STUDIES ON PERMEABILITY IN RELATION TO NERVE FUNCTION

III. PERMITTIVITY OF BRAIN CORTEX SLICES TO GLYCIN AND ASPARTIC ACID*

by

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INTRODUCTION

In the two preceding papers[1,2] the importance of the permeability of axonal membranes has been discussed. The problem is of special interest in relation to nerve function since it is known that conduction is associated with permeability changes, affecting ion movements across the active membrane[2,3,4]. In addition there are other membranes surrounding the active membrane of the axon and possibly the cell body. This fact is essential not only for the analysis and understanding of nerve function, but also for the interpretation of the pharmacodynamic action of many compounds. *E.g.*, the inability of compounds like acetylcholine and curare to affect conduction in contrast to their well known action on the synapse has been explained by the demonstration of a structural barrier around the axon.

The investigations mentioned were carried out on the giant axon of squid. This preparation is an unusually favorable material for certain problems due to its simplicity. However, for the same reason it does not give information with regard to the more complex situation prevailing in other nervous tissues. Studies have now been initiated on the rate of penetration of amino acids into brain cells and the factors influencing this process.

The use of amino acids to determine the nature of the permittivity, surface and metabolic relationships of the neuronal membrane has considerable theoretical possibilities. One can employ for the study of these membrane characteristics a great variety of structurally distinct amino acids: of different molecular volume, carbon chain length, and polar groups. In view of these qualities the passage of amino acids into such biological systems as plant and red blood cells has been investigated[5,6,7]. In the nervous system very little work has been done with the amino acids.

Experiments with the giant axon of squid have been initiated[8]. In mammalian

* These investigations were supported by a research grant from the Atomic Energy Commission.

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forms no such suitable system is available. In the absence of a preparation containing isolated and similar cells in which the processes of permeability and metabolism may be simultaneously studied, the use of tissue slices appears to serve this purpose. Although removed from the physiological situation where extrinsic regulatory mechanisms continuously affect their functioning, the cells of the tissue slice do preserve their neighboring structural associations and evidently their fundamental metabolism. There are other factors which limit the tissue slice technique. The cortical slice lacks the homogeneity of a suspension of red blood cells or of the single axon. Within the cortex there are various types of nerve cells, the processes of these cells and glial cells. Moreover, an interstitial space through which substances must diffuse to reach the parenchymal cell is included within the system. Notwithstanding these deterrents valuable information regarding permeability may be expected from work with tissue slices.

The investigations presented in this paper are concerned with the permeability of tissue slices of rabbit cortex to the amino acids glycine and aspartic acid.

METHODS AND MATERIALS

Adult rabbits weighing 2 to 3 kg were decapitated quickly. The brain was divested of its leptomeningeal coverings and removed within three minutes. The specimen was submerged in an oxygenated solution of the following composition: 110 parts 0.9% NaCl; 4 parts 1.15% KCl; 3 parts 1.22% $CaCl_2$; 1 part 2.11% KH_2PO_3; 1 part 3.82% $MgSO_4$ H_2O; 3 parts 1.3% $NaHCO_3$; 3 parts 12% Na_2HPO_4 buffer; 5 parts 5.4% glucose at 23° C[9]. After cutting the brain into convenient portions the lateral aspects of the cerebral cortex were sectioned with the Stadie-Riggs instrument into slices 0.450 mm thick. Only a single slice was made through any area of the cortex so as to avoid incorporating underlying white matter. These cortical slices were pooled and kept at room temperature 20 minutes prior to the experiments to permit equilibration with the medium[10].

1. *Measurement of the interstitial space of the tissue slice*

Both the total volume of the interstitial space and the rate of diffusion of inulin out of the space have been evaluated by the following method.

A tissue slice was immersed for forty minutes in KREBS' medium to which inulin had been added. The time of immersion was found to exceed that required to saturate the interstitial space. The slice was then removed, quickly washed and placed in a known volume of inulin-free KREBS solution. At suitable intervals a small quantity of the bath fluid was pipetted for determination of inulin*,[11]. On termination of the experiment the slice was blotted on a hard filter paper and weighed.

The ratio of interstitial space to total volume has been calculated as follows:

$$I_T \cdot \frac{1000}{W} = I_S$$

$$\frac{I_S}{I_O} \cdot 100 = \% \text{ interstitial space}$$

I_T = inulin contained in slice as determined by the amount diffusing out into inulin-free KREBS solution of known volume
W = wet weight of slice in mg
I_S = concentration of inulin mg/g of tissue
I_O = concentration of inulin in solution with which slice first equilibrated.

In this manner the time required for the inulin of the interstitial space to equilibrate with the inulin-free outer solution was determined. As a consequence the rate of diffusion of inulin out of the slice and the capacity of the intercellular compartment were obtained. The data concerning the rate of diffusion out of the tissue slice apply equally to the rate of the reverse process[12].

2. *Experiments with ^{15}N labeled glycine and aspartic acid*

Tissue slices previously equilibrated at room temperature in oxygenated Krebs solution were

* We are greatly obliged to Dr STANLEY BRADLEY for the determinations of inulin and the demonstration of the method employed.

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placed in 2.5 to 4.0 ml of a similar solution in which ^{15}N labeled glycine or aspartic acid had been added. Isotonicity was preserved by replacing part of the NaCl with an osmotically equivalent amount of the amino acid added. The final concentration of the amino acid was in each instance 0.03 *M*. The p_H of the medium was 7.2–7.4. Aside from the experiments designed to determine the temperature coefficient, the solutions were maintained at 37° C. Depending on the nature of the study, the individual tissue slices were placed either in WARBURG vessles or in stoppered test tubes and gently agitated. At appropriate times, the tissue was removed from the test mixtures and twice washed in isotonic sodium chloride for 3 to 5 seconds. The excess fluid was wiped off by applying the slice to a smooth absorbent paper. The tissue was weighed and instilled in a KJELDAHL flask to be digested at a later time for the determination of ^{15}N*.

Experiments to ascertain the temperature coefficient were run at 37° and 23° C.

3. *Experiments with* HCN

Tissue slices were placed in solutions of 0.02 *M* HCN in KREBS' medium at 37° C for 30 minutes and then inserted into the amino acid solutions. All solutions were adjusted to isotonicity in the usual manner. The controls were treated similarly. The remainder of the experiment was carried out as above. The degree of inhibition of respiration of the tissue slice was determined by the method of ROBBIE[13].

4. *Experiments with* DFP *and eserine*

Tissue slices were exposed to diisopropyl fluorophosphate (DFP) (4 mg/ml) and eserine (4–6 mg/ml) in KREBS solution adjusted to isotonicity, at 37° C for 30 minutes. They were then inserted into the amino acid solutions and subjected to the routine procedure. In each case, as for every experiment, corresponding controls were made.

5. *Experiments with* ^{15}N *labeled urea*

These experiments were performed in a similar manner except that 2 mg/ml of ^{15}N labeled urea replaced the amino acids in the test solutions. In this case there was no replacement of NaCl in the KREBS solution.

In a third of the experiments the total nitrogen of each slice was determined. The specimen was then recollected and the ^{15}N content subsequently determined. Since there was agreement between the calculated and the experimentally found total nitrogen, it was thought unnecessary to perform total nitrogen determinations on each tissue slice. Before equilibration in KREBS medium the N content of the tissue slice was 1.35–1.45% wet weight. After 20 minutes of equilibration the N content decreased to 0.85–1.0% wet weight because of imbibition of water by the slice.

The figures are constructed from representative experiments chosen from the general data without preference except for the following. Most of these experiments were selected because they extended 60 minutes and so the full curve of the rate of penetration could be analyzed and unusual deviations indicating changes in the slice readily detected.

RESULTS

I. Interstitital space

The cortical tissue slice consists of neurons, the supporting glial net, blood vessels and juxtagriseal white matter that inadvertently may be incorporated into the section. The entire mass is interlaced by vascular, perivascular and pericellular channels which cumulatively represent the intercellular or interstitial space. Tissue slices of the cerebral cortex of rabbit contain an interstitial compartment varying between 19 and 34% of the total volume, as determined from slices from various areas of the cortices of different rabbits. The average value was 30%. The time required to equilibrate the intercellular space with the outer milieu in the first instance is about 7 minutes, and in the second, 11 minutes. The total time required for filling of the interstitial space depends on the usual factors involved in the process of diffusion and so varied from section to section according to the capacity of the space. The rate of filling the interstitial spaces by inulin

* We are most grateful to Dr DAVID RITTENBERG AND Mr I. SUTURE for the mass-spectrographic analysis of ^{15}N.

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is, however, independent of the capacity, as may be seen from Fig. 1. It must be expected that under the experimental conditions the time for diffusion of any substance from the exterior to the interior of the cell will include that period during which the interstitial compartment is saturated.

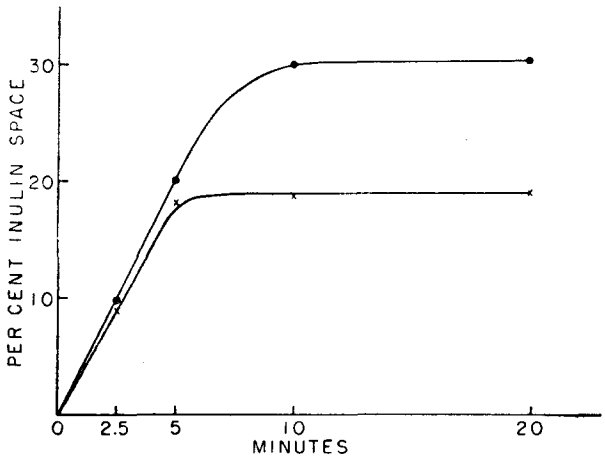

Fig. 1. The rate of penetration of inulin into cortical slices of rabbit brain indicating the percentage of the intercellular space. The two curves represent the extreme values between which the percentage varied

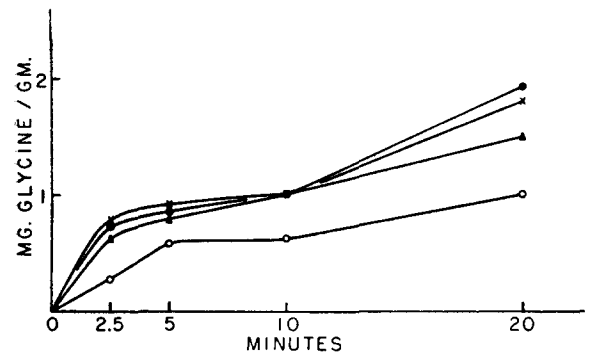

Fig. 2. The rate of penetration of ^{15}N labeled glycine into cortical slices of rabbit brain. Outside concentration of glycine was 2.25 mg/ml. The upper three curves represent experiments at 37° C, the lower at 23° C. Although the rates of inter- and intracellular penetration are overlapping, the distribution is apparent

II. The diffusion of glycine and aspartic acid

The diffusion of the amino acids under study into the interstitial space appears to occupy the first 2.5 to 5 minutes following exposure. As seen in Fig. 2, glycine at 37° C diffuses in this interval rather rapidly. During the time, 5 to 10 minutes after exposure there is a persistant though less rapid increase in the amount of substance which diffuses into the tissue slice. It is probable that the rate of this first period is determined mainly by the entry of the amino acid into the interstitial space. As the concentration within this compartment increases, an appreciable amount of glycine begins to pass intracellularly. This is suggested by the increase of rate after about 10 minutes exposure. After 20 minutes the rate decreases considerably and equilibrium between the outer fluid and the slice is attained one hour after the beginning of the experiment.

The maximum concentration of amino acid in the interstitial space may be calculated, assuming the average value of the space to be 30%. In Table I*a* the interstitial content of glycine at various periods of the first 20 min of exposure to the amino acid is expressed as a percentage of the amount determined experimentally for the entire slice. It is seen that at the end of 5 min 80% of the total glycine is in the intercellular space. At 10 minutes, and more strikingly at 20 minutes, the preponderant intracellular location of glycine becomes evident. These data appear to conform with the implications drawn from the curves of the rate of penetration of glycine into the tissue slice. They also indicate that although the rate-governing process of the first 5 minutes is diffusion

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into the free space, a small fraction of the glycine (10 to 20%) has entered the cell.

TABLE I

THE PENETRATION OF GLYCINE AND UREA INTO THE CORTICAL SLICE AS A FUNCTION OF TIME

The amount of compound found (column 3) is compared with the maximum amount of compound calculated to be present interstitially assuming this space represents 30% of the total volume of the slice. Column 5 is the per cent of compound within the slice which is intercellular.

Time min	Tissue weight mg	Total found mg/g	Interstitial calc. mg/g	Interstitial per cent of total
		a. Glycine		
2.5	52	0.735	0.675	92
5.0	47	0.850	0.670	79
10.0	58	1.03	0.670	65
20.0	35	1.94	0.640	33
		b. Urea		
2.5	109	1.04	0.59	57
5.0	54	1.18	0.60	50
10.0	70	1.56	0.60	38
20.0	55	1.66	0.58	35
40.0	122	1.74	0.60	34
90.0	158	1.92	0.60	31

The results for the diffusion and penetration of aspartic acid into tissue slices are similar to those found with glycine (Fig. 3). However, in the case of aspartic acid equilibrium between the external fluid and the tissue slice was achieved only in a few instances and required 90 minutes. A comparison of the average obtained in eight experiments demonstrates the similarity in the form of the diffusion processes for glycine and aspartic acid into cortical sections (Fig. 4).

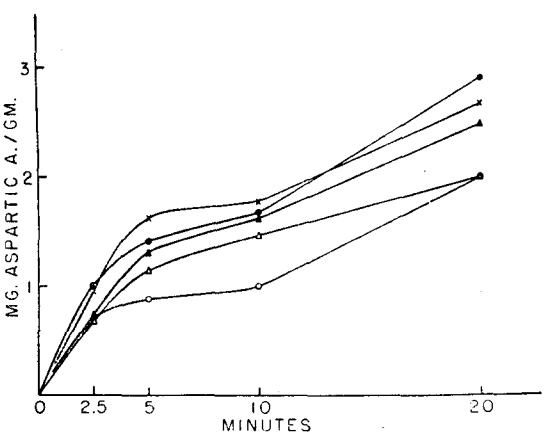

Fig. 3. The rate of penetration of ^{15}N labeled aspartic acid. Outside concentration of aspartic acid was 4 mg/ml

III. Effect of Temperature

In spite of the apparent parallelism there are evidently different mechanisms involved in the passage of the two amino acids into the cortical cells. The first 10 minutes during which the tissue slices are exposed to the amino acids are occupied mainly by their diffusion into the free space, whereas the subsequent period is concerned with penetration of these substances into the cells. Fig. 5 shows that the temperature coefficient, Q_{10}, of glycine measured from 10 to 40 minutes is of the order of 1.0, indicating that its diffusion into the cells is not

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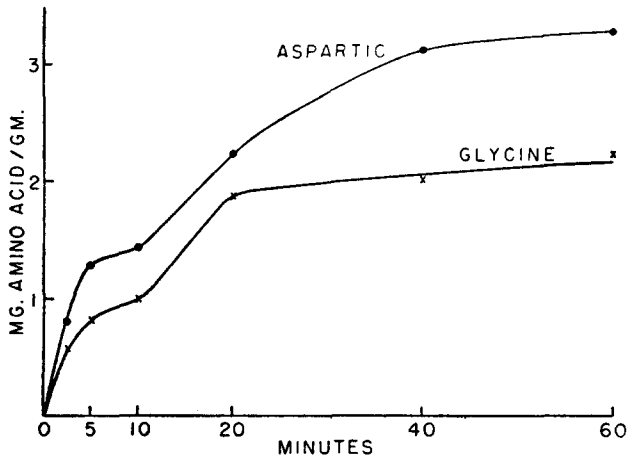

Fig. 4. Comparison of the penetration rates of glycine and aspartic acid into cortical slices. Average of 8 experiments. The shape of the curves is similar. In the slices exposed to glycine equilibrium was reached in about 40 min, whereas with aspartic acid it was not attained within 60 min

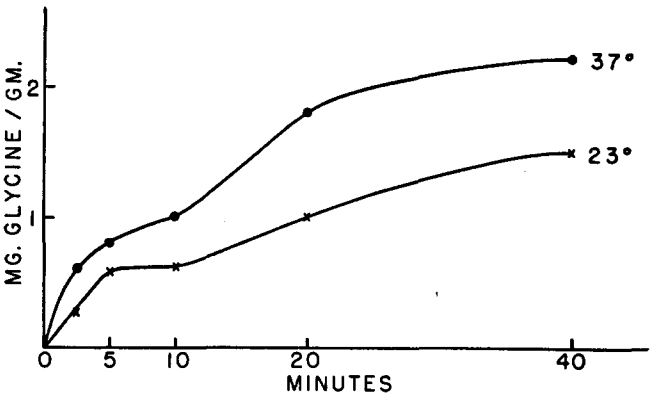

Fig. 5. The rate of penetration of glycine as a function of temperature. Q_{10} measured between 10 and 40 min is about 1.0. Average of 3 experiments

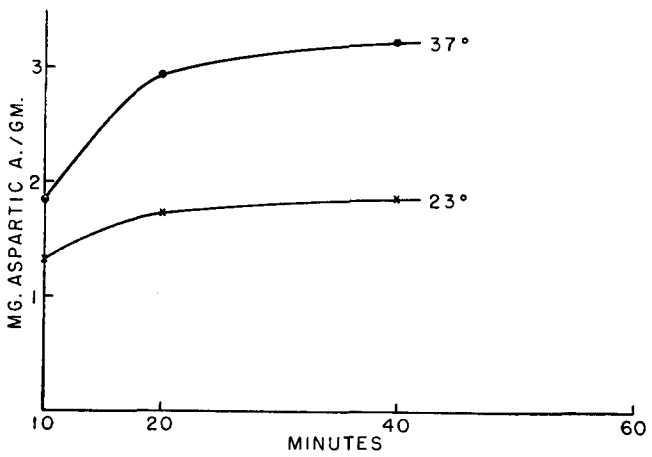

Fig. 6. The rate of intracellular penetration of aspartic acid as a function of temperature. Q_{10} measured between 10 and 40 min is about 2.4. Average of 2 experiments

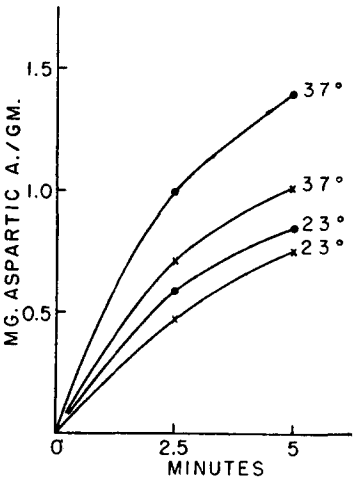

Fig. 7. The rate of penetration of aspartic acid into the intercellular space as a function of temperature. Q_{10} is close to 1.0

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associated with reactions having a high heat of activation. In contrast, the comparable temperature coefficient for the penetration of aspartic acid is about 2.4, suggesting that its entry into the neuron is linked to an active process. Fig. 6 shows the rate of intracellular penetration of aspartic acid as a function of temperature. In Fig. 7, the temperature coefficient of diffusion of aspartic acid chiefly into the interstitial compartment is seen to be 1.0. This supports the belief that the experimentally determined distinction between the first interval of ten minutes and the following period are representative of different phases of the total diffusion. In the case of aspartic acid it also points to the separation of these two phases.

IV. Rôle of Respiration

The rôle of respiration in the process studied was tested by the use of HCN. The amount applied was sufficient to suppress respiration of the tissue slices for the duration of the experimental period, as was determined manometrically. The inhibition of respiration has a distinctly different effect upon the penetration of the two amino acids tested. Inward diffusion of glycine was little or not at all affected, whereas aspartic acid penetration was decreased 25% (Figs 8 and 9).

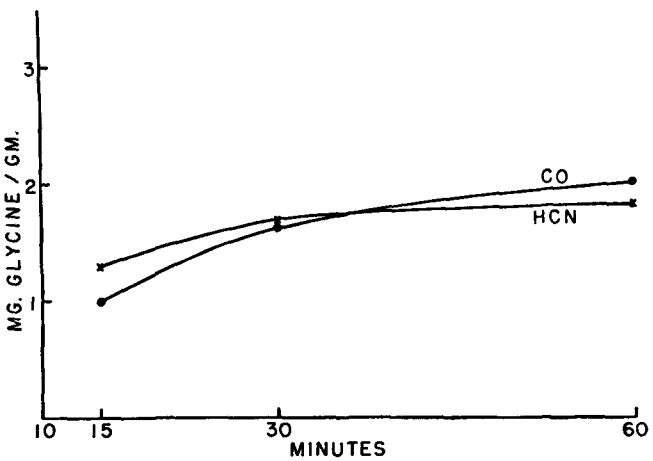

Fig. 8. The effect of inhibition of respiration by HCN (0.02 *M* final concentration) upon the rate of penetration of glycine. *CO* = controls; t = 37°. Average of 3 experiments

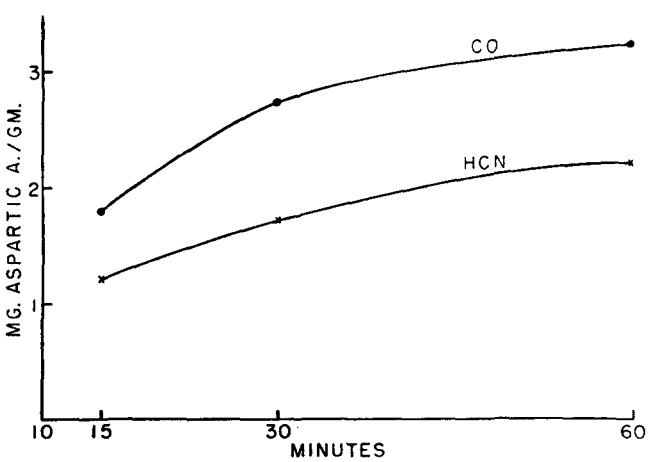

Fig. 9. The effect of inhibition of respiration by HCN (0.02 *M* final concentration) upon the rate of penetration of aspartic acid. *CO* = controls; t = 37°. Average of 3 experiments

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V. Effect of inhibitors of acetylcholinesterase

No significant effects of eserine and DFP on the neuronal permeability to glycine and aspartic acid were observed. As indicated in Figs 10 and 11, there was perhaps a slight tendency to lowered final concentration of these amino acids intracortically. The rates of penetration, however, are not significantly different from the control values (Figs 10 and 11).

The extent of the effect of the inhibitors DFP and eserine on the acetylcholinesterase activity of the tissue slice cannot be determined accurately. Previous experiments indicate that at the times when the function of conduction of intact nerves exposed to DFP is abolished only 0.1% of the concentration of outside DFP has entered the cellular interior[14]. If the tissue is them ground and the structural barriers destroyed, the inhibitor can more easily combine with the now directly accessible enzyme. For this reason the values of acetylcholinesterase thus determined do not reflect the enzymatic activity in the intact slice. The situation is essentially the same in the case of eserine. However, from previous experience in this Laboratory the amounts of inhibitors employed and the duration of exposure of the cells to them justify the conclusion that the acetylcholinesterase is virtually inactivated[15, 16].

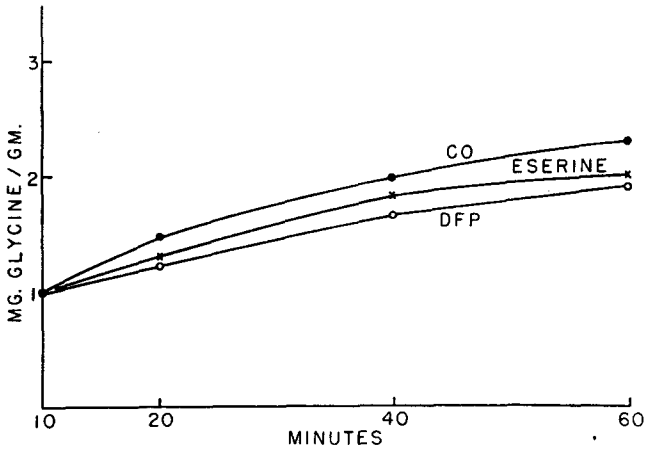

Fig. 10. Effect of inhibitors of acetylcholinesterase upon the rate of penetration of glycine. Final concentration of eserine 6 mg/ml, of DFP 4 mg/ml

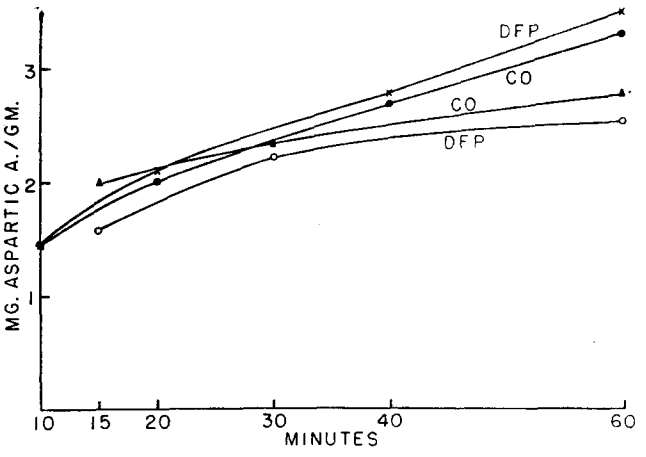

Fig. 11. Effect of DFP (4 mg/ml) on the rate of penetration of aspartic acid

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VI. Penetration of urea

Urea has long been thought to diffuse freely through tissue cells. There is evidence that this is not a general condition prevailing in all biological material[17]. In the cortex there is a rapid saturation of the interstitial space and marked penetration into the cells within 2.5 minutes (Table I*b*). The diffusion process proceeds at a diminished rate from 2.5 to 10 minutes. It then appears to very gradually approach a steady state which is reached only after 90 minutes (Fig. 12).

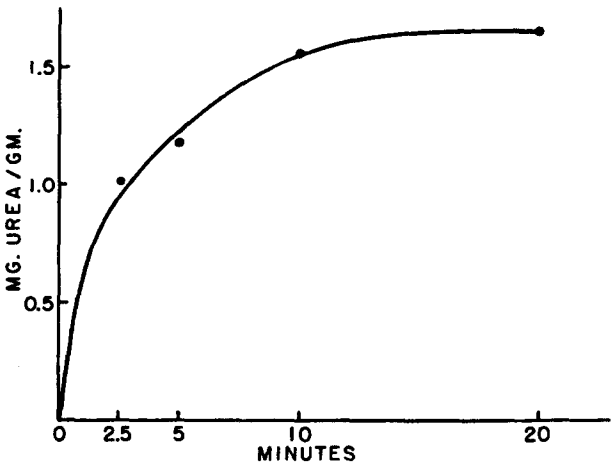

Fig. 12. The rate of penetration of urea into cortical slices. The outside concentration of urea was 2 mg/gm. No clear distinction is apparent between intra- and intercellular penetration. Average of 2 experiments

DISCUSSION

The data presented require several comments. The preservation of the cortical slice during the course of the experiment depends on environmental factors in addition to oxygenation, isotonicity and appropriate salts in the medium. The factors determining decay of its functional efficiency are not well known to us. The experience from the work described, as well as that of others, suggests that optimal conditions within the slice at 37° C prevail during the first hour[18]. While most of the experiments performed were within this temporal limit, a few exceeded it. The alterations in the usual shape of the curves indicating the penetration of substances into the tissue reveals the presence of apparently unexplained conditions. In some instances, for example, the amino acid diffused into the slice steadily. However after a certain time a plateau of its concentration in the tissue was reached, although equilibrium with the external fluid was not yet attained. This discrepancy was greater than the possible experimental error. The nature of this retardation of further penetration remains unexplained. An additional factor normally influencing permeability, and which may or may not be present in the cells of the cortical slice, is believed to be the rapid and frequent fluctuations in the state of the active membranes of the neuron.

While it is possible to investigate systematically the size of the intercellular compartment in relation to cortical region and cerebral weight, the experiments were not designed for this purpose. Rather, average values were assumed to operate. As noted

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in Figs 2 and 3, the concentration of amino acids attained within the slice after 5 minutes exposure is somewhat variable because it depends on the volume of the interstitial space. The penetration into the intracellular space during that time is quite limited.

It is possible to approximate the diffusion coefficients of glycine and aspartic acid if one considers the passage of these substances into the interstitial space is a consequence of and in accord with the process of simple diffusion in which the boundary conditions are identical[12]. For these conditions the following equation applies:

$$Q_{o,t} = \mu_o AH\left[\left(1 - \frac{8}{\pi^2}\right)\left(e^{-\frac{\pi^2 Dt}{H^2}} + \frac{1}{9}e^{-\frac{9\pi^2 Dt}{H^2}} + \ldots\right)\right]$$

Q = amount of amino acid in M/ml within slice
μ = concentration in M/ml of external fluid
AH = volume of slice: A = area; H = thickness
D = diffusion constant
t = time in seconds

Using the first member of the series an adequate approximation may be made by dropping all the terms other than the first. This yields:

$$[Q - (\mu AH)] = -\frac{8\mu AH}{\pi^2}\left(e^{-\frac{\pi^2 Dt}{H^2}}\right)$$

$$\ln[Q - (\mu AH)] = -\ln\frac{8\mu AH}{\pi^2} - \frac{\pi^2 Dt}{H^2}$$

By plotting $\ln[Q - (\mu AH)]$ against t the slope obtained may be equated to $\frac{\pi^2 D}{H^2}$. If one substitutes the diffusion constants of the amino acids in this equation the calculated thickness of the tissue slice is found to be about 5 times the experimentally determined thickness. The interstitial path for diffusion is therefore considerably greater than the measured thickness of the slice. However, if one assumes that the route of diffusion is not so markedly increased beyond the actual thickness of the slice, apparent diffusion constants tor the amino acids under these conditions can be calculated. They are of the order of $^1/_8$ to $^1/_4$ of those referred to standard conditions[19, 20, 21]. This may indicate that under the conditions of the intercellular milieu diffusion proceeds at a slower rate. It is interesting to note that HARRIS AND BURN, determining the diffusion constant of Na^+ from its rate of penetration into the interstitial space of muscle, reported a value of $^1/_4$ that seen in free solution diffusion of Na^+[22]. The rate of diffusion tends to be less in biological than in aqueous solutions. The magnitude ot this change is difficult to state since physical experiments have not been done under conditions comparable to those of biological studies.

Once the intercellular compartment has been saturated, the intracellular penetration of the amino acids depends on the particular mechanisms utilized for transport across the cell boundary and the connection of the amino acids with the metabolism of the cell. The entry ot a substance into the cell, although contingent on the general factors that influence diffusion, may be associated in many cases with specific cellular activities[18, 23, 24]. Indirectly this is borne out by the effect of temperature on the penetration of aspartic acid. The temperature coefficient for aspartic penetration is 2.4. There is a significant

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effect of temperature in accelerating the cellular uptake of aspartic acid beyond that consistent with the effect of temperature on a simple diffusion process (Figs 6 and 7). In the case of aspartic acid one may suppose its intracellular penetration is coupled to an active process, one with a high energy of activation.

A further support for the association of the penetration of aspartic acid with an active process is the markedly reduced rate of penetration in the absence of measurable tissue respiration. There is evidence that the penetration of aspartic acid into bacteria likewise depends on an active process[25]. In contrast, under similar conditions glycine diffuses into the cortical cells without significant alteration. The results of these experiments furnish no indication that glycine is conveyed intracellularly by a mechanism other than diffusion. However, in other tissues, *e.g.*, kidney, the competitive effect of compounds such as creatine in decreasing the reabsorption of glycine from the renal tubules makes possible the assumption that its penetration into the cell likewise depends on a chain of chemical events[24].

Cyanide does not affect permeability directly but most likely by interference with special chemical reactions requiring oxidative energy. It is possible that these specific chemical reactions vary from one group of substances to another. Moreover, energy-yielding reactions do not appear necessary to maintain the permeability of the membrane to all substances. In these experiments the interference with respiration of the tissue affects those mechanisms connected with the uptake of aspartic acid and not of glycine, and so distinguishes between these processes.

Eserine and DFP, applied in concentrations and for sufficient time to inhibit more than 90% of the acetylcholinesterase of the tissue slice, appear to have little or no influence on the permeability of the cell to the amino acids. It is believed that the acetylcholinesterase-acetylcholine system may be associated with permeability characteristics of the active neuronal membrane[26]. The slow inward diffusion of the amino acids into neurons may depend on the presence of another membrane surrounding the active membrane. However, it is possible that the permeability changes of the active membrane do not affect the amino acids. The diffusion of amino acids into the cells may rather serve a nutritional purpose. Adequate amounts of amino acids may not diffuse from the cell soma to its extension but rather enter along the course of these cellular processes, the necessary transport system existing in their axonal membranes.

Urea, which appears to be freely diffusible, has been used for comparison. However, even in this case it is uncertain whether chemical reactions interfere with its free penetration.

A comparison of the data for the rates of penetration of glycine and aspartic acid between the giant axon of the squid and rabbit cortex is of interest. In thirty minutes at 23° C 16% of the external concentration of glycine and 5% of aspartic acid are found within the axoplasm[8]. At corresponding temperatures and concentrations relative to total molarity of the exterior fluid, 40% of glycine and 13% of aspartic acid were intracellular. It is interesting that the ratio of glycine/aspartic acid is in both cases about 3:1, although the absolute amounts found are much greater in the parenchymal cell than in the axonal process. The results raise the general problem whether the penetration into cell bodies occur at a higher rate than into the axons.

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ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr DAVID NACHMANSOHN for his encouraging guidance and stimulating advice.

SUMMARY

Cortical tissue slices of rabbit were exposed to ^{15}N labeled glycine, aspartic acid, and urea. The rates of penetration of these substances into the interstitial compartment and the neuronal cells were determined. Factors, such as temperature, respiratory inhibition, and acetylcholinesterase inactivation were studied with regard to their effect on the permeability of the tissue cells to the amino acids.

The following results were obtained:

1. The capacity of the interstitial space as determined by the inulin method was found to vary between 19 and 34% and to average 30% of the total volume.

2. The Q_{10} of the passage of glycine into the cells was close to 1.0; in contrast, that of aspartic acid about 2.4.

3. Respiratory inhibition by cyanide diminished the rate of entry of aspartic acid into the cells; in contrast, that of glycine was unaffected.

4. Inhibitors of acetylcholinesterase did not exert a significant effect on the cellular permeability to amino acids.

RÉSUMÉ

Nous avons exposé des tranches de tissu cortical à de la glycine, de l'acide aspartique et de l'urée marqués à ^{15}N. Les vitesses de pénétration de ces substances dans le compartiment interstitiel et dans les neurones ont été déterminées. Des facteurs, tels que la température, l'inhibition respiratoire et l'inactivation par l'acétylcholinestérase ont été étudiés en ce qui concerne leur action sur la permiabilité des cellules tissulaires aux acides aminés.

Nous avons obtenu les résultats suivants:

1. La capacité de l'espace interstitiel déterminée par la méthode à l'inuline varie de 19 à 34% (moyenne 30%) du volume total.

2. Le Q_{10} du passage de la glycine dans les cellules était près de 1.0; par contre, celui de l'acide aspartique était environ 2.4.

3. L'inhibition respiratoire par le cyanure diminue la vitesse de pénétration de l'acide aspartique dans les cellules; par contre, celle de la glycine n'est pas affectée.

4. Les inhibiteurs de l'acétylcholinestérase n'exercaient pas d'effet significatif sur la perméabilité cellulaire aux acides aminés.

ZUSAMMENFASSUNG

Scheiben von Cortexgewebe vom Kaninchen wurden der Einwirkung von mit ^{15}N markierten Glycin, Asparaginsäure und Harnstoff ausgesetzt. Die Penetrationsgeschwindigkeiten dieser Substanzen in den interstitiellen Raum und in die Neuronen wurden bestimmt. Der Einfluss von Faktoren wie Temperatur, Atmungshemmung und Acetylcholinesterase-Inaktivierung auf die Permiabilität der Gewebezellen für Aminosäuren wurden untersucht.

Die folgenden Ergebnisse wurden erhalten:

1. Die Kapazität des interstitiellen Raumes, mit Hilfe der Inulinmethode bestimmt, varierte zwischen 19 und 34% (Durchschnitt 30%) des Gesamtvolumens.

2. Der Q_{10} der Penetration von Glycin in die Zellen war nahezu 1.0; dagegen war er bei Asparaginsäure ungefähr 2.4.

3. Die Atmungshemmung durch Cyanid setzte die Penetrationsgeschwindigkeit der Asparaginsäure in die Zellen herab; dagegen war diejenige von Glycin unverändert.

4. Hemmstoffe der Acetylcholinesterase übten keinen bedeutenden Einfluss auf die Permeabilität der Zellen für Aminosäuren aus.

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Received February 15th, 1951