Studies on Possible Correlations between Cholinesterase Levels in Mouse Brain and Behavior

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STUDIES ON POSSIBLE CORRELATIONS BETWEEN CHOLINESTERASE LEVELS IN MOUSE BRAIN AND BEHAVIOR

A Dissertation Submitted to the Faculty of the Department of Pharmacology, Loyola-Stritch School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy
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BIOGRAPHY

Chen-ho Lin was born to Mr. and Mrs. Jian-Shian Lin on September 22, 1940 in Tainan, Taiwan.

She graduated from Taiwan Provincial Tainan Girls' Middle School on 1959 and subsequently from the School of Pharmacy, Kaohsiung Medical College, Taiwan in 1964.

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Publications:

Lin, Chen-ho (1967)
Morphine Administration to Pregnant Rats and Behavioral Effects on the Offspring. Master's Thesis; School of Pharmacy, University of Mississippi, Oxford, Mississippi.

Davis, W. M., and C. H. Lin (1972)
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To the gang —- The Institute Fellows —- the author would like to express her gratitude for their warm friendship and aid which she received during her stay at the Institute.

Finally, to my parents who made all of this possible, the author would like to express the most special thanks, respect, and love for all the sacrifices they made for her and for the infinite confidence which they always have had in her.
The basic assumption underlying this thesis is that ACh is one of the transmitter substances in the central nervous system which mediate the neuronal activity in the brain. Behavior generally per se can be regarded as an expression of the interaction of the external state of the environment and the internal state (neural activity) of the animals which through evolution generally achieves the highest homeostatic efficiency for the animal and species. Hence it seems logical to assume that a relationship exists between central cholinergic systems and animal behavior (cf. Central Cholinergic Involvement in Behavior, Chapter I). Since AChE is the enzyme responsible for the destruction and inactivation of ACh, it is reasonable to presume further that such a relationship might also exist between ChE and behavior. The purpose of this study, therefore, was to attempt to establish such a relationship, by studying 1) ChE activities in various species and strains of mice which differ widely in behavior and ecological profiles (Scudder et al., 1966a; Scudder et al., 1966c; Scudder et al., 1967), 2) circadian rhythm in ChE activity in relation to behavior, 3) drug effects on brain ChE level, and finally 4) the effects of environmental manipulation on brain ChE activity. The first two approaches appeared to be more appealing as they are more naturalistic in the sense that they occur within the normal
physiological state; however, the two latter approaches also proved to be extremely useful in the establishment of such relationship.
Chapter I. General Review of the Literature

Since Loewi (1921) and Loewi & Navratil (1926) demonstrated that vagal stimulation of a perfused frog's heart resulted in the release of an inhibitory substance in the perfusing fluid which had the property similarly to vagal stimulation of inhibiting the beat of another frog's heart, the term "Neurohumoral Transmission" has come into existence and ACh has been confirmed to be the neurohumoral transmitter at his particular autonomic effector site.

Subsequently the work done on the neuromyal junction (Dale & Feldberg, 1934; Dale, Feldberg & Vogt, 1936), on superior cervical ganglion (Kibjakow, 1933; Feldberg & Guddum 1934; Feldberg & Varitiainen, 1934), and also on the submandibular and inferior mesenteric ganglia (Emmelin & Muren, 1950; Barsoum, Gaddum & Khayyal, 1934) further confirmed the cholinergic nature of neuromyal and ganglionic transmission. In addition, various electrophysiological, electron-microscopic, biochemical, and histochemical studies also contributed invaluable evidence to the establishment of peripheral as well as central chemical transmission. These included the finding of synaptic vesicles in the skeletal neuromyal junction (Palade, 1954; Robertson, 1956), in the ganglia (De Robertis & Bennett, 1954; De Robertis & Bennett, 1955), as well as in the central nervous system (Palade, 1954; Palay, 1954 & 1956; Gray & Guillery, 1966; Bloom & Aghajanian, 1966; Aghajanian & Bloom, 1967). It has been shown that these
synaptic vesicles contain various substances such as acetylcholine (ACh), norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT) and histamine. Enzymes concerned with the synthesis of these substances have also been found to be present in either the vesicle fraction or in the mitochondria. Of importance in regard to neurotransmitter was the theory of the miniature potentials and their relationship to the synaptic vesicles (Castillo & Katz, 1956; Katz, 1958; Katz, 1962). These spontaneous miniature potentials recorded at quiescent synapses were shown to be related to the liberation of ACh from ACh-containing synaptic vesicles located in the nerve terminal and were considered as quanta of chemical activity for the nervous system. Miniature potentials also have been found in quiescent ganglia (Blackman, Ginsborg & Ray, 1962; Nishi & Koketsu, 1960) as well as in the central nervous system.

I. Central Cholinergic Transmission

The investigation of the central nervous system transmission has long been described as a very tedious task. This is due to the fact that vertebrate central synapse differs from the peripheral synapse in several major properties and hence special problems arise from the study of this structure. First of all the central synapse differs from the peripheral synapse by not having a sharp and easily discernible focalization of the synaptic regions as does the muscle endplate. Secondly, the well-
known phenomenon of the blood-brain barrier makes the movements of drugs from capillaries to neuronal surfaces uncertain. This has been circumvented by the use of multibarrelled micro-pipette for the study of actions of drugs on single neurons (Curtis & Eccles, 1958a & b, Curtis, 1965), by the application of permanent implanted cannulae, either single or multiple, for the intraventricular injections of drugs and regional perfusion of cerebral ventricles (cf. Feldberg, 1963; Feldberg & Fleischhauer, 1965), and by the administration of various precursors such as 5-hydroxytryptophan and beta-(3,4-dihydroxyphenyl)-L-alanine (DOPA), etc. Thirdly the fact that each central nervous system neuron may have more than one transmitter acting on its surface and one transmitter may have different types of receptor sites for its transmission at this cell (cf. Werman, 1966; Votava, 1967) further complicates and perplexes the study of the CNS transmission. Finally, the inaccessibility of the central synapses and the difficulty of identifying specific cells also greatly reduce the possibility of successfully collecting an identifiable transmitter or understanding its action.

Various substances have been reviewed recently as being possible candidates for central transmitters (Hebb, 1970; Vogt, 1969). These include ACh; monoamines such as norepinephrine, dopamine and 5-HT; neutral amino acids (gamma-aminobutyric acid, taurine, alpha- and beta-alanine and glycine) as inhibitory
transmitters; and acidic amino acids (glutamate and aspartate), as excitatory transmitters. A variety of approaches have been developed to identify the central transmitter substances. An article by Werman (1966) provides a useful guide for this purpose. Of the eight criteria he discussed, only six of them which are more generally accepted for the identification of central cholinergic transmission will be presented in the following section. The six criteria are Identity of Action, Pharmacological Identity, Distribution of ACh in the Central Nervous System, Presence of Synthesizing Enzyme, Presence of Degradating Enzymes and Collectability of ACh.

A. Identity of Action

The criterion of Identity of Action states that a suspected transmitter should produce the same effects as does the physiological transmitter. This criterion is the most fundamental and also has always been the most basic criterion for the identification of a suspected transmitter.

B. The Criterion of Pharmacological Identity

This criterion states that drugs which interact with physiological synaptically active chemicals will interact with the suspected transmitter in the same way, or more specifically and correctly, drugs which interact at postsynaptic structures with the natural transmitter should interact with the suspected transmitter in the same way.
Both the above criteria -- Identity of Action and Pharmacological Identity -- can be met in experiments in which responses of single neurons are studied by the multi-barelled microelectrode technique which allows one to record single cell activities while the neurons are subjected by iontophoresis to as many as four different suspected agents. Generally, when cholinceptive neurons are found, their response to \( \text{ACh} \) is compared to that of excitatory amino acids and analyzed pharmacologically by means of various cholinergic substances such as physostigmine, atropine and dihydro-beta-erythroidine (DHE). If both of the above criteria are met, the presence of cholinergic transmission at the synaptic site in question is very likely.

1. Cholinergic Transmission in the Spinal Cord

Although much research has been carried out on the possibility of central cholinergic transmission in the CNS, the only cholinergic pathway thus far confirmed is that of the motor axon collateral to the Renshaw cells which are situated in the ventral medial area of the spinal cord. This relationship was established by Eccles et al. (1954) in their elegant experiments in which the electrical potential changes in the cell bodies of individual motoneurons and of the interneurons were recorded and the parallel effects of different chemical substances on the potential changes in the motoneurons were compared with their effects on the discharges of the interneurons on the other. Briefly, this experiment clearly demonstrated that \( \text{ACh} \) mediates...
the excitation of Renshaw cells by impulses in the collaterals of motor axons. Furthermore, it has been demonstrated that the cholinoceptive receptors upon the Renshaw cells are that of the "nicotinic" category, i.e. exhibit rapid onset and short duration of action; intra-arterial injection of nicotine produced a prolong discharge of Renshaw cell which was much more effective than that induced by ACh, DHE greatly and atropine to a lesser extent depressed the sensitivity of Renshaw cells to both nicotine and ACh, while antiChE such as eserine and tetraethylpyrophosphate (TEPP) greatly increased the effectiveness of injected ACh (Eccles et al., 1954; Eccles et al., 1956). Later Curtis et al. (1958a & b), Curtis et al. (1961) and Curtis (1965) by utilizing microelectrophoretic technique, not only confirmed the findings that Renshaw cells are spontaneously active and the rate of this spontaneous discharge is increased by ACh, nicotine and related compounds, but also demonstrated the existence of certain diffusional barrier around these central synapses. Moreover, recent experiments have provided evidence of the existence of muscarinic receptors as well as nicotinic receptors on the Renshaw cells (Curtis & Ryall, 1964).

2. Cholinoceptive Neurons in the Brain Stem

In a general survey of the cholinoceptive neurons in the cat medulla, up to 29% of the neurons investigated were found to be sensitive to ACh electrophoretic application, of which 22% exhibited an excitatory effect while 7% showed a depressant
action (Salmoiraghi & Steiner, 1963; Bradley & Wolstencroft, 1963). This ratio of cholinoceptive neurons varied with areas investigated; 35% excited and 12% inhibited neurons were present in certain region of the medulla (Bradley, Dhawan & Wolstencroft, 1964). In general, those cells excited by ACh in the brain stem exhibited both nicotinic and muscarinic properties, and when they were compared to responses of cholinoceptive neurons in other regions of the brain, they appeared to be more muscarinic than Renshaw cells, but less so than cortical neurons (Bradley & Wolstencroft, 1965; Bradley et al., 1966). However, the cells inhibited by ACh in the brain stem were similar to those excited by ACh in the cerebral cortex in that they were muscarinic in nature (Bradley & Wolstencroft, 1965; Bradley et al., 1966). AntiChE agents exhibited strong excitatory effects of their own in addition to their potentiation of the action of ACh; atropine, hexamethonium and gallamine antagonized both the excitatory and inhibitory effect of ACh, whereas DHE antagonized only excitatory responses (Salmoiraghi et al., 1963; Bradley et al., 1964; Bradley et al., 1965; Bradley et al., 1966). A combination of iontophoretic, histochemical, physiological as well as pharmacological techniques may in time map out the possible cholinergic synapses within this brain area (Bradley et al., 1965). According to the histochemical studies of Shute & Lewis (1963) these cholinoceptive neurons in the medulla and pons might belong to the cholinergic reticular activating system.
3. Cholinoceptive Neurons in Cerebellum

A possible cholinergic mechanism in the cerebellum based on iontophoretic, histochemical, as well as subcellular fraction techniques, has been proposed by McCance & Phillis (1964a & c). They reported that iontophoretic application of ACh to cells of the cerebellar cortices produced an excitant effect. Pharmacological analysis of this cellular response to ACh revealed that DHE abolished the spontaneous discharge as well as the excitation induced by ACh and nicotine, atropine and to a lesser extent gallamine, mecamylamine and d-tubocurarine also antagonized the excitant action of ACh, while antiChE agents exhibited powerful excitatory actions. These ACh-sensitive cells in the cerebellar cortices have been identified as granular layer cells. These findings together with those obtained from histochemical studies (Austin et al., 1964; Shute & Lewis, 1965; Koelle, 1954), as well as the data derived from subcellular fractionation (Austin et al., 1965) indicating that AChE was concentrated in the granular cell layers and was present largely in synaptosomes, led to the conclusion that transmission between afferent mossy fibers to the cerebellum and granule cells was mediated by ACh, while excitation of Purkinje cells by cholinergic substances was a result of stimulation of cells in the granular cell layer or by a direct action of ACh on excitatory receptors on the Purkinje soma (McCance & Phillis, 1964c; Phillis, 1965). This proposal, however, is not supported by the work recently carried
out by Crawford et al. (1966). They demonstrated, in contrast to those of McCance & Phillis, that only Purkinje cells were excited by cholinomimetics; the ACh receptors were muscarinic in nature. Moreover, intravenous administration of atropine and DHE did not depress the synaptic excitation of cerebellar neurons evoked by impulses either in mossy, climbing, or parallel fibers. Thus they concluded that ACh is unlikely to be an excitatory transmitter within the feline cerebellum despite the presence of relatively high levels of AChE in this brain area.

4. Cholinoceptive Neurons in the Thalamus and Hypothalamus

ACh sensitive units have also been found in the thalamus and hypothalamus. In the thalamus, these cholinoceptive neurons are identified as thalamocortical relay neurons located within the ventrobasal complex of thalamus, lateral geniculate neurons, as well as thalamic interneurons (Curtis & Andersen, 1962; Curtis & Davis, 1963; Andersen & Curtis, 1964a & b; Curtis, 1965). The ACh receptors within the thalamus and lateral geniculate nucleus are of an intermediate type between those of cortex and Renshaw cells. They resemble the former by a slow onset and prolonged duration of firing upon iontophoretic ejection of ACh; they resembled the latter by being readily depressed by DHE. While these experiments successfully demonstrated the presence of cholinoceptive neurons in the thalamus and lateral geniculate, they failed to determine which afferent pathways to these regions of the brain are cholinergic since pharmacological investigation
demonstrated that DHE and atropine, which readily reduced the response of thalamic neurons to ACh, did not influence the firing of these cells by impulses initiated in cutaneous sensory fibers (Andersen, 1964b); 5-HT which suppressed the synaptic excitation of lateral geniculate neurons by optic nerve volleys, did not affect the excitatory action of ACh (Curtis & Davis, 1963). Thus, it is unlikely that the transmitter released from the medial lemniscus pathway or optic nerve terminals is ACh or closely related compounds. Recently Curtis (1965) suggested that this pathway might be derived from the reticular activating system in the brain stem which is presumably cholinergic in nature.

In the hypothalamus, in their study of the reactions to various stimuli of neurons within or near supraoptic nuclei, Brooks et al. (1962) found that intracarotid injection of ACh caused an acceleration of neuron firing. Bloom et al. (1963a & b), using microelectrophoretic technique, provided more direct evidence for the presence of cholinceptive neurons in the hypothalamus. They found that many ACh sensitive units are encountered in paraventricular and ventral median nuclei. They were essentially distributed diffusely throughout the hypothalamus. These cholinceptive cells responded either by increasing or decreasing their spontaneous rate of discharge when tested. They were considered to be a part of the ventral tegmental pathway by Shute & Lewis (1966).
Cholinoceptive Neurons in the Caudate Nucleus

The caudate nucleus is one of the regions which contains the highest concentrations of ACh (MacIntosh, 1941), of choline acetylase (Fedldberg & Vogt, 1948; Hebb & Silver, 1956), and of AChE (Burgen & Chipman, 1951). Hence there has been considerable interest in the possible cholinergic transmission of this nucleus. Bloom et al. (1964 & 1965) studied the responsiveness of individual neurons of the caudate nucleus in unanesthetized, decerebrate cats and barbiturate anesthetized cats to ACh, NE, and dopamine. They reported that the majority of cells encountered in unanesthetized animals were spontaneously active and most of these spontaneously active cells responded to ACh by an increase in rate of firing, while certain other cells responded in the opposite direction. These facilitatory responses to ACh were greatly reduced by the depressant effects of NE and dopamine. Moreover unit facilitation by ACh was reversibly diminished or abolished by small to moderate doses of short-acting barbiturates or electrophoretically administered procaine. No effects of these anesthetic agents on ACh-induced depression or responsiveness to NE, dopamine, and glutamate were observed. These findings were substantiated by succeeding experiment (McLennan & York, 1966) in which unit cell firing within the caudate nucleus to microelectrophoretic application of ACh was compared with firing produced by stimulation of the nucleus ventralis anterior thalami (VA). Both types of neurons responded either by excita-
tion or depression, following ACh and VA stimulation. Nicotinic stimulants were found to be ineffective, while acetyl-beta-methyl-choline was as effective as ACh. Moreover the responses evoked by VA stimulation or ACh iontophoretic application were prevented by atropine and hence lend to the conclusion that cholinceptive neurons in the caudate nucleus bear a close similarity to those of the cortex, i.e. they are primary "muscarinic" in character. Taken all together on the basis of the anatomical and electrophysiological evidence as well as the presence of high levels of choline-acetylase, AChE, and also the enhanced release of ACh from caudate nucleus upon VA stimulation, McLennan & York (1966) suggested that the final synapse in the VA-caudate pathway is cholinergic in nature.

6. Cholinoceptive Neurons in Cerebral Cortex

An excitant action on some cortical neurons of ACh applied iontophoretically has been described in cats by Krnjevic & Phillis (1961, 1962, 1963a, b, & c), Krnjevic (1964) and Crawford (1970). These sensitive units were found to be distributed mainly in the primary somatosensory, visual, and auditory receiving areas; the greatest concentrations were seen in the cat primary visual area. These neurons tended to occur in groups, and were characterized by spontaneous activity related to slow waves of the electrocorticogram. The Betz cells in the sensory-motor cortex of cats anesthetized with Dial compound (allo-barbitone + urethane) have been found to be more sensitive to
ACh iontophoresis than to L-glutamate application. It has been suggested that all cortical cells which are excited by ACh are activated synaptically by cholinergic fibers from a common source, and that Betz cells are especially likely to have a supply of these postulated fibers. Generally the excitatory cholinceptive neurons found in the cerebral cortex are characterized by a slow onset of firing and long lasting action, and the ACh receptors responsible for their activation have strong muscarinic properties. This action of ACh on cortical cells has been directly correlated with awareness (Krnjevic, 1967). All the ACh sensitive units are excited by a number of cholinomimetic drugs. The action of ACh can be potentiated by antichE agents, on the one hand, and prevented by atropine or hyoscine, and, in many cases, by gallamine on the other. In keeping with these findings, Spehlmann (1963) demonstrated, in cat visual cortex, that ACh could increase the rate of spontaneous firings. It also facilitated neuronal discharges evoked by illumination of the eye and facilitated the short-latency response to stimulation by epicortical electrical shocks given with subthreshold intensity. In addition, by slightly varying the positions of the multi-barrelled electrode, he was able to demonstrate that ACh is effective only when applied to certain sites. These observations that ACh is effective only at the receptor site, together with other evidence, seem to favor the hypothesis that ACh acts like a transmitter substance.
Possible cholinergic pathways in the cortex have been suggested by Krnjevic et al. (1963b & 1965a) on the basis of the histochemical distribution of AChE in the cat forebrain. A distinct, tangential system of AChE-containing fibers was found in the neocortex of the cat. This system was best seen under the sulci, where bundles of AChE stained fibers are in U- formations. In the gyri these fibers ascended on either side of the relatively unstained central cord, which contained the main afferent, efferent, and commissural pathways; these ChE containing fibers gave off branches which formed a complex network in the deeper half of the cortex, with rather diffuse endings, especially in relation to pyramidal cells of layer V. The main subcortical connections of these AChE-containing fibers were traced to the corpus striatum and the septal region. In addition to these U-fibers of subcortical origin, there were many AChE-containing fibers arising in the cortex to reach adjacent areas. The cells of origin for these fibers were believed to be the spindle (or polymorph) cells of layer VI. This entire system of AChE-containing fibers in the forebrain has been suggested as the final corticopetal link in the ascending pathway from the midbrain reticular formation mediating arousal (Shute & Lewis, 1963; Shute & Lewis, 1966; Shute & Lewis, 1967; Krnjevic, 1965). It may also be the identical pathway responsible for projection activity and repetitive responses (Krnjevic, 1965; Morrison & Morrison & Dempsey, 1943).
Besides the excitant effects on cortical neurons of the cat, ACh also exerts a depressant action on a certain percentage of cortical neurons, ranging from 5.5% to 46% of the cells tested, depending upon the areas of the cortex investigated (Randic et al., 1964; Phillis & York, 1967; Phillis & York, 1968; Phillis & York, 1968). Some of these cholinceptive cells were innervated by ascending fibers of the reticular system; others were innervated by cholinergic inhibitory interneurons lying wholly within the cortex. These cholinergic inhibitory interneurons may be identical to those described by Krnjevic & Silver (1965).

7. Cholinceptive Neurons in the Hippocampus

About 50-60% of hippocampal neurons tested electrophoretically were excited by ACh (Biscoe & Straughan, 1966; Slamoiraghi & Stefanis, 1967; Steiner, 1968). Characteristically this excitation developed slowly over many seconds and persisted after stopping electrophoresis; i.e. there were typical central muscarinic responses similar to those seen in the cerebral cortex. Most cholinceptive units were found to be concentrated in the superficial layer of the cortex corresponding to the hippocampal pyramidal cells and their main dendritic processes. It was thought that an afferent volley either via the commissural, the septal (Lewis, Shute & Silver, 1964; Lewis & Shute, 1967), or the local afferents, activated both the pyramidal axons and their axon collaterals through the activation of the presumably cholinergic pyramidal cells. These synapses of the axon collaterals...
excite a set of inhibitory interneurons, the basket cells, which in turn activated their inhibitory synapses around the cell bodies of a much larger number of pyramidal cells than were excited initially (Eccles, 1969).

The presence of a possible cholinergic mechanism in the hippocampus is also substantiated by the fact that the response of hippocampal neurons to photic stimulation was enhanced by local application of ACh; even during phenobarbital depression the photic responses were restored by ACh microelectrophoresis (Steiner, 1968).

8. Cholinoceptive Neurons in Olfactory Bulb

The presence of cholinoceptive neurons in the olfactory bulb was reported by von Baumgarten et al. (1963) and Bloom, Costa & Salmoiraghi (1964). They found that a small portion of olfactory neurons in decerebrate rabbits responded to ACh by increasing their rate. Most of the responsive neurons, however, decreased their rate of discharge when tested with ACh. It was suggested that ACh may be involved in the function of an olfactory inhibitory synaptic pathway (cf. Yamamoto et al., 1963; Eccles, 1969).

C. Distribution of ACh in the Central Nervous System

The presence of ACh in the central nervous system has been well documented (MacIntosh, 1941; Quastel, 1962; Toru & Aprison, 1966; Holmstedt, 1967; Aprison, Kariya, Hingtgen & Toru, 1968; Sobotka, 1969). Although ACh is present in almost all areas
of the brain, some areas such as the cerebral cortex, the basal ganglia, midbrain, certain parts of the corpus callosum and of the internal capsule, and the superficial layers of the pons are relatively rich in ACh, while the dorsal columns in the medulla, pyramids, and cerebellum contain hardly any ACh. In the spinal cord of the dog, ACh occurs in the grey matter, and in those parts of the white matter containing only afferent axons (MacIntosh, 1941). Tower et al. (1952) in their studies of the activity of the ACh system in the cerebral cortex of various mammalian species further discovered that ACh, ChE, and choline acetylase (ChAc) decreased fairly regularly with ascending order on the phylogenetic scale. The decrease was related to the average total brain weight for the measurements -- ACh, ChE & ChAc. A decrease in the average number of neurons per unit volume of cortex with increasing brain weight ran parallel to the decrease in these parameters of the ACh system.

D. Presence of Synthesizing Enzyme

Choline-acetylase (ChAc) is the enzyme responsible for the synthesis of ACh from choline and acetyl-CoA. A systemic survey of the distribution of ChAc in the CNS has been carried out in dog by Feldberg & Vogt (1948), in man, dog, cat, rabbit, pig, sheep, and guinea pig by Hebb & Silver (1956), and in rabbit by McCaman (1963), McCaman & Aprison (1964), McCaman & Hunt (1965), and Goldberg & McCaman (1967). In general these studies showed that cortical choline-acetylase activity is related inversely
to the degree of cortical development. The greatest species differences in this synthesizing enzyme were shown by areas of neocortex while the least species difference were shown by areas of rhinencephalon or allocortex. The visual cortex of man showed the lowest values. Within the central nervous system of the seven species studied the head and body of the caudate nucleus, supraoptic nuclei, and cornu ammonis showed the highest activity of choline acetylase with little species variation occurring in the caudate nucleus. The highest activities were among those of the anterior roots and of the anterior horns and the hypoglossal and vagal (motor) nuclei where the spinal and cranial motor nerves originate. The thalamus showed a considerably lower value than that of the basal ganglia, nevertheless this was a relatively high level of activity. The values for hypothalamus were less. Low activity occurred in the cerebellum with higher values in the cerebellar peduncles. Little or virtually no enzyme was found in dorsal spinal roots and optic nerves. Moderate concentrations of choline-acetylase were present in the superior colliculus and the concentration in this center correlated positively with the retinal concentration in different species. Moderate amounts of ACh were also synthesized by the olfactory bulbs in all species with less enzyme in the olfactory tract than the bulb in man. Relatively high values were found in medulla, pons, hippocampus (Lewis, Shute & Silver, 1964).

E. Presence of Degrading Enzymes
Another criterion for identifying the central synaptic role of a suspected substance is the presence of its destructive enzyme or enzyme system within the central nervous system. This criterion is by no means conclusive by itself, however, it is one of those most useful as a subsidiary means for speculation about a possible site of central cholinergic transmission. Numerous investigators contributed a wealth of knowledge in this field (Kolle, 1954; Gerebtzoff, 1959; Foldes et al., 1962; Torack & Barrnett, 1962; Eranko, 1967). The term ChE, from now on, will be described as an entity of enzymes including AChE, BuChE, as well as the whole family of isozymes. Nachmansohn (1940) in his survey of the distribution of ChE in the central nervous system in rabbit, dog, ox, and man, first reported that there is a characteristic, uneven distribution of enzyme in the brain areas studied. A close correlation of brain function and enzyme activity was observed; for example, in the spinal cord, high ChE activity was obtained in the gray matter with little activity in the white matter. Among the various brain centers studied, the ChE activity is highest in the basal ganglia, high in retina, relatively high in tuberculum quadrigemina anterior, pons, low in cortex, cerebellum and tuberculum quadrigemina posterior and intermediate in the thalamus opticus. Species variation, however exists, for instance, in man and dog there is relatively high value of enzyme activity in the cerebellum compared to that of
rabbit and ox. Later Burgen & Chipman (1951) carried out a rather thorough and detailed enzyme analysis of AChE and BuChE in a large number of regions of dog's brain and spinal cord, and further confirmed the findings of Nachmansohn. Again, caudate nucleus showed the highest activity, the cerebellar cortex showed a high activity, the thalamus was considerably less active than either of these with the massa intermedia more active than the dorso-lateral nucleus, the hypothalamus showed moderate activity, while the cerebral cortex gave low values (McCaman et al., 1964). In the optic pathway there was found a very low value for the optic nerve, a somewhat higher value for the optic tracts, a still higher value in the lateral geniculate body and the highest value in the superior corpus quadrigeminum. There was a sharp fall to a very low value in the occipital subcortical white matter and a low value in the visuosensory cortex. In the motor tracts, there is moderate activity in the motor cortex, very low in the subcortical white matter, high in the spinal grey matter and moderately high in the anterior spinal roots. In the sensory pathway the enzyme activity was generally low except in nucleus gracillis, cuneatus, and thalamus. A rather uniform enzyme activity was observed in the olfactory bulb, medial geniculate body, and inferior corpus quadrigeminum. The distribution of BuChE, bore a fairly constant relationship to that of AChE in the cortical areas, however, in the hypothalamus it is higher. A comparison of ChE activity with ChAc and ACh content
shows a good correlation between them among the various brain regions investigated except in the cerebellar hemisphere and anterior spinal roots.

In addition to these large systemic surveys on brain ChE distribution, numerous studies have been carried out in detail in discrete brain areas. Pope (1952) described the enzyme distribution in the rat cerebral cortex in relation to its architectonic layers; he claimed that AChE activity is high in layer I, the junction of layers II & III, and in layers III, V, and VI, this pattern of enzyme distribution correlated well with the degree of fractionation of the cortical plexus and hence suggested that this enzyme is located at the surfaces of dendrites and axons including synaptic terminals. Comparable to Pope's study on rat cerebral cortex, Okinaka and his associates (1961) undertook their experiment on human tissues and found that ChE activity is high particularly in the motor and premotor areas and in hippocampic region of the limbic lobe. Compared to the basal ganglia, the cerebral cortex showed markedly low activity, although in the first layer of the regions, anterior and posterior to the central sulcus, the enzyme activity in the ground substance was somewhat higher than in other layers. The ChE activity in the cerebral cortex was found mainly within the cytoplasm of the nerve cells, and activity is strong in the nerve cells of the third and 5th layers. An architectonic description of enzyme distribution similar to that of Pope (1952) was presented.
by Mathisen & Blackstad (1964) for the hippocampus. They suggested that the high enzyme activity around the neuronal soma of the hippocampus and of the fascia dentata was mainly contributed by a plexus of short axons, chiefly from basket cells, which surround pyramidal and granular cells and that the axo-somatic synapses here may be cholinergic in nature.

The presence of ChE in sites other than cerebral cortex has also been reported. Pavlin (1963 & 1965) in his study of ChE activity in single nerve cells from the reticular formation found that only 43% of the examined cells showed AChE activity whereas 94% of the examined cells showed butyryl ChE activity. He suggested that there may exist a possible functional relationship between enzyme activity and ACh-sensitive cells in this brain region and also raised the question of the possible function of BuChE in these nerve cells. So far as cerebellum concerned, Austin et al. (1964) reported that in the feline cortex, little or no BuChE was observed whereas relatively high AChE was concentrated in the granular cell layer. A well-defined network of ChE containing fibers was found traveling along the deep surface and extending into the granular cell layer especially the deeper layer. Considering the icntophoretic studies and ChAc distribution, these authors postulated that the transmission at synaptic junctions between granule axons, Purkinje cell dendrites, and stellate cells in the molecular layer as well as transmission between granular cells and Golgi cells in the
granular cell layer are mediated by ACh. A more recent study on cerebellar ChE in several species was carried out by Goldberg & McCaman (1967) in which the high enzyme activity in the feline granular layer reported by Austin (1964) was confirmed. A marked variation in enzyme activity from species to species, however, was demonstrated. A comparison between ChE and ChAc showed that there was no apparent correlation between the levels of these two enzymes in the various cerebellar layers or nuclei.

Recently a comprehensive series of experiments on ChE distribution have been undertaken by Shute & Lewis (1963; 1965; 1966; & 1967) and Lewis & Shute (1967). In these studies possible cholinergic pathways in the CNS were ruled out by the combining technique of histochemical and surgical methods. The determination of the polarity of the AChE-containing fiber is made possible by the observations that the enzyme was found to accumulate in the cut ends of axons on the cell-body side of the lesion and to disappear from the axons and their terminals on the opposite side. Briefly, two main cholinergic systems - the "cholinergic reticular activating system" and the "cholinergic limbic system" - were described. At the forebrain level, the cholinergic reticular activating system was subdivided into two subsystems; 1) the dorsal tegmental pathway which arises from the nucleus cuneiformis situated in the dorsolateral part of the mesencephalic reticular formation, and distributes to the tectum, pretectal area, geniculate bodies and to the non-specific and specific
nuclei of the thalamus, 2) the ventral tegmental pathway which arises mainly from the substantia nigra and ventral tegmental area of the midbrain, and traverses the hypothalamus and subthalamus to reach the basal forebrain areas and there continues to many regions of the cerebral cortex and the olfactory bulb. This ascending cholinergic reticular system has been suggested to be responsible for the electrocortical arousal and to be identical to the "ascending reticular activation system" described by neurophysiologists. At the hindbrain level, the cholinergic reticular activating system was found to provide cholinergic innervation to the cerebellum and cochlear nuclei. The cholinergic limbic system included the cholinergic neurons of the medial septal nucleus and the nucleus of the diagonal band which innervate the hippocampal formation (hippocampus and dentate gyrus). Hippocampal efferents traveling by way of the fornix then project, directly or indirectly onto ChE-containing neurons in the hippocampal commissure, anterior thalamus, habenular and interpeduncular nuclei, and the midbrain tegmentum. These cholinergic neurons (presumably), in turn, project to medial cortex, to nuclei of the ascending cholinergic reticular system, and to the subfornical organ and supraoptic crest (Lewis & Shute, 1967).

F. Collectability of ACh

The criterion of collectability of the transmitter states that during stimulation the transmitter substance should be de-
etectable in extracellular fluid collected from the region of the activated synapses. However, the structure of the central nervous system is extremely tortuous and complicated and this criterion has met only limited success. Nevertheless, due to the development of new techniques such as the collecting cup (McIntosh & Oborin, 1953), push-pull cannulae (Gaddum, 1961), regional perfusion of cerebral ventricles (Carmichael et al., 1964), and concentric glass micropipettes (Mitchell, 1964), the release of ACh from the surface of the brain has been demonstrated. Richter & Crossland (1949) first described a correlation of rat brain ACh with various physiological states. They found that brain ACh level varies inversely with the degree of activity of the brain; it is increased in pentobarbital anesthesia and in sleep and it is decreased in emotional excitement, in electrical stimulation, and in convulsions. The authors suggested that there might be a relationship between ACh levels and convulsive activity. Later Gaddum (1961) in order to gain more convincing evidence on central cholinergic transmission, using plastic cups and a push-pull cannulae method, found that ACh is spontaneously liberated from the cerebrum of sheep and cats. The rate of liberation was increased by stimulating various nerves. Beleslin et al. (1964), by utilizing a different technique - regional perfusion of cerebral ventricles in the presence of an antiChE - were able to determine the site of origin of the ACh appearing in the perfusate. They showed that the greatest amount of ACh
comes from structures lining the anterior horn of the lateral ventricle (caudate nucleus, the olfactory grey matter, and the septum) while the smallest amount comes from structures lining the ventral half of the third ventricle (nuclei of the hypothalamus). Deepening the chloralose anesthesia decreases the amount of ACh released in the effluent. A similar spontaneous and evoked release of ACh from the caudate nucleus of cats both resting and in a state of stimulation of the thalamic nucleus ventralis anterior, has also been described (McLennan, 1964). A relatively extensive investigation of the central release of ACh has been carried out by Mitchell (1963 & 1966), Collier & Mitchell (1966 & 1967), in which the effects; 1) of direct cortical or specific afferent stimulation, 2) of anesthetics, 3) of other drugs (eg. atropine, leptazole), 4) of brain lesions, and 5) of states of consciousness were studied. In all instances the releases of ACh were observed in the presence of either anti-ChE or atropine. Direct stimulation of the cortex or excitation by transcallosal or peripheral stimulation resulted in an increase in the rate of ACh release from the primary somatosensory cortex. A maximal release of ACh per stimulus was seen in sensory nerve stimulation while a minimum release occurred in transcallosal stimulation. Direct cortical excitation was intermediate as to its ACh releasing effect (Mitchell, 1963; Celesia et al., 1966). In cases of lateral geniculate stimulation (unilateral)
and medial geniculate stimulation a marked increase in the release of ACh from the corresponding primary visual and auditory areas of the cortex was observed. Also a widespread but smaller increase from other areas including the contralateral visual area was reported (Collier et al., 1966; Hemsworth et al., 1969). These results lead the authors to the conclusions that there exist two ascending cholinergic systems: 1) the ascending reticular formation which is responsible for the general increase in ACh release and 2) the specific afferent sensory pathway (visual and auditory in these instances) which, in turn, is responsible for localized release of ACh from the stimulated cortex (Kanai et al., 1965). This assumption was later supported by a lesion study in which a vertical lesion which separated the lateral geniculate from midline structures did not alter cortical release of ACh in response to lateral geniculate stimulation; on the other hand, a horizontal section which separated the nucleus from the lower brain centers, affected the release of ACh from the contralateral visual area by stimulation of the lateral geniculate. This operation did not affect the spontaneous release of ACh (Collier et al., 1967). Drugs such as atropine caused an increase in output while chloralose abolished it (Mitchell, 1963; Celesia et al., 1966). Increasing the depth of anesthesia reduced the rate of ACh released (Mitchell, 1966; Celesia et al., 1966; Kanai et al., 1965). Other evidence for the central release of ACh upon nervous activity were provid-
ed by Kanai (1965) & Szerb (1967). A marked increase in ACh output from the cortex upon stimulation of the mesencephalic reticular formation, hypothalamus, medial thalamus and the septum was observed. Atropine completely abolished the EEG arousal evoked by reticular activation without preventing the increase in ACh. This result was interpreted as postsynaptic blockade of the effect of ACh by atropine on the central cholinergic site responsible for EEG arousal.

II. Central Cholinergic Involvement in Behavior
A. Gross Central Effects of Cholinergic Drugs

The administration of large doses of atropine in man (larger than 10 mg) produces a syndrome characteristic generally of the central action of anticholinergic agents. These effects include the impairment of thoughts, attentional changes, disturbances of recent memory (Ostfeld et al., 1959; Ostfeld, 1960; Migdal & Frumin, 1963), drowsiness (Callaway, 1958; Forrer, 1951), non-aggressive excitement, ataxia, delirium, hallucination (Miller, 1956; Forrer, 1956), and increased liveliness and restlessness (Feldberg et al., 1954). Scopolamine, which is more sedative than atropine in certain aspects (Ostfeld et al., 1959), produces excitatory syndromes similar to those seen with large doses of atropine except that a smaller dose of scopolamine is required (Ostfeld et al., 1959). In contrast to these behaviorally excita-
tory effects observed with atropine and scopolamine, a depressant action of ACh and antiChE is observed in animals receiving these drugs intraventricularly, intrathalamically, or systemically. Emmelin et al. (1945) reported that intrahypothalamic administration of ACh, eserine, and prostigmine produced central effects which are opposite to those observed after intravenous injection, i.e. apnea, inhibition of the motility and tone of the gut and bladder and some other effects which are similar to those seen in electrical stimulation of definite hypothalamic regions. They suggested that ACh, eserine, and prostigmine excite hypothalamic cells, which constitute a sympathetic center at this level. By permanently implanting a cannula into the lateral ventricle of the cat, Feldberg & Sherwood (1954a) reported that the intraventricular administration of ACh produced retching, high-pitched phonation and a state resembling an akinetic seizure followed by a condition in which the cat is subdued and appears stuporous (unanesthetized cat). Along this line of observations, Feldberg & Sherwood (1954b) recorded the effects of the antiChEs eserine and diisopropylfluorophosphate (DFP) administered by the same route. They found that three stages of behavior can be obtained after 10–100 micrograms of eserine sulfate or 100 micrograms of DFP. The first stage suggests that the animal suffers severe itching and irritation, the second stage includes changes in gait, and posture, and finally, during the third stage there
is an alteration of awareness, including the development of stupor with signs of "catatonia". Large doses of ACh, by this route, produced one or two convulsions which lasted for about 2 minutes, followed by a deep stupor sometimes reaching the full picture of "catatonia" for a short time. The effects obtained with antiChE were considered to result from inhibition of ChE and accumulation of ACh in the region of periventricular grey matter. Furthermore, the effects such as nightmares, confusion and hallucination observed in myasthenic patients treated with DFP were suggested to be due to a paralyzing action of excess and persisting ACh on structures of the ventricular wall. This paralyzing action of excess ACh can thus account for the similarity of effects produced by this agent and by an anticholinergic drug, atropine (cf. Feldberg, 1963). More recently Zetler (1968), by using various combinations of cholinergic agonists, antagonists, and antidepressants, came to the same conclusion as Feldberg, namely muscarinic stimulation of the central nervous system causes catalepsy.

B. Drinking Behavior

Lesions or stimulation of two locations in the hypothalamus have an overt effect on feeding; first, bilateral lesions in or along the lateral border of the ventromedial nuclei causes hyperphagia and obesity (Hetherington & Ranson, 1942), second, lesions in the same coronal plane, but in the lateral hypothalamic area, cause aphagia and adipsia (Anand and Brobeck, 1951). Electrical
stimulation of the lateral hypothalamus of cats (Delgado & Anand, 1953) increases food intake. Thus the hypothalamus appears to contain a lateral "feeding center" and a medial "satiety center". Application of epinephrine and NE to the lateral hypothalamus increases food intake both in satiated and hungry rats and also causes a decrease in water consumption in hungry rats. In contrast to this, application of ACh (together with eserine to delay its destruction by enzymes in the brain) or carbachol decreases food intake in thirsty rats and increases water intake both in satiated and thirsty animals (Grossman, 1960 & 1962). Atropine and ethomoxane specifically blocked the ACh and NE induced "drinking" and "eating" respectively (Grossman, 1962). In addition, injection of atropine methyl nitrate, which does not readily cross the blood-brain barrier, produces much less reduction in drinking by water-deprived rats than does an injection of atropine sulfate, which readily gets into the brain. Finally injection of eserine into the preoptic area of the brain of a rat very slightly deprived of water will increase the amount of water consumption during the next thirty minutes (Miller, 1965). Therefore a cholinergic component seems to be involved in thirst.

C. Appetitive and Adversive Behavior

Olds (1958) and Olds et al. (1960) demonstrated that electrical stimulation of the medial forebrain bundle (MHB) of the lateral hypothalamus and of the medial hypothalamus would produce
appetitive and advesive behavior in the animals respectively, i.e. the MFB and the medial hypothalamus serve as a reward and a punishment center respectively. Electrical stimulation of the reward center produced pleasure and hence caused the animal to maintain a certain type of behavior such as lever pressing for self-stimulation. On the other hand, stimulation of the punishment center produced the opposite effect, i.e. pain or displeasure for the animal and thus caused the animal to cease to maintain a certain type of behavior or to cease to press the lever thereby terminating the punishment. The electrical stimulation itself in the first case appears to serve as a maximal source of positive reinforcement whereas in the second as a negative reinforcement. Therefore it appears that the effects of administering a positive reinforcement is identical to that of terminating a negative reinforcement. The animal will continue to press a lever for self-stimulation if the maintenance of this behavior will produce reward. Similarly the animal will continue to press a lever if the maintenance of this behavior will prevent punishment. Hence it appears that there are two ways of maintaining a specific type of behavior; either administering a positive reinforcement or terminating a negative reinforcement. More recently it was found that medial hypothalamus stimulation yields ambivalent reinforcement while median forebrain bundle (MFB) yields pure positive reinforcement (Poschel, 1966).
Stark & Boyd (1963), Jung & Boyd (1966), and Domino & Olds (1968) administered various cholinergic drugs including tertiary and quarternary antiChE and anticholinergics to dogs and rats, and found that physostigmine significantly depressed the self-stimulation response rate while neostigmine which does not penetrate the blood-brain barrier did not demonstrate such effect. Furthermore the inhibitory action of physostigmine was blocked by the prior administration of atropine capable of penetrating the blood-brain barrier but not by methyl-atropine which is presumed incapable of penetrating the blood-brain barrier. These findings lead to the speculation that there exists in the brain a cholinergic system which can inhibit intracranial self-stimulation in animals with electrodes at various loci (Jung et al., 1966; Domino & Olds, 1968) and which also inhibits various types of behavior (Carlton, 1963). These findings were confirmed by Olds & Domino (1969) in their later experiment on the differential effects of cholinergic agonists on self-stimulation and escape behavior. They found that escape behavior is not depressed by cholinergic agonists in doses which produce a profound depression of self-stimulation behavior. Finally it may be pertinent to mention that in contrast to the inhibitory effect of cholinergic mechanism in the reward system, Poschel & Ninteman (1963 & 1966), Stein (1964a), and Stein et al. (1967; 1969 & 1970) have recently proposed a possible excitatory effect of NE in the same system. They
found that self-stimulation behavior was greatly facilitated by the administration of combination of a monoamine oxidase (MAO) inhibitor; tranylcypromine, and a catecholamine depleter, alpha-methyl-meta-tyrosine. Similarly blockade of NE biosynthesis caused a marked suppression of the self-stimulation behavior while administration of methamphetamine reinstated this behavior (Poschel & Ninteman, 1963 & 1966).

D. Adversive Circling Syndrome

It is a well-known phenomenon that unilateral intracarotid injections of DFP in cats, dogs, rabbits, and monkeys will induce contraversive circling, i.e. turning away from the injection side, unless the dose is too high, in which case convulsions occur. Among DFP treated animals both circling and non-circling subjects showed a profound and precipitous decrease in ChE activity in the caudate nucleus and the cortex on the injected side (Essig et al., 1950; Harwood, 1954). Slightly greater doses of DFP than that necessary to produce circling behavior will cause generalized convulsions associated with a state of very low ChE activity in both hemispheres. The circling and convulsions were both blocked by the administration of atropine and scopolamine and thus established a cholinergic origin for the forced circling behavior (Essig et al., 1950). Later Aprison et al. (1954) demonstrated that although forced turning away from the injected right side ("lefters") is the pattern usually observed, reversion of the direction of turning sometimes occurred, i.e. toward the inject-
ed side ("righters"), and in some cases the animal does not exhibit any compulsory behavior pattern ("neutrals"). A characteristic AChE pattern in the cortex and caudate nucleus associated with each behavioral response was obtained (Aprison et al., 1954 & 1956). In all instances of circling the decrease in AChE activity was very much greater on the right side of the brain than on the left irrespective of the direction of circling. No such asymmetry of enzyme activity was observed, however, in the "neutrals". A similar cholinergic involvement in circus movement has been demonstrated in White's (1956) experiment in which persistent contraversive turning was found to occur in response to an intracerebral injection of DFP directly into the caudate nucleus in doses which decrease ChE activity there to 20–40% of normal. A local application of atropine corrected this forced circus movement. This cholinergic involvement was further confirmed by Aprison et al. (1956) by injecting ACh and methacholine (mecholyl) into the right carotid artery producing animals which turned to the right.

E. Aggressive Behavior

Aggression is a very complex behavioral syndrome. There are several types of aggression which are often defined situationally. A drug which is effective in antagonizing one type of aggression may not be effective for another (Loia, 1969). Tedeschi (1959) described a type of aggression which is induced by exposing a pair of mice to a mild but continuous electric
foot-shock (electric shock-induced fighting). Brady and Nauta (1953) described another type of aggression elicited in rats by lateral lesioning of the septal area of the brain ("septal rats"). Still another type of aggression was found to exist in rats when a mouse was introduced into the rat's cage and the rats almost immediately killed the mouse by biting the animal through the cervical cord ("killer rat", Horovitz et al., 1965). In our laboratory a type of aggression was found to present in the various genera and strain of mice when they were placed into a semi-natural field condition, i.e. the "Mouse City" (Scudder et al., 1969). Finally, there is a type of aggression which is induced in standard albino mice by prolong isolation (Valzelli et al., 1967, Valzelli, 1969). Therefore when one speaks about aggression one must qualify and delineate its nature since there seem to be different types. Janssen et al. (1960) and DaVanzo et al. (1965 & 1966) demonstrated that scopolamineHBr and other anticholinergic agents were effective blockers of isolation-induced aggressive behavior. Related observations were reported by Karczmar & Scudder (1969a) that administration of several doses of the anticholinergic agent scopolamine or of the anticholinesterase physostigmine produced biphasic, dose-dependent effects. At relatively small doses (0.01-0.05 mg/Kg), physostigmine increased aggression. Scopolamine, at low doses increased, and high doses decreased aggression (0.3-1.0 and 2-10 mg/Kg), respectively. Methionine sulfoximine, a cholinergic drug which produces a build
up of ACh leading ultimately to convulsions, exhibited biphasic
actions; it produced a marked block of aggression occurring after
a 4-hour delay. From these data, it was suggested that ACh is
cconcerned with certain inhibitory mechanisms (Karczmar et al.,
1968a), i.e. cholinergic block may induce disinhibition and
fighting.

F. Thermoregulation

The hypothesis that a cholinergic, atropine-sensitive link
is involved in the central mechanism controlling dissipation and
decreased production of body heat has been suggested by investi­
gations with muscarinic agonists and with antagonists in mice,
rats, and man (Everett, 1956; Henderson & Wilson, 1936; Lomax &
Jenden, 1966; Spencer, 1965; Kirkpatrick & Lomax, 1967; Zetler,
1968; Friedman & Jaffe, 1969). It has been found that mice and
rats responded to tremorine, a cholinomimetic, parkinson inducing
drug, by a profound fall in body temperature (Everett, 1956).
Micoinjection of carbachol or oxotremorine into the anterior
hypothalamic preoptic area of rats will rapidly reduce rectal
temperature by several degrees centigrade (Lomax & Jensen, 1966).
These cholinergic hypothermic effects are antagonized by systemic
atropine and scopolamine but not by quarternary muscarinic block­
ing agents methyl atropine and methscopolamine (Spencer, 1965;
Friedman & Jaffe, 1969). Similar cholinergic hypothermic effects
were obtained in Miller's study in which carbachol was adminis­
tered to the anterior hypothalamus in cat via a double-cannula
system implanted in the cat brain (Miller, 1965). It should be pointed out, however, that in opposition to this hypothesis a thermogenic action for ACh has also been postulated by Dutta (1948) in mice and by Meyers & Yaksh (1968 & 1969) in rats and monkeys.

g. Wakefulness and Sleep

Much data has supported the hypothesis that cholinergic mechanisms are involved in states of wakefulness and sleep. Rinaldi & Himwich (1955a & 1955b) and Longo (1955) demonstrated that intracarotid administration of ACh produced EEG arousal response in the rabbits, diminished the voltage and increased the frequency of the EEG activity. AntiChE, physostigmine and DFP, produced the same effect (EEG activating pattern) in the experimental animals (Bradley & Elkes, 1953; Wescoe et al., 1948). Both the EEG alerting response induced by ACh or antiChE agents were blocked by atropine (Rinaldi & Himwich, 1955a & 1955b; Wescoe et al., 1948). Conversely, the EEG picture of synchronization caused by atropine or scopolamine was antagonized by the administration of eserine (Bradley & Elkes, 1953; cf. Longo, 1966). By using the technique of brain stem sections at different levels, together with various pharmacological analysis, Rinaldi & Himwich (1955a & b) and Illyuchenok (1962) were able to show that ACh, eserine and arecoline still produced an arousal reaction in cerveau isolé preparation but not in a preparation which was cut at a line connecting the front edge of the corpora quadrigemina and
touching the brain base behind the corpus mamillare (Illyuchenok, 1962) or in an isolated hemisphere preparation in which the cerebral cortex is deprived of all its neuronal connections with the rest of the brain while maintaining its vascular connections (Rinaldi & Himwich, 1955b). These evidences lead Rinaldi & Himwich (1955a & b) to postulate that the alerting reaction is produced by activating those mesodiencephalic structures with diffuse projections over the entire cortex, such as the midbrain reticular formation of Moruzzi and Magoun and the thalamic diffuse projection system of Jasper. Indeed the latter system has been later proved to be the final corticopetal link with a cholinergic function both from EEG analysis and histochemical studies (Cuculic, Himwich, 1968; Krnjevic & Silver, 1963 & 1965a). However, this does not preclude the existence of adrenergic synapses in the same system as postulated by Rothballer (1956) and Illyuchenok (1962), (also cf. Longo et al., 1957). Recently Domino, Yamamoto & Dren (1968) offered interesting data on the muscarinic and nicotinic nature of the cholinergic mechanisms involved in wakefulness and sleep. They studied the effects of various muscarinic and nicotinic cholinergic agonists and antagonists on the awake-sleep cycle of cats with indwelling brain electrodes in various neocortical and limbic areas. They found that atropine pre-treatment blocked EEG activation induced by ACh, arecoline, pilocarpine, and physostigmine, but only reduced that produced by 1,1-dimethyl-4-phenyl-piperidinium iodide (DMPP) and nicotine.
Atropine also blocked nicotine induced hippocampal theta wave activity. Methyl atropine, a muscarinic cholinergic antagonist with predominant peripheral effects, markedly antagonized EEG activation by ACh, but did not block EEG activation induced by other muscarinic or nicotinic cholinergic agonists. The nicotinic ganglionic cholinergic antagonists, mecamylamine and trimethidinium, had no significant effects on EEG activation induced by muscarinic cholinergic agonists while the actions of nicotinic cholinergic agonists such DMPP and nicotine were completely blocked by mecamylamine. Trimethidinium blocked EEG activation of DMPP but reduced only slightly that of nicotine. Moreover hemicholinium (HC-3), a drug which decreases ACh synthesis by interfering with choline transport, produce initially in acute dog preparations spiking in the amygdala and a blockade of the hippocampal theta wave activity without affecting neocortical activation when given intraventricularly in total doses up to 5 mg. Eventually neocortical slow waves appeared. Exogenous choline produced a delayed and transient reversal of the HC-3 effects. Arecoline, pilocarpine, and physostigmine caused EEG activation following HC-3, whereas nicotine, epinephrine and d-amphetamine were either much less effective or their EEG actions were completely blocked.

In addition to arousal, cholinergic mechanisms are also found to be involved with sleep in a limbic forebrain-limbic midbrain hypnogenic circuit (Hernández-Peón & Chavez-Ibarra, 1963; Her-
Evidence has accumulated that sleep is not the result of a passive deactivation of the neuronal structures responsible for wakefulness, but, instead, sleep is induced by an active inhibitory process which requires the activity of specific hypnogonic structures. By using both permanently implanted cannulae and a simultaneously recording electrode in cats, Hernández-Peón identified a sleep system which consists of 2 components: 1) a descending component with corticofugal projections from the pyriform cortex, the orbital surface of the frontal lobe and the anterior part of the gyrus cinguli which converge upon the limbic midbrain circuit extending down to the ponto-mesencephalic tegmentum (also cf. Akert, 1965), and 2) an ascending component which, originating in the spinal cord, joins the descending component at the pontine level. Another important hypnogenic area is the thalamic regions lateral to the massa intermedia (Hess, 1965; Hernández-Peón, 1965; cf. Akert, 1965). Local application of ACh alone, ACh plus eserine, or carbamylcholine on the aforementioned hypnogenic areas produced the typical behavioral and electrophysiographic manifestations of both the synchronized and the desynchronized stages of sleep identical to those effects produced by the electrical stimulation of the same regions (Hernández-Peón et al., 1963a & b, Hernández-Peón, 1965). The antiChE eserine, alone, when applied to the hypnogenic preoptic region induced sleep, the muscarinic anti-
cholinergic agent, atropine, produced a state of alertness accompanied by the typical persistent EEG desynchronization and high voltage "arousal" discharge. Moreover, when atropine was applied locally to caudal segments of the limbic midbrain hypnogenic circuit, cholinergic stimulation of a previously activated hypnogenic point in the preoptic region became ineffective in inducing sleep. Similar observations were obtained with electrolytic lesion studies. These findings lend support to the hypothesis that atropine blocked the action of ACh normally released at presynaptic terminals of hypnogenic neurons along the limbic midbrain hypnogenic pathway and that the activity along that neuronal system is transmitted from the forebrain down to the midbrain (Hernández-Peón, 1965; Velluti et al., 1963). However, it should be pointed out that this hypothesis is in contrast to that of Jouvet (1967) in which no discrete but rather a continuous hypnogenic mechanism, presiding over the periodic succession of the states of sleep, was stressed (also cf. Karczmar et al., 1970).

Finally, an important and frequently mentioned phenomenon of antiChE and cholinolytic agents on EEG and behavior has to be pointed out. Although close correlation between the EEG and behavioral manifestations has been found both in man and in lower mammals, i.e. low voltage fast activity is usually associated with wakefulness and high voltage slow waves are commonly associated with sleep, exceptions to this relationship may occur. Wikler (1952) reported that atropinized dogs displayed high vol-
tage slow waves and "spindle burst" EEG activity regardless of whether concomitantly the animals were alert or drowsy behaviorally. This EEG and behavior "dissociation" or "divorced" phenomenon was later confirmed by many investigators (Longo, 1956; Bradley & Elkes, 1953; also cf. Long, 1966). In light of the results obtained in animals prepared with chronically implanted electrodes and trained to perform various kinds of learned task, Longo (1966) suggested that the EEG modifications induced by anti-cholinergic drugs correspond to alterations of "behavior-related" systems subserving learning, perception, and memory rather than to changes in gross motor performance or to the induction of sleep and wakefulness.

H. Conditioning, Learning, and Retention

The effects of cholinergic drugs, on conditioning, learning, and retention is an area of active research. Numerous results have been reported during the last 20 years. However, due to the difficulties of research in this field, originating from the confounding of effects of individual differences in the reactions of the animal even of the same age, breed, and sex, and of techniques and of dosages used, entirely different results to the same drug, often the same dose, occur according to the different CNS states of the animals. In spite of these difficulties, some generalities about the cholinergic drug effects on spontaneous activity, conditioning, learning and retention can still be drawn. Rats when allowed two consecutive, unrewarded runs in a T-maze...
typically alternate their choice between the arms of the T-maze ("spontaneous alteration", cf. Meyers & Domino, 1964). This spontaneous alteration has been demonstrated to be blocked by the administration of cholinergic blocking drugs, atropine and scopolamine (Meyers & Domino, 1964; Parkes, 1965). Parkes (1965) suggested that scopolamine and related agents exert their effects through interference with the organization of sensory information which normally familiarizes an animal with a situation as a result of inspection. In addition to suppression of spontaneous alternation, another kind of unlearned behavior has also been reported to be affected by cholinergic blocking agents. Tapp (1965) reported that animals after atropine treatment showed a significant increase of gross activity level in an open-field test and also an increase in spontaneous response rate in operant bar pressing.

The effects of cholinomimetics on avoidance conditioning is somewhat controversial. While Pfeiffer & Jenney (1957), Bureš et al. (1965) demonstrated that arecoline, pilocarpine, eserine and related antiChE agents exert inhibitory effects on avoidance conditioning, Dilts & Berry (1967) and Russell et al. (1961) reported no significant effects of pilocarpine, arecoline, and antiChE on avoidance conditioning. An enhancing effect of eserine has been shown by Stratton et al. (1963) and Russell (1954), (also cf. Carlton, 1963). Recently Banks & Russell (1967), by using serial problem-solving situations, found that the impairment of serial problem-solving behavior induced by systox (00-diethyl-S-ethyl
mercaptoethanol thiophosphate) occurred only after chronic re-
duction of AChE activity below a critical level of 40-60% of the
enzyme's normal activity. Furthermore by utilizing a one trial
passive avoidance technique Bureš et al. (1962 & 1964) discovered
that physostigmine completely suppressed the acquisition and re-
trieval of a passive avoidance reaction without exerting any ad-
versive effect (0.5 mg/Kg) on an overlearned either passive or
active avoidance reaction. These overlearned avoidance reactions
were only partly impaired by a high dose of physostigmine (1 mg
/Kg). While there are controversial results obtained after treat-
ment with pilocarpine, arecoline and antiChE agents, another cho-
linomimetic, nicotine, seems to produce clear facilitating effects
on learning in rats. This can be seen in the avoidance condition-
ing experiment in which nicotine treated animals produced a signi-
ficantly higher performance than the control animals. This faci-
litation effect is even more evident when the control and the
treated groups are compared on the basis of a series of learning
criteria of increasing difficulty (Bovet & Gatti, 1965). However,
it should be noted that this effect of nicotine on rats does not
hold for mice in which only poor learners were found to be faci-
litated, while good learners were impaired by nicotine (Bovet et
al., 1966).

As far as the acquisition and retention of conditioned res-
penses is concerned, anticholinergic agents seem to yield more
generalized data than those obtained after cholinomimetic treat-
First of all, the administration of cholinergic blocking drugs significantly impair instrumental and operant reward conditioning and maze performance. Hernstein (1958) demonstrated that rats trained on a 4-ply multiple schedule, consisting of a food-reinforcement component, an avoidance component, and two other components during which responses had no explicitly programmed consequence, showed a disruption of discrimination after a range of doses of scopolamine (0.05 mg/Kg - 0.8 mg/Kg). Later Hearst (1959) reported that the administration of scopolamine in rats trained to make a different lever response to each of two auditory stimuli in order to obtain a water reward, resulted in an increase in number of incorrect responses to the stimuli, a large increase in the number of lever-presses in the silent periods, and an increased tendency for subjects to make successive responses on the same lever rather than to alternate responses between the two (behavioral perseveration). A disruption of the extinction process after scopolamine has also been observed. Similar results of anticholinergic drug administration were obtained by Boren et al. (1959) upon fixed-interval and fixed-ratio behavior in a multiple schedule. A decrease in the ability of the rats to solve the maze problem after cholinergic blocking agents has also been reported by Domer et al. (1960), Sadowski et al. (1962) and McGaugh et al. (1963), by training rabbits to pull a ring with the mouth when a buzzer sounded in order to obtain a piece of food found that scopolamine at small doses (0.025 - 0.05
0.1 mg/Kg) abolished the instrumental reward conditioned response and also blocked discrimination to a conditioned stimulus from a discrimination stimulus (non-rewarded). Carlton (1961 & 1963) also reported that atropine and scopolamine caused a disruption of the operant reward conditioning when rats were trained to alternate lever pressing or to discriminate multiple choice. In experiments involving avoidance conditioning, cholinergic blocking agents again seem to exert adverse effects, although not so frequently as seen in those of the instrumental reward conditioning. A decrease in avoidance conditioning is sometimes observed but more frequently it is unchanged. In most cases the drugs augmented response rate. This has been attributed to the increase of general activity, to disruption of timing behavior, or to changes in aversion level. Meyers et al. (1964) reported that in active avoidance conditioning, scopolamine and atropine retarded acquisition at low doses, while virtually abolished it at higher doses. These effects, however, were not observed with methyl atropine and methyl scopolamine treatment (peripherally active drugs). An increase in spontaneous activity and no effect on retention by either atropine, scopolamine or peripherally acting cholinergic blocking agents have also been obtained. The authors (Meyers et al., 1964) suggested that a deficit of recent memory might account for the disruption of the acquisition, but not retention. A disruption of performance after cholinergic blocking agents was also described by Morpurgo (1965) in a three-
chambered discrimination box using rats. Studies done on monkeys revealed the same finding (Samuel et al., 1965).

Another important feature of cholinergic blocking agents is the marked impairment of learning in animals administered these drugs. Ricci et al. (1965) in their studies of EEG correlates of avoidance conditioning in the monkey demonstrated that animals treated with atropine exhibited a clearcut impairment of the acquisition of a new conditioned response as well as disruption of the performance of a well-conditioned response. A series of studies on passive avoidance conditioning also confirmed these data (Burešová et al., 1964; Bureš et al., 1964; Meyers, 1965; Dilts & Berry, 1965 & 1967). In addition to disrupting learning, Bureš (1964) and Burešová (1964) further demonstrated that atropine lengthened the extinction period of a previous learned task, affected adversely retrieval of liminal (threshold conditioned) reactions but not those of over-learned response. A hypothesis was proposed for passive avoidance tasks by Meyers (1965) that learning is normally "recorded" in cholinergic systems, but, in the presence of a cholinergic blockade, the memory trace can also be stored in non-cholinergic systems (also see Burešová et al., 1964; Bureš et al., 1964; Ricci & Zamparo, 1965). Another hypothesis concerning behavior and cholinergic mechanism was advanced by Carlton (1963 & 1968), who suggested that the neurotransmitter ACh mediates the effects of non-reward, that is, attenuation of cholinergic function is correlated with the attenuation of the
normal consequences of non-reinforcement; and blockade of the central cholinergic system will thence result in a release of normally inhibited, non-reinforced responses. Generally under normal conditions when an animal is subjected to a stimulus or stimuli which resulted in non-reward responses, the animal will eventually respond to the stimulus or stimuli by no response after several such trials. And it is in this process that ACh has been suggested to be implicated. Therefore, in such a sense, ACh acts as inhibitory substance which inhibits the occurrence of non-reward responses, or similarly acts by selecting or channeling the firing of certain central neuronal circuitry which in turn is responsible for the appearance of certain specific behavior observed. He also suggested that these inhibitory cholinergic systems are in reciprocal balance with adrenergic systems which are excitatory behaviorally. While this hypothesis seems to fit fairly well in many experimental situations, negative data do occur and it has been criticized by Longo (1966) and by Karczmar (1970).
SECTION I

INTERGENERIC WHOLE BRAIN LEVELS OF CHOLINESTERASE IN
SIX GENERA AND THIRTEEN STRAINS OF MICE
Chapter II. Introduction

A. Evidence for a Genetic Predisposition of Brain ChE Level and for its Relationship with Behavior

The concept of a biochemical correlate of behavior has been well documented (Rosenzweig et al., 1958 & 1960; Russell, 1964 & 1966). This correlation is not only due to a genetic factor but also results from various conditions coincident with experimental manipulations within the same species or strain of animals. In cases of genetic differences, Bennett et al. (1958a & b), Krech et al. (1959), Bennett et al. (1960) & Roderick (1960) have successfully demonstrated that significant differences in cortical and subcortical ChE and ACh activities were observed between two different strains of mice, and a relationship between the level of ChE activity in rat cortex and adaptive behavior was also indicated (Krech et al., 1954; Rosenzweig et al., 1958). Bennett et al. (1960) further reported that the genetic mechanisms controlling ChE activity and ACh concentration in the rat brain were independent of each other. This phenotypic variation in brain ChE activity in various strains of mice was also reported by Broadhurst et al. (1964), Pryor (1968), and Al-Ani et al. (1970). A series of studies have been carried out in this laboratory in which different genera and strains of mice, which differ widely in ecological and behavioral profiles, have been studied. They have been found to be characterized by distinctive biochemical, pharmacological, as well as neurophysiological patterns (Scudder...
et al., 1966a & c; Karczmar et al., 1968; Sobotka et al., 1968; Scudder et al., 1969a & b; Karczmar et al., 1969a). It was suggested that mouse types requiring mother protection and mother defense, which exhibit good exploration and motor activity, and which are good learners, may be associated with high aggression or at least with a capacity for aggression in certain conditions. Moreover, the early experience in the wild or inherited characteristics of certain strains may play an important role in limiting this tendency in many conditions. In addition to these behavior patterns, various neurochemical parameters have also been measured and correlated with various behavior profiles wherever possible. It has been found that ACh levels in the whole brains of the various genera are directly proportional to the amine and serotonin levels; and an optimum level of ACh activity is necessary for the maximum expression of certain behavior patterns such as exploration, aggression, as well as learning. Either increasing or decreasing the ACh levels resulted in disruption of these behaviors (Sobotka, 1969). Bovet et al. (1966) in their study on the effects of nicotine on avoidance conditioning of inbred strains of mice found that distinctly different learning curves existed among mice of different strains. This genetic attribution in animal behavior was also demonstrated by the fact that nicotine facilitated learning in slow learners and impaired learning in good learners. Lindzey (1951) also described a genetic difference in five inbred strains of mice with regard to emotion-
ality and audiogenic seizure susceptibility.

B. Genetic Correlations of Biogenic Amines and Other Substances with Behavior

In addition to ChE, other enzymes and biologically active substances such as 5-hydroxytryptamine (5-HT or serotonin), norepinephrine and dopamine have also been studied in relation to genetic variation. Mass (1962 & 1963) described a relationship between 5-HT and fearful and exploratory behavior as well as motor activity within two strains of mice. Sudak & Mass (1964) found a significant negative correlation between serotonin values in the limbic portion of the brain and ambulation scores evaluated in the open-field test in reactive and non-reactive strains of rats. Schlesinger et al. (1965) also demonstrated a negative correlation between brain serotonin and norepinephrine and audiogenic seizure susceptibility among three strains of mice. Scudder and his associates in their studies of biochemical correlates of behavior further offered a possible correlation between biogenic amine levels and various types of animal behavior. They suggested that strains with high brain norepinephrine and/or serotonin levels were those with long convulsive latencies and also with stereotyped, repetitive, and catatonic states of behavior, and vice versa, i.e. animals with short latencies have lower amine levels and a lesser degree of inhibition, leading to a greater behavioral variability and to rapid homeostatic adjustment to repeated environmental stimuli (Scudder et al., 1966a; Bourgault et
Chapter III. Material and Methods

A. Animals

The mice used in this study were as follows: four strains of *Mus*: *Mus musculus* C57BL/6J, *Mus musculus* CF1, *Mus musculus* SKL, and *Mus musculus* "Missouri", *Microtus ochrogaster*, *Onychomys leucogaster*, *Peromyscus maniculatus* Bairdii; *Reithrodontomys raviventris* raviventris; two strains of *Dipodomys*: *Dipodomys merriami*, and *Dipodomys deserti*. All the mice were adult, male mice. Ten animals per genus or strain were studied in this experiment unless otherwise specified.

1. Sources

The inbred *Mus musculus* C57BL/6J and *Peromyscus maniculatus* Bairdii were obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, while CF1 mice were purchased from Carworth Farm, SKL from Scientific Small Animal Feeds in Arlington Height, Chicago. *Mus musculus* "Missouri", *Microtus* and *Onychomys* mice were live-trapped by collectors, *Microtus* and *Mus* in the field in the vicinity of Columbia, Mo., and *Onychomys* in the arid area of Tucson, Arizona*. The trapping period lasted for some 2 weeks; as soon as trapped, the males were isolated from females. *Reithrodontomys raviventris* raviventris were also trapped by collectors.

* We are indebted to D.M. Cameron, Jr., Dept. of Zoology, Univ. of California, Davis, Calif., for taxonomic identification of these strains from skulls and pelts which we provided.
they were found in the salt marshes of the San Francisco Bay area. Dipodomys merriami and Dipodomys deserti were purchased from Pet Coral, Tucson, Arizona. All the mice were shipped to this laboratory by air express. Upon arrival, the mice were paired off and treated routinely for parasites. Following this two week post-reception period, they were placed, still mated, in an environment room under controlled conditions of temperature (23.9° ± 2.8° C) and humidity, and a 14-hour light cycle running from 6:00 A. M. to 8:00 P.M. They were housed, two per cage, with sawdust on the cage floor. Regular laboratory mouse pellets were provided in hampers, water was supplied ad libitum. The cages were cleaned weekly. All the animals were accommodated to the animal room for at least 2 weeks prior to the beginning of the experiments.

2. Phylogenetic Description

Phylogenetically, Onychomys, Peromyscus and Reithrodontomys are closely related genera, they belong to the same subfamily, Cricetinae (Hall, 1902). Microtus is a near relative of the above genera, also of the family Cricetidae. Mus and Dipodomys, on the other hand, are distantly related genera, belonging to the Muridae and Heteromyidae respectively. All of the above genera are mammals of the order, Rodentia and the sub-orders, Sciuromorpha (Dipodomys) and Myomorpha (Onychomys, Peromyscus, Reithrodontomys & Microtus).
3. Ecological Diversification and Specialization

Mus musculus C57BL/6J and Mus musculus CFl are highly inbred strains. Peromyscus maniculatus Bairdii, the white footed-deer mouse, timid in field conditions (Brant & Kavanau, 1964), is a grassland animal (Getz, 1965) and a seed eater found in the Northern and Central United States. Mus musculus "Missouri" is a grassland and wild strain from Columbia, Missouri, and omnivorous. Microtus, the meadow vole, is also a grassland or prairie genus (Harris, 1952), herbivorous, relatively heavy and fearless (Scudder, Karczmar, & Lockett, 1967). Onychomys, the grasshopper mouse (Bailey et al., 1929), is a desert form, and can be herbivorous or omnivorous depending on conditions (Hall, 1902); both use the ground runways of other rodents and are desert animals found in Southern and Western United States. Reithrodontomys is a small herbivorous mouse (Walker, 1964, vol.2), very specialized ecologically. It lives in salty swamps within a rather warm climatic zone and is nocturnal and active throughout the year. Mus musculus SC1, the laboratory white mouse, is essentially a separately bred strain derived from Mus musculus CFl (Scudder et al., 1966a & c).

B. Dissection Procedure

For whole brain samples, the animals were decapitated (at approximately 9:30 A.M.) with a pair of scissors with a minimum of handling so as to minimize any excitement that might occur in the animals. The blood was then washed off the head and the skin
was peeled back to expose the bare skull. Two parallel incisions were then made with a pair of small curved scissors, starting at the open end of the skull (pons-medulla region) and cutting anteriorly to a point between the eyes and just ahead of the olfactory lobes. The top of the cranium was then lifted and the brains were exposed and gently pushed out with a spatula after severing the cranial nerves. The removed brains were immediately dropped into liquid nitrogen for the subsequent biochemical analysis. The elapsed time from decapitation until freezing was roughly 20-25 seconds.

C. Biochemical Analysis

The method employed for the determination of brain ChE activity is that of Tammelin and Strindberg (1952) in which an automatic titration procedure is performed in a Radiometer "Titrator" and "Titrigraph". This technique has also been used by Jensen-Holm et al. (1959), Delaunois (1962), Nabb and Whitefield (1967) and many other investigators. Essentially the principle is to titrate the amount of acetic acid liberated from the substrate ACh during the process of enzyme hydrolysis against a certain amount of known concentration of alkali solution (NaOH 0.025N). The enzyme activity which is a measure of the rate of hydrolysis, is estimated at 38° C and pH=7.40, being expressed as micromoles per gram tissue per minute. Since the substrate used in this thesis was ACh, the enzyme activities measured were that of both AChE and pseudocholinesterase.
Immediately after the brain was removed from liquid nitrogen, it was weighed and homogenized in a saline solution (0.9% NaCl solution) at a homogenate concentration of 10% by weight. The homogenate was kept in an ice bath and stored in the refrigerator during most of the experiment. 0.6 ml of the homogenate was then pipetted into 12.9 ml of the saline-MgCl₂ solution (0.073 gram MgCl₂ in 100 ml saline solution) which was pre-warmed to 38°C in the reaction vessel. The stirring motor was switched on and a continuous flow of nitrogen gas, at a pressure of 10 psi, was blown over the surface of the reaction mixture during the whole period of titration procedure to exclude any acid formation from carbon dioxide in the air (Ballantyne, 1968a). The reaction mixture was allowed to run and titrate for ten minutes to serve as a control. At the end of this period, 1.5 ml of 10⁻¹ M ACh solution (Merck 100 mg ACh chloride per ampoule in 5.5 ml distilled water or Sigma 150 mg ACh chloride per vial in 8.26 ml distilled water) was injected into the reaction mixture. The substrate solution was prepared freshly and was kept in an ice bath throughout the experiment. After the addition of the substrate, the reaction mixture was then allowed to run for at least 20 minutes in order to obtain a suitable length of curve. From this curve five measurements of the slope were obtained. The enzyme activity was derived from the amount of NaOH used according to the following equation:
\[ \text{ChE} = \frac{\text{(number of milliliters of NaOH used)}}{\text{(titration time in min.)}} \times \frac{\text{(number of micromoles of NaOH/ml)}}{\text{(weight of tissue in solution)}} \]

The five slope measurements, each over a three-minute interval, were determined in order to obtain the slope value with maximum accuracy. The mean of these five measurements was then taken as the final value of enzyme activity for that specific enzyme determination. A duplicate study was carried out for every brain. Since the curve was almost linear, the variation between consecutive slopes from any experiment was slight.

Chapter IV. Results

A. Species and Strain Differences in Brain ChE Activity

The differences in total brain ChE activity among the various genera and strains of mice studied are given in Table I and Fig.I. All values are obtained from ten animals per genus or strain of mice (duplicate readings were obtained for each animal) with the exception of three strains of Peromyscus, i.e. Peromyscus maniculatus "Colorado", Peromyscus maniculatus gracillis and Peromyscus leucopus in which only one animal per strain was studied (pilot study). The results show that up to more than six-fold differences in total brain ChE activity were observed among the various genera and strains studied. Mus musculus "Missouri" occupies the highest and Dipodomys deserti occupies the lowest position. The genera may be divided into three groups, i.e. the high, the intermediate, and the low brain ChE activity groups. Reithrodontomys raviventris raviventris, Peromyscus maniculatus Bairdii, Mus
musculus "Missouri", Mus musculus SCl, Mus musculus CF1 and Mus
crusculus C57BL/6J belong to the high enzyme activity group. Within
the genus of Mus, there are essentially no significant differ-
ences among the four strains of mice studied although Mus musculus
"Missouri" does show the highest activity. Onychomys leucogaster,
Peromyscus maniculatus "Colorado", Peromyscus leucopus, Peromys-
cus maniculatus gracillis, Microtus ochrogaster are among the
intermediate group. Again, there are no significant differences
in brain ChE activity among the various strains of Peromyscus in
question with the exception of Peromyscus maniculatus Bairdii
which is ranked among one of the high enzyme activity group.
Finally the two strains of Dipodomys, i.e. Dipodomys merriami and
Dipodomys deserti, give the lowest enzyme activity and also showed no significant difference between the species. The overall
pattern of enzyme activity seems to indicate clear-cut inter-
generic differences without any significant intrageneric variation.
B. Intergeneric Comparisons of Brain Weights and Body Weights

The mean and standard error of whole brain weights as well
as of body weights of the various genera and strains of mice are
also given in Table I and Figures II & III. For the whole brain
weights the two strains of Dipodomys, Dipodomys deserti and Dipo-
domys merriami, have the heaviest brains, Microtus ochrogaster,
Onychomys leucogaster and two strains of Peromyscus; Peromyscus
maniculatus gracillis and Peromyscus leucopus have medium brain
weight, while the four strains of Mus; Mus musculus SCl, Mus
Peromyscus maniculatus "Colorado" as well as Reithrodontomys raviventris have the lightest brains. For body weight, the sequences of decreasing order of magnitude are: Dipodomys deserti, Dipodomys merriami, Microtus ochrogaster, Mus musculus SC1, Mus musculus CF1, Onychomys leucogaster, Mus musculus C57BL/6J, Peromyscus maniculatus gracillis, Peromyscus leucopus, Peromyscus maniculatus Bairdii, Peromyscus maniculatus "Colorado", Mus musculus "Missouri", Reithrodontomys raviventris raviventris.

Chapter V. Discussion

A. Genetic Variation in Brain ChE Activity and Behavior

The results of these studies point up the problem of the difficulties found when correlating specific behavior with biochemical systems. While these studies successfully demonstrate the differences in certain substances such as brain ChE activities in different genera and strains of mice, they fail to demonstrate definite correlation between these enzymes and various types of naturally as well as artificially induced behavior. This is especially true when one considers together all the possible bioactive substances such as ACh, norepinephrine, serotonin, DOPA and dopamine etc. (Sobotka, 1969; Bourgault et al., 1963; Scudder et al., 1966a). There is practically no unitary pattern
<table>
<thead>
<tr>
<th>Type of Mouse</th>
<th>ChE Activity Mean ± S.E.</th>
<th>Whole Brain Weight Mean (gm) ± S.E.</th>
<th>Body Weight Mean (gm) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mus m. C57BL/6J</td>
<td>25.40 ± 1.06</td>
<td>0.45 ± 0.01</td>
<td>27.4 ± 1.0</td>
</tr>
<tr>
<td>Mus m. CFL</td>
<td>28.31 ± 0.93</td>
<td>0.49 ± 0.01</td>
<td>35.0 ± 1.1</td>
</tr>
<tr>
<td>Mus m. &quot;Missouri&quot;</td>
<td>34.05 ± 0.98</td>
<td>0.42 ± 0.01</td>
<td>19.7 ± 1.5</td>
</tr>
<tr>
<td>Mus m. SCl</td>
<td>28.89 ± 1.25</td>
<td>0.48 ± 0.01</td>
<td>36.0 ± 1.4</td>
</tr>
<tr>
<td>Microtus ochrogaster</td>
<td>16.03 ± 0.61</td>
<td>0.68 ± 0.02</td>
<td>39.8 ± 1.8</td>
</tr>
<tr>
<td>Onychomys leucogaster</td>
<td>19.20 ± 0.46</td>
<td>0.60 ± 0.01</td>
<td>29.9 ± 1.5</td>
</tr>
<tr>
<td>Peromyscus maniculatus Bairdi</td>
<td>26.85 ± 1.45</td>
<td>0.46 ± 0.01</td>
<td>20.0 ± 1.3</td>
</tr>
<tr>
<td>Peromyscus m. &quot;Colorado&quot;</td>
<td>19.00</td>
<td>0.54</td>
<td>18.0</td>
</tr>
<tr>
<td>Peromyscus m. gracill</td>
<td>17.00</td>
<td>0.69</td>
<td>26.0</td>
</tr>
<tr>
<td>Peromyscus leucopus</td>
<td>17.43</td>
<td>0.61</td>
<td>22.0</td>
</tr>
<tr>
<td>Reithrodontomys r. raviventris</td>
<td>32.64 ± 0.91</td>
<td>0.42 ± 0.01</td>
<td>11.9 ± 0.5</td>
</tr>
<tr>
<td>Dipodomys deserti</td>
<td>5.40 ± 0.23</td>
<td>1.55 ± 0.02</td>
<td>76.4 ± 2.2</td>
</tr>
<tr>
<td>Dipodomys merriami</td>
<td>6.55 ± 0.50</td>
<td>1.12 ± 0.01</td>
<td>47.5 ± 1.6</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram/min. 
2. 10 animals/genus or strain of mouse, duplicate readings for each brain. 
3. Pilot study (1 animal/strain) 
4. ACh Chloride Anhydride 100 mg/ampoule (Merck) was used.
Mus musculus
Reithrodontomys ravinventris ravinventris
Mus musculus SCl
Peromyscus maniculatus Bairdi
Mus musculus C57BL/6J
Mus musculus C57BL/6J
Mus musculus C57BL/6J
Peromyscus leucopus
Peromyscus maniculatus "Colorado"
Peromyscus leucopus
Mus musculus "Missouri"
Dipodomys merriami
Dipodomys deserti
Peromyscus leucogaster
Peromyscus maniculatus gracilis
Onychomys leucogaster
Microtus ochrogaster

Figure 1: Brain ChE activity in different genera and strains of mice (mean ± S.E.)

(micromoles acetic acid/gram wet weight/min.)
Whole Brain Weights (grams)

Dipodomys deserti
Dipodomys merriami
Peromyscus maniculatus gracilis
Microtus ochrogaster
Peromyscus leucopus
Onychomys leucogaster
Peromyscus maniculatus "Colorado"
Mus musculus CF1
Mus musculus SCl
Peromyscus maniculatus Bairdii
Mus musculus C57BL/6J
Mus musculus "Missouri"
Reithrodontomys raviventris raviventris

Figure II. Comparison of Whole Brain Weights in Different Genera and Strains of Mice (Mean ± S.E.)
Dipodomys deserti
Dipodomys merriami
Microtus ochrogaster
Mus musculus SCl
Mus musculus CF1
Onychomys leucogaster
Mus musculus C57BL/6J
Peromyscus maniculatus gracilis
Peromyscus leucopus
Peromyscus maniculatus Bairdii
Mus musculus "Missouri"
Peromyscus maniculatus "Colorado"
Reithrodontomys raviventris raviventris

Body Weights (grams)

Figure III. Comparison of Body Weights in Different Genera and Strains of Mice (Mean ± S.E.)
Figure IV. Comparison of Brain ACh and ChE Activities in Different Genera and Strains of Mice (Mean $\pm$ S.E.)

Dipodomys deserti
Mus musculus C57BL/6J
Microtus ochrogaster
Mus musculus SCl
Mus musculus "Missouri"
Mus musculus CFl
Peromyscus maniculatus "Colorado"
Dipodomys merriami
Peromyscus maniculatus gracilis
Reithrodontomys raviventris raviventris
Perognathus longimembris bailli
Peromyscus maniculatus Bairdii
Onychomys leucogaster
Peromyscus polionotus

ACh Concentration
(micrograms/gram brain weight)

ChE Activity
(micromoles acetic acid/gram brain weight/Min.)

1. Values for whole brain ACh concentrations were taken from Sobotka (1969) with the investigator's permission.
Figure V. Brain ACh\textsuperscript{1}, ChE, Serotonin\textsuperscript{1} and Norepinephrine\textsuperscript{1} Activity in Different Genera and Strains of Mice

Values for whole brain ACh concentrations were taken from Sobotka (1969) while those for serotonin and norepinephrine were taken from Richardson (unpublished data) with the investigator’s permission.
or correlation between the various transmitters or related substances and the specific behavioral traits exhibited by the various types of mice although there does seem to exist certain types of correlation in certain types of mice which may be fortuitous.

1. Lack of Parallelism Between ACh and ChE in Various Genera and Strains of Mice Studied

First of all, from Figure IV in which a comparison between whole brain ACh and ChE activities among the various mouse types was made, one can easily see that there exists a lack of any simple correlation between the transmitter substance and its degrading enzyme. However, a general direct relationship exists between ACh and various biogenic amines in these species (Karczmar et al., 1969a, see Figure V). In general, the various strains of Mus have relatively low levels of ACh activity while they have the highest brain ChE concentration; Dipodomys merriami which has intermediate concentration of ACh, is one of the members of the lowest ChE activity group, Onychomys leucogaster, on the other hand, has relatively high ACh concentration but only possesses intermediate enzyme activity. This finding is in contrast to that reported by Tower et al. (1952); Hebb & Silver (1956); (cf. also Friede, 1966) in which a general parallelism of AChE, choline acetyltransferase (ChAc), and ACh was found to exist among the various brain regions within the same species. However, it is similar to the finding that the genetic mechanisms controlling the enzyme ChE and ACh might be independent of each other (Rosen-
zweig et al., 1960; Roderick, 1960). A lack of correlation between the levels of ChE and ChAc in the various cerebellar layers or nuclei was also reported by Goldberg & McCaman (1967).

2. Variability of Brain ChE Activity versus That of ACh Levels in the Various Genotypes of Mice Investigated

The study of whole brain ChE activities in various genotypes of mice indicates more than six-fold differences between different types (Table I & Fig. I). When a similar comparison for brain ACh was made for the different genera and strains of mice studied, it was found that only approximately four-fold differences differentiated the two extreme mouse types, Dipodomys deserti (low ACh level) and Peromyscus polionotus (high ACh level) (Sobotka, 1969; also cf. Fig. IV). That there was a generally greater variation in ChE activity compared to that of ACh level (Burgen & Chipman, 1951; Friede, 1966) is confirmed in this study as seen by a six-fold vs. four-fold differences for ChE and ACh respectively.

3. Inverse Relationship Between ChE Activity and Brain Weight

An inverse relationship between brain ChE activities and brain weights is evident in these experiments by a value of the Spearman Rank Correlation Coefficient (Siegel, 1956) of 0.934 which is significant at 0.01 level. For whole brain ChE activities, the sequences of increasing order of magnitude are: Dipodomys deserti, Dipodomys merriami, Microtus ochrogaster, Peromyscus maniculatus gracilis, Peromyscus leucopus, Peromyscus maniculatus "Colorado", Onychomys leucogaster, Mus musculus C57BL/6J,
Peromyscus maniculatus Bairdii, Mus musculus CFl, Mus musculus SCl, Reithrodontomys raviventris raviventris, Mus musculus "Missouri" (Table I & Fig. I). For whole brain weight, a generally inverse sequence is seen: Dipodomys deserti > Dipodomys merriami > Peromyscus maniculatus gracilllis > Microtus ochrogaster > Peromyscus leucopus > Onychomys leucogaster > Peromyscus "Colorado" > Mus musculus CFl > Mus musculus SCl > Peromyscus maniculatus Bairdii > Mus musculus C57BL/6J > Mus musculus "Missouri" > Reithrodontomys raviventris raviventris (Table I & Fig. II). The four Mus subspecies, Mus musculus "Mo", Mus musculus SCl, Mus musculus CFl, and Mus musculus C57BL/6J, are among the high enzyme activity group; in fact the wild strain of Mus, Mus musculus "Mo", is the highest thus far investigated with regard to this enzyme activity. Yet, they possess small brains in comparison to mice of other genera or strains (Table I & Fig. I & II). Similarly, Reithrodontomys raviventris raviventris which is among the high enzyme activity animals possesses the smallest brain size. On the other hand, the two strains of Dipodomys, Dipodomys deserti and Dipodomys merriami, have the lowest enzyme activities but they possess the highest brain weights. With the exception of Peromyscus maniculatus Bairdii which is among the high enzyme activity group and has small brain weight, the subspecies of Peromyscus, Peromyscus m. gracilllis, Peromyscus leucopus, Peromyscus m. "Colorado", Microtus ochrogaster as well as Onychomys leucogaster, are found to be intermediate in enzyme activities and in brain weights.
This inclination of an inverse relationship between brain ChE activity and brain weight is in agreement with those reported by Tower and Elliot (1952), Ishii (1957a) and Friede (1966) who all also claimed an inverse relation between AChE activity in cerebral cortex, whole brain, and subcortical structures and corresponding brain mass. However, it is contrary to data reported by Krech et al. (1959) in which no such negative correlation was found to exist.

4. Lack of Correlation either Between ChE Activity and Body Weight or Between Brain Weight and Body Weight

In contrast to the inverse relation between ChE activities and brain weights, a lack of correlations either between brain ChE activities and body weights or whole brain weights and body weights was found in these animals (Table I & Fig. I, II, & III). In general, although the four strains of the Mus mice have high enzyme activities they possess body weights ranging from relatively high (Mus musculus SCl & CFL) to relatively low (Mus m. C57BL/6J & Mus m. "Mo"). Reithrodontomys raviventris raviventris has the second highest enzyme activity with the lowest body weight, Microtus ochrogaster, on the other hand, has intermediate enzyme activity with heavy body weight. The subspecies of Peromyscus, with the exception of Peromyscus maniculatus Bairdii which has relatively high enzyme activity, have intermediate brain ChE activities and small body weights. Onychomys leucogaster has intermediate enzyme activity and body weight. Finally, the two
strains of Dipodomys have the heaviest body weights but possess the lowest enzyme activities (Table I & Fig. I & III). This lack of correlation between enzyme activity and body weight is in contrast to the data obtained by Friede (1966), Ishii (1957a), and Tower & Elliot (1952) in which an inverse relation was found to exist not only between enzyme activity and brain weight but also between enzyme activity and body weight.

As seen in the case of enzyme activity and body weight, a similar lack of correlation exists between body weight and brain weight (Table I & Fig. II & III). The two strains of Dipodomys mice (Dipodomys deserti and Dipodomys merriami) and Microtus ochrogaster have heavy body weights as well as heavy brain mass. Mus musculus Scl and Mus m. CFl have relatively high body weights but possess relatively low brain weights, Mus m. C57BL/6J is intermediate in body weight but small in brain weight. The subspecies of Peromyscus, on the other hand, possess generally low body weights, however, have intermediate brain mass. Finally, Reithrodontomys raviventris raviventris has low body weight and brain weight (Table I & Fig. II & III). Therefore it is apparent from this study that there is a lack of correlation between brain weight and body weight among these mice.

5. Marked Intergeneric versus Slight Intragenetic Variation in Brain ChE Levels

As mentioned previously in the results (Chapter, IV, A) the overall picture of enzyme activities in the various types of mice
studied seems to indicate a clear-cut intergeneric variation with little differences between brains of animals of the same genus. Again this seems to be in contrast to ACh situation; in this case, a wide variation was found to exist between strains of the same genus (Sobotka, 1969). The only exception to this general rule are Mus m. "Mo" and Peromyscus maniculatus Bairdii. The former is wild strain of Mus, which differs from the other highly inbred strains of Mus by having a particularly high enzyme activity. This difference in enzyme activity within Mus between the wild and inbred forms may be attributed either to genetic variation possibly due to inbreeding influences on this mouse type. The speculation that brain ChE activities are genetically predetermined is substantiated by histochemical studies on AChE distribution in the brains of different genera and strains of mice; for the most part, the amount and distribution of AChE within the same species were found to be identical (Betti, 1969). Since the enzyme activities investigated in this study are on the basis of whole brain measures no information concerning region or regions of the brain especially rich in enzyme activity can be drawn. However, studies using a histochemical technique have revealed that, in general, in the species of Mus, structures such as lamina glomerulosa bubli olfactorii, nucleus septi medialis, nucleus habenulae lateralis, stria medullaris thalami, nucleus posterior thalami as well as hypothalamus are particularly rich in this enzyme activity. The high concentration of AChE found in nuclei habenulae lateralis
was related by Betti (1969) to the greater olfactory sense of these animals. Other structures of high AChE concentration may be related to other specific types of behavior as will be seen later in this discussion. Within the species of *Peromyscus*, two strains of mice were studied: *Peromyscus maniculatus Bairdii* and *Peromyscus maniculatus "Colorado"*. These were found to have practically an identical amount and distribution of AChE throughout the various brain regions investigated with structures such as globus pallidus, lamina glomerulosa bubli olfactorii, nucleus caudate, tractus olfactorius lateralis, nucleus posterior thalami as well as stria medullaris thalami having the highest enzyme concentration. *Microtus ochrogaster* which had the largest volume of caudate nucleus was found to have the highest concentration of AChE located in this structure. Other structures rich in ChE activity in this genus of mice are those of nucleus amygdaloides, hypothalamus, stria medullaris thalami as well as stria terminalis (Betti, 1969).

6. Biochemical Correlates of Behavior

Behaviorally, the four strains of *Mus* were described as relatively fearless, extremely curious, highly exploratory as well as aggressive animals (Scudder, Karczmar, & Lockett, 1967; Scudder et al., 1969b). These behavior traits have been demonstrated in various behavioral studies such as curiosity, first fifteen-minute activity in the photoactometer, isolation-induced intrageneric and intergeneric aggression as well as "Mouse City"
These animals also exhibited high adaptive behavior, they showed good learning ability in the penta-level climbing screen (conditioning avoidance apparatus) and exhibited a lack of stereotypic or freezing behavior in wheel cage as well as in the "Mouse City" study (Scudder et al., 1969a; Scudder et al., 1965; Scudder, Karczmar, & Lockett, 1967). In addition, this genus of mouse has a relatively slow rate of growth and generally high and long-lasting maternal interest compared to the other mice studied here. This behavioral profile in particular has been related to the relatively high degree of aggression seen in Mus. With these behavioral characteristics on one hand, and distinct neurochemical patterns on the other, one can easily draw the correlation that a high brain ChE activity is associated with a high flexibility of the animals to respond to their environmental input as well as a high learning ability or intelligence. However, one should bear in mind that the existence of such a correlation does not necessary indicate the simultaneous existence of a causal relationship between the two parameters studied; the interpretation of such a relationship should be made with great caution. While the measurements of whole brain activity failed to reveal the quantitative differences in ChE activity in various brain regions, histochemical techniques have clearly demonstrated that the highest concentration of AChE in the Mus genus was generally located in the hypothalamus, and since this anatomical structure is so intimately involved in rage, reward and punishment behavior it was suggested that the
high functional activity of this structure might be responsible for the high aggression and the good learning behavior seen in this genus of animals (Betti, 1969).

Peromyscus, another genus of mouse, is also characterized by distinct behavioral traits. These mice grow and mature rapidly which is typical for field animals. They also show little maternal interest toward their pups. The most characteristic behavior exhibited by this genus of mouse is that of "freezing" and stereotypic behavior as seen in the "Mouse-City" situation as well as in its wheel cage activity respectively (Scudder et al., 1969a; Scudder, Karczmar, & Lockett, 1967). Because of the tendency of these mice to exhibit stereotypic behavior as well as to freeze under stressful conditions, they were generally described as a timid, emotional animals. They are nocturnal (Emlen et al., 1963) with regard to photoactometer, wheel cage and "Mouse-City" activity. Unlike Mus, Peromyscus exhibited little orienting reflex in the photoactometer, little curiosity, as well as little exploration and almost no aggression in the "Mouse-City". This genus of mouse when subjected to the conditioning-avoidance apparatus showed good ability to learn although they did not perform as well as Mus initially. This genus was considered as endowed with high learning capacity based on some other test conditions (Kavenau, 1967). The high degree of stereotypic and freezing behavior of Peromyscus may well explain the fact that they did not come out as well as Mus in the penta-level conditioning apparatus since it
was noted that they occasionally embarked upon certain types of stereotypic activity which interfered with their conditioning during the experiments (Scudder et al., 1969a). Neurochemically, *Peromyscus* was ranked in the intermediate ChE activity group, however, within the intermediate group they are among the relatively high activity members. This is especially true for the strain *Peromyscus maniculatus Bairdii* which was the strain of this genus subjected to the conditioning avoidance study and which indeed showed identical brain ChE activity to that of the *Mus*. Therefore, the relationship between brain ChE activity and learning ability, again, seems to be relatively well correlated. However, the dissociation of curiosity, exploration, aggression, as well as stereotypic behavior from learning ability seen in this genus can not be explained solely on the basis of this enzyme activity since the brain ChE activities were identical in *Mus* (except *Mus m. "Mo"*) and *Peromyscus maniculatus Bairdii*. The relatively high activity of ACh as well as other biogenic amines found in this genus of mice may account for these differences, especially that of the stereotypic behavior (Karczmar & Scudder, 1967; Scudder et al., 1966a).

*Onychomys leucogaster* is carnivorous. These mice grow rapidly and develop slowly with regard to various behavioral traits such as torso twisting, supporting their weight, walking, self-righting, eye opening, and jumping etc. They were characterized by high maternal defensive behavior. It has been suggested that
with a longer period of socialization and learning in the nest, and with a highly protective mother, the pups of the *Onychomys* may acquire or learn during their prolonged pre-puberal period some of the skills necessary for a successful predatory life. In the "Mouse-City", they exhibited little aggressive behavior unless they were trained to kill, however, the female of this genus is unique in that these females exhibit the highest maternal aggressive behavior thus far investigated. They were avoided by most mouse types except *Mus m. CFl* which attacked all genera indiscriminately. They resemble *Peromyscus* by being nocturnal but in contrast to them they were relatively inactive with regard to photoactometer activity. They differed from *Peromyscus* also in the incidence of stereotypic behavior exhibited in the "Mouse-City" study. They exhibited the lowest incidence in this behavior among the various genera and strains of mice investigated. Again, they showed little exploration and curiosity as did *Peromyscus*. When they were subjected to the "penta-level" apparatus, they showed poor or no learning ability although they did learn to escape. They never learned to avoid the shock in this test condition. Since these mice have been shown to learn such activities as aggression (Bailey & Sperry, 1929), it was suggested that this testing situation might not be appropriate for the measurement of learning capacity in this genus of mouse (Scudder et al., 1969a). If this is indeed the case, then, here again, the learning ability and brain ChE activity might be related to certain extent, i.e.
a mouse type with intermediate brain ChE activity might still be associated with certain degree of learning although the extent of learning is lower than that of high ChE activity animal. While the differences in brain ChE activities between Onychomys and Peromyscus were able to differentiate the learning ability between these two genera of mice, they failed to explain the similarity such as lack of curiosity, exploration etc. seen in these animals. This might be explained on the basis of the similar concentration of ACh and biogenic amines found in these mice. The high levels of maternal aggressive behavior exhibited by the female mice of this genus, on the other hand, may be related to hormonal influence and should not be considered synonymous with other types of aggressive behavior (Scudder et al., 1969b).

Finally Microtus, the meadow vole, is a herbivorous, grassland, field form as is Peromyscus. These mice were found to grow and to develop various behavioral traits rapidly. Similar to Onychomys, Microtus exhibited high maternal interest and maternal defensive behavior, indeed, the latter behavior was rated the second highest, next to that of the female Onychomys (Scudder, Karczmar, & Lockett, 1967). These mice are characterized by lacking good avoidance conditioning. They did not show any improvement in the performance during the period of conditioning avoidance training, although they did respond to pemoline Mg(OH)₂ by a dose-related change in performance. However an analysis of the
initial performance to that of the final performance revealed no
learning had occurred in these animals (Scudder, Avery & Karczmar,
1967; Scudder, Karczmar & Lockett, 1967). Other characteristic
of these animals is that of hoarding behavior (storage of food)
and that of development of social hierachic system under corwded
conditions. They showed little exploration and curiosity although
they were rated the highest in total locomotor activity as measured
in photoactometer study. Although a field form similar to Pero-
myscus and high in general motor activity, they contrast to Pero-
myscus by being low in stereotypic behavior. Altogether this
genus of mice seems to be characterized by relatively simple be-
havior patterns associated with a lack of behavioral flexibility
or variability in response to the environmental changes. These
qualities might be indicative of lack of intelligence in these
animals. With this behavioral profile characterizing this genus
of mice, it is interesting to note that the brain ChE activity
of these mice was the lowest among the intermediate enzyme activi-
ty group. Therefore, a low brain ChE activity is associated with
a low learning ability, exploration, curiosity etc. A low con-
centration of ACh, 5-HT and catecholamine were also found in these
mice.

For the two strains of Dipodomys as well as the genus Rei-
throdontomyx, behavioral data are not presently available. It
would be interesting to see whether a lack of learning ability
is associated with the genus Dipodomys while a good adaptive be-
Behavior is correlated with *Reithrodontomys* since the former was found to have the lowest and the latter to have the second highest brain ChE activities so far investigated.

It might be postulated at this point that flexible, exploratory, mobile behavior, good learning ability, as well as "Mouse-City" aggression is associated with high ChE activity and relatively low levels of ACh, catecholamines and of serotonin such as seen in the various strains of *Mus* while the opposite is true for *Microtus* which has in general low enzyme activity as well as low levels of various transmitter substances. The stereotypic behavior, on the other hand, might be related to high levels of ACh, catecholamines and 5-HT as evident in *Peromyscus* (Karczmar et al., 1969a; Sobotka, 1969). It has been suggested that the high levels of catecholamines may relate to certain cortical inhibitory circuits (Bourgault et al., 1963; Scudder et al., 1966a) and the low levels of ACh may relate to low levels of inhibition (Karczmar, 1969b; Karczmar et al., 1969a). Such postulation, is indeed highly speculative and the relative behavioral significance of these substances and related enzyme systems remains unsolved until more experimental evidences can be accumulated.
SECTION II

CHOLINESTERASE LEVELS IN BRAIN HALVES OF MUS MUSCULUS SCI AND BRAIN PARTS OF DIPODOMYS DESERTI MICE
Chapter VI. Introduction

Numerous investigators have dealt with the characteristic distribution of ChE in the various regions of the brain (Nachmansohn, 1940; Burgen & Chipman, 1951; Pope, 1952; Okinaka et al., 1961; Betti, 1969). Nevertheless, none of them compared the possible differences of this enzyme between the left and right parts of the brain in mice. It is a well known phenomenon that there exists a "cerebral dominance" in the human brain, i.e. one hemisphere appears to be the "leading" one in certain higher functions believed to be cortical in nature (Joynt et al., 1964). This "cerebral dominance" was found to be most complete in relation to the complex and highly evolved aspects of language. Handedness also was related to cerebral dominance (Henschen, 1926). It appeared, therefore, interesting to see if there is any difference with regard to this enzyme, ChE, between the left and right brain halves as well as among brain parts in SCl and Dipodomys deserti respectively.

Isolation for at least a period of two weeks causes changes in both behavioral measures as well as brain chemistry in laboratory white mice (cf. Section V, Chapter XVIII, C). Therefore it is also interesting to see whether a difference in brain ChE activity between the left and right brain halves as well as among brain parts, if any, exists in the isolates as it might in the aggregates.

Chapter VII. Materials and Methods
A. Animals

The animals used in this experiment were male, adult mice of *Mus musculus* SC1 and *Dipodomys deserti*. For brain-half study, SC1 mice both aggregates and isolates were used (5 animals per group). For brain parts, only normal grouped *Dipodomys deserti* were employed (6 animals per group).

The aggregated animals were obtained by housing 20 to 25 SC1 mice per cage (6x10x18 inches). The isolated animals, on the other hand, were isolated one per cage (3.5x3.5x7.5 inches) for a period of at least 2 weeks (2 to 3 weeks). *Dipodomys deserti* were housed in a normal grouped condition of 2 per cage (3.5x5.5 x 7.5 inches). All animals were under constant humidity, illumination cycle (light running from 6:00 A.M. to 8:00 P.M.) and temperature control (23.9° ± 2.8° C). Food and water were provided ad libitum.

B. Dissection Procedure

For brain-half as well as brain-part samples, the removed brains, instead of being immersed into liquid nitrogen immediately, were placed on a piece of paper towel wet with water. Several divisions were then made on the whole brain with a spatula. For brain-half samples, a longitudinal incision was made anterior-posteriorly along the fissura longitudinalis cerebri. For brain part samples further subdivisions were made. The cerebrum samples were obtained by gently lifting the cerebral hemispheres and making a section at the stria terminalis which connects the telen-
cerebrum and the midbrain-diencephalon. The second brain sample, the cerebellum, was then removed from the rest of the brain. The midbrain-diencephalon was obtained by making an incision at a point immediately posterior to corpus quadrigemina and anterior to the pons. The remaining brain sample was the pons-medulla. All brain parts were immersed in liquid nitrogen as soon as they were dissected and were weighed thereafter. The entire procedure for decapitating the animal, taking out the brain and placing it into liquid nitrogen takes no more than 20 seconds for the whole brain and less than a total of 30 seconds for the brain parts.

g. Biochemical Analysis

The biochemical analysis used in this study was identical to that described in Section I, Chapter II, C.

h. Statistical Evaluation

The statistical method employed for the analysis of experimental data is that of the Student-t test (Snedecor, 1956). The following formulae are used for an equal number as well as a different number of animals used respectively:

1) Comparison Between Two Groups of Equal Size:

\[ t = \frac{(\bar{x}_1 - \bar{x}_2) \sqrt{n \cdot \frac{x(n-1)}{\sum x^2}}}{\sqrt{n_1 n_2}} \]

\( n_1 = n_2 = n \): number of animals used per group

\( \bar{x}_1, \bar{x}_2 \): means of \( n_1 \) and \( n_2 \) observations respectively

\( \sum x^2 = \text{pooled sum of squares} \)

\[ = \sum_{i_1=1}^{n_1} (x_{i_1} - \bar{x}_1)^2 + \sum_{i_2=1}^{n_2} (x_{i_2} - \bar{x}_2)^2 \]
where \( x_{i1} \) = individual observations in \( n_1 \)
\( x_{i2} \) = individual observations in \( n_2 \)

b) Comparison Between Two Groups of Different Size:

\[
t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{n_1 + n_2 \sum x^2}}}
\]

All notations are identical to those described in a) except \( n_1 \neq n_2 \).

Chapter VIII. Results

A. Cholinesterase Activity in Brain Half of Mus musculus SCl Mice

The ChE activities in brain halves and whole brains of SCl male adult mice, both in isolated and aggregated groups, are shown in Table II. The corresponding brain-half weights, whole brain weights, and body weights are also given in the same table. The results indicate that there are no significant differences in either ChE activities or brain weights between the left and the right halves of the brain when they are compared within the same aggregated or isolated groups. However, a slightly higher ChE activity is found in the isolated group when they are compared between the aggregates and isolates. This is true in the brain-half as well as in the whole brain measures although these differences do not reach statistical significance. Conversely, while there is an increase in ChE activities in the isolated animals, a drop in brain weights, both in brain halves and whole brain,
is seen in these subjects. Again, these differences are not statistically significant with the exception of the whole brain which is statistically significant at 0.05 level. For the body weights, the data show that there are no significant differences between the isolates and aggregates.

B. Cholinesterase Activity in Brain Parts of Dipodomys deserti

The ChE activities in various brain parts of Dipodomys deserti are given in Table III. The brain parts studied are cerebrum, midbrain-diencephalon, cerebellum and pons-medulla. The values on both left and right side of the various brain regions are also shown in the table. Again, as with the previous brain-halves experiment in SCl mice (Table II), the results indicate no significant differences in either ChE activities or tissue weights between the left and the right side of the brain parts investigated. However, when measurements are compared on the basis of regional differences instead of the left-and right-side differences of the corresponding brain parts, significant differences are found to exist both in ChE activities and tissue weights. This is true not only for comparisons among the same side of the brain parts but also for those among the whole brain. The sequences of ChE activities in increasing order of magnitude are as follows: cerebrum, midbrain-diencephalon, cerebellum, pons-medulla.

Chapter IX. Discussion
Table II  ChE Activity\(^1\) in Brain Halves of SCl Mice\(^2\)

<table>
<thead>
<tr>
<th>Brain Treatment</th>
<th>Left Brain Halves</th>
<th>Right Brain Halves</th>
<th>Whole Brain</th>
<th>Body Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ChE (gm)</td>
<td>Wt. (gm)</td>
<td>ChE (gm)</td>
<td>Wt. (gm)</td>
</tr>
<tr>
<td>Aggregates</td>
<td>27.35 ± 0.76</td>
<td>0.26 ± 0.01</td>
<td>27.90 ± 1.45</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>0.65</td>
<td>0.01</td>
<td>1.01</td>
<td>0.49</td>
</tr>
<tr>
<td>Isolates</td>
<td>29.75 ± 1.95</td>
<td>0.24 ± 0.01</td>
<td>28.65 ± 1.10</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>0.95 ± 0.01</td>
<td>0.01</td>
<td>1.10</td>
<td>0.01</td>
</tr>
<tr>
<td>% Change from Aggregates</td>
<td>+8.8</td>
<td>-7.7</td>
<td>+2.7</td>
<td>-8.4</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram brain wet weight/minute.
2. Five animals/group, duplicate readings for each brain were used.
* Significant at 0.05 level.

ACh Chloride Anhydride 150 mg/vial (Sigma) was used.
<table>
<thead>
<tr>
<th>Brain Parts</th>
<th>Left</th>
<th>Right</th>
<th>Whole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ChE</td>
<td>Wt. (gm)</td>
<td>ChE</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>1.86</td>
<td>0.47</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>±</td>
<td>0.08</td>
</tr>
<tr>
<td>Midbrain-diencephalon</td>
<td>5.13</td>
<td>±</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td>0.65</td>
<td>±</td>
<td>1.01</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>5.55</td>
<td>±</td>
<td>5.65</td>
</tr>
<tr>
<td></td>
<td>0.69</td>
<td>±</td>
<td>1.10</td>
</tr>
<tr>
<td>Pons-Medulla</td>
<td>7.27</td>
<td>±</td>
<td>7.55</td>
</tr>
<tr>
<td></td>
<td>0.77</td>
<td>±</td>
<td>1.46</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram brain wet weight/minute.

2. Six animals/group, duplicate readings for each brain were used.

ACh Chloride Anhydrous 150 mg/vial (Sigma) was used.
Figure VI. Regional ChE Activity and Tissue Weight in *Dipodomys deserti* (Mean ± S.E.)

**Brain ChE Activity**

<table>
<thead>
<tr>
<th>Region</th>
<th>L: left</th>
<th>R: right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telencephalon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midbrain-Diencephalon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pons-Medulla</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Tissue Weight (gram)**

<table>
<thead>
<tr>
<th>Region</th>
<th>L: left</th>
<th>R: right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telencephalon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midbrain-Diencephalon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pons-Medulla</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lack of Differences in Enzyme Activities of the Left and Right Brain Regions of SCl Mice and Dipodomys deserti

The results obtained in this experiment indicate that there is no significant differences between the left and the right brain halves as well as brain parts investigated (Tables II, III, Fig. VI). This findings seem to suggest that in the central nervous system, structures which are physiological and anatomical counterparts exhibit identical neurochemical constituents—at least those studied here—regardless of the spatial difference these structures. Therefore, one might speculate that, similar identical values of substances such as ACh, norepinephrine, HT....etc. between the brain halves of the same animals might found if such experiments are undertaken. One might also advance a step further to expect identical neurochemical comments among various symmetrical structures of the brain such as the left and the right brain parts of cerebrum, midbrain-encephalon, cerebellum as well as pons-medulla etc. as seen in this experiment (Tables II & III, & Fig. VI). However, it would be pointed out that definite conclusion for this speculation can only be drawn after careful experimental evidence is gathered.

Regional Distribution of Cholinesterase in Dipodomys deserti

The pattern of regional distribution of brain ChE in Dipodomys deserti (Table III & Fig. VI), in general, is in accord with such distributions reported by various authors on other
species of animals (Nachmansohn, 1940; Burgen & Chipman, 1951; McCaman et al., 1964; Bennett et al., 1966), i.e. little activity in the cerebrum, a general high activity in the midbrain-diencephalon, a still higher activity in the cerebellum. The high activity of ChE in the cerebellum is also noted by other investigators irrespective of the fact that there is present relatively low activity with regard to the synthesizing enzyme ChAc in this brain area (Austin et al., 1964; Goldberg & McCaman, 1967; Hebb & Silver, 1956; Phillis, 1965; Austin & Phillis, 1965; Friede et al., 1964). The enzyme activity in the pons-medullary region of this genus is distinctive in that it has the highest values among the various brain regions investigated. These results confirmed the uneven distribution of ChE in the central nervous system and also necessitate the regional study of this enzyme in order to reveal any small but significant biochemical changes in the brain which otherwise will be masked by the whole brain measure. Another point of interest concerns the brain weights. A negative correlation between enzyme activity and the brain weights is also evident here even among the various regions of the same brain (Table III & Fig. VI). This data support that obtained in the genetic studies (Table I).
SECTION III

CIRCADIAN RHYTHMS IN BRAIN CHOLINESTERASE LEVELS IN TWO GENERA OF MICE — MUS MUSCULUS SCI AND DIPodomys Merriam
Chapter X. Introduction

It is a well-established phenomenon that a regular physiological (expresses as locomotor activity) oscillation of approximately 24-hour duration exists in mammals (Cloudsley-Thompson, 1961; Richter, 1965). Recently this relationship has been extended to various biologically active substances and correlations might exist between these rhythms and other physiological or behavioral states.

A. Circadian Fluctuation in Locomotor Activity in Mammals

Emlen et al. (1963) described a distinct activity rhythm in *Peromyscus maniculatus Bairdii*. This finding was confirmed in our laboratory where *Peromyscus* and *Onychomys* were both described as nocturnal animals as compared to other genera or strains of mice (Scudder, unpublished data). Holmquest et al. (1966) in their study of effects of random lighting on circadian rhythms in rats also noted the existence of physiological fluctuation of group running activity and adrenal steroid secretion in these animals. They also found that simple alteration of only the temporal aspect of the light environment, without alteration of the net photoperiod, resulted in modifying and in some cases nullifying the important effect of light on biological rhythms in mammals.

B. Circadian Rhythms in ACh, ChE, and Corticosteroid Levels

Dixon (1957) described a circadian variation in human cholinesterase activity. He took four observations per day and
noted that although there was only a slight variation in erythrocyte AChE, there was a statistically-significant diurnal rhythm in plasma pseudocholinesterase. A maximum activity near noon and a minimum near midnight was observed. Naidu (1969) in his study of heart beat of scorpion found that the level of ChE activity of the heart muscle followed a regular circadian rhythm like that of the heart beat, with the maximum enzyme activity at about 20:00 hours and the minimum enzyme activity at about 08:00 hours.

The only information available regarding the periodicity of central ChE activity is that reported by Venkatachari et al. (1968) on ventral nerve cord of scorpion. Peak value of this enzyme activity was found to be at 16:00 hours and trough value at 04:00 hours. The possible relationship of this rhythm to electrical cortical activity has been suggested. More recently twenty-four rhythms in ACh levels were determined by Friedman and Walker (1969 & 1972) in rat midbrain and caudate nucleus and mouse whole brain. Peak ACh levels were found in the former at 24:00 hours, in the latter, at 06:00 hours. Trough values occurred at 12:00 hours, in rat midbrain and at 18:00 hours in rat caudate nucleus and mouse brain.

Krieger et al. (1967) found a circadian pattern of plasma 17-hydroxycorticosteroid which begins to rise at 8:00 PM and reached its peak at 12:00 midnight. This rise of plasma hydroxy-corticosteroid level could be prevented by the administration of atropine just prior to the rise but not at other times of the
cycle. They therefore suggested a cholinergic component in the release of ACTH and that this activation of the hypothalamus-pituitary-adrenal axis reflected by the rise of 17-hydroxycorticosteroid level occurs only during a "critical time period" in the circadian cycle. Dixit et al. (1967) also reported a circadian change in plasma corticosterone and brain 5-HT in the rat.

C. Circadian Periodicity in Biogenic Amines and Physiological States

Quay (1966) reported a periodic fluctuation in pineal 5-HT and hydroxyindole-O-methyl transferase in the primate, he found that high 5-HT activity occurred at the light phase of the illumination period while high hydroxyindole-O-methyl transferase existed at night. Friedman and Walker (1968), in addition to reporting the existence of circadian rhythms in biogenic amine levels in rat mid-brain and caudate nucleus, further speculated on a possible relationship between sleep and 5-HT and also between wakefulness and histamine and norepinephrine.

D. Circadian Rhythm in Drug Sensitivity

Numerous studies have been done on drug effects in relation to circadian rhythms. Davis (1962) described a day-night periodicity in pentobarbital response in mice, he found that the duration of anesthesia to pentobarbital was greater during the light period than during the dark period, and the exact peak and trough of the 24-hour response curves appear to be variable about the mid-points of light and dark periods respectively. Similar find-
ings in rat were also reported by Friedman and Walker (1969). Scheving et al. (1968), instead of using mice, used rats and found that rats remained anesthetized to pentobarbital sodium for longer periods of time between the hours of 16:00 and 22:00 than at other times of the day when they were adapted to alternating light-dark cycle of 12 hours with light phase ranging from 06:00-18:00 hours. This difference between crest and trough of the curve was about 100%. Millichap et al. (1966) dealt with the hyperkinesic rather than the anesthetic effects of pentobarbital in mice for a period of 24 hours and in hamsters for 1-8 days. They found that doses of 100-150 mg/Kg of pentobarbital caused stimulation, 1 and 5 mg/Kg doses were followed by suppression of activity, and 200 mg/Kg resulted in a biphasic, suppression-stimulant effects. This hyperkinetic response occurred after administration either in day or at night, and artificial light failed to abolish the circadian rhythmic increase in activity at night in both treated and untreated animals. The circadian periodicity of central side effect of lidocaine has also been described by Lutsch et al. (1967). They reported that a quantitative circadian rhythm with maximal convulsant activity occurred at 21:00 hours which was approximately a fourteen fold increase over the values obtained at 15:00 hours. Recently Walker and Friedman (1968) and Friedman and Walker (1969 & 1972) studied twenty-four rhythms in the toxicity of cholinomimetics in mice and found that the median lethal dose for I.V. administered ACh
was lowest at 04:00 hours and highest at 12:00 hours. This toxicity rhythm was related to brain ACh levels, i.e. peak toxicity occurred when brain ACh levels were maximal, minimal toxicity occurred when brain ACh levels were minimal. Oxotremorine, pilocarpine, and neostigmine had a similar toxicity rhythm corresponding to ACh while carbachol displayed toxicity patterns at twelve hour intervals. Tertiary antiChE physostigmine gave both its peak and trough toxicity rhythms in the dark period of illumination cycle. Cholinolytic drugs, atropine and scopolamine, showed toxicity rhythms which were mirror image of that of cholinomimetics.

Chapter XI. Materials and Methods

A. Animals

The animals used in this study were male adult mice, Mus musculus SC1 and Dipodomys merriami. For SC1 mice, ten animals per group were housed in a cage of 6x10x8 inches. For Dipodomys merriami, the subjects were paired in group of two per cage (3.5 x5.5x7.5 inches). All the animals were maintained under identical food, water and environmental conditions as that described in Section I, Chapter III, A, 1.

B. Experimental Design

The circadian rhythm of brain ChE activity was determined at every three-hour interval beginning at 00:00 hour and ending at 24:00 hours; i.e. 00:00 hour, 03:00 hours, 06:00 hours, 09:00 hours, 12:00 hours, 15:00 hours, 18:00 hours, 21:00 hours and
24:00 hours. For Mus musculus SCI, six mice per group, for Dipodomys merriami, five animals per group, were used in the experiment. All the animals belonging to the same group were sacrificed at the same time at which the enzyme activity was to be determined and the brains were stored in liquid nitrogen until biochemical analysis was made. Duplicate readings were determined for each brain and the means of the six or five animals (SCI and Dipodomys respectively) were then taken as the final values for each specific time interval.

C. Dissection Procedure and Biochemical Analysis

The dissection procedure (whole brain samples) as well as biochemical analysis used in this experiment were identical to those mentioned in Section I, Chapter III, B & C.

D. Statistical Evaluation

The statistical method employed in this experiment is identical to that described in Section II, Chapter VII, D.

Chapter XII. Results

A. Circadian Rhythm in Brain Cholinesterase Activity in SCI Mice

The rhythms of whole brain ChE activities over a 24-hour period in two genera of mice are given in Table IV & V and Fig. VII & VIII. The results show that a bimodal pattern of whole brain ChE activity is present in the SCI mice (Table IV & Fig. VII). The peak values are found to be at 03:00 hours and 15:00 hours, while the trough values at 00:00 hour and 09:00 hours. Statistical analysis reveals that significant differences exist
between the peak values and trough values (p < 0.05), however, no differences are found either between the two peak values, the two trough values, or values obtained at other time of the day and the trough values.

B. Circadian Rhythm in Brain Cholinesterase Activity in *Dipodomys merriami*

A different type of circadian rhythm is found in *Dipodomys merriami*. The rhythm appears to be a trimodal one, the highest reading is found to be at 12:00 hours, the other two peak figures occur at 03:00 hours and 21:00 hours (Table V & Fig. VIII). Statistical analysis reveals that there are significant differences between the peak values; i.e. at 03:00 hours (p < 0.05), 12:00 hours (p < 0.001), or 21:00 hours (p < 0.001), and the two trough values at 06:00 hours and 18:00 hours. Again, there is no significant difference between the two trough values. Among the three peak values, the difference between 12:00 hours and 21:00 hours is significant at 0.05 level while that between 12:00 hours and 03:00 hours does not reach statistical significance.

Chapter XIII. Discussion

A. Trimodal Circadian Rhythm in Brain Cholinesterase in *Dipodomys merriami*

In the course of this study attempts were made to correlate the possible relationship of this enzyme rhythmicity to that of their corresponding motor activities as evaluated both in photocellometers and wheel cages in two genera of mice, *Mus musculus*
Table IV  Circadian Rhythm in Brain ChE Activity\textsuperscript{1} in SCl Mice\textsuperscript{2}

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>00:00</th>
<th>03:00</th>
<th>06:00</th>
<th>09:00</th>
<th>12:00</th>
<th>15:00</th>
<th>18:00</th>
<th>21:00</th>
<th>24:00</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChE</td>
<td>29.59</td>
<td>32.57</td>
<td>31.07</td>
<td>30.10</td>
<td>31.82</td>
<td>33.94</td>
<td>32.86</td>
<td>31.64</td>
<td>30.75</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>0.95</td>
<td>0.66</td>
<td>1.21</td>
<td>0.41</td>
<td>1.10</td>
<td>1.04</td>
<td>1.17</td>
<td>0.59</td>
<td>1.02</td>
</tr>
<tr>
<td>% Change</td>
<td>---</td>
<td>10.1*</td>
<td>5.0</td>
<td>1.7</td>
<td>7.5</td>
<td>14.7*</td>
<td>11.1</td>
<td>6.9</td>
<td>3.9</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram brain wet weight/minute.
2. Six animals/group, duplicate readings for each brain.
   * Significant at 0.05 level as compared to the trough value at time 00:00 hour.
   ACh Chloride 100 mg/ampoule (Merck) was used.
Table V  Circadian Rhythm in Brain ChE Activity\(^1\) in *Dipodomys merriami*\(^2\)

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>00:00</th>
<th>03:00</th>
<th>06:00</th>
<th>09:00</th>
<th>12:00</th>
<th>15:00</th>
<th>18:00</th>
<th>21:00</th>
<th>24:00</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChE</td>
<td>4.27</td>
<td>5.24</td>
<td>3.23</td>
<td>6.24</td>
<td>7.04</td>
<td>6.38</td>
<td>3.82</td>
<td>5.30</td>
<td>4.58</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>0.31</td>
<td>0.70</td>
<td>0.14</td>
<td>0.25</td>
<td>0.51</td>
<td>0.70</td>
<td>0.35</td>
<td>0.04</td>
<td>0.43</td>
</tr>
<tr>
<td>% Change</td>
<td>32.2</td>
<td>62.2</td>
<td>---</td>
<td>93.1</td>
<td>117.8</td>
<td>97.5</td>
<td>18.3</td>
<td>64.2</td>
<td>41.8</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram brain wet weight/minute.

2. Five animals/group, duplicate readings for each brain.

* Significant at 0.05 level as compared to the trough value at time 06:00 hours.
** Significant at 0.01 level as compared to the trough value at time 06:00 hours.
*** Significant at 0.001 level as compared to the trough value at time 06:00 hours.

ACh Chloride Anhydride 150 mg/vial (Sigma) was used.
Figure VII. Circadian Rhythm in Brain ChE Activity in SCl Mice

(Mean ± S.E.)

1. n=6, duplicate readings/brain
2. The hatched lines indicate the dark period of the illumination cycle.
Figure VIII. Circadian Rhythm in Brain ChE Activity in *Dipodomys merriami* Mice (Mean ± S.E.)

1. ChE activity is expressed in term of micromoles acetic acid/gram/Min.
2. n=5, duplicate readings/brain
3. The hatched lines indicate the dark period of the illumination cycle.
SCl and Dipodomys merriami, which possess relatively high and low brain ChE activity respectively. Since the behavioral data concerning circadian periodicity is not available as yet for the genus Dipodomys merriami (due to the difficulty of supply of these animals), a possible correlation can not be made at the present time. Dipodomys merriami is found to exhibit a trimodal pattern in brain ChE activity with peak values occur at 12:00 hours, 21:00 hours and 03:00 hours and trough values at 06:00 hours and 18:00 hours (Table V & Fig. VIII). It should be pointed out, however, that due to the difficulty of supply of these mice, the total completion of these series of experiment lasted for more than 3 months (approximately 3½ months). Since it has been reported that there is a seasonal variation in human BuChE activity (Sakaino, 1955), it is not clear, whether such factor also plays a role in AChE activity and contributes to the data obtained here. Possibly it is purely a genus specific circadian rhythm displayed by these mice. The behavioral as well as the physiological significance of this enzyme fluctuation in this genus of mouse await further information.

B. Bimodal Pattern in Brain Cholinesterase in SCl Mice and Biochemical Correlation with Behavior

For the Mus musculus SCl mouse, which is an inbred strain of Mus musculus CFl and resembles the latter in brain chemistry (Karczmar & Scudder, 1969), a description of the circadian rhythm with respect to motor activity and brain ChE level is possible.
In the photoactivity studies, *Mus musculus* CF1 showed a higher exploratory or orienting reaction (first 15-minute readings in the photoactometer) in the afternoon (2,000 counts/hour at 4:30 p.m.) than in the morning (1,500 counts/hour at 9:30 A.M.) (Scudder, unpublished data). The corresponding values for brain ChE activity are 33.9 (3:00 P.M.) and 30.10 micromoles acetic acid/gram brain weight/minute (9:00 A.M.) which correspond to high and low enzyme activity seen in the circadian rhythm (Table IV & Fig. VII). These differences both in exploration and enzyme activity are significant at 0.05 level (Fig. IX). Therefore, there seems to exist a correlation between high enzyme activity and high orienting reaction and vice versa. The data showing a biochemical correlate to behavior obtained in this circadian study seems to be in agreement with correlation found in the intergeneric experiment in which *Mus* was found to exhibit high exploration and high ChE activity as compared to the other genera of mice. For general motor activity, a comparison of the day and night enzyme (ChE) activities (32.18 and 31.23 micromoles acetic acid/gram brain weight/minute respectively) with the corresponding day and night photoactivity (220 counts/hour and 400 counts/hour respectively; Scudder unpublished data) fails to reveal any significant correlation between these two measures. The day enzyme activity is obtained by averaging the enzyme activities measured from 09:00 hours to 18:00 hours while that of the night activity is obtained by averaging ChE activities determined from
Figure IX. Relationship Between Brain ChE Rhythm and Exploratory Rhythm in *Mus musculus* SCl Mice

1. ChE activity is expressed in term of micromoles acetic acid/gram tissue weight/Min.
2. Exploratory score is expressed in term of counts/hour.
21:00 hours to 06:00 hours (see Table IV & Fig. VII). The lack of correlation between enzyme activity and general motor pattern indirectly supports the behavioral data obtained in this laboratory that there is no direct relationship between exploratory behavior and general motor activity (Scudder, unpublished data). This is indicated in Mus which in general has high exploratory activity and low total activity, while Microtus has low exploratory activity and high motor activity, Onychomys has low exploratory as well as low motor activity. Since it has been shown that curiosity, exploration, aggression and learning ability might be associated with each other (Scudder, Richardson & Karczmar, 1969b), it would be interesting to study the circadian periodicity with respect to these behavioral parameters and to compare them to that of the brain ChE levels. In the wheel cage study, a lack of a biochemical-behavioral correlation is also observed in SCl mice which exhibit higher wheel cage activity in the night than in the day but no significant difference in ChE activity with regard to day and night is observed. One point has to be stressed, however, that the lack of correlation between brain ChE activity and motor activity measured in the wheel cage and that evaluated in photoactometer can not be considered equal since these two behavioral measurements represent two entirely different behavioral parameter; the former is a self-rewarding, perpetuating behavior while the latter is not (Scudder et al., 1967).

Altogether, it is desirable to conduct circadian behavioral
studies using the same time intervals as those used for the brain ChE determinations in order to facilitate a direct comparison of these variables.

C. Circadian Rhythm in Brain ChE Activity in Relation to Other Pharmacological and Physiological Functions

Finally it may be of interest to point out that the circadian rhythm of brain ChE activity in laboratory white mice might be associated with the toxicity rhythm of a tertiary antiChE, physostigmine, which gave toxicity rhythms comparable to that of the brain ChE rhythm seen in this experiment, i.e. a low LD50 value for physostigmine may be correlated with a period of low brain ChE activity (cf. Friedman & Walker, 1969 & 1972). Whether this rhythm also plays a role in cerebral electrical activity, as suggested by Venkatachari et al. (1968), is not known at the present time.
SECTION IV

DRUG-INDUCED CHANGES IN CENTRAL CHOLINERGIC TRANSMISSION

AND BEHAVIOR
Numerous studies regarding the central cholinergic system and behavior have been conducted by artificially manipulating the balance of this system with regard to other systems in the CNS (see Cholinergic Transmission and Behavior in General Introduction). These approaches can be achieved by administering specific drugs alone or in combination to the experimental animals.

A. Central Cholinergic System and Physiological States

Aprison et al. (1958), by injecting DFP unilaterally into the right carotid artery of the rabbits, described a direct correlation between the rate of compulsive circling and the asymmetric accumulation of ACh in the right and left cortices of rabbits. This was true irrespective of the direction of the circus movement.

Takahashi et al. (1964) reported that there was a decrease in total ACh levels in whole brain of the animals during pentylenetetrazole induced convulsion. Toru et al. (1966) studied the relationship between regional ACh concentrations in rat brains and three states of avoidance behavior; normal, depression, and excitation induced either by administration of specific drugs alone or in combination to animals working on the same or different schedules of reinforcement. They found that lowered ACh concentrations existed in three brain areas (telencephalon, diencephalon plus mesencephalon and pons plus medulla oblongata) of
rats exhibiting an increased avoidance behavior response rate induced by pretreatment of iproniazid (50 mg/Kg) followed by 2 mg/Kg tetrabenazine 16 hours later. Conversely, in rats injected with 2 mg/Kg of tetrabenazine and exhibiting markedly depressed responding rates only, the ACh concentration in diencephalon plus mesencephalon increased. These data were subsequently confirmed by Aprison et al. (1968). They found that ACh concentration in all three brain areas, telencephalon, diencephalon-mesencephalon, and pons-medulla oblongata, decreased and returned to normal levels at different time in rats exhibiting behavioral excitation induced by the same procedures described by Toru et al. (1966). In addition, they reported that the time course of increased response rates correlated best with the ACh levels in the telencephalon. Both the 5-HT and norepinephrine concentrations remained similar to the iproniazid control values during the period of behavioral excitation. However, the norepinephrine concentration in the midbrain showed a continuously decreasing trend toward naive control levels. They, therefore, suggested that changes in a cholinergic system in the telencephalon and an adrenergic system in the midbrain maintain behavioral excitation. In contrast to this speculation that the in vivo level of ACh in brain varies inversely with the state of its functional activity, Giarman and Pepeu (1962) in their study of the correlation between drug induced behavioral changes in the rat and total brain ACh, reported that an intraperitoneal injection of 20 mg/Kg phenyl-
cyclohexylperidine, which produced excitation, tremors and ataxia in the rats, showed an increase of 15% total brain ACh whereas lysergic acid diethylamine (0.2 mg/Kg) or iproniazid (100 mg/Kg) plus DOPA (250 mg/Kg) resulted in excitation although ACh levels did not vary significantly from control values. The discrepancies of these findings were explained on the basis of 1) differences in decapitation method used, 2) the definition of behavioral excitation (in the present experiment, the excitation refers to increases in general or unlearned activity whereas in those of Toru & Aprison, the excitation refers to increases in the rate of specific learned response), 3) use of whole brain samples rather than specific areas for ACh assay, and 4) the possible involvement of other biochemical systems in the "production" of the excitation noted in these animals (Toru et al., 1966).

B. Central Cholinergic System and Animal Behavior (Conditioning and Extinction)

A series of studies regarding ChE activity and operant extinction have been carried out by Glow and his associate (Glow & Rose, 1965; Glow & Rose, 1966). They found that a reduction of ChE in the CNS through the use of the peripheral ChE reactivator C_43_4 (NN-trimethylene (1,3)-bis (pyridinium-4-aldoxime) bromide) resulted in little change in extinction responding. When the activity levels of ChE were varied systemically in brain and muscle, a reduction to 40% of the control ChE activity level was required to produce changes in extinction. Furthermore the re-
istance to extinction was obtained only with an acute injection of DFP but not with the chronic administration of an organophosphate. The initial depression in the number of responses made on the first few days of the extinction period was presumably due to a peripheral "weakness" of greater or lesser degree, and the differences in degree of this "weakness", in turn, were attributed to the striking differences in muscle AChE activity levels between the DFP + H₂O and DFP + C₄₃₄ groups. They suggested that a strong peripheral component was involved in the central organization of behavioral extinction. This finding that the speed of extinction of an avoidance conditioning response was slower when ChE activity was reduced by DFP was also reported by the use of systox (Russell et al., 1961).

A study on response control after DFP has also been reported by Glow et al. (1967) who found that rats which were subjected to a double level conditioning procedure showed a strong tendency to make unnecessary responses after chronic reduction of ChE activity, i.e. there was a loss of response control as a consequence of a reduction in the concentration of ChE. Banks et al. (1967) described a relationship between brain AChE activity and learning ability. He suggested that a decrease of AChE activity below a critical level of 40-60% of the enzyme's normal activity was associated with a progressive increase in total error scores in a serial problem-solving situation.

Deutsch (1966) has interpreted pharmacological-behavioral...
evidences as showing that, during and after learning, a set of
synapses increases its capacity to emit ACh and that this ca­
pacity later declines. After rats learned to escape to whichever
arm of a Y-maze that was lighted on a given trial, performance
could be disrupted by an injection of DFP, into the hippocampus.
This type of amnesia dissipated over a few days (reversible am­
nesia). The same DFP treatment that impaired a well-learned
habit was found to enhance the habit when it was still weak or
if it has been almost forgotten (Deutsch & Leibowitz, 1966; Deu­
tsch & Lutzky, 1967). This has been explained on the basis that
if DFP is used to inactivate AChE and prevent it from rapidly
hydrolyzing ACh, then liberation of ACh in large amounts may pro­
duce a level that will block synaptic transmission; on the other
hand, when the amount of ACh liberated is too small to mediate
behavior, the behavior occurs if the ACh activity is effectively
increased by DFP-inactivation of AChE. It was predicted that
opposite effects would be realized by the use of the anti-ACh
agent scopolamine; that is, the learned behavior would be blocked
by the drug when levels of ACh were relatively low but could
appear if liberation of ACh was high (Rosenzweig, Bennett, &
Ramond, 1967). Results supporting this prediction were obtained
by Deutsch et al. (Deutsch & Deutsch, 1966). Deutsch inferred
that learning involves changes at synapses already in use rather
than changes at previously ineffective loci (Deutsch, 1966).

Chapter XV. Materials and Methods
A. Animals

The subjects used in these experiments included both aggregated and isolated male, adult laboratory white mice (SCL) weighing from 35-40 grams (Tables VI & VII). The procedure for aggregating and isolating the animals were identical to that described in Section II, Chapter VII, A.

B. Drug Preparations

All drugs were prepared in 0.9% NaCl solution except DFP which was dissolved in peanut oil. The drug concentrations were such that hundredths of a milliter volume correspond to grams of the animal body weights were administered, providing the following dosages: scopolamine.HBr, 2 mg/Kg; physostigmine salicylate 0.001 mg/Kg and 0.01 mg/Kg; DFP 0.001 mg/Kg, 0.05 mg/Kg, 0.1 mg/Kg and 1 mg/Kg. All drugs were given intraperitoneally in the first series of drug experiments (Tables VI & VII) and enzyme activities were determined either 15 minutes or 2 hours after the drug administration as specified in the tables (Tables VI & VII).

C. Experimental Design

In these series of drug effect experiment (Tables VI & VII), five animals (SCL mice) per group were used in either the saline control or drug-treated groups (scopolamine, physostigmine and DFP). A completely randomized design was conducted for the control and the experimental subjects.

Usually duplicate analysis were made daily for each of six brains. Samples from each drug treated group and from saline or
peanut oil control groups were analyzed daily and also the animals were sacrificed at the same time of the day each day (approximately 9:30 A.M.).

**d. Dissection Procedure and Biochemical Analysis**

Both dissection procedure (whole brain) and biochemical analysis were identical to those described in Section I, Chapter III, B & C.

**e. Statistical Evaluation**

The statistical method employed in this experiment is identical to that described in Section II, Chapter VII, D.

Chapter XVI. Results

A. Effects of Scopolamine and Physostigmine on Brain Cholinesterase Level

The effects of various cholinergic drugs, cholinomimetics as well as cholinolytic agents on whole brain ChE activities, both in isolated and aggregated laboratory white mice (SCl) are presented in Table VI. The results indicate that neither 15 minutes nor two hours after intraperitoneal administration of 2 mg/Kg scopolamine.HBr was there a significant difference in brain ChE activities between treated and control animals both in aggregates and isolates. However, at this dose level and at these time intervals, scopolamine tends to obliterate the slight and consistent increase in brain ChE activity (although not statistically significant) which was usually found in isolated animals (see Section V, Chapter XX, A & Chapter XXI, A). The administra-
tion of 0.001 mg/Kg and 0.01 mg/Kg of physostigmine salicylate resulted in a decrease in brain ChE activity (both aggregates and isolates) as measured 15 minutes after the I.P. administration of the drug. Again, with the exception of the 0.01 mg/Kg physostigmine in the isolated group (p<0.05) these differences are not statistically significant. As can be seen from the results, the difference between scopolamine and physostigmine is that while the former obliterates the slight difference between the isolated and aggregated animals, the latter tends to preserve this difference although there are corresponding decreases in brain ChE activities due to the administration of this drug both in aggregated and isolated subjects.

B. Effects of DFP on Brain ChE Activity

The effects of DFP on brain ChE activity both in isolates and aggregates are given in Table VII. As mentioned previously the brain ChE activity is slightly higher in isolated control animals than in aggregated control animals although this difference is not statistically significant (see Section V, Chapter XX, A & Chapter XXI, A). In general, administration of DFP (I.P.) at dose levels of 0.001 mg/Kg and 0.05 mg/Kg resulted in a dose-related decrease in brain ChE activities in both isolated and aggregated animals. Thereafter an increase in dose to 0.1 mg/Kg and 1 mg/Kg resulted in a progressively smaller degree of enzyme inhibition! The differences between DFP 0.05 mg/Kg; both in aggregates (p<0.05) and isolates (p<0.05), as well as DFP 0.1 mg/Kg;
again both in aggregates (p<0.01) and isolates (p<0.05), and their corresponding controls are all statistically significant. However, the differences between 0.05 mg/Kg and 0.1 mg/Kg, are not statistically significant within the same aggregated or isolated groups. Finally, as seen in the previous experiment with physostigmine, DFP caused a decrease in brain ChE activity both in aggregates and isolates, but did not obliterate the effects of isolation on brain ChE level; i.e. in all instances, the ChE activities are higher in isolates than in aggregates irregardless of the different dose levels of DFP used. The usually non-significant difference observed between isolates and aggregates reached a statistically significant level (p<0.01) after a dose of 0.1 mg/Kg of DFP.

Chapter XVII. Discussion
A. Effects of Scopolamine in Reference to Brain Cholinesterase Activity and Behavior

Scopolamine.HBr 2 mg/Kg, either 15 minutes or 2 hours after intraperitoneal administration, shows no significant changes in enzyme activity, this is true for both aggregated and isolated animals (Table VI). Behaviorally scopolamine at this dose level produced learning and memory deficits in the aggregated animals (Meyers et al., 1964; Morpurgo, 1965; Carlton, 1961 & 1963; Scudder, unpublished data) and abolished aggression in the isolated animals (Karczmar & Scudder, 1969a). A point of interest emerges here, although there seems to be no correlation between learning
Table VII  Effects of DFP (I.P.) on Brain ChE Activity\(^1\) in SCl Mice

<table>
<thead>
<tr>
<th>Treat.</th>
<th>peanut oil control</th>
<th>DFP 0.001 mg/Kg (15' after I.P.)</th>
<th>DFP 0.05 mg/Kg (15' after I.P.)</th>
<th>DFP 0.1 mg/Kg (15' after I.P.)</th>
<th>DFP 1.0 mg/Kg (15' after I.P.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>N</td>
<td>Mean ± S.E.</td>
<td>N</td>
<td>Mean ± S.E.</td>
<td>N</td>
</tr>
<tr>
<td>Aggregates</td>
<td>20(^2)</td>
<td>30.41 ± 1.26</td>
<td>5</td>
<td>27.45 ± 1.39</td>
<td>5</td>
</tr>
<tr>
<td>% Change</td>
<td>---</td>
<td>-9.7</td>
<td>-23.3*</td>
<td>-19.8**</td>
<td>-4.9</td>
</tr>
<tr>
<td>Isolates</td>
<td>20(^2)</td>
<td>32.47 ± 1.06</td>
<td>5</td>
<td>29.47 ± 1.17</td>
<td>5</td>
</tr>
<tr>
<td>% Change</td>
<td>---</td>
<td>-9.2</td>
<td>-15.2*</td>
<td>-12.1*</td>
<td>-7.2</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram brain wet weight/min.
2. There were no significant differences among the peanut oil controls for any single group.

Therefore, the value of the mean ChE activity and the number of animals used in the peanut oil control are polled from four separate control experiments.

* Significant at 0.05 level as compared to their corresponding control.
** Significant at 0.001 level as compared to their corresponding control.

ACh Chloride Anhydride 100 mg/ampoule (Merck) was used.
ability, memory deficit and brain ChE activity in the aggregated subjects after this drug, there seems to exist a trend for scopolamine to decrease the enzyme activity of the isolated animals bringing them toward that of the aggregated controls. In light of the fact that this enzyme persistently differentiate the isolated, aggressive subjects from the aggregated, non-aggressive animals throughout this thesis by a slight and consistent but statistically non-significant increase in enzyme activity (see Section V, Chapter XX, A, and Chapter XXI, A), it is of interest to comment on the possibility that scopolamine might abolish isolation-induced aggression by obliterating the slight increase in ChE activity seen in the isolated animals. A strategic analysis of this enzyme by brain region might reveal a more clear and pronounced response. It should be pointed out, however, that concomitant neurochemical changes in ACh were found in these isolated mice, i.e. an increase in telencephalic while a decrease in midbrain-diencephalic ACh was observed. Scopolamine (2 mg/Kg) was found to abolish isolation-induced aggression at a time which caused a significant decrease in telencephalic ACh without producing significant changes of this substances in other areas of the brain (Sobotka et al., 1968; Sobotka, 1969). Since the telencephalic portion constitutes the bulk of the brain, the increase in whole brain ChE activity seen in the isolated animals might be indicative of an increase of telencephalic ChE activity as well. If this is so, then scopolamine might abolish aggression by de-
creasing both ChE activity and ACh level in telencephalon. However, in view of the fact that a considerable extent of brain ChE inhibition is noted before the changes in behavioral patterns can be observed (Russell, 1961; Kling et al., 1965; Russell, 1969; cf. Karczmar, 1969b), it is suggested that such speculation should not be overemphasized. No changes in various biogenic amines were found in these isolated mice (Scudder; unpublished data).

B. Effects of Physostigmine and DFP with regard to Brain ChE Activity and Behavior

Physostigmine has been reported to exert either inhibitory (Bures et al., 1962 & 1964; Herz & Yacoub, 1964; Goldberg et al., 1965) or facilitatory effects (Stratton et al., 1963; Russell, 1954) on avoidance conditioning. In our laboratory, however, physostigmine was found to exert a biphasic effect on learning in mice. It was found that at very small doses (0.001 mg/Kg – 0.01 mg/Kg) physostigmine increased learning while higher doses disrupted it (Scudder, unpublished data). In the present study at the doses employed (0.001 mg/Kg & 0.01 mg/Kg) physostigmine was found to facilitate learning in the aggregated mice, but produced no statistically significant changes in brain ChE activity in these mice although a decrease in enzyme activity did occur after this drug. Nevertheless only a few readings reach a statistically significant level. Whether a regional analysis of the enzyme might reveal a significant difference in brain ChE activity is not known in the present study. A few words regarding this lack
of effects of physostigmine on brain ChE activity at the dose levels employed, however, have to be made. It should be noted that the doses of physostigmine used in these studies were extremely small as compared to the conventional dose of this drug (0.1 - 1.0 mg/Kg) employed in the behavioral studies. And hence physostigmine is a reversible anticholinesterase, the possibility exists that, at these low dose levels, the amount of enzyme inhibition might indeed be reversed by the process of homogenation and dilution, to such an extent, that no enzyme inhibition can be measured by this technique.

For isolated animals, physostigmine exhibited an effect different from that of scopolamine, i.e. while physostigmine caused a dose-dependent decrease in brain ChE activity on these subjects, it did not obliterate the slight and consistent, although not statistically significant, difference in brain ChE activity between the aggregates and isolates (higher brain ChE activity in the isolates). This finding that physostigmine at the doses employed did not decrease the enzyme activity of the isolates to that of the aggregates on one hand, and enhanced post-isolation aggression on the other (Scudder, unpublished data) seems to be in agreement with the data obtained after the administration of scopolamine which obliterated the difference in enzyme activity and also abolished aggression in these animals. It should be pointed out, however, the possibility exists that an optimal level of brain ChE inhibition may be necessary for the abolition of
isolation-induced aggression to occur since both scopolamine and phystostigmine caused a decrease in enzyme activity in the isolates and phystostigmine was found to decrease the enzyme activity to a greater extent than scopolamine. Therefore, it might appear that scopolamine abolished aggression by decreasing brain ChE activity to that optimal level which is necessary for aggression to be abolished while phystostigmine, which due to its ability to decrease the enzyme activity further below the optimal level caused an increase in aggressive behavior. Again, as mentioned previously in the effects of scopolamine on isolation-induced aggression, this speculation of the effects of phystostigmine on post-isolation aggression is highly speculative and can not be regarded as conclusive data since none of these drug effects reached a statistically significant level (except that of phystostigmine 0.01 mg/Kg in the isolates).

Chronic administration of DFP has been reported to impair learning (Banks et al., 1967) and to cause changes in extinction rate in rats (Russell et al., 1961; Glow et al., 1965 & 1966). In our laboratory, DFP was found to be similar to phystostigmine which at very small doses (0.001 mg/Kg - 0.01 mg/Kg) improved learning in the aggregates and enhanced aggression in the isolates while at higher doses it depressed animals (both aggregates and isolates) prior to convulsive seizures (Scudder, unpublished data). Again as seen in the drug effects of scopolamine and phystostigmine, no biochemical correlates of this enzyme and learning ability can
be drawn. At a dose level of 0.001 mg/Kg, DFP was found to improve learning in mice and produced 9.7% enzyme inhibition, while at higher dose levels (0.05 mg/Kg, 0.1 mg/Kg, & 1 mg/Kg) it was found to impair learning and caused 23.3%, 19.8%, and 4.9% enzyme inhibition respectively. If it is speculated that learning improvement by DFP can only occur at a dose level which slightly decreases brain ChE activity, it would appear difficult to explain the data that DFP 1 mg/Kg, while causing a smaller degree of enzyme inhibition than that of 0.001 mg/Kg (both are not statistically significant different from each other) produced an effect opposite to that of 0.001 mg/Kg, i.e. learning deficit in these animals. It should be stressed, however, that the order of magnitude of enzyme inhibition among the various doses of DFP in unexpected; i.e. 0.05 mg/Kg > 0.1 mg/Kg > 1 mg/Kg. In the isolated animals, small doses of DFP exerted an effect similar to that of physostigmine; it caused a dose-related decrease in enzyme activity without obliterating the slight increase in brain ChE activity (statistically non-significant) seen in the isolates on one hand, and enhanced aggression on the other. However, it should be pointed out that such difference in brain ChE activity between aggregates and isolates also existed at higher dose levels of DFP which abolished aggression. Also there was a similarity in this isolate data to that of the aggregates (DFP 1 mg/Kg caused an approximately equal amount of enzyme inhibition as that of 0.001 mg/Kg, yet produced an opposite behavioral effect to that
of 0.001 mg/Kg; i.e. learning impairment instead of learning improvement); DFP 1 mg/Kg produced an approximately similar amount of enzyme inhibition as that of 0.001 mg/Kg and also preserved the differential effects of isolation on brain ChE activity yet produced an entirely opposite behavioral effect - aggression abolishing action. Therefore, it appears that no single correlation between either learning ability or aggressive behavior and brain ChE level can be drawn from this study.
SECTION V

ENVIRONMENTAL MANIPULATIONS
Chapter XVIII. Introduction

A. Environmental Influences on Animal Behavior

One of the most useful and conventional approaches to behavioral research is to manipulate artificially various environmental factors which will affect the animals' behavior and search for biochemical correlates of this behavior. Although a causal relationship between these two measures cannot be established through this type of study, it is extremely useful, especially at the present stage of our knowledge, to provide correlates with various behavioral patterns.

Thiessen et al. (1962) reported that mice housed in groups and receiving extra-stimulation showed both the shortest running time in the "hole-in-wall" test and an increase in adrenal weight and subcortical ChE activity. Moreover both cortical and subcortical ChE activity correlated positively with the adrenal weights under all stimulus conditions, and all three measurements, viz. adrenal weight, cortical, and subcortical ChE levels, in turn correlated well with the behavioral measure under most stimulus conditions. Therefore, they suggested that housing conditions can markedly influence both behavior and physiological states and that a relationship exists linking the endocrine response, brain chemistry, and behavior.

Bovet-Nitti et al. (1968) reported that cross-fostering did not affect the usual patterns of avoidance behavior (shuttle-box avoidance) in DBA2J mice but decreased performance in C3H/He mice.
The latter effects were suggested to be due to the appearance of high levels of freezing behavior. These findings supported the hypothesis that non-genetic factors can also contribute to the development of some strain-specific patterns of behavior. In particular, modifications of the pre-weaning environment affect the emotional behavior while variations of learning patterns are only due to the concomitant appearance of freezing behavior.

Denenberg et al. (1962) described a handling effect on animal behavior. They reported that subjects handled in infancy were significantly less emotional than non-handled controls as evaluated by activity and defecation measures in open-field tests. Subjects with free-environment experience were significantly less emotional than subjects kept in laboratory cages. Furthermore, the handled subjects, especially the females, were benefited by the mixing of the two infantile-experience groups while the non-handled subjects were impeded by it.

Kling and his co-workers (1965) reported effects of handling and another environmental factor, light stimulation, on the developing rat brain. They found that, in general, the effect of environmental stimuli (handling and light stimulation) in the pre-weaning period was a suppression of the enzymatic (ChE) development in the cortical structures and an acceleration of that in the subcortical structures. Related studies have been carried out by Liberman (1962) and Glow et al. (1964). Liberman (1962) found that rats that are raised in the dark from birth to 17 weeks
of age have significantly lower AChE activities in the retina than control rats raised under standard conditions. No such differential effects were observed with pseudocholinesterase and the glycolytic enzymes. Using a different technique, Glow et al. (1964) fitted a moulded opaque polyethylene corneal-scleral contact lense over the right eye in rats and studied the effects of light and dark on the AChE activity of the retina. They found that de novo synthesis of AChE after DFP treatment is consistently and significantly depressed in the eye which is deprived of light. They suggested a possible substrate regulated mechanism for this enzyme in the retina.

B. Environmental Complexity, Behavior and Brain Measures

A series of experiments concerning environmental complexity, behavior and various brain measures such as brain ChE, AChE, brain weight, brain morphology, as well as intelligence have been conducted by the California investigators. In general, the standard experiment consisted of immersing one set of animals from weaning age (25 days), in an enriched environment (ECT group) and the littermate brothers of these animals in an impoverished environment (IC group). After 80 days in these environments the animals are sacrificed for anatomical and chemical analysis of brain. Briefly the ECT condition provided the animals with an environment as rich as a rat can accept. It includes group housing, daily handling, toy playing and maze training etc. Their IC littermates, on the contrary, receive no such environmental rich-

ness but rather live in an isolated cage in a quiet and secluded room without contact or sight of other animals. The results revealed that, after 80 days of exposure in such differential complexity of the environment, significant changes in cerebral measures of these animals occurred. The general patterns of cerebral changes between the ECT and IC littermates are: 1) a significant increase in tissue weight in whole brain, total cortex, as well as certain region of the cortex in ECT animals in comparison to their IC littermates. A concurrent increase in thickness and depth of the cortex in ECT animals also occurred; 2) chemically, a significant increase in total AChE in the cortex of the ECT subjects as compared to their IC brothers was obtained. This increase in AChE activity in the cortex of ECT subjects, however, always lagged behind the increase in tissue weight of the cortex, hence a decrease in specific AChE activity (AChE/tissue weight) results consistently in the ECT subjects. Conversely, for the enzyme butyrocholinesterase (BuChE or pseudoChE), while there is a similar increase in total ChE activity in the subcortical structures parallel to the increase in AChE in the cortex, an increase in specific activity for this enzyme in the subcortex was observed in the ECT animals; this is due to the fact that there is a greater increase in total ChE activity in the subcortex than the weight increase in the same brain structures (Krech et al., 1960; Rosenzweig et al., 1962; Rosenzweig et al., 1964a & b; Krech et al., 1964; Bennett et al., 1964; Krech et al., 1966; Rosenzweig, 1966a;
The increase in total AChE activity in the cortex was later confirmed to be due to an increase in the volume of perikaryon and of nuclei in the neural tissue of the ECT subjects while an increase in the number of glial cells was found to account for the increase in pseudocholinesterase in the subcortex (Diamond et al., 1964; Diamond et al., 1965; Altman et al., 1964). The speculation that the bulk of brain tissue which was gained in the ECT subjects in response to enrich experience is relatively non-cholinergic in nature is also supported by the findings that there is a concomitant increase in the diameter of cerebral blood vessels in the ECT animal (Krech et al., 1966; Rosenzweig et al., 1962 & 1968).

An attempt at finding behavioral correlate of enriched experience has also been carried out (Krech, Rosenzweig, & Bennett, 1963a; Hymovitch, 1952). It was found that rats which underwent 30 days of enriched environmental experience were significantly superior to their corresponding IC littermates on a reversal discrimination schedule in the Krech Hypothesis Apparatus (Krech et al., 1963).

These effects of environmental complexity on cerebral measures were then subjected to various types of analysis in an attempt to analyze the factor or factors which are most important and responsible for these cerebral changes. It was found that social grouping as well as the enriched environmental experience rather
than isolation stress, visual stimulation, or neurological injury are essential for these changes since sighted rats brought up in a totally dark environment were found to have identical or even greater ECT-IC effects than their enucleated, blinded littermates; and SC rats (standard colony group) which are identical to IC rats in environmental conditions except they are housed in a standard colony environment were found to be closer or identical to IC littermates in all cerebral measures. Furthermore, the ECT-SC differences were found to be similar to that of ECT-IC effects (Krech et al., 1963b). Handling and formal training seems to play a small to insignificant role in these results (Krech et al., 1960). Moreover, this cerebral plasticity in response to differential experiences was found to occur in different strains of rats (Krech et al., 1960; Rosenzweig et al., 1964b), at different ages (Rosenzweig et al., 1964a; Krech et al., 1960), in both sexes and was also a function of differential environmental complexity (Krech et al., 1960; Rosenzweig et al., 1962; Bennett et al., 1964; Krech et al., 1966).

In the study of the time-course of these environmental effects on cerebral changes, three types of curves were found to exist; one, changes in response to an enriched environment were found to increase and persist after the cessation of the exposure (such a change was seen in the weight of the occipital cortex); two, some changes were found to exist only during or immediately after the exposure but subside rapidly thereafter (weight differ-
ences in the somesthetic samples); and three, there was a combination of both permanent and temporary changes in which a greater effect was found to occur during or after the exposure followed by a decline but persistent effect thereafter (weight differences in remaining dorsal cortex). These transitory changes that occur in the brain as a consequence of experience have been referred to as the process of consolidation of memory. It is suggested that during the time when learning is occurring and stable traces (of some sort) are being laid down in the brain, temporary modifications of brain tissue such as enlargement of cell bodies and proliferation of the surrounding glial cells, formation of altered ratio of chemical products within the nerve cells etc., which reflect increases in metabolic and synthetic activities, may well form the physical substrate of memory. Once learning was completed and the resultant process of consolidation had subsided, then the changes needed to support this extra activity would regress, and only the structural correlates of long-term memory would remain to differentiate the trained from untrained brain (Rosenzweig et al., 1966b; Rosenzweig et al., 1967). It should be pointed out, however, that the changes in enzyme activities measured in these experiments were within the range of 5% or so and have been highly criticized by Karczmar (1969b), and Klüver (1958).

Related experiments concerning environmental complexity and brain chemistry have also been reported by Zolman & Morimoto.
(1962 & 1965), and Geller et al. (1965) in rats, and LaTorre (1968) in mice. Generally the findings are similar to that of the California investigators. One exception was reported by LaTorre (1968) in mice correlated ChE activity with environmental enrichment. She found that instead of changing inversely as was seen in the rats, the changes in ChE activity with enriched experience generally appeared to parallel the changes in AChE activity.

Pryor et al. (1967) attempted to determine whether diffuse stimulation of the brain would alter its morphology and biochemistry as does exposure to an enriched environment. They found that chronic electroconvulsive shock (ECS) led to increases in brain weight and to changes in AChE and ChE activities. However, the pattern of ECS-induced changes was different from that induced by enriched experience; ECS led to changes chiefly in the ventral cortex and to a fall rather than a rise in ChE activity. The ECS treatment also led to poorer maze performance, whereas enriched experience led to improved problem-solving behavior. Some of the effects of ECS on brain weight and brain chemistry have been replicated by Vernadakis et al. (1967).

C. Effects of Isolation on Animal Behavior and Brain Chemistry

Studies relating to biochemical correlates of isolation-induced aggression have been well documented. The involvement of androgens in aggressive behavior is beyond doubt (Levy & King, 1953; Bevan et al., 1960; Sigg et al., 1966; Suchowsky et al.,
1967; Bronson et al., 1968; Abbatiello et al., 1968; Abbatiello, 1969). Other biologically active substances such as norepinephrine and 5-HT have also been investigated (Welch & Welch, 1968; Consolo et al., 1965; Welch & Welch, 1965; DaVanzo et al., 1966; Valzelli, 1967; Valzelli et al., 1968; Giacalone et al., 1968). In general, no differences in biogenic amines; brain serotonin or norepinephrine between isolated (aggressive) and grouped mice were reported (Consolo et al., 1965; DaVanzo et al., 1966; Valzelli, 1967; Scudder, unpublished data). Related studies on the activity of the enzyme involved in the biosynthesis and metabolism of catecholamines have also been conducted (Axelrod et al., 1970).

It was reported that there was a significant decrease in adrenal tyrosine hydroxylase and phenylethanolamine N-methyl-transferase (PNMT) activity in the socially-deprived mice (isolated mice) while a marked elevation of these substances as well as MAO activity in the adrenals of psychologically stimulated (grouped) mice (Axelrod et al., 1970).

In addition to androgens and biogenic amines, other biochemical substances may also be involved in aggressive behavior. Marcucci et al. (1968) reported that there was a significant lowering of N-acetyl-L-aspartic acid (NAA) in the brain of isolated male mice which develop aggression, but not in the brain of isolated female mice which do not develop aggression. Agrawal et al. (1967) in their study of the neurochemical and behavioral effects of isolation-rearing in the dog found that young puppies reared
in partial isolation for one week at 4-5 weeks of age showed some behavioral abnormality, hyperactivity, and diffuse reactions. These behavioral changes were associated with significant changes in the concentration of free amino acid in the subcortical areas with practically no changes in the cerebral cortex. However, partial social deprivation had a pronounced effect on glutamic acid and gamma-amino-butyric acid (GABA) content of thalamus-hypothalamus and caudate nucleus with glutamic acid increasing in the former and decreasing in the latter. A decrease in glutamine content in the superior colliculi and caudate nuclei was also apparent and significant in the isolated animals.

Finally the cholinergic system which is of the primary interest in this thesis has been shown to be implicated in isolation-induced aggression. Janssen et al. (1960) and DaVanzo (1965 & 1966) reported an effective anti-aggressive action of scopolamine. Her and other anticholinergic agents. Karczmar & Scudder (1969a) found biphasic, dose-dependent effects from the administration of either scopolamine or physostigmine. Another cholinergic drug, methionine sulfoximine, has also been reported to produce biphasic actions on aggressive behavior, i.e. initial increase followed by a decrease in aggression (Karczmar et al., 1968a).

More recently Sobotka (1969) studied ACh levels in the brain parts of isolated SCI mice and found that there existed a small, but significant decrease in the midbrain-diencephalic ACh concentrations of the isolated mice compared to the aggregates. The de-
crease in midbrain-diencephalic ACh levels was interpreted as a
decrease in cholinergic activity at this region of the brain and
has been related to the process of habituation (cf. Scott &
Fredericson, 1951; Carlton, 1963 & 1968), i.e. reduction of cho-
linergic activity at this specific brain area might be associated
with a regression of habituation to sensory input, which, in turn,
results in an exaggerated response consequent to the presentation
of a mild sensory input such as introduction of a strange male
mouse into the isolation cage (Sobotka, 1969). Finally the de-
crease in cortical AChE and increase in subcortical pseudocholin-
esterase activity in rats and mice raised in enriched environment
has already been mentioned previously (Rosenzweig, 1970; LaTorre,
1968; Geller et al., 1965). It is, therefore, of interest to
study and confirm the differences in brain ChE activity in re-
ference to isolation-induced aggression.

D. Effects of Learning on Animal Behavior and Brain Chemistry

Adams et al. (1967), by varying the baseline rate of bar
pressing on which one trial learning (foot shock) was super-
imposed, demonstrated that one ECS (electro-convulsive shock)
caused a decrement in retention if baseline rate is low but not
if it is high. He, therefore, concluded that ECS causes dis-
inhibition rather than disruption of memory traces in this ex-
perimental procedure. The hypothesis that ECS caused disinhibi-
tion and decrement in retention by altering the activity of
cholinergic neurons was then investigated in a follow-up study
(Adams et al., 1969). They showed that ECS resulted in an immediate increase in whole brain AChE levels which return to pre-ECS level within 96 hours. When attempts were made to eliminate or augment the retention deficit caused by ECS through the use of scopolamine and eserine, they found that scopolamine partially eliminated the decrements in retention while eserine in combination with ECS caused an even greater decrement, thus suggesting that ECS treatments might influence learning and memory through mechanisms of neurochemical changes, especially AChE activity levels.

Sklyarov et al. (1963) described a relationship between food reflexes and ChE activity of cortical tissue in dogs. They found that the activity of ChE was decreased when dogs were under the influence of unconditioned and conditioned food reflexes. These experimental results were explained by the assumption that ACh takes part in the realization of conditioned-reflex reactions and the decrease in ChE activity is secondary to the liberation of ACh during excitation, which allowed ACh to be preserved in a larger quantity or for a longer time.

Chapter XIX. Materials and Methods
A. Animals

The animals used in this series of experiments were male, adult SCJ mice, weighed approximately 35 ± 5 grams. The living conditions, again, were identical to that described in Section I, Chapter III, A, 1.
For environmental effects (aggregation vs. isolation) three separate experiments were conducted; one **ChE Activity in Brain Halves of SCI Mice** (see Section II, Table II), two, **Drug Effects on Brain ChE Activity on SCI Mice** (see Section IV, Table VI), and three, **Effects of DFP on Brain ChE Activity in SCI Mice** (see Section IV, Table VII). The animals were either aggregated 20 to 25 per cage (6x10x18 inches) or isolated one per cage (3.5x3.5x7.5 inches) for at least a period of 2 weeks (cf. Section II, Chapter VII, A). The ChE activities were determined either on brain halves (Section II, Table II) or whole brain (Section IV, Tables VI, VII). For number of groups, number of animals per group, dose of drug etc. please see Section II, Chapter VII and Section IV, Chapter XV for references.

For the learning and frustration experiment, three groups of ten animals (SCI) each were employed, i.e. naive control; learning group; and frustration group. The behavioral procedure for learning and frustration is described in the following paragraph. The animals were housed in normal condition of 10 per cage (6x10x8 inches) in this experiment and only whole brain ChE activities were determined.

**B. Behavioral Procedure**

For learning and frustration, the animals were subjected to a penta-level conditioning climbing screen (Scudder et al., 1969). Briefly the apparatus consists of five base chambers with electrified grid floors. The chambers are connected to each other
by means of a 35° inclined runway. Solenoid-operated gates, in turn, communicate each base chamber to and from the inclined runways. A gate is opened 5 seconds before the animal is shocked and the electric shock is then applied at 10 second intervals to the four consecutive sections of the runway grid to force the animal to climb to the next base chamber. The time which the mouse spends in each base chambers as well as in inclined runway is recorded. After the animals undergo ten trials in the apparatus, a total of 50 readings per animal for base-chamber time and climbing time are obtained. Since total of 10 animals per group are used, the values obtained in the learning curves of these animals are the means of 50 readings (base chamber time) per trial. This number of trials is chosen because it has been established that SCl mice exhibit significant degree of learning after this procedure (Scudder et al., 1969). In order to confirm that the animals were well-learned in these studies, a 20-trial procedure was also performed.

To cause "frustration", animals were subjected to the same apparatus and received approximately the same number of shocks but they were not allowed to escape, i.e. the doors were not opened either during the visual stimuli or during electroshock period. The animals were sacrificed either immediately or 24 hours after these procedures and brain ChE activities were determined.

C. Dissection Method, Biochemical Analysis, and Statistical Evaluation
The dissection procedures for brain half and whole brain are identical to those described in Section II, Chapter VII, B, and Section I, Chapter III, B respectively whereas the biochemical analysis is identical to that in Section I, Chapter III, C and statistical evaluation identical to that in Section II, Chapter VII, D.

Chapter XX. Results

A. Differential Effects of Environment on Brain Cholinesterase Activity

The effects of environmental influences (isolation vs. aggregation) on brain ChE activity on SCl mice were presented in Section II, Table II (ChE Activity in Brain Halves of SCl Mice); Section IV, Table VI (Drug Effects on Brain ChE Activity on SCl Mice); and Section IV, Table VII (Effects of DFP on Brain ChE Activity in SCl Mice). The results indicate that in all instances there is a slight and consistent increase in ChE activity ranging from 7-9% (ChE/gram wet weight) in the untreated, isolated control as compared to that of the untreated, aggregated control although this difference is not statistically significant. This is true both for enzyme activity measured either on brain half or whole brain basis.

B. Effects of Learning and Frustration on Brain ChE Activity

The effects of learning (foot-shock avoidance conditioning) and frustration (foot shock without allowing for escape) on brain ChE activity are shown in Table XII. The animals were subjected
to a ten-trial training procedure in the penta-level climbing screen as described by Scudder et al. (1969a). The brain ChE activities either immediately or 24 hours after the ten-trial conditioning training and frustration stress were determined and the percentage difference from their corresponding controls are given in Table XII. The results indicate that neither immediately nor 24 hours after either a ten-trial conditioning or an encounter with frustration foot shock were there significant changes on brain ChE activity as compared to corresponding normal controls (non-conditioned, unstressed animals). Although among the conditioned group sacrificed 24 hours later there was a slightly increase in the brain ChE activity over its normal control. This difference, however, was not statistically significant. When one considers the fact that, in the conditioned subjects, in addition to the changes in performance (improvement) that occurred in these animals, concomitant changes of some sort induced by foot shock might also occur, it would appear reasonable to assume that the frustration stress group would be a better control for the conditioned group. This comparison, however, also indicated no significant differences between the conditioned and frustration groups either immediately or 24 hours after the foot shock experiment. Another study in which ten animals were subjected to 20 trials of frustration foot shocks and sacrificed 24 hours later was carried out to confirm the effects of frustration on brain ChE level. These findings are in agreement with those
reported in the above experiment, i.e. no difference was found between normal control and frustration group with regard to brain ChE activity.

Chapter XXI. Discussion

A. Differential Effects of Environmental Influences on Brain Cholinesterase Activity

One point of interest comes up in three separate experiments (Tables II, VI, & VII) during the course of this thesis investigation. This is, that there exist a slight and consistent increase in brain ChE activity in the isolated animals when they are compared to that of the aggregated subjects although this difference is not statistically significant. These findings are in agreement with those reported by the California investigators (Bennett et al., 1964; Krech et al., 1964 & 1966; Rosenzweig et al., 1962; Rosenzweig et al., 1964a; Rosenzweig et al., 1966b; Rosenzweig, 1970) as well as other authors (Geller et al., 1965; LaTorre, 1968; Zolman et al., 1962 & 1965) in which the environmentally enriched subjects have a lower AChE activity per unit tissue weight than their impoverished isolated controls although the former has a higher total cortical ChE activity than the latter. The lack of statistical significance observed in the experiments presented here can be accounted for on the basis of several factors; 1) the use of whole brain instead of strategic brain parts which may mask the slight but significant changes in response to environmental influences, 2) the measure of enzyme
<table>
<thead>
<tr>
<th>Time Sacrificed</th>
<th>Group</th>
<th>Control</th>
<th>Conditioned</th>
<th>Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Mean ± S.E.</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immed. after Shock</td>
<td>20</td>
<td>26.95 ± 0.83</td>
<td>10</td>
<td>26.58 ± 0.87</td>
</tr>
<tr>
<td>% Change</td>
<td></td>
<td>---</td>
<td>-1.4</td>
<td></td>
</tr>
<tr>
<td>24 hours after Shock</td>
<td>20</td>
<td>24.99 ± 0.92</td>
<td>10</td>
<td>27.08 ± 1.31</td>
</tr>
<tr>
<td>% Change</td>
<td></td>
<td>---</td>
<td>+8.4</td>
<td></td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in terms of micromoles acetic acid/gram wet weight (whole brain)/minute.

ACh chloride Anhydride 150 mg/vial (Sigma) was used.
activity in terms of total enzyme level without the differentiation between specific and non-specific enzyme activity may also affect the variance, since it is believed that the two enzymes are functionally different from each other; the former is related to neuronal activity while the latter is concerned with the metabolism of glial cells, 3) the difference in the variations of environmental complexity may play an important role in explaining the discrepancy observed in these studies. In the experiments of other investigators the animals were under considerable environmental richness; they received daily handling, various types of toy playing, informal training as well as formal maze learning, on the other hand, those carried out in this study are only under a social grouping of 20 to 25 animals per cage with all the other conditions identical to that of the isolated subjects. These factors may also explain the lack of differences in brain weights and body weights between the isolated and aggregated subjects in the present experiment. In spite of the lack of statistical significance, these studies are important in that they imply that the brain is capable of responding to environmental pressure neurochemically or possibly anatomically -- a criterion demanded by physiological theories of learning and memory (Bennett et al., 1964). Information concerning learning ability in these mice is not available in the present studies. However, if it is true that there is a correlation between enriched environment, low AChE activity per unit weight and better learning ability (Hymvitch,
In addition to learning ability, an important behavioral syndrome developed in the mice after two weeks of isolation in the present experiment is that of aggressive behavior (DaVanzo et al., 1965 & 1966; Valzelli, 1967; Valzelli, 1969). Due to the limited information provided in this experiment, it is impossible to attribute the concomitant neurochemical changes (slight but insignificant changes in brain ChE activity) seen in these animals compared to their aggregate control to either the effects of isolation or aggregation or to both of them, i.e. whether there may be an increase in isolates or a decrease in aggregates or
en both conditions occurred simultaneously. An increase in ACh
tivity in telencephalon while a decrease in midbrain-diencephalon was described for these isolated aggressive mice, and a
dcrease in cholinergic function in midbrain which in turn allows
the expression of aggressive behavior has also been proposed
(Sobotka, 1969).

Effects of Learning and Frustration on Brain ChE Activity

The data presented in this experiment indicate that there are
significant differences in brain ChE activity in animals either
jected to avoidance conditioning or to inescapable foot shock
stration. This is true not only immediately after the electro-
ck experiment but also holds for measurements made 24 hours
er as well as to determinations performed immediately after
trials of inescapable foot shock (Table XII). This finding is
agreement with those reported by Pryor et al. (1967); Pryor
Otis (1969) in which no significant changes in brain ChE
ivity per unit weight were observed although there was a trend
ard higher activities in the chronically electroshock subjects.
ever, in Pryor's study when the enzyme activities were express-
in terms of total activity a significantly higher enzyme level
electroshocked subjects was observed. It therefore appears
at the increase of this enzyme in excess of the increase of
ue weight to chronic electroshock is relatively small. Since
adakis et al. (1967) reported that rats receiving shocks after
th for various periods of time developed higher AChE activity
in the hypothalamus, but no differences in cerebral cortex, it appears that varied conditions and different measurement techniques such as specific regional definitions may reveal a more pronounced response. The lack of changes in brain weight in shocked animals in the present experiment is different from those mentioned above; however, this can easily be explained on the basis of the difference in duration of treatment. In the present study, the animals received only either 10 or 20 trials training in the conditioning climbing screen while in those experiments reported by others, the animals received daily shock for at least a period of 4 weeks (Pryor et al., 1969; Vernadakis et al., 1967). It might appear that a certain limit of duration of treatment is necessary for the brain to develop significant changes with regard to these cerebral measures.

That learning produced no significant changes in brain ChE activity is contrary to data reported by Sklyarov et al. (1963) in dogs. They found that a decrease in cortical ChE activity occurred in dogs in response to unconditioned, and conditioned food reflexes. A direct comparison between Sklyarov's et al. report and those presented here is not appropriate, however, due to the fact that different experimental conditions were employed. In the present experiment, the conditioning involves some sort of instrumental learning mechanism from the behavioral acts of the animal evoked by the shocks, i.e. the animals either escape the shocks by leaving the chamber before the shocks are administered
receive the shocks without leaving the chamber after the door is opened. In the Sklyarov's study a different type of feedback mechanism is involved, i.e. Pavlovian conditioning; no matter what kind of behavioral acts is displayed by the animals, the sound of the bell is always associated with the food. Such differentiation between experimental conditions might account for the discrepancy seen in these studies. While ChE activity failed to differentiate the behavior patterns produced by avoidance conditioning and inescapable footshock, the ACh content has been found to be different in these two groups of animals (Sobotka, 1969). It has been reported that an increase in ACh activity occurred in the telencephalon of the frustrated mice but not in the conditioned mice (Sobotka et al., 1968, Sobotka, 1969). In view of the fact that the behavior of the conditioned animals is identical to that of the unshocked animals while those of the frustrated subjects after a period of jumping, biting, squealing ....etc. remain quietly receiving the shocks without displaying any behavior acts, i.e. become maladaptive or neurotic, it might be suggested that a homeostatic state is re-established in the former but not in the latter, and this expression of internal states might be reflected in the differential changes in the neurohumors observed in these animals. Such temporary neurohumoral fluctuation in turn might be, as suggested by Scudder (1971), necessary for learning process to occur.
Chapter XXII. Summary and Conclusion

The whole brain levels of ChE in six genera (13 strains) of mice as well as regional ChE activity in Mus musculus ScI and Dipodomys deserti mice were determined. The whole brain enzyme activities of Mus musculus ScI were also measured in various experimental conditions, in different environments (isolation, aggregation, learning, frustration etc.), and in drug-induced states. Changes in brain ChE levels during a 24 hour period (circadian rhythm) were reported in two genera of mice; Mus musculus ScI and Dipodomys merriami.

The enzyme levels were determined by an automatic titration technique (Tammelin & Strindberg, 1952) in which the amount of acetic acid liberated as a result of enzymatic activity on the substrate (ACh) was titrated against an alkali solution (0.025 N NaOH).

Possible biochemical correlates of behavior were discussed in reference to learning ability, exploration, general motor activity, curiosity, aggression, stereotypic behavior as well as developmental traits. Up to six-fold differences in brain ChE activity were found to differentiate the two extreme mouse types investigated; Mus musculus "Missouri" (highest) and Dipodomys deserti (lowest). No single unitary correlation was found to exist between this enzyme system and various types of behavior although there may be a trend that high brain ChE level (ChE/weight) is associated with better learning ability as well as high
exploratory behavior.

A species specific circadian rhythm in brain ChE level was also found to exist in Mus musculus SCl and Dipodomys merriami mice; in the former, a bimodal circadian pattern was found; peak values were obtained at 03:00 hours and 15:00 hours, trough values were found at 09:00 hours and 24:00 hours; while in the latter, a trimodal pattern was reported; peak values were measured at 03:00 hours, 12:00 hours, and 21:00 hours, trough values at 06:00 hours, 18:00 hours, and 24:00 hours.

A characteristic uneven distribution of ChE activity in the CNS was confirmed in Dipodomys deserti. The sequences of decreasing order of magnitude are: pons-medulla, cerebellum, midbrain-diencephalon, telencephalon.

Isolation for at least a period of two weeks was found to cause a slight and consistent increase in brain ChE activity in SCl mice; however, this increase in brain ChE activity did not reach a statistically significant level.

Physostigmine and diisopropyl fluorophosphate (DFP), in general, caused a dose-dependent decrease in enzyme activity but these drugs did not obliterate the slight and consistent but statistically non-significant increase in brain ChE activity seen in the isolated animals. Scopolamine, on the other hand, tended to decrease the higher enzyme activity seen in the isolates as compared with that of the aggregates. Few of these effects, however, reached a statistical significant level (for example DFP at
Dose levels of 0.05 mg/Kg and 0.01 mg/Kg both in aggregates and isolates, and physostigmine at 0.01 mg/Kg in isolates).

Altogether, the ChE activity as measured in whole brain, in the genetic study, in studies on circadian rhythm, and drug effects, as well as in the various forms of environmental manipulations sheds little light on the understanding of biochemical correlates of behavior. It appears that these enzymes are either present in excess as compared to that of the synthetizing enzyme (Aprison et al., 1964; cf. Friede, 1966) or it is relatively inaccessible to environmental influences which affect possible neurohumoral substances such as ACh, 5-HT and norepinephrine etc. However, in view of the fact that changes in enzyme activities did occur consistently in the same direction in some instances (such as seen in the isolated animals in which a slight and consistent but statistically non-significant increase in brain ChE activity was observed in three separate experiments; Section II, Table II, Section IV, Tables VI & VII), it might be safe to speculate that while the enzyme is capable of responding to environmental pressure it is operating within a considerably wide safety factor. This point of view has been widely accepted by various investigators (Bullock et al., 1947; Wilson et al., 1953; Nachmansohn et al., 1947; White, 1956; Russell et al., 1961; cf. Russell, 1969; Karczmar, 1969b). Bullock et al. (1947) and Wilson et al. (1953) stated that a reduction of ChE activity below 25 per cent or more of normal value was necessary for a conduction failure to
occur. Behaviorally, Russell et al. (1961) reported that a reduction of brain ChE activity below a "critical level" of 60 to 65 per cent of normal value was required to produce changes in extinction (also cf. Russell, 1969 & Karczmar, 1969b). Glow et al. (1965 & 1966) also reported that a reduction of ChE activity to 40 per cent of the control was necessary for such behavioral changes to occur. Similarly, Banks et al. (1967) suggested a decrease of AChE activity below 40 to 60 per cent of the enzyme's normal level was necessary for learning impairment to occur in a serial problem-solving situation. If this is indeed the case then it would not be surprising to find that there are no statistically significant differences in this enzyme (ChE) as a consequence of various relatively mild and durationally short environmental pressures such as seen in this thesis.

While certain environmental and pharmacological manipulations successfully demonstrated the changes in physiological substances in the organisms, the mechanisms by which such neurohumoral substances as well as related enzyme systems operate in modifying or modulating behavior is not known at the present time. Nevertheless, it might be speculated that the fluctuations of these biologically active substances in response to environmental input are a necessary process for the generation of certain behavioral patterns as suggested by Scudder (1971) and these changes might modify neural activity by facilitating or inhibiting the organization or patterning of the neural circuitry in some way similar to that of the
neuro-endocrine control over the conditioned reflex and basic emotional behavior in higher animals (Lissak and Endroczi, 1961).
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### Table XII. Effects of Learning and Frustration on Brain ChE Activity in SCl Mice
Glossary

1. Cross Fostering
   after being delivered the youngsters are grouped with and
   fed by mother other than the one to whom they are born
   (Bovet-Nitti et al., 1968)

2. Exploratory or Orienting Reaction
   a type of reaction exhibited by the animals when they are
   exposed to a novel situation; these include higher initial
   readings in visual, tactual, as well as motor activities
   ... etc., it is different from the measurement of general
   motor activity (Scudder et al., 1969)

3. Freezing Behavior
   a type of behavior exhibited by the animals when they are
   exposed to novel situations or under stress conditions,
   they appear motionless, standstill, freezed.... etc.
   (Scudder et al., 1969a)

4. Frustration
   an unsoluble situation, in this thesis it was produced
   by giving the mice electroshocks without allowing them
   to escape (see Section V, Chapter XIX, B)

5. Hole-in-Wall Test
   a behavioral test designed to measure the activation level
   in animals. The device consists of two compartments se-
   parated by a partition containing a hole that can be un-
   covered by a guillotine door. Running time was recorded
   as the amount of time it took the subject to pass through
   the hole in all four feet. Lower score in the apparatus
   is associated with higher levels of activation (Thiessen
   et al., 1962)
6. Maternal Defensive Behavior

-- a type of aggressive behavior developed by the mothers when their youngsters are disturbed; the mothers appear protective and aggressive in such a sense that they will attack whatever subjects approaching their offspring (Scudder, Karczmar, & Lockett, 1967)

7. Mouse-City

-- a pseudo-field condition developed in our laboratory for the study of animal behavior. The apparatus consists of six small chambers, each of which connects to a central communal chamber by way of tubular runway. The floor were covered with sawdust, and food and water was present in the main chamber. The experiment was run under red light. Fifteen minutes after the mice were placed in the chambers, the tubular runways were opened and the mice were permitted to interact. The behavior of each mouse at observation was categorized as follows: contactual behavior, digging, stereotypic behavior, freezing, ingestion, being groomed, grooming self, grooming others, sleeping, exploration, & aggression...etc. (Scudder et al., 1969)

3. Penta-Level Apparatus

-- an avoidance conditioning apparatus which consists of five base chambers with shocking grid floors. Each chamber was connected to another by a 35-degree inclined tunnel, the floors of which were also electrified by means of four consecutive grids. The door from each chamber opened 5 sec. before the animal was shocked and the wave of shock was carried progressively in segments of the grid up to the next chamber at 10-sec. intervals forcing the animal to climb. The mean time the animals spent in the base chambers (base times) and climbing (climbing times) were recorded by means of timers (Scudder et al., 1965)
The purpose of these studies is to investigate the dose-response and time-course effects of DFP on two different strains of mice, Mus musculus SC1 and Mus musculus C57BL/6J. These experiments are presented here in the appendix due to the difficulties which arose during the course of this investigation. It was found that a profound and prolonged enzyme inhibition by DFP occurred at the dose levels employed. Even after careful washing and rinsing the electrodes and reaction vessel in 1N NaOH solution, the enzyme activities progressively decreased at the same dose level of DFP and in the peanut oil control, i.e. on the first day of the series of experiment, the ChE activities measured, either for the peanut oil control or for the DFP treated animals, were within normal range of enzyme level. On the second day, however, after high doses of DFP (e.g. 1.59 mg/Kg & 2.51 mg/Kg which were carried out on the first day), the enzyme activities measured for the control and for the same dose levels of DFP were considerably lower than those determined on the first day and so forth. Therefore it seems there was a carried-over effect of the high dose of DFP on the electrodes and the reaction vessels etc. causing an additive enzyme inhibitory effects upon successive brain ChE measurements. This carried-over effect of DFP was present both for the control and for the drug treated groups which were sacrificed on the same day, since both the control and
the experimental animals were presumed to show approximately the same amount of additive enzyme inhibitory effects and comparisons were relative to control values, the conclusions are not invalid.

Animals

In this series of drug effect experiments including dose-response and time-course study of DFP (Tables VIII, IX, X, & XI), both male, adult SCl and C57BL/6J mice were employed. All animals were provided with food and water ad libitum and were under identical laboratory conditions as those described in Section I, Chapter III, A, 1.

Drug Preparation

DFP was given subcutaneously and the dosages employed were as follows: DFP 0.63mg/Kg, 1.0 mg/Kg, 1.59 mg/Kg, and 2.51 mg/Kg for the dose-response study (Table VIII) and 0.79 mg/Kg for the time-course study (Table IX) in SCl mice; while 0.22 mg/Kg, 0.28 mg/Kg, 0.36 mg/Kg, 0.39 mg/Kg, and 1.0 mg/Kg for the dose-response experiment (Table X) and 0.28 mg/Kg for the time-course experiment (Table XI) in C57BL/6J mice. The doses used in the dose-response curve were chosen to cover the entire range of brain ChE inhibition (0-100% enzyme inhibition) while the doses employed in the time-course experiments were chosen to produce approximately 50% enzyme inhibition.

Experimental Design

In these series of experiments, 3 animals (both SCl &
C57BL/6J) per group were employed for the drug-treated groups and 6 animals (both SC1 & C57BL/6J) per group for the peanut oil control groups.

For the dose-response study, both in SC1 and C57BL/6J, the whole brain enzyme activities were determined 30 minutes after the subcutaneous administration of DFP (Tables VIII & X). For the time-course study of DFP effects, the brain ChE activities were measured at 30 minutes, 1 hour, 2 hours, and 3 hours in SC1 mice (Table IX) and 1 hour, 2 hours, and 3 hours in C57BL/6J (Table XI) after subcutaneous administration of DFP.

Results

1. SC1 Mice

The dose-response and time-course studies of DFP on SC1 mice are shown in Table VIII & Table IX respectively. The results indicate that there is a proportional decrease in brain ChE activity as measured at 30 minutes after 0.63 mg/Kg, 1.0 mg/Kg, 1.59 mg/Kg, and 2.51 mg/Kg of DFP in SC1 mice, i.e. 85.5%, 22.5%, 8.2%, and 3.9% of the control value respectively (Table VIII).

In the time-course study, the results show that the peak effects of DFP (0.79 mg/Kg) which was 5.8% of the control occurred at one hour after subcutaneous injection, thereafter the enzyme activity rose. At two hours after a subcutaneous injection, the enzyme activity rose to 32.8% of the control and by the end of 3 hours the enzyme activity returned to 44% that of the control (Table IX).
Table VIII DFP Dose-Response Study on SCl Mice
(30 minutes after subcutaneous injection)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DFP 0.63 mg/Kg</th>
<th>DFP 1.0 mg/Kg</th>
<th>DFP 1.59 mg/Kg</th>
<th>DFP 2.51 mg/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChE Activity¹</td>
<td>20.26</td>
<td>17.16</td>
<td>4.51</td>
<td>1.64</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>+ 17.89</td>
<td>+ 0.50</td>
<td>+ 1.97</td>
<td>+ 1.34</td>
<td>+ 0.65</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>% of Control</td>
<td>---</td>
<td>85.5</td>
<td>22.5</td>
<td>8.2</td>
<td>3.9</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram wet weight/minute, duplicate readings for each brain.

ACh Chloride Anhydride 150 mg/vial (Sigma) was used.
Table IX  Time-Course Study of DFP on SC1 Mice  
(0.794 mg/Kg, S.C.)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>30' after s.c.</th>
<th>1 hr. after s.c.</th>
<th>2 hrs. after s.c.</th>
<th>3 hrs. after s.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChE Activity1</td>
<td>18.16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mean + S.E.</td>
<td>1.44</td>
<td>1.06</td>
<td>5.95</td>
<td>8.03</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>% of Control</td>
<td>7.9</td>
<td>5.8</td>
<td>32.8</td>
<td>44.3</td>
<td></td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram brain wet weight/min.,
duplicate readings for each brain.

2. There were no significant differences among the control for any experimental intervals.
   Therefore, the value of the mean ChE activity and the number of animals used in the control
   are polled from the four time intervals.
2. C57BL/6J Mice

The dose-response and time-course studies of DFP on C57BL/6J are shown in Tables X & XI respectively. In the dose-response study, a simple correlation between the dose of DFP and the decrease in brain ChE activities was not observed. At dose level of 0.22 mg/Kg, DFP caused a 40% decrease in brain ChE level, while at a higher dose level (0.28 mg/Kg), it caused only a 16% decrease in this enzyme activity as measured at 30-minute interval after a subcutaneous injection of DFP, however, this difference in enzyme activity between 0.22 mg/Kg and 0.28 mg/Kg is not statistically significant. As the doses were further increased, there was a dose-related decrease in the enzyme activity, i.e. at 0.36 mg/Kg the enzyme activity was 65.3% of the control; at 0.39 mg/Kg, 15.5% of the control; and at 1.0 mg/Kg, 0% of the control (Table X).

In the time-course study, the peak effects of DFP (0.28 mg/Kg) on C57BL/6J mice was found to be at 2 hours after subcutaneous injection and at this time interval the enzyme activity was found to be zero per cent of the control. The enzyme activity remained completely inhibited at 3 hours after subcutaneous administration of this dose of DFP (Table XI).

Discussion

In view of the fact that the unusual, continuous inhibition of the enzyme activity seen during the course of this study as well as the limited number of animals employed, it is suggested
that the data reported here serve as a preliminary rather than a conclusive experiment. In spite of this fact, however, an overall picture can still be drawn from this study. In general, C57BL/6J mice seem to exhibit a higher sensitivity to DFP than do SCl mice. This can be seen both from the dose-response study as well as the time-course experiment (Tables VIII, IX, X, & XI). The ED50, i.e. the dose of DFP which produced approximately 50% inhibition of brain ChE activity, for both strains of mice, C57BL/6J and SCl mice, are 0.28 mg/Kg and 0.79 mg/Kg respectively, while those for ED100 (dose of DFP produced 100% enzyme inhibition) are 1.0 mg/Kg and 2.51 mg/Kg respectively (Tables VIII & X). Therefore C57BL/6J mice appear to be approximately 2.5x more sensitive to DFP than are SCl mice. This difference in drug sensitivity can also be seen in the time-course study when the relative enzyme inhibition instead of the absolute enzyme inhibition are compared. Comparison of absolute values of enzyme inhibition is not possible due to the continuous, additive inhibition which occurred in these experiments. For instance, enzyme inhibition of more than 50% was not expected to occur in either of the two time course studies since the doses used in these experiments were chosen to produce approximately 50% enzyme inhibition. At an equi-potent dose, the peak effects of DFP on brain ChE inhibition occurred at 2 hours after subcutaneous injection in C57BL/6J mice and this level of enzyme inhibition (100% inhibition) continued even 3 hours after drug administration (Table XI); on the other hand, in SCl mice
Table X  DFP Dose-Response Study on C57BL/6J Mice  
(30 minutes after S.C.)

<table>
<thead>
<tr>
<th></th>
<th>Peanut Oil Control</th>
<th>DFP 0.22mg/Kg</th>
<th>DFP 0.28 mg/Kg</th>
<th>DFP 0.36 mg/Kg</th>
<th>DFP 0.39 mg/Kg</th>
<th>DFP 1.0 mg/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChE Activity 1</td>
<td>12.86</td>
<td>7.73</td>
<td>10.77</td>
<td>8.40</td>
<td>2.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>0.63 ± 1.12</td>
<td>1.52 ± 1.08</td>
<td>0.55 ± 0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>% of Control</td>
<td>---</td>
<td>60.1</td>
<td>83.8</td>
<td>65.3</td>
<td>15.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram brain wet weight/minute, duplicate readings for each brain. ACh Chloride Anhydride 150 mg/vial (Sigma) was used.
Table XI Time-Course Study of DFP on C57BL/6J Mice
(0.282 mg/Kg, S.C.)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 hr. after S.C.</th>
<th>2 hrs. after S.C.</th>
<th>3 hrs. after S.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChE Activity</td>
<td>13.76+</td>
<td>1.06+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>0.99</td>
<td>0.87</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N</td>
<td>6²</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>% of Control</td>
<td>---</td>
<td>7.7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1. ChE activity is expressed in term of micromoles acetic acid/gram wet weight/minute, duplicate readings/brain.
2. There were no significant differences among the peanut oil controls for any single group. Therefore, the number of animals used in the control are polled from the various time intervals after S.C. injection of DFP. ACh Chloride Anhydride 150 mg/vial (Sigma) was used.
the peak effects (approximately 94% inhibition) occurred at one hour after subcutaneous administration thereafter the enzyme recovered gradually. By the end of 3 hours, the enzyme activity returned to 44% that of the control (Table IX). This higher sensitivity of C57BL/6J mice to drugs in general seems to be in agreement with that reported by Bourgault et al. (1963) in which this strain of mice was also shown to have a higher sensitivity than did SCl mice to chlorpromazine, reserpine, phenobarbital and probably to pentylenetetrazole. It is interesting to postulate that the rapid recovery of brain ChE activity after DFP in SCl mice might be due to the presence of either a rapid re-synthesis mechanism or a rapid re-activation system or even both in these animals. A correlation between high activity, long electroshock latency, high brain amine levels and high sensitivity to both stimulant and depressant agents was postulated for C57BL/6J (Bourgault et al. 1963). Neurochemically C57BL/6J has a lower brain ChE activity than that of SCl mice. While it is impossible to make a biochemical correlate of behavior in this experiment (due to successive enzyme inhibition) this study did successfully demonstrate the pharmacogenetic differences in response to drug effects on these two different strains of mice (cf. Fuller, 1970).

SUMMARY

Dose-response and time-course studies of DFP were conducted on two strains of laboratory mice, Mus musculus SCl and Mus
musculus C57BL/6J mice. The ED50 (dose of DFP which produced 50% inhibition of brain ChE activity) for both strains of mice, C57BL/6J and SCl mice, are 0.28 mg/Kg and 0.79 mg/Kg respectively, while those for ED100 (dose of DFP produced 100% enzyme inhibition) are 1.0 mg/Kg and 2.5 mg/Kg respectively. For time-course study, at an equi-potent dose, the peak effects of DFP on brain ChE inhibition occurred at 2 hours after subcutaneous injection in C57BL/6J mice and this level of enzyme inhibition (100% inhibition) continued even 3 hours after drug administration; on the other hand, in SCl mice the peak effects (approximately 94% inhibition) occurred at one hour after subcutaneous administration, thereafter the enzyme recovered gradually. By the end of 3 hours, the enzyme activity returned to 44% of the control. It is concluded that C57BL/6J mouse has a higher drug sensitivity to DFP than has SCl mouse. The differences in response to DFP are suggested to be due to either the presence of a faster re-synthesis mechanism or a faster re-activation system or even both in the SCl mice as compared to that of the C57BL/6J mice.
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The dissertation submitted by Chen-Ho Lin has been read and approved by six members of the faculty of Loyola-Stritch School of Medicine.

The final copies of the dissertation have been examined by the director of the examining committee and the signature which appears below verifies the fact that all necessary changes have been incorporated and that the dissertation is now given final approval with reference to content, form and mechanical accuracy.

The dissertation is, therefore, accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

October 24
Date

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