td>
<td>$52$</td>
</tr>
</tbody>
</table>

A, Intact preparation; B, nerve cord disrupted before exposure; C, nerve cord disrupted after exposure, but before efflux.
Penetration barriers in cockroach ganglia

Let us consider first the total radioactivity present prior to efflux, as computed by measuring all that effluxed and adding the amount retained at the end of the experiment (last column of Table 1). It will be seen that this quantity is about the same in conditions A and C, as one would expect, since in both cases uptake was by an intact cord. The effect of sheath disruption on uptake, given by the difference between B and, for instance, C [the most valid comparison] is seen to be small for butanol or butyrate; but for butyltrimethylammonium disruption caused a 3.5-fold increase in uptake. These findings simply confirm the conclusion from the influx studies above, showing that the sheath plays a role in cation uptake only, but that this effect cannot account for all of the ion-limiting phenomena.

Next let us consider effects upon efflux rates in Table 1 and Text-figs. 3 and 4. The fast efflux rate (constant $k_f$) is not greatly affected by sheath disruption either prior to or just after uptake, suggesting that the sheath is not rate limiting for fast release. By contrast the slow rate ($k_s$) is more variable, and was either increased or remained unaffected. These findings are surprising, because our earlier interpretation (in harmony with Treherne, 1961 a) had been that $k_f$ measures the rate of movement from extracellular space to the washing solution, whereas $k_s$ measures the movement across cell membranes. We had therefore expected disruption to affect $k_f$ and not $k_s$. The fast pool sizes are in every case substantially increased by sheath disruption; by 2-5-fold for butanol, 2-3-fold for butyrate and 1-8-fold for butyltrimethylammonium (in each case these are the maximal effects for each compound). In addition to the increase in pool size, i.e. in the proportion that effluxes rapidly, there is almost a doubling of the total amount that effluxed (Table 1). Sheath disruption therefore increases the proportion and amount of fast-effluxing material, without affecting its efflux rate.

One interpretation of the data is as follows. The fast-pool material is that which is exposed to the washing solution, i.e. is effectively external. The effect of disruption is to expose additional material (the volume of the ganglion increases on sheath disruption, and therefore the surface is correspondingly increased). Thus disruption creates extra fast-effluxing material (i.e. increases the fast pool size) but without affecting the rate $k_f$. The slow-effluxing component is all that is within the sheath, and includes intracellular and extracellular material.

An alternative interpretation, which involves an extension of Treherne's hypothesis, is as follows. The rate constant $k_f$ represents the rate of transfer of material from extracellular space (inside the sheath) to the washing solution. It is analogous to a desorption constant, and is independent of the status of the sheath, and hence its rate is unaffected by sheath disruption. The constant $k_s$ represents the rate of transfer from intracellular to extracellular space. On sheath disruption many cell walls are broken, so that some material, formerly intracellular, is made extracellular, and the fast-effluxing pool is thereby increased. In this interpretation, material adsorbed on the outside of the sheath is indistinguishable from extracellular fast-effluxing material within the sheath.

Finally, let us examine the situation (D) when one disrupts in mid-efflux, as shown in Text-fig. 5. One then obtains a new burst of fast-phase efflux; the values for the $k_f$ initially and the $k_f$ for this new burst were 0.28 and 0.31 for butanol, 0.21 and 0.43 for
butyrate, and 0.30 and 0.69 for butylammonium. After these minutes of disturbance the efflux settles down to its former $k_e$ rate. The extra material thus fast-effluxed amounts to approximately 18% for butyrate and 13% for butanol and butyltrimethylammonium. These findings could be interpreted in accordance with either of the above two hypotheses. One thing that is certain is that in intact cords it is not the case, as one might have thought, that at the end of fast-efflux some special pool has been entirely emptied. Clearly a pool remains that can be revealed by sheath disruption.

\[ \begin{align*} 
\text{Text-fig. 5. Effect of de-sheathing during efflux of butanol (○), butyrate (×), and butyltrimethylammonium (○). Arrows indicate the time at which de-sheathing was carried out. The points represent mean values of triplicate determinations.} 
\end{align*} \]

Effect of 2,4-dinitrophenol on influx

DNP has been found to decrease the influx of fatty acids which are metabolized in the nerve tissue (Eldefrawi & O'Brien, 1967a; Toppozada & O'Brien, 1967), and also the influx of Na$^+$ in the abdominal nerve cord of the stick insect Carausius morosus (Treherne, 1965). The data in Text-fig. 6 show that while butyrate influx decreases by 30–40% in the presence of DNP, the influx of the unmetabolizable butyltrimethylammonium is completely unaffected and the influx of butanol is only slightly affected. To explain further the relation between metabolism, influx and the effect of DNP, the metabolism of butyrate by the nerve cord in the presence and absence of DNP was compared. The chromatograms show that butyrate is metabolized rapidly in the cockroach nerve cord, for 90% had disappeared in 1 hr. (Text-fig. 7), but in the presence of $5 \times 10^{-4}$M DNP metabolism is slowed down, although not entirely eliminated; butyrate appears in the tissues, representing 32% of the total radioactivity. The partial metabolism may explain why butyrate even in the presence of $5 \times 10^{-4}$M DNP still shows a higher influx rate than the alcohol or the cation (Text-fig. 5). The implication is that if metabolism did not occur (e.g. if metabolic blockade was complete, or if an
Penetration barriers in cockroach ganglia

unmetabolizable anion such as a sulphate was employed), then the influx values of anions and butyltrimethylammonium would be quite similar.

These findings strongly suggest that the DNP effect noted in this and earlier experiments employing organic compounds is not caused by an effect of DNP on some energy-requiring active transport system. On the contrary, DNP simply inhibits metabolism in some cases, and hence prevents the 'metabolic effect' whereby interior removal of the diffusing species maintains a high in–out gradient. A precise analogue is the case of acetylcholine, whose influx is greatly retarded when its metabolism is blocked by eserine (Treherne & Smith, 1965; Eldefrawi & O'Brien, 1967a). Presumably the effect of DNP on Na\(^+\) influx involves other factors than those considered here.

**Histological studies**

Our point of departure was the early work on the vertebrate blood–brain barrier, which utilized vital dyes such as trypan blue; after injection of the intact vertebrate, dissection revealed that most tissues, but not the brain, were stained (Schmid, 1931; Lundquist, 1942; Bakay, 1947). However, staining of the brain occurred if 'the barrier' was disrupted by treatments such as severe radiation (Clemente & Holst, 1954) or poisoning with various agents (Broman & Lindberg-Broman, 1945). Radio-

![Text-fig. 6. Influx of butyrate (●), butanol (x) and butyltrimethylammonium (O) in the absence (—) and presence (---) of 5 × 10\(^{-4}\)M DNP. Vertical lines represent the range of triplicate determinations.](image-url)
autography has been used in an analogous way to indicate a blood–brain barrier effect (Herlin, 1954; Hansson & Schmiterlöw, 1961).

Methylene blue was selected for the present study; it is widely used to stain nerve fibres, and is a large, polar, cationic compound which would be expected to be subject to a clear barrier effect.

The terminology to be used is as follows (Smith & Treherne, 1963): The nerve cord consists of ganglia joined by connectives. Both are invested by the neural lamella, which is a tough, laminated sheath of connective tissue. In the ganglia there is an inner sheath, the perineurium, which consists of a layer of interdigitating glial cells. Within these sheaths lies a cortex, made up of numerous nerve cell bodies and their axons, invested by the ramifying plasma membranes of the glial cells. Within the cortex lies the neuropile, consisting of axons and dendrites and characteristic axodendritic synapses. Fine arborizations of glial cells invest the neuronal material.

In the connectives, perineurium cells do not form a distinct layer, nor is there a cortex. The interior consists of axons invested by glial cells. There is a boundary zone between the ganglion and its connectives, in which the sections lose their radial symmetry and a centre line of glial cell bodies appear.

When intact nerve cords were exposed to methylene blue as described under Methods, sections showed that the dye colour was much more intense in the fatty tissue than in the ganglia (Pl. 1, 2) or connectives. Wigglesworth (1960) reported a similar finding for the negatively charged dye, trypan blue; and Eldefrawi (unpublished results) found that butyltrimethylammonium achieved levels in cockroach fat body which were, after 1 hr. exposure, twice as high as in muscle and five times higher than in nerve cord. In ganglia the neural lamella was most heavily stained, then the nuclei of the perineurium, then the glial and (to a lesser extent) neuronal cell bodies of the cortex. No dye was seen in the axons, either of the ganglia or of the connectives. No dye was seen in the neuropile itself. The absence of dye in the neuropile cannot be accounted for purely on the grounds of a gradient of dye between the periphery and the centre (which might arise because of inadequate equilibration) because the nuclei of the midline glial cells in the boundary zone invariably showed a stain.

There are two possible explanations for the absence of the dye from the neuropile and the cortical axons. Either the dye failed to reach them because of a barrier effect* or the dye reached them but had inadequate affinity to combine with and stain them. An attempt was made to distinguish between these possibilities: sections were cut of a frozen, unexposed nerve cord, then drops of methylene blue solution were placed on the sections for 5 min., the sections were rinsed several times for a total of about 20 sec., until no colour appeared in the rinsing water, then dried. When examined, there was distinct blue coloration in every region of these sections, and they retained much more dye than the sections made of the nerve cord that was exposed intact for 90 min. to the dye. The cortical nuclei had the highest concentration of the dye, followed by the neural lamella, perineurium and glial cells. The axoplasm itself showed little or no dye, but the glial processes investing the axons of neuropile, cortex and connectives were clearly stained. Consequently, the absence of staining within the neuropile cannot be accounted for purely on the grounds of a gradient of dye between the periphery and the centre (which might arise because of inadequate equilibration) because the nuclei of the midline glial cells in the boundary zone invariably showed a stain.

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* The term 'barrier effect' is used functionally, without necessarily implying a discrete anatomical structure.
neuropile, observed in the intact preparations described above, was not due to a lack of affinity, and must have been due to a barrier effect.

The findings of these histological studies should be compared with the observation reported above that the net influx of anions and cations into the abdominal nerve cord was only slightly modified by severe damage to the elastic neural lamella and peri-neurium. Consequently, the fairly intense staining of the neural lamella observed herein probably reflects its affinity for the polar dye rather than some sort of blockade of entry. It is therefore likely that the relatively general staining of dyed slices, as compared with the more selective staining of dyed whole ganglia, reflects a partial exclusion of polar materials by the cortex and sheaths of the intact ganglion (due to the overall apolar character of the ganglion) rather than by any specific barrier layer. Wigglesworth (1960) found results with the anion, trypan blue, which were essentially

Text-fig. 7. Effect of DNP on metabolism of sodium butyrate by nerve cord tissue. A, Reference chromatogram of sodium butyrate. B, Acetone extract of nerve cord exposed to $10^{-4}$M sodium butyrate for 1 hr. C, Acetone extract of nerve cord exposed to $10^{-4}$M sodium butyrate and $5 \times 10^{-4}$M DNP for 1 hr.
the same as ours with the cation, methylene blue. His conclusion was that the neural lamella was permeable to ions, but the perineurium was not. We disagree, in part because of the small effect of damage on influx, and in part because the ‘barrier effect’ is seen clearly in the connectives, which lack an organized perineurium.

**DISCUSSION**

The results from the influx and efflux studies are compatible with the histological studies: they suggest that the external sheath accounts in part for the slowing of cation penetration, but not for the slowing of penetration by anionic molecules. Certainly there is no evidence for a discrete anatomical barrier which, by itself, accounts for slowing of penetration. This opinion is fortified by the finding that the inhibition of influx DNP is not attributable to an effect upon an active transport mechanism. The conclusion that emerges is that most of the ‘barrier system’ effect for anions, and a lesser part of it for cations, is a reflection of the net properties of the ganglion. For cations only, the sheath plays a small but significant role. The histological work implies that progressively greater protection from external cations is enjoyed as one nears the centre of the ganglion. The neuropile itself, which contains the synapses and is therefore potentially the most sensitive area, may be additionally protected, either by the cells bordering the neuropile or by the glial or neuronal membranes of the neuropile itself. Further evidence for this additional protection is required, however.

The interpretation of the significance of the fast and slow phases seen when molecules of any type efflux from the cord remains obscure. The earlier view, that the fast phase is extracellular, may require revision.

**SUMMARY**

1. The nature and location of the barrier system which partially protects the nervous system in insects has been investigated in the American cockroach by studying the fluxes of $^{14}$C-butanol, $^{14}$C-butyrate, $^{14}$C-butylamine and $^3$H-butyltrimethylammonium.

2. Disruption of the sheath covering the ganglia has little effect on the influx of butanol and butyrate, but increased that of butylamine and butyltrimethylammonium.

3. 2,4-Dinitrophenol slowed the influx of only those compounds whose metabolism it blocks.

4. Effluxes show a fast and a slow component. Disruption of the sheath increases the amount but not the rate of the fast component; it raises the rate of the slow component for butanol, butyrate and butyltrimethylammonium, but not always.

5. Nerve cords stained with methylene blue and freeze-sectioned show dye concentrated in the fat tissue surrounding the nerve cord, then in the neural lamella; a little penetrated the cell bodies of glia and neurones, but the neuropile was unstained.

6. These findings suggest that the external sheath plays some part in restricting cation influx; apart from this the barrier system is a function of the whole ganglion, except that the neuropile may enjoy special protection.
REFERENCES


EXPLANATION OF PLATES

PLATE 1

Ganglion from nerve cord bathed in $10^{-4}$M methylene blue for 1.5 hr. Magnification, $\times 225$.

Fig. 1. Section photographed with phase optics to visualize unstained as well as stained components.

Fig. 2. Same section photographed with light optics to visualize only stained components. Note heavy stain in neural lamella, and light stain in glial cell nuclei.

PLATE 2

Connective from nerve cord bathed in $10^{-4}$M methylene blue for 1.5 hr. Magnification, $\times 225$.

Fig. 1. Section photographed with phase optics.

Fig. 2. Same section photographed with light optics. Note stain in neural lamella.