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## I. Introduction

Our understanding of the biochemical and physiological effects of drugs within the central nervous system (CNS) has lagged behind that of drug actions in peripheral nervous structures. This situation derives as much from the prodigious heterogeneity of CNS tissues as from their physical and chemical inaccessibility. Among the approaches employed to circumvent the inherent difficulties of studying CNS function *in vivo* is the use of the brain slice. The rapidly expanding application of this preparation to investigations of drug actions on substances which may serve as mediators of central synaptic transmission is the subject of this review.

While the brain slice presents such problems as the presence of damaged cells [estimated by Hill (1932) to involve only about 0.2% of the total cell surface], lack of circulating fluid, loss of normal innervation and heterogeneity of cellular constitutents, the preparation affords a unique opportunity to study a complex tissue in isolation and in a precisely controlled environment (cf. McIlwain, 1966). When incubated in a suitable medium, brain slices

retain many of the *in vivo* attributes of cerebral tissue for several hours. These include (1) respiration and glycolysis (McIlwain, 1966); (2) uptake and metabolism of a broad range of compounds; (3) recovery and maintenance of normal intra- and extracellular ionic concentration gradients (McIlwain, 1966); and (4) maintenance of resting membrane potentials which may be reversibly displaced by appropriate electrical stimulation (Hillman *et al.*, 1963; Richards and McIlwain, 1967). Studies employing brain slices have specific advantages over *in vivo* investigations of the metabolism of compounds believed to mediate central neurotransmission. Not only is the problem of penetration of these substances across the blood-brain barrier (Weil-Malherbe *et al.*, 1961; Bertler *et al.*, 1966) circumvented, but quantitative estimates of catabolite formation become feasible. Such estimates cannot be carried out *in vivo* because these compounds pass from the CNS into the general circulation at differing rates (Glowinski and Baldessarini, 1966; Werdinius, 1967).

For more than a century (cf. Bernard, 1856), it has been recognized that synaptic transmission is peculiarly sensitive to modification by drugs, possibly owing to the fact that the process is mediated by specific chemical substances. The influence of pharmacological agents on compounds presumed to play a role in central neurotransmission may be studied in brain slices at one or more of the following stages (cf. Bloom and Giarman, 1968; Glowinski and Baldessarini, 1966; Carlsson, 1965, 1966): (1) precursor uptake, (2) sequential biosynthesis of the substance, (3) storage of the active compound, (4) release of the substance upon neuronal depolarization, and (5) termination of action of the substance by reuptake into the presynaptic neuron or by enzymatic degradation. The literature pertaining to the use of brain slices for studies of putative neurotransmitters will be discussed in relation to these stages. Although a number of compounds have been implicated in the mediation of neurotransmission within the CNS, this review will be limited to a consideration of the biogenic amines (norepinephrine, dopamine, and serotonin), certain amino acids (especially $\gamma$-aminobutyric acid), and acetylcholine.

## II. Uptake and Storage

The availability of radioactively labeled acetylcholine (ACh), biogenic amines, and amino acids (AA) of high specific activity has facilitated *in vitro* studies of the accumulation of these pharmacologically active substances by cerebral tissue. Although simple diffusion dominates the uptake process when the substances are added in high concentrations to the incubation medium, active mechanisms may be demonstrated when lesser concentrations are employed. Under the latter circumstances a portion of the accumulation

appears to occur in nerve terminal structures normally containing the endogenous material. The use of brain slices has enabled studies of the ionic requirements for the uptake of putative neurotransmitters into CNS tissue and provided insight into drug effects on reuptake mechanisms which may play a role in terminating the postsynaptic action of neurotransmitters released into the synaptic cleft.

### A. Catecholamine Uptake

The ability of mammalian cerebral slices to actively accumulate norepinephrine (NE) from a suitable incubation medium is now well established (Dengler *et al.*, 1961, 1962; Dengler, 1965; Hamberger and Masuoka, 1965; Haggendal and Hamberger, 1967; Snyder *et al.*, 1968; Ross and Renyi, 1966a; Jonason and Rutledge, 1968a). It would appear that the exogenous amine is largely taken up by noradrenergic terminals within CNS tissues. Dengler *et al.* (1962) have shown that slices of cat brain, heart, and spleen, organs which are rich in adrenergic nerve endings, attain a 4:1 tissue-to-medium ratio of NE-$^3$H, whereas slices prepared from adrenergic-poor organs such as liver, kidney, or skeletal muscle fail to achieve concentrations of labeled NE greater than those in the medium. Hamberger and Masuoka (1965) and Hamberger (1967) have applied the fluorescent histochemical technique of Falck (1962) to study the uptake of the monoamine into rat cerebral cortex slices. They observed that NE was largely confined to varicose axons, indistinguishable from those which contain endogenous NE, as well as to nonvaricose, more proximal portions of the axon. Lenn (1967) using electron microscopic radioautography to localize NE-$^3$H accumulated by brain slices, concluded that most of the tritium was taken up in relation to small unmyelinated axons (40%), nerve endings (30%), and axons (10%). The radioautographic study of Ishii and Friede (1968) performed in human cerebral autopsy specimens, which showed strong binding of NE-$^3$H to membrane sites known to contain high endogenous NE levels, further supports the contention that exogenous NE is accumulated at central adrenergic terminals. Considerably more binding of NE was found at the surface membrane of pigmented neurons in substantia nigra, nucleus coeruleus, and nucleus dorsalis vagi than at various nonpigmented nuclei. Some accumulation may, however, occur in nonadrenergic structures inasmuch as the amount of labeled NE taken into slices from various brain regions (Snyder *et al.*, 1968; Iversen and Snyder, 1968) does not correspond precisely to endogenous NE levels or to NE accumulations after intraventricular injection of the labeled amine (Glowinski and Iversen, 1966).

Although the analysis of NE uptake into brain slices is complicated by ongoing metabolism and varying rates of efflux of the accumulated catechol-

amine, two components may be identified: an active process which approximates Michaelis-Menten kinetics and simple diffusion (Dengler *et al.*, 1961, 1962; Titus and Dengler, 1966; Ross and Renyi, 1966b; Snyder *et al.*, 1968; Iversen and Snyder, 1968; Haggendal and Hamberger, 1967; Jonason and Rutledge, 1968a). Dengler *et al.* (1962) observed that the accumulation of NE exhibited the characteristics of active uptake at amine concentrations in the incubation medium of about $10^{-7}$ $M$, but that simple diffusion predominated at higher concentrations. The view that the accumulation of NE by brain slices occurs by an active process derives from the observations that uptake is temperature dependent, requires oxygen and glucose, is retarded by inhibitors of cell metabolism, occurs against a concentration gradient, and depends on sodium in the external medium (Dengler *et al.*, 1961, 1962; Dengler, 1965; Ross and Renyi, 1964, 1966a). Omission of calcium or magnesium ions from the incubation medium has little effect on NE uptake in brain slices (Hamberger, 1967).

Drugs which inhibit the uptake and storage of labeled NE in the brain of the living animal usually have a similar effect on the accumulation of NE by CNS tissues *in vitro* (Dengler *et al.*, 1961, 1962; Dengler, 1965; Hamberger and Masuoka, 1965; Hamberger, 1967; Ross and Renyi, 1964, 1966a). These drugs include ouabain, cocaine, phenoxybenzamine, chlorpromazine, imipramine, desmethylimipramine, reserpine, amphetamine, and related sympathomimetic amines. Most of these agents are effective at concentrations of the order of $10^{-4}$ $M$. Lysergic acid diethylamide and haloperidol are without effect at this concentration. Drugs such as cocaine, and desmethylimipramine, which are thought to impair NE uptake by inhibiting the cell membrane pump, exert a greater effect on uptake when a short incubation period is used (Ross and Renyi, 1966a). This observation suggests that uptake by the neuronal membrane is the initial dominant factor at a time when storage mechanisms play a minor role. Prolongation of incubation time leads to an increasing effect of reserpine, presumably due to a time-dependent increase in the importance of storage mechanisms. Although it cannot be excluded that binding of amines to intraneuronal storage sites may have some significance for the initial uptake of amines by brain slices, it seems probable that the results of kinetic analysis of cocaine sensitive uptake (Ross and Renyi, 1966a; Dengler *et al.*, 1962; Snyder *et al.*, 1968) reflect mainly the cell membrane pump.

The uptake of the NE precursors tyrosine, dopa, and dopamine (DA) has been described (Neame, 1961a; Guroff *et al.*, 1961; Dengler *et al*, 1962; Ross and Renyi, 1966b; Yoshida *et al.*, 1963a,b, 1965). The active accumulation of DA into brain slices resembles that of NE (Ross and Renyi, 1964, 1966a), although differences have been described regarding regional variations in uptake as well as in the catabolism of the two catecholamines (Jonason and

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Rutledge, 1968a). Guroff *et al.* (1961) have shown that rat brain slices actively concentrate tyrosine from the suspending medium and can attain intracellular concentrations more than four times that of the medium. Tyramine and α-methyl-*m*-tyrosine are also concentrated, while *p*-hydroxyphenylacetic acid appears to be taken up only passively. The uptake of tyrosine is impaired by a variety of metabolic inhibitors and enhanced by glucose and other hexoses; excess calcium or magnesium increases uptake whereas potassium decreases it. Differences in the accumulation of catecholamine precursors between brain slices and cerebral tissue *in vivo* are suggested by the finding that L-tyrosine enters the brain of the living animal more rapidly than D-tyrosine, while in brain slices D- and L-tyrosine are concentrated at equal rates (Chirigos *et al.*, 1960).

The accumulation of L-dihydroxyphenylalanine (L-dopa) by brain slices by an energy-dependent process has also been reported (Yoshida *et al.*, 1963a,b). As with other active uptake systems, this uptake has been shown to be temperature dependent, to require oxygen and glucose, and to be impaired by metabolic inhibitors. Omission of sodium, potassium, or magnesium retards the accumulation of dopa by brain slices, but calcium omission enhances it. Ouabain and proveratrine (Yoshida *et al.*, 1963a,b) as well as protamine (Yoshida *et al.*, 1965) reduce dopa uptake.

### B. Serotonin Uptake

Studies of the accumulation of serotonin (5-HT) by cerebral slices (Schanberg, 1963; Ross and Renyi, 1967; Blackburn *et al.*, 1967; Pletscher and Bartholini, 1967) illustrate the dependence of uptake mechanisms on the concentration of the exogenous amine. Although Schanberg (1963) and Pletscher and Bartholini (1967) were unable to demonstrate active transport of 5-HT into brain slices, the maximum amount of amine entering the tissue by a carrier mechanism at the high incubation medium concentrations used (above $10^{-7}$ *M*) would be small in comparison to that entering by passive diffusion. It is thus not surprising that at these concentrations, little difference between the uptake of labeled 5-HT into brain slices or slices of kidney, liver, or spleen, could be demonstrated. Blackburn *et al.* (1967), however, observed that brain slices incubated with 5-HT-$^{14}$C at a concentration of $2 \times 10^{-8}$ *M* accumulated the labeled amine by a mechanism showing all the characteristics of active transport. The kinetic constant ($K_m$) estimated by Blackburn *et al.* (1967) for 5-HT uptake by rat brain slices resembles that for 5-HT uptake by platelets (Hughes and Brodie, 1959; Weissbach *et al.*, 1960) and choroid plexus (Tochino and Schanker, 1965) and for NE uptake by brain slices (Dengler *et al.*, 1962; Snyder *et al.*, 1968). Approximately 60% of the 5-HT accumulated in the experiments reported by Blackburn *et al.* (1967)

was recovered in the nerve ending (synaptosomal) fraction and 40% in the supernatant after fractionation of the tissue homogenates on a sucrose density gradient. The subcellular distribution of 5-HT-$^{14}$C taken up into brain slices is thus similar to that of endogenous 5-HT (Schanberg and Giarman, 1962) and NE (Potter and Axelrod, 1963).

A number of drugs have been found to alter the uptake of 5-HT into brain slices. Imipramine, desmethylimipramine, ouabain, cocaine, and chlorpromazine substantially inhibit 5-HT-$^{14}$C uptake at a concentration of $10^{-5}$ *M* (Blackburn *et al.*, 1967; Ross and Renyi, 1967). Reserpine does not appear to influence the transport of 5-HT-$^{14}$C into cerebral slices, but, as with other amines, it does act to reduce their capacity for binding 5-HT (Schanberg, 1963; Ross and Renyi, 1966b, 1967; Blackburn *et al.*, 1967). Iproniazid, which has no effect on 5-HT uptake, inhibits the reserpine-induced depletion of 5-HT-$^{14}$C from brain slices by blocking serotonin degradation (Ross and Renyi, 1967). These findings agree with previous evidence that reserpine produces a depletion of biogenic amines *in vivo* by interfering with vesicular storage rather than by inhibition at the membrane pump (Carlsson, 1965) and that reserpine pretreatment does not alter transport of 5-HT into brain *in vivo* (Palaic *et al.*, 1967).

D,L-Amphetamine, a potent inhibitor of the accumulation of NE by brain slices (Hillarp and Malmfors, 1964; Dengler *et al.*, 1961), exerts a less powerful inhibitory influence on the accumulation of 5-HT (Ross and Renyi, 1967; Schanberg, 1963). The impairment of 5-HT uptake by drugs which also inhibit the uptake of NE suggests a similarity between uptake mechanisms for the two biogenic amines. NE interferes with 5-HT uptake, but inasmuch as the concentration of NE required to produce significant inhibition is approximately 100 times greater than the $K_m$ for NE uptake (Titus and Dengler, 1966; Dengler *et al.*, 1962), it would seem that 5-HT and NE are normally accumulated at different sites. This is in contrast to results of experiments performed with rabbit choroid plexus, where there appears to be a common mechanism for the active accumulation of relatively large amounts of NE and 5-HT (Tochino and Schanker, 1965).

The uptake of 5-HT-$^{3}$H into brain slices is also inhibited by several tryptamine derivatives (Ross and Renyi, 1967). One of the most potent of these compounds is bufotenine which is effective at concentrations as low as $3 \times 10^{-6}$ *M*. *N*,*N*-Dimethyltryptamine is somewhat less active, while lysergic acid diethylamide, when given intraperitoneally one hour prior to the experiment, had a negligible effect. In the studies of Blackburn *et al.* (1967) uptake of 5-HT-$^{14}$C was inhibited by tryptamine ($10^{-5}$ *M*); dopamine was a less effective inhibitor and L-NE or D,L-5-HTP (both at $10^{-4}$ *M*) had relatively little effect.

Facilitated transport of 5-hydroxytryptophan (5-HTP), the immediate

precursor of serotonin, has been demonstrated in cerebral slices from several species (Wartburg, 1962; Schanberg, 1963; Smith, 1963). The accumulation of 5-HTP does not appear to be associated with the activity of 5-HTP decarboxylase, since α-methyldopa, a potent inhibitor of this enzyme fails to alter the uptake of 5-HTP (Schanberg, 1963). That the mechanism of uptake into slices and the decarboxylating enzyme are not linked is further suggested by the observation of Smith (1963) that under optimal conditions *in vitro*, brain homogenates will decarboxylate about 20 times more 5-HTP than is decarboxylated by brain slices. Only a small portion of the 5-HTP-$^{14}$C accumulated by rat brain slices is decarboxylated to the amine (Smith, 1963).

The uptake of 5-HTP into slices of cerebral tissue is retarded by the naturally occurring neutral amino acids L-tryptophan (although not by its D- isomer), L-phenylalanine, L-tryrosine, L-dopa, L-leucine, L-isoleucine, and L-proline when added to the incubation medium at a concentration of $2 \times 10^{-6}$ *M* (Schanberg, 1963). Since in brain the transport of an AA is generally inhibited by structurally related analogs (Neame, 1968; Blasberg, 1968), it would appear that 5-HTP fits the classification of a neutral amino acid. The uptake of 5-HTP-$^{14}$C into brain slices is not influenced by the presence of other biogenic amines such as histamine ($10^{-6}$ *M*), NE ($10^{-6}$ *M*), epinephrine ($10^{-6}$ *M*), or by γ-aminobutyric acid (GABA) ($5 \times 10^{-2}$ *M*) (Schanberg, 1963). Although as already noted dopa partially blocks 5-HTP uptake, α-methyldopa, when given to rats (400 mg/kg) 45 minutes prior to sacrifice and added to the medium ($10^{-4}$ *M*), fails to reduce 5-HTP$^{-14}$C uptake into brain slices. This loss of inhibitory activity by α-methyl analogs was also observed in the tryptophan series (Schanberg, 1963).

Many drugs which exert profound central actions, when administered to rats in concentrations sufficient to influence behavior, fail to alter 5-HTP uptake into brain slices subsequently prepared from these animals. These agents include lysergic acid diethylamide, chlorpromazine, imipramine, iproniazid, pheniprazine, morphine, phenobarbital, and reserpine (Schanberg, 1963; Wartburg, 1962), suggesting that the possible influences of these drugs on serotonergic mechanisms do not derive from an effect on precursor uptake.

### C. Amino Acid Uptake

The extensive literature describing the movement of AA into mammalian brain slices has been the subject of several recent reviews (Lajtha, 1968; Blasberg, 1968; Neame, 1968). At low bath concentrations AA flux occurs mostly through mediated transport mechanisms rather than by simple diffusion (Chirigos *et al.*, 1960; Lajtha, 1964; Lajtha and Toth, 1961, 1963). Many properties of these transport mechanisms such as substrate specificity (Neame, 1961a; Abadom and Scholefield, 1962b; Blasberg and Lajtha, 1966),

stereospecificity (Lajtha and Toth, 1963), energy requirements (Elliott and van Gelder, 1958; Neame, 1961b; Abadom and Scholefield, 1962a; Lajtha, 1967), kinetic constants, and drug effects (Lajtha and Toth, 1965) have been examined in detail.

It is generally agreed that brain slices accumulate labeled AA from an incubation medium until a concentration gradient is established such that intracellular levels are severalfold those of the medium. Since there appears to be relatively little metabolism of AA taken up into brain slices during a 30–60-minute period of incubation (Blasberg and Lajtha, 1965; Oversen and Neal, 1968), transport rather than metabolism is probably the major determinant of this steady state. Transport constants for the influx of amino acids into brain slices must, however, be determined in short-term experiments which essentially measure the initial unidirectional flux; transport constants for influx cannot be derived from steady state experiments inasmuch as amino acid exodus from brain cells is also a mediated process and demonstrates saturation kinetics. Both the rate of AA uptake and release are concentration dependent (Levi *et al.*, 1965), influx depending on AA concentration in medium and efflux on AA concentration in the intracellular water. Net amino acid accumulation will continue until an intracellular concentration is attained at which influx and efflux are equal and a new steady state is attained.

Amino acid transport in brain slices appears to involve a number of carrier systems which may be partially characterized by the particular group of AA they predominantly mediate across the cell membrane. Comparison of representative small neutral, large neutral, large basic and acidic amino acids ($\alpha$-aminoisobutyric acid, L-phenylalanine, L-arginine, and L-aspartate, respectively) indicates that at least four transport systems are involved with the passage of AA across brain cell membranes (Blasberg, 1968). These systems evidently do not possess absolute specificity because a number of AA seem to have some capacity for transport by carrier systems other than those which primarily mediate their passage into the cell.

Regional differences in the concentration of endogenous free AA have been compared with AA uptake by slices from the same brain areas (Levi and Lajtha, 1965; Kandera *et al.*, 1968). The distribution pattern of the free pool varies with the AA and with the region analyzed. Similarly the uptake of AA into slices prepared from various regions of brain vary with the AA tested. In general, steady state accumulations attained *in vitro* parallel the physiological levels found in the living animal. Amino acids occurring at high concentrations *in vivo* are usually accumulated to higher levels in brain slices (Levi *et al.*, 1967). It thus appears that transport mechanisms play a major although not exclusive role in determining the regional distribution of AA in living brain.

The site of accumulation of exogenous AA by brain slices remains uncertain. Since uptake decreases upon slice swelling, at a time when the extracellular

space presumably increases, it is more probably intracellular than extracellular (Lahiri and Lajtha, 1964). Furthermore, nonspecific binding does not appear to be involved since omission of sodium from the incubation medium, which abolishes concentrative uptake, is unlikely to affect all specific binding sites (Lahiri and Lajtha, 1964).

Uptake sites for GABA may be unique to central nervous tissue (cf. Curtis and Watkins, 1965), because slices of rat liver under similar conditions fail to accumulate GABA-$^{3}$H (Iverson and Neal, 1968; Elliott and van Gelder, 1958). Varon *et al.* (1965) have shown that a considerable portion of the labeled GABA taken up by mouse brain particulate preparations is contained in synaptosomal particles, suggesting that GABA uptake sites may be associated with nerve terminals in brain. Iversen and Snyder (1968) have recently examined different synaptosomal populations storing exogenous catecholamines and GABA in homogenates prepared from rat brain slices. Slices from hypothalamus and striatum were incubated with labeled NE, DA, or GABA, homogenized and placed on sucrose gradients. Each of these labeled substances was found in the soluble supernatant fraction and in a particulate fraction having the density characteristics of synaptosomal particles. The peak of NE-$^{14}$C, however, occurred at a denser level of sucrose than that of GABA-$^{3}$H. A similar contrast between DA-$^{3}$H and GABA-$^{14}$C was observed, while DA-$^{3}$H and NE-$^{14}$C were found in the same particulate fraction. These observations suggest the existence of different populations of brain synaptosomes subserving different putative transmitters. Overlapping populations of synaptosomes remain to be separated.

The effects of the ionic composition of the incubation medium on AA uptake have been the subject of several investigations. An absolute requirement for sodium has been demonstrated for GABA uptake into cerebral slices (Iversen and Neal, 1968; Lajtha, 1968). The absence of potassium, calcium, magnesium, or phosphate ions inhibit uptake of GABA only slightly, while excess potassium or phosphate are strongly inhibitory. In their studies of AA in mouse brain slices, Lahiri and Lajtha (1964) noted that slices swelled greatly in the absence of sodium, but not if sodium was replaced by lithium or choline. Tsukada *et al.* (1963) observed that the uptake of D- or L-glutamatic acid was accompanied by marked potassium accumulation and cell swelling. In contrast, GABA or $\beta$-alanine, neutral amino acids, were accumulated actively without changes in electrolyte distribution or concomitant swelling of cells. In these studies, omission of potassium inhibited, while omission of calcium ions tended to enhance, AA uptake. In a potassium-rich media, GABA and $\beta$-alanine uptake was slightly inhibited, while that of D- and L-glutamic acid was markedly increased.

The effects of various substrates on uptake of GABA, L-glutamic acid, L-histidine, and glycine have been compared by Barborosa *et al.* (1968).

The presence of glucose, pyruvate, lactate, and oxaloacetate favored maximal uptake of these AA into brain slices, while in the presence of succinate and fumarate, uptake was only slightly higher than in the absence of substrate. Hypoxia and metabolic inhibitors diminished uptake, confirming the dependence of uptake on oxidative metabolism.

Several drugs have been reported by Lajtha and Toth (1965) to influence the accumulation of AA by brain slices. The uptake of L-lysine or cycloleucine is markedly reduced by ouabain ($10^{-5}$ *M*), reserpine ($10^{-3}$ *M*), and pentobarbital ($10^{-3}$ *M*). Chlorpromazine exerts a diphasic effect; relatively high concentrations ($2 \times 10^{-3}$ *M*) strongly inhibit while lower concentrations ($2 \times 10^{-5}$ *M*) slightly enhance uptake. Morphine ($10^{-3}$ *M*) has only a mild inhibitory action, while pentylenetetrazole ($10^{-3}$ *M*) and cocaine ($10^{-3}$ *M*) have no significant influence on the accumulation of L-lysine or cycloleucine. The uptake of α-aminoisobutyric acid was also found to be reduced by ouabain ($10^{-5}$ *M*) and chlorpromazine ($2 \times 10^{-3}$ *M*). Presumably these drug effects on AA transport *in vitro* are mediated by more than one mechanism.

### D. Acetylcholine Uptake

Although a variety of early studies failed to demonstrate a significant accumulation of ACh by brain slices, it is now apparent that the active uptake of this substance into cerebral tissue does occur *in vitro* in the presence of a suitable cholinesterase inhibitor. In brain slices as well as in nerve ending particles, Burton (1964) and Guth (1962) found negligible uptake of ACh in the absence of anticholinesterase agents. Similarly, uptake experiments in which eserine (physostigmine) was used to inactivate cholinesterase yielded negative or nearly negative results (Mann *et al.*, 1938; Elliott and Henderson, 1951; Schuberth and Sundwall, 1967), due to the strong inhibitory action of this drug on ACh uptake mechanisms (Polak and Meeuws, 1966). In the presence of the irreversible organophosphorus inhibitors, soman (pinacolyl methylphosphonofluoridate), sarin (isopropyl methylphosphonofluoridate), or paraoxon (diethyl-4-nitrophenyl phosphate), however, a significant accumulation of ACh has been observed (Polak and Meeuws, 1966; Schuberth and Sundwall, 1967; Liang and Quastel, 1969a; Polak, 1969; Heilbronn, 1969). The major fraction of this uptake occurs against a concentration gradient, requires oxygen, and is impaired by substances interfering with energy metabolism. The role of active uptake appears to depend on the concentration of ACh in the incubation medium according to Michaelis-Menten kinetics (Schuberth and Sundwall, 1967; Polak and Meeuws, 1966; Lang and Quastel, 1969a).

Preliminary experiments reported by Schuberth and Sundwall (1967) on the subcellular distribution of ACh-$^3$H actively accumulated in brain slices suggest that 75% is located in the supernatant while 25% is in the

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