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PERMEABILITY OF THE ABDOMINAL NERVE CORD OF THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA* L. TO QUATERNARY AMMONIUM SALTS*

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INTRODUCTION

For several years it was accepted that the central nervous system of insects was protected from a variety of cations, including K^+, acetylcholine and cationized drugs and toxicants, by virtue of an ion-barrier. The evidence included the insensitivity to exogenous K^+ (Hoyle, 1953) or acetylcholine (Twarog & Roeder, 1957) and the pH dependence of penetration of the base Amiton (*O,O*-diethyl *S*-diethylaminoethyl phosphorothiolate) into the American cockroach nerve cord, which was compatible with ready penetration of the unprotonated but not the protonated form (O'Brien, 1959). However, Treherne (1961*a*) showed that K^+ in fact penetrated readily into the cockroach nerve cord, and he attributed the K^+ insensitivity of the nerve cord to a Donnan effect. Treherne & Smith (1965*a*) showed that acetylcholine penetrated readily into the same preparation, and that its extremely rapid hydrolysis therein accounted for the physiological insensitivity to exogenous acetylcholine (Treherne & Smith 1965*b*).

We have recently shown that fatty acids ions move very rapidly into and out of the cockroach nerve cord (Eldefrawi & O'Brien, 1966). The present paper explores the problem of whether there is any cation barrier in that preparation; it seemed plausible that although small cations (such as K^+) and perhaps other physiologically important cations (such as acetylcholine) might penetrate, nevertheless large exotic cations might not.

Quaternary ammonium compounds offered considerable advantages for our study. They are readily labelled isotopically; series increasing either in length or minimal diameter may readily be designed; they are metabolically stable (Williams, 1959) yet bear close structural relations to physiologically interesting molecules such as acetylcholine.

MATERIALS AND METHODS

Two series of tritiated quaternary alkylammonium iodide salts were prepared. Series I had the general structure $RN^+(CH_3)_3I^-$, where $R = C_2H_5$, C_4H_9, C_6H_{13}, C_8H_{17} and $C_{10}H_{21}$. Series II had the general structure $C_2H_5N^+(R')_3I^-$, where $R' = CH_3$, C_2H_5, C_3H_7, C_4H_9 and C_5H_{11}. Synthesis of series I salts was accomplished using tritiated methyl iodide (purchased from Nichem, specific activity 50 mc./mM.) and the corres-

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ponding dimethyl *N*-alkylamines. The latter were synthesized by treating alkylamines with formaldehyde and formic acid according to Clark, Gillespie & Weisshaus (1933). Series II salts were prepared by using tritiated ethyl iodide (purchased from Nichem, specific activity 50 mc./mM.) and the corresponding trialkylamines.

The synthesis was accomplished by adding the tritiated alkyl iodide to the trialkylamine in a 2 ml. glass ampoule using benzene as a solvent. The glass ampoule was then sealed and floated in an oil bath held at 70° C. The upper stem of the ampoule while floating in an upright position with only the bottom 1 cm. immersed in the oil, acted as an air-cooled reflux column. The upright position of the ampoule was established by placing it in a circular cork holder which floated on the surface of the oil. All products were colourless solids insoluble in benzene, and precipitated upon formation. The reaction time was 6 and 12–18 hr. for the salts of series I and series II respectively. After the reaction was completed, the ampoule was broken open and the solid products were washed with benzene and ether to remove any traces of unreacted alkylamines or iodides. Yields ranged from 70 to 85%. Specific activity of the different quaternary salts ranged from 7·5 to 8·5 mc./mM. The purity of the products was established by comparing the melting-points of the products as well as by paper chromatography using the method of Bregoff, Roberts & Delwiche (1953).

The octanol–water partition coefficients of the different cations were determined. A quantity of each compound representing 2–3 million counts/min. was transferred into a 15 ml. test tube and water added to make a volume of 1·03 ml. Three samples of 0·01 ml. were taken from each tube to determine the exact counts/min. per ml. of water. Five ml. of octanol were added to each test tube and the contents of the tube were well mixed by placing it for 1 min. on a vibrator mixer. The tubes were then placed in a centrifuge and spun at 5000 rev./min. for 20 min. to accomplish complete separation of the two layers. Three 0·1 ml. samples were then taken from the upper octanol layer. The water and octanol samples were counted in a liquid scintillation counter using Bray's (1960) dioxane cocktail. The octanol–water partition coefficients were calculated by the equation

$$P = \tfrac{1}{5}x/(y-x),$$

where y = original counts/min. per ml. water, and x = total counts/min. per 5 ml octanol and $y-x$ = total counts/min. left in 1 ml. of water after partitioning.

The abdominal nerve cord preparation used in this study and the steps followed to measure influx or efflux were the same as those described previously (Eldefrawi & O'Brien, 1966). The concentration of the quaternary ammonium salts as well as ACh and eserine in the Ringer solution bathing the abdominal nerve cord ranged between 0·6 and $1{\cdot}7 \times 10^{-4}$ M.

RESULTS AND DISCUSSION

For reasons previously discussed (Eldefrawi & O'Brien, 1966) it was convenient to express the penetration at any given time as 'molar ratio', defined as the ratio of the internal to the external concentration; this value would be 1 if simple equilibrium was achieved.

Our data for acetylcholine penetration (Fig. 1) are qualitatively identical with those of Treherne & Smith (1965*a*); both show achievement of an equilibrium in an eserinized preparation, and an almost first-order continuous penetration in the absence of

eserine, undoubtedly due to the fact that such a preparation can hydrolyze internal acetylcholine extremely fast (Treherne & Smith, 1965*b*). Calculations from the data of Treherne & Smith (1965*a*) show a molar ratio of about 0·48 at equilibrium (in the eserinized preparation) compared to our value of 0·42, while their molar ratio for acetylcholine after 50 min. in the non-eserinized preparation is 2·6 compared to our value of 2·1.

Each of the two series of cations contains a group of compounds differing progressively in molecular weight of alkane substituents, and hence also in related physical

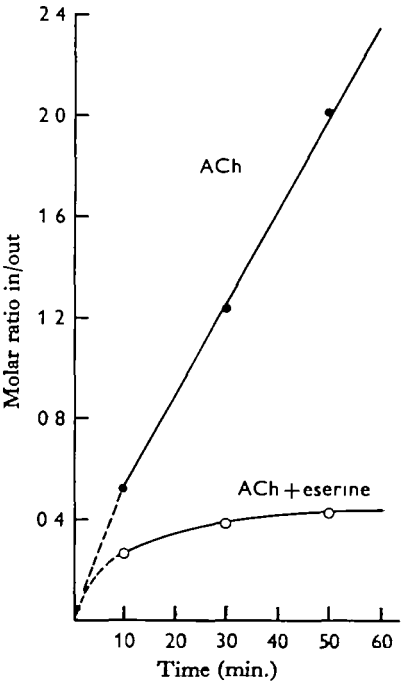

Fig. 1. The relation between time and influx of acetylcholine (alone and in the presence of eserine) in the abdominal nerve cord. The points represent mean values of a minimum of two tests.

properties, such as molecular volume and apolarity (as measured by the octanol–water partition coefficients). The two series differ in that increasing molecular weight in series I is achieved without increasing the minimal cross-sectional area of the molecule, whereas in series II this area increases progressively. The intent in selecting two series with such a difference was to permit expoloration of the role of molecular size in diffusion.

Influx. Figs. 2 and 3 show that the influx of the ammonium cations is approximately first order and continuous, only the highest member of series I showing two-phase influx (in an anionic series, two phase influx was more usual, Eldefrawi & O'Brien, 1966). Consequently, a first-order rate constant for influx may be calculated, with results shown in Table 1. These constants or their equivalents may then be plotted as a function of potentially significant parameters, such as partition coefficient (Fig. 4), molecular volume (Fig. 5) or size of *N*-trialkyl group 'cationic head' (Fig. 6). The first

two parameters essentially parallel each other. Because the most striking effect is an increase in rate with partition coefficient and with molecular volume (as was also seen in the anionic series—Eldefrawi & O'Brien, 1966), and because it is implausible that increasing size would assist penetration into or through any system, it is most likely that the increasing partition coefficient (i.e. liposolubility) is the factor responsible for increasing influx. There are two anomalies: the lowest member of series I enters faster than the higher homologue. It is possible that this compound (trimethylethylammonium) is so close an analogue of choline, and therefore of acetylcholine and phosphatidylcholine, that it interacts with some macromolecule which normally binds such

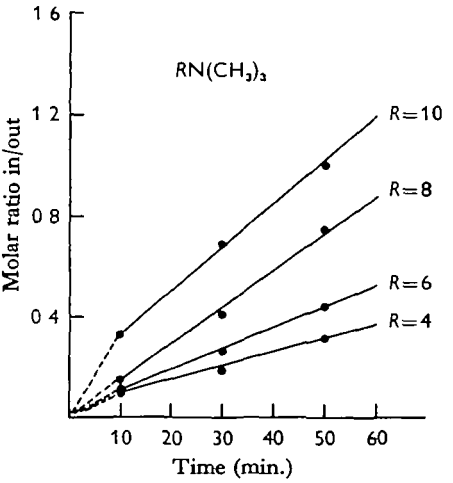

Fig. 2

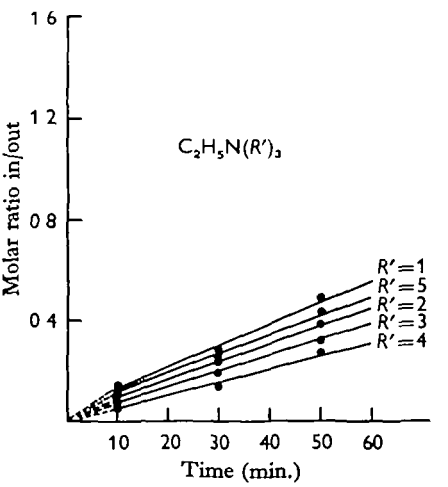

Fig. 3

Fig. 2. The relation between time and influx of *N*-alkyltrimethyl ammonium iodides into the abdominal nerve cord. The points represent mean values of a minimum of two tests.

Fig. 3. The relation between time and influx of *N*-ethyltrialkyl ammonium iodides into the abdominal nerve cord. The points represent mean values of a minimum of two tests.

Table 1. *Physical properties of the quaternary ammonium cations and their influx rates*

Quaternary compound	Molecular volume	Min. and max. diameter of *N*-trialkyl group (Å.)	Octanol–water partition coefficient	Influx rate, k min.$^{-1}$
$RN^+(CH_3)_3$				
$R = C_2H_5$	144	5·3–6·7	$9{\cdot}9 \times 10^{-4}$	0·013
$= C_4H_9$	188	5·3–6·7	$2{\cdot}49 \times 10^{-3}$	0·006
$= C_6H_{13}$	232	5·3–6·7	$1{\cdot}46 \times 10^{-2}$	0·009
$= C_8H_{17}$	276	5·3–6·7	$8{\cdot}55 \times 10^{-2}$	0·015
$= C_{10}H_{21}$	320	5·3–6·7	0·69	0·020
$C_2H_5N^+(R')_3$				
$R' = CH_3$	144	5·3–6·7	$9{\cdot}9 \times 10^{-4}$	0·013
$= C_3H_5$	210	7·1–8·6	$1{\cdot}5 \times 10^{-3}$	0·010
$= C_3H_7$	276	8·7–11·0	$6{\cdot}5 \times 10^{-3}$	0·008
$= C_4H_9$	342	9·5–13·5	5×10^{-2}	0·006
$= C_5H_{11}$	408	10·7–16	0·6	0·009
Acetylcholine	197	5·3–6·7	0·56	0·04

compounds. The second 'anomaly' is that in series II the dependence of influx on partition coefficient is small, and tends to be negative for the first four members, with an upturn for the fifth. The reason may be that increasing the size of the smallest cross-section tends to decrease influx, whilst increasing partition coefficient tends to increase it; in series I only this second factor is operative because the increase in size

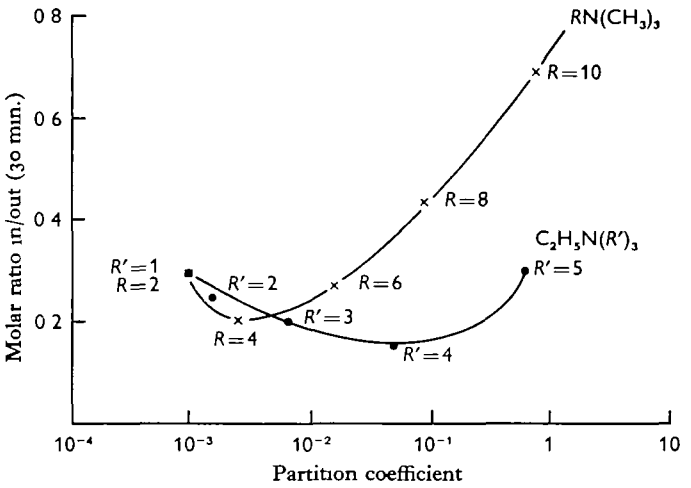

Fig. 4. The relation between octanol–water partition coefficients and uptake of the quaternary ammonium salts by the abdominal nerve cord in 30 min.

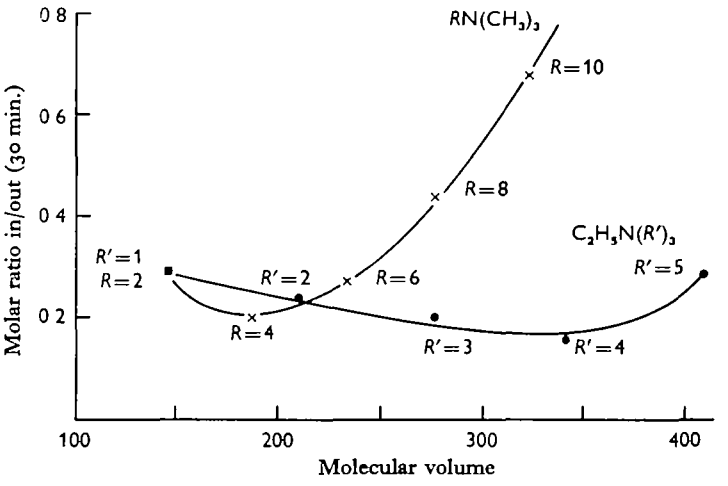

Fig. 5. The relation between molecular volume and uptake of the quaternary ammonium salts by the abdominal nerve cord in 30 min.

is all in length rather than cross-section. In series II the size effect predominates up to tributyl compound, after which further increase to tripentyl has a relatively small effect (presumably because as the alkyl size increases, the 'head' size is less accurately represented by the fully extended alkyl chains, whose flexibility becomes a more important factor) and the increase in partitioning is the predominant factor.

Metabolism of a compound by nervous tissue can greatly increase the rate of penetration of radioactivity into the tissue by continously removing the penetrating species and thus eliminating its back diffusion. However, simple metabolism, e.g. to a single labelled fragment which cannot be utilized and which moves within the tissue as readily as the penetrating species, would not assist accumulation of radioactivity, for the back reaction with respect to the labelled fragment could be as fast as that of the unmetabolized compound. It is even conceivable that metabolism could slow the accumulation of radioactivity, by conversion of the diffusing species to a compound whose polarity leads to a very fast efflux. But whenever metabolism leads to incorpora-

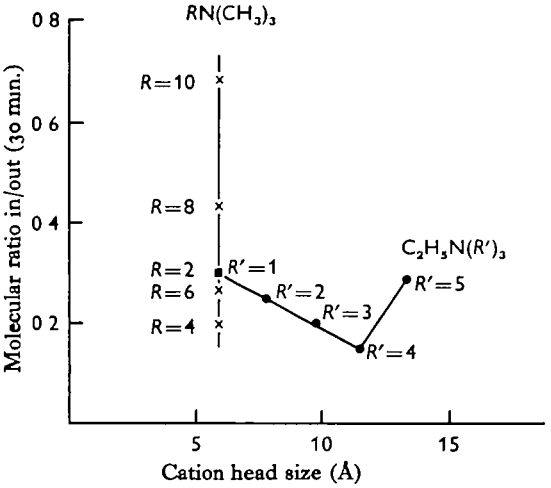

Fig. 6. The relation between the diameter of the *N*-trialkyl groups (Å) and uptake of the quaternary ammonium salts by the abdominal nerve cord in 30 min.

tion of the labelled fragment into relatively non-diffusible compounds, or converts the diffusing species into many compounds, the gradient of any one of which is correspondingly small, then metabolism will indeed enhance accumulation of radioactivity in the tissue. And indeed blockade of acetylcholine metabolism by eserine leads to greatly reduced influx (Fig. 1), corresponding to the complex utilization of the products of its metabolism in cockroach nervous tissue (Treherne & Smith, 1965*b*).

The fact that the quaternary ammonium compounds are not expected to be much involved in metabolic activity (Williams, 1959) may be a contributing factor to their low influx rates in the cockroach C.N.S. The metabolism of butyltrimethylammonium and decyltrimethylammonium salts in the cockroach was investigated. Abdominal nerve cords exposed for 1 hr. to each of these cations were digested in nitric acid, the acid was evaporated under a stream of warm air, the residues were redissolved in water and chromatographed. The chromatograms were cut into $\frac{1}{2}$ in. strips, which were placed in vials with 10 ml. Bray's dioxane cocktail (1960) and counted in a liquid scintillation counter. Fig. 7 shows that neither cation was metabolized in the nerve tissue of the cockroach.

The rates of influx of the different cations expressed as moles that penetrate 1 cm.^{2} of nerve membrane per sec. was calculated using the equation of Keynes (1951), and

presented in Table 2. The value for Na^+ is that reported for the cockroach by Treherne (1961 *b*). These values imply that influx of Na^+ is approximately 100 times faster than that of the ammonium cations, but the concentration of Na^+ in the outside solution

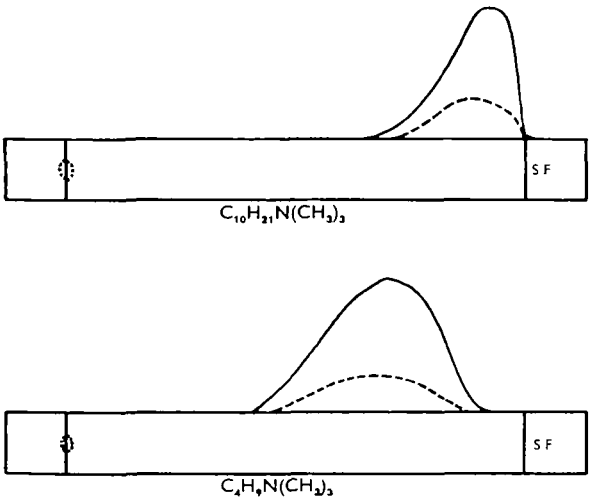

Fig. 7. Radiochromatograms of two alkylammonium cations extracted from nerve tissue after 1 hr. exposure. The solid tracings are those of the reference compounds treated with HNO_3 and the broken line tracings represent the extracted radioactivity from the nerve cord digested with nitric acid.

Table 2. *Influx rates of the alkyl ammonium cations into the abdominal nerve cord*

Cation	Influx rate moles cm^{-2} $sec.^{-1}$	k $min.^{-1}$	molar conc. of cation in test solution
$RN^+(CH_3)_3$			
$R = C_2H_5$	$1{\cdot}34 \times 10^{-13}$	0·013	$1{\cdot}65 \times 10^{-4}$
$= C_4H_9$	$1{\cdot}00 \times 10^{-13}$	0·006	$1{\cdot}0 \times 10^{-4}$
$= C_6H_{13}$	$1{\cdot}25 \times 10^{-13}$	0·009	$0{\cdot}95 \times 10^{-4}$
$= C_8H_{17}$	$1{\cdot}40 \times 10^{-13}$	0·015	$0{\cdot}6 \times 10^{-4}$
$= C_{10}H_{21}$	$1{\cdot}66 \times 10^{-13}$	0·020	$1{\cdot}01 \times 10^{-4}$
$C_2H_5N^+(R')_3$			
$R' = CH_3$	$1{\cdot}34 \times 10^{-13}$	0·013	$1{\cdot}65 \times 10^{-4}$
$= C_2H_5$	$1{\cdot}08 \times 10^{-13}$	0 010	$1{\cdot}4 \times 10^{-4}$
$= C_3H_7$	$1{\cdot}15 \times 10^{-13}$	0·008	$1\ 65 \times 10^{-4}$
$= C_4H_9$	$0{\cdot}97 \times 10^{-13}$	0·006	$1{\cdot}35 \times 10^{-4}$
$= C_5H_{11}$	$1{\cdot}22 \times 10^{-13}$	0·009	$1{\cdot}45 \times 10^{-4}$
*Na^+	$13{\cdot}9 \times 10^{-12}$	0·04	$1{\cdot}32 \times 10^{-1}$

* From Treherne (1961 *b*).

was 132 mM compared to 10^{-4}M in the case of the quaternary ammonium salts, and these influx figures are dependent on the total concentration of the cation in the nerve tissue, which is directly affected by the outside concentration. Therefore it would seem that the rate constant k ($min.^{-1}$), that is used in this study, represents a better parameter for comparing data of different workers, and use of this figure indicates that Na^+ enters from 2 to 7 times faster than the quaternary ammonium cations.

Efflux. The efflux of the quaternary ammonium salts is characterized by a two-stage

process with simultaneous rapid and slow phases, which we attribute to loss from two effective pools. The properties of the two pools characteristic of the efflux of series I and series II cations are listed in Tables 3 and 4 respectively. It is noticed that k_1 (0·28–0·35) and k_2 (0·006–0·01) values for the different cations are higher than those reported earlier for the fatty acids, 0·13–0·2, and 0·005 respectively (Eldefrawi & O'Brien, 1966), but are still lower than those for Na^+ (1·25 and 1·58) or glucose (2·25 and 0·071) calculated from Treherne (1960, 1961*b*). However, the k_1/k_2 ratios for all the cations,

Table 3. *Properties of the two pools characteristics of the efflux of N-alkyl trimethyl ammonium cations from the abdominal nerve cord*

	Fast pool			Slow pool			% retained by nerve cord after	
Cation	$t_{0.5}$ min.	k_1 min.$^{-1}$	% size	$t_{0.5}$ min.	k_2 min.$^{-1}$	% size	1 hr. efflux	k_1/k_2
$C_4H_9N^+(CH_3)_3$	2·3	0·30	35	95	0·0073	65	41	41·1
$C_8H_{13}N^+(CH_3)_3$	2·5	0·28	38	68	0·0101	62	33	27·8
$C_8H_{17}N^+(CH_3)_3$	2·5	0·28	32	95	0·0073	68	42	38·4
$C_{10}H_{21}N^+(CH_3)_3$	2·5	0·28	37	80	0·0086	63	36	32·6

Table 4. *Properties of the two pools characteristics of the efflux of N-ethyl trialkyl ammonium cations from the abdominal nerve cord*

	Fast pool			Slow pool			% retained by nerve cord after	
Cation	$t_{0.5}$ min.	k_1 min.$^{-1}$	% size	$t_{0.5}$ min.	k_2 min.$^{-1}$	% size	1 hr. efflux	k_1/k_2
$C_2H_5N^+(CH_3)_3$	2·3	0·30	37	75	0·0092	63	35·0	32·6
$C_2H_5N^+(C_2H_5)_3$	2·3	0·30	40	95	0·0073	60	38·1	41·0
$C_2H_5N^+(C_3H_7)_3$	2·5	0·28	30	110	0·0063	70	48·5	44·5
$C_2H_5N^+(C_4H_9)_3$	2 5	0·28	36	95	0·0073	64	38·5	38·2
$C_2H_5N^+(C_5H_{11})_3$	2·0	0 35	39	70	0·0098	61	30·0	35·6

fatty acids and glucose are similar, ranging between 23 and 44; while the ratio is only 9 for Na^+, perhaps because of its very small size and the presence of a sodium pump in the C.N.S. of insects (Treherne, 1961*c*). If the slow pool represents the inside cellular structures of the C.N.S. while the fast pool represents the extracellular spaces as was suggested by Treherne (1961*b*), then k_1 and k_2 represent the rates of penetration through the nerve sheath and the cellular membranes respectively. Thus the ratio is a reflexion of the relative permeability of the two membranes.

The free diffusion rates (D' and D'') for the different cations are calculated by use of the equation $t_{0.5} = 0{\cdot}118r_0^2/D'$ according to Treherne (1961*b*). Considering that the abdominal nerve cord may be represented by a cylinder whose $r_0 = 0{\cdot}2$ mm., the D' values for the different cations are calculated for the fast and slow effluxes directly using the above equation and substituting for $t_{0.5}$ the corresponding values in seconds measured for the cations (Tables 3 and 4). The D'' values are obtained by substituting $t_{0.5}$ with the expression $2{\cdot}3 \log 2/k$, where k is the influx rate per sec. The diffusion rates of efflux and influx are listed in Table 5. The free diffusion of many organic compounds such as sugars and amino acids was found to range between 5 and 10×10^{-6} cm^2./sec. (Longsworth, 1953). If one assumes that the ammonium cations

would have similar values for free diffusion in solutions, then one may assume that the smaller values of D' and D'' reflect the magnitude of the resistance offered by the different membranes of the C.N.S. which the cations cross during their fluxes.

Table 5. *Diffusion rates of the different cations across the C.N.S. membranes during influx and efflux*

	Diffusion constants (cm.^{2} sec.$^{-1}$)		
	Efflux D'		Influx D'
Compound	Fast	Slow	
$RN^+(CH_3)_3$			
$R = C_2H_5$	$1{\cdot}8 \times 10^{-7}$	$5{\cdot}5 \times 10^{-9}$	$1{\cdot}46 \times 10^{-8}$
$= C_4H_9$	$1{\cdot}8 \times 10^{-7}$	$4{\cdot}4 \times 10^{-9}$	$0{\cdot}68 \times 10^{-8}$
$= C_6H_{13}$	$1{\cdot}65 \times 10^{-7}$	$6{\cdot}1 \times 10^{-9}$	$1\ 02 \times 10^{-8}$
$= C_8H_{17}$	$1{\cdot}65 \times 10^{-7}$	$4{\cdot}4 \times 10^{-9}$	$1{\cdot}68 \times 10^{-8}$
$= C_{10}H_{21}$	$1{\cdot}65 \times 10^{-7}$	$5{\cdot}2 \times 10^{-9}$	$2{\cdot}24 \times 10^{-8}$
$C_5H_5N^+(R')$			
$R' = CH_3$	$1{\cdot}8 \times 10^{-7}$	$5{\cdot}5 \times 10^{-9}$	$1{\cdot}46 \times 10^{-8}$
$= C_2H_5$	$1{\cdot}65 \times 10^{-7}$	$4{\cdot}4 \times 10^{-9}$	$1{\cdot}14 \times 10^{-8}$
$= C_3H_7$	$1{\cdot}65 \times 10^{-7}$	$3{\cdot}8 \times 10^{-9}$	$0{\cdot}92 \times 10^{-8}$
$= C_4H_9$	$1{\cdot}65 \times 10^{-7}$	$4{\cdot}4 \times 10^{-9}$	$0{\cdot}68 \times 10^{-8}$
$= C_5H_{11}$	$1{\cdot}65 \times 10^{-7}$	$5{\cdot}9 \times 10^{-9}$	$1{\cdot}02 \times 10^{-8}$
Acetylcholine	$1{\cdot}8 \times 10^{-7}$	$2{\cdot}4 \times 10^{-10}$	$4{\cdot}58 \times 10^{-8}$
*Na^+	$5{\cdot}7 \times 10^{-7}$	$7{\cdot}3 \times 10^{-8}$	$4{\cdot}58 \times 10^{-8}$

* From Treherne (1961*b*).

It is noticed that the average size of the fast pool for these cations (36 %) is larger than the approximate size of the extracellular spaces estimated for the cockroach nerve cord, 20 % (Smith & Treherne, 1963), and also that for the fatty acids, 24 % (Eldefrawi & O'Brien, 1966). This discrepancy suggests the possibility of a different explanation for the two pools; in the case of cations, it may be that only the extracellular space is occupied, and the slow pool represents the fraction that is bound to extracellular mucopolysaccharides. Whichever explanation is offered, it seems at first surprising that the efflux diffusion rates (D') of the fast pool are more than ten times faster than the corresponding influx diffusion rates (D''); however, the D' for slow efflux is roughly 10 times slower than D''. The second explanation helps one to understand why in Figs. 2 and 3 those cations enter with no sign of saturation, even at molar ratios near 1, suggesting that equilibrium would eventually be achieved at very high molar ratios. Thus the thermodynamic activity of these compounds is less inside the cord than out, a stabilizing phenomenon that could be due to binding.

Although there is evidence that cation penetration into the insect's C.N.S. is somewhat hindered, it is clear that cations do indeed penetrate, some of them reaching equilibrium (i.e. molar ratio = 1) in about 1 hr. Therefore the hypothesis that the nerve sheath acts as an ion barrier should be modified accordingly. It may even be concluded from the present data that the nerve sheath is a weak cation barrier. This conclusion conflicts with at least three reports. Winton, Metcalf & Fukuto (1958) stained pieces of nerve from the American cockroach for cholinesterase, using the Koelle technique, and reported that the stain was only effective where the sheath was

damaged, a finding which they attributed to the failure of the acetylthiocholine, used in the procedure, to penetrate intact perineurium. However, their procedure involved drying the pieces of nerve at 38° C. for 15 min. prior to staining. In this time such a tiny piece of tissue dries out almost completely, and the properties of its components could be severely affected. O'Brien (1959) reported evidence that the free base form of Amiton penetrated intact nerve cords of the American cockroach far more readily than the protonated form (as judged by the pH dependence of the penetration) or than the quaternarized form, whose penetration was poor and independent of pH. Penetration was assayed by the resultant cholinesterase inhibition. Along similar lines, Kolbezen, Metcalf & Fukuto (1954) found that when *m*-dimethylaminophenyl methylcarbamate was quaternerized, its anticholinesterase activity was greatly increased, but its toxicity to insects was almost abolished. These data, originally postulated as being the consequence of the nerve sheath acting as an ion-barrier, could be interpreted in other ways. One possibility is that cholinesterase is primarily in the neuropile and around the perikarya as shown by Smith & Treherne (1965) and that cations move poorly from the extracellular spaces and the periphery of the nerve cord towards the inside, either because of a barrier effect as represented by the several cellular membranes which the cation has to cross to get inside or perhaps because of binding to the numerous carboxylate groups of the extracellular space (Ashhurst, 1961). It is entirely possible that all the events in the radio-isotope studies are occurring in a thin peripheral layer. Indeed, Rosenberg & Hoskin (1965) found that acetylcholine, labelled with ^{14}C on the *N*-methyl group, penetrated readily into the periphery of the isolated giant axon of the squid, but that the concentration achieved in the axoplasm was only 1 % of that achieved in the periphery. Hindrance to centripetal movement could be caused either by large numbers of mesaxonic, glial and neuronal membranes, each of which by itself presents a slight resistance to movement; or by a more discrete barrier located within the ganglion. All these possibilities are under investigation and will be tested by suitable radioautographic techniques.

In all these discussions it is not easy to separate the effects of charge *per se* from the effect of liposolubility; if one compares a charged molecule and its uncharged analogue, the charge introduces both a coulombic effect and (as a consequence) a change in liposolubility. One might elucidate these effects by comparing a multiply hydroxylated molecule (e.g. glucose) with a singly cationized but unhydroxylated analogue of similar molecular volume, thus obtaining molecules with similar polarity, but differing in charge. The k (min.$^{-1}$) for influx of the smallest ammonium cation is 0·01 while that for glucose (calculated from Treherne, 1960) is 0·036. Glucose, however, is metabolized rather rapidly while the cation is not metabolized at all (Fig. 7), so the 'true difference' due to charge is probably less than 3·6-fold.

The restrictions on cation penetration into the C.N.S. of vertebrates and invertebrates appear to be similar. A system appears to exist which particularly restricts cation movement in invertebrates and both cations and anions in vertebrates. This system, however, is easily by-passed by very small cations such as Na^+. The k (min.$^{-1}$) for Na^+ into the brain and cerebrospinal fluid (C.S.F.) from the blood calculated from Davson (1955) is 0·04 which is identical with Na^+ influx into the abdominal nerve cord of the cockroach (Table 2). Goldsworthy, Aird & Becker (1954) reported brain/blood ratios for several ionized and nonionized drugs in the rat. The ratios ranged from 0·06 to

1·0 which is similar to the molar ratios found for the cations in this study (Figs. 2, 3). This would indicate that the regulating systems in vertebrates and invertebrates have similar properties. The term 'brain barrier system' was suggested by Lajtha (1962) to replace the 'blood-brain barrier'. If by an ion barrier one means a certain anatomical structure which prevents ions from moving into the C.N.S., then such a barrier does not exist in insects. On the other hand, if the restrictions of movement of ions is due to several properties of the ion (e.g. liposolubility, size, metabolism charge) then 'barrier system' may be a better term, especially since a system implies a complex made up of several factors, one or more dominating in a particular situation.

It is hard to know where to draw the line separating compounds that penetrate readily from others that penetrate less readily. As an example, if one compares the influx of series I cations one finds that the cation of highest liposolubility attains a molar ratio of 1 in 1 hr., while the smallest and highly water-soluble cation can attain only half that value in the same period. Acetylcholine attains twice the value within 1 hr., but only a quarter of that in the eserinized preparation.

On the basis of this and the preceding paper, there appears to exist a 'regulatory' system' in the C.N.S. of insects that controls the movement of molecules, in that it tends to restrict the influx of large, charged, unmetabolizable, polar molecules.

SUMMARY

1. The influx and efflux of two series of quaternary alkylammonium cations in the abdominal nerve cord of the American cockroach have been studied. The data are interpreted as showing that increasing liposolubility tends to increase penetration, and increasing size (with respect to the smallest cross-sectional area) decreases it.

2. The influx rates of the quaternary alkylammonium cations into the cockroach central nervous system (C.N.S.) is 2–7 times lower than Na^+ when equimolar concentrations are compared.

3. Acetylcholine penetrates into the C.N.S. rapidly due to its metabolism, since in the presence of eserine its influx rate becomes similar to that of the analogous alkylammonium cation which is unmetabolized.

4. The alkylammonium cations that penetrate into the C.N.S. appear to be distributed into fast and slow pools, as judged by their relative rates of efflux. These may represent the distribution of cations between extracellular and intracellular spaces or possibly free and bound cations in the extracellular spaces.

5. Although the alkylammonium cations penetrate the C.N.S., they apparently encounter a regulatory system that discriminates against large size, positive charge and polarity.

REFERENCES

ASHHURST, D. E. (1961). An acid mucopolysaccharide in cockroach ganglia. *Nature, Lond.* **191**, 1224–5.

BRAY, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**, 279–85.

BREGOFF, H. M., ROBERTS, E. & DELWICHE, C. C. (1953). Paper chromatography of quaternary ammonium bases and related compounds. *J. Biol. Chem.* **205**, 565–74.

CLARK, H. T., GILLESPIE, H. B. & WEISSHAUS, S. Z. (1933). The action of formaldehyde on amines and amino acids. *J. Am. Chem. Soc.* **55**, 4571–81.

DAVSON, H. (1955). A comparative study of the aqueous humour and cerebrospinal fluid in the rabbit. *J. Physiol., Lond.* **129**, 111–33.

ELDEFRAWI, M. E. & O'BRIEN, R. D. (1966). Permeability of the abdominal nerve cord of the American cockroach to fatty acids. *J. Insect Physiol.* **12**, 1133–42.

GOLDSWORTHY, P. D., AIRD, R. B. & BECKER, R. A. (1954). The blood-brain barrier—the effect of acidic dissociation constant on the permeation of certain sulfonamides into the brain. *J. Cell Comp. Physiol.* **44**, 519–26.

HOYLE, G. (1953). Potassium ions and insect nerve muscle. *J. Exp. Biol.* **30**, 121–35.

KEYNES, R. D. (1951). The ionic movements during nervous activity. *J. Physiol., Lond.* **114**, 119–50.

KOLBEZEN, M. J., METCALF, R. L. & FUKUTO, T. R. (1954). Insecticidal activity of carbamate cholinesterase inhibitors. *J. Agric. Fd Chem.* **2**, 864–70.

LAJTHA, A. (1962). The 'brain barrier system'. In *Neurochemistry, The Chemistry of Brain and Nerve*, pp. 399–430. Ed. K. A. C. Elliott, I. H. Page and J. H. Quastel. (2nd edition). Springfield, Ill., U.S.A.: Charles C. Thomas.

LONGSWORTH, L. G. (1953). Diffusion measurements at 25° C. of aqueous solutions of amino acids, peptides and sugars. *J. Am. Chem. Soc.* **75**, 5705–9.

O'BRIEN, R. D. (1959). Effect of ionization upon penetration of organophosphates to the nerve cord of the cockroach. *J. Econ. Ent.* **52**, 812–6.

ROSENBERG, P. & HOSKIN, F. C. G. (1965). Penetration of acetylcholine into squid giant axons. *Biochem. Pharmac.* **14**, 1765–72.

SMITH, D. S. & TREHERNE, J. E. (1963). Functional aspects of the organization of the insect nervous system. *Advanc. Insect Physiol.* **1**, 401–84. Eds. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth. London: Academic Press.

SMITH, D. S. & TREHERNE, J. E. (1965). The electron microscopic localization of cholinesterase activity in the central nervous system of an insect, *Periplaneta americana* L. *J. Cell. Biol.* **26**, 445–65.

TREHERNE, J. E. (1960). The nutrition of the central nervous system in the cockroach, *Periplaneta americana* L. The exchange and metabolism of sugars. *J. Exp. Biol.* **37**, 513–33.

TREHERNE, J. E. (1961*a*). Sodium and potassium fluxes in the abdominal nerve cord of the cockroach *Periplaneta americana* L. *J. Exp. Biol.* **38**, 315–22.

TREHERNE, J. E. (1961*b*). The kinetics of sodium transfer in the central nervous system of the cockroach *Periplaneta americana* L. *J. Exp. Biol.* **38**, 737–46.

TREHERNE, J. E. (1961*c*). The movements of sodium ions in the isolated abdominal nerve cord of the cockroach *Periplaneta americana* L. *J. Exp. Biol.* **38**, 629–36.

TREHERNE, J. E. & SMITH, D. S. (1965*a*). The penetration of acetylcholine into the central nervous tissues of an insect, *Periplaneta americana* L. *J. Exp. Biol.* **43**, 13–21.

TREHERNE, J. E. & SMITH, D. S. (1965*b*). The metabolism of acetylcholine in the intact central nervous system of an insect, *Periplaneta americana* L. *J. Exp. Biol.* **43**, 441–54.

TWAROG, B. M. & ROEDER, K. D. (1957). Pharmacological observations on the desheathed last abdominal ganglion of the cockroach. *Ann. Ent. Soc. Am.* **50**, 231–7.

WILLIAMS, R. T. (1959). *Detoxication Mechanisms*, pp. 147–8. New York: John Wiley and Sons, Inc.

WINTON, M. Y., METCALF, R. L. & FUKUTO, T. R. (1958). The use of acetylthiocholine in the histochemical studies of the action of organophosphorus insecticides. *Ann. Ent. Soc. Am.* **51**, 436–41.