Contrasting Behavioral, Pharmacological, Neurophysiological and Biochemical Profiles of C57B1/6 and SC-1 Strains of Mice

Priscilla Bourgault
Loyola University Chicago

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Contrasting Behavioral, Pharmacological, Neurophysiological and Biochemical Profiles of C57Bl/6 and SC-I Strains of Mice.

by

Priscilla C. Bourgault

A Dissertation
Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Priscilla C. Bourgault was born in Winooski, Vermont, on January 1, 1928.

She received a Bachelor's Degree in Chemistry from Trinity College, Burlington, Vermont, in 1950.

She was a Research Assistant in the Department of Pharmacology of Albany Medical School, Albany, New York, from 1950 to 1951.

Between 1951 and 1956 she was a Research Assistant in the Department of Pharmacology, Sterling Winthrop Research Institute.

From 1956 to 1958 she served on the staff of Stanford Medical School as an Instructor and Demonstrator.

Since 1958 she was a graduate student and research assistant in the Department of Pharmacology, Loyola University Graduate School. While at Loyola she was also a graduate assistant.

**PUBLICATIONS**


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GENERAL INTRODUCTION

Two strains of mice, C57Bl/6 and SC-I, were investigated in this laboratory while screening tranquilizing agents (Bour­gault and Karczmar, 1961). In the course of these attempts, marked behavioral differences between these two strains became apparent (Bourgault et al., 1963). Observation of C57Bl/6 mice revealed that they were more reactive than SC-I mice and that they squirmed or squealed more when handled. It was thought, therefore, of interest to use these two strains of mice, each relatively homogeneous biologically, to study the relationship of behavioral, pharmacological, neurophysiological, and biochemical characteristics. The major intent was comp­arative and descriptive in hope that an understanding of many aspects of these two different living systems would throw light on the biological basis of behavior. The parameters studied were aggressive behavior; escape and avoidance behavior, motor activity; drug effects, electroshock and pentyl­enetetrazol convulsions; and brain levels of serotonin, nor­epinephrine and acetylcholine.

Although as comprehensive an analysis as this one has not been attempted before, many separate aspects of the present investigation have been explored. It was shown for instance that inherent differences in aggression, avoidance, and motor activity exist between different strains of animals (Yerkes, 1916; Dawson, 1932; Scott, 1942; Rundquist, 1933). Other investi­gators (Kornetsky et al., 1957; Kornetsky, 1963; Shagass, 1960; Shagass and Jones, 1958; Shagass et al., 1956, 1959) have attempted to correlate behavioral parameters with pharma­
cological effects, while still other workers (Maas, 1962, 1963; Caspari, 1960) have tried to correlate behavioral and biochemical parameters. Similarly, studies of Toman (1958) indicate a relationship between the adrenergic system and latency of electroshock response. Generally, in this type of study, the interrelationship of various parameters has not been compared in different strains of animals.

The strains of mice used in this study, the C57Bl/6 and the SC-I mice have not been compared previously. However, other strains of mice and also other species have been studied in a manner which relates to separate aspects of the proposed problem and which will be discussed under separate topic headings. For example, King (1957b) has compared the agonistic behavior of two genera, Mus musculus, C57Bl/10 strain, and the Peromyscus maniculus bairdii. Maas (1962, 1963) and Caspari (1960) have measured brain levels of serotonin and norepinephrine and have attempted to correlate them with aggression in mice of C57Bl/10 and CF-I strains.

Finally, the various aspects of the present problem have been investigated separately, and are discussed in this thesis under separate chapter headings.
CHAPTER I

AGGRESSION

A. LITERATURE SURVEY

Genetics and Social Factors

The first study in mental heredity undertaken in the laboratory, where conditions under which subjects lived could be controlled, is attributed to Yerkes (1913, 1916). He showed that wildness, savageness and timidity were qualities that were inherited in the rat.

Coburn (1922), a student of Yerkes, undertook a similar study in mice. The characteristics studied were also savageness and wildness. These terms referred to conflict behavior of animals in relation to experimenter. Savageness refers to tendency of animal to attack while wildness refers to tendency to escape. Yerkes (1913) had found that there were two types of savageness in rats, offensive and defensive, while Coburn observed that only defensive savageness occurred in mice. Coburn's cross breeding experiments indicated that qualities of savageness and wildness were dominant over tameness (absence of conflict behavior). His results were achieved from observations only. Savageness was difficult to separate from wildness, since the response of the mouse was dependent upon the gentleness of experimenter since most mice will bite when hurt. Rating scales and graded subjective impressions were used. Later workers (Scott, 1958a, b) studied characteristics of aggressiveness and timidity, terms used to describe conflict behavior within a strain or species.

Allee and Ginsburg (1941) studied the social hierarchy in
mice and found that in small groups of male mice, the hierarchy was based on aggressiveness and general fighting ability. He also found that once a mouse was dominant it was relatively easier to condition down in the social hierarchy, than to take a submissive mouse and to train it to become dominant. The longer and more severe the conditioning the more lasting the results. In 1942 Ginsburg and Allee studied three inbred strains of mice in order to investigate inherited aggressive behavior. The C57Bl/10 was found to be more aggressive than C3H and Bagg C albino. In the same year, Scott studied the same strains and found that the C57Bl/10 was the least, and the C3H strain the most aggressive against the Bagg C albinos. Bauer in 1956 found C57Bl/10 more aggressive than Balb/C. Fredericson and Birbaum in 1954 observed while testing for competitive fighting that, when no competition was involved, Balb/C mice frequently fought and killed their partners whether of their own strain or of the C57Bl/10 strain. In 1955 (b) Fredericson et al. tested these same strains for spontaneous fighting and substantiated these observations which were contrary to Bauer's findings that Balb/C mice were more aggressive in a non-competitive situation than C57Bl/10 in both intensity and fighting latency. The discrepancies in the results of: Ginsburg and Allee compared to Scott; and those of Bauer compared to Fredericson, may be due to the adaptability of the C57Bl/10 strain. Some indications of this has been demonstrated by Calhoun.

Calhoun (1950b) observed the behavior of pregnant DBA and C57Bl mice in large cages with free access to food, water, and nesting material. DBA mice were quite stereotypic in their behavior and only gradually did new or modified behavior patterns emerge, but once assumed, a behavior pattern was quite in-
variable. The C57Bl were quite labile in behavior. They readily adjusted to new situations as they arose. Among DBA mice, each encounter between individuals was followed by an exhibition of social position, usually by fighting. This led to development of a rigid hierarchy. The C57Bl mice rarely fought. Two individuals seeking the same goal alternately relinquished priority to it. This relinquishing of priority essentially replaces the hierarchal organization seen in DBA mice. In a study by Arnold et al. (1959) C57Bl were found to be more variable in motor activity than DBA.

It seems then that the differences in results observed by different investigators concerning aggression in C57Bl mice may be dependent on the high degree of adaptability of this strain. It is likely that this strain is pacific in a normal environment, but that with changing environment it can become a very efficient fighter.

**Effect of Sex**

Factors involved in fighting were investigated by many workers. Since spontaneous fighting referred to above occurs in male mice and very rarely in female mice, the effect of androgens have been studied by several workers. Androgens are known to stimulate aggressive behavior in vertebrate males, and in females who normally have a high endogenous level (Collias, 1944).

In strains of Mus Musculus evidence indicates that androgens increase aggressive behavior in male but not in female mice. Beeman (1947) studied the effect of testosterone in both C57Bl/10 and Balb/C strain. Castration of immature or adult male mice resulted in the failure to display aggressive behavior. Implanted testosterone pellets induced castrate.
mice of both strains to display aggressive behavior that is similar qualitatively and quantitatively to behavior of normal male mice. Removal of pellets resulted in almost immediate cessation of aggressiveness in the majority of mice. Immature males but not female mice will fight when given testosterone (Levy and King, 1953; Levy, 1954). Gonadectomized females (Tollman and King, 1956) receiving testosterone did not exhibit a greater degree of aggression than intact females. Tollman and King (1956) suggest that these sex differences are probably not due to different rates of detoxification of androgens since organic changes of the uterus and adrenals resulting from androgens occur and are progressive and do not correspond to peak aggressive response when present in the female.

Although the sex hormones are a major factor in the differences between the sexes, Bayre (1952) suggests that this may be due to structural differences between male and female CNS which Beach (1947) believes results in a greater susceptibility of male CNS to sensitizing action of testosterone.

The aggressive response of male mice usually emerges in the presence of another male and not a female. The female may not present olfactory or behavioral stimuli necessary to induce aggressive behavior (Tollman and King, 1956). It was observed in the C57Bl/10 that the presence of a female may inhibit the aggressiveness of one male towards another. This was supported by Fredericson et al. (1955b) who found that the fighting latency time of the above strain was delayed in presence of a female but not in presence of an extra male. He also found that sexual experience caused a drop in aggressiveness in this strain. Balb/C mice were not affected in either situation. Gustafson and Vinokur (1960) found that neither sexual satiation or female hormone had an effect on aggressive behavior.
of the male Balb/C.

**Effect of Isolation**

Most investigators testing aggression in mice isolate their subjects for various periods of time. The exact mechanism of the effect of these isolation periods on aggression is not known. King (King et al., 1954; King, 1957a) studied the effect upon later aggression of male mice isolated for different periods after weaning. C57Bl/10 and Balb/C strains were isolated at 15 to 45 days after birth until tested at 110 days. Balb/C were not affected in their aggressive behavior by these different periods of early isolation. However, C57Bl/10 that were isolated at 20 to 25 days of age were less aggressive than those which were isolated between 30 to 45 days indicating that in this strain isolation before mice reach 30 days of age inhibits aggressive behavior. The inhibition of aggression due to isolation during this critical period was not due to prohibition of overt fighting and competition for food, water and space since a group of these mice isolated at 20 days but separated by a screen partition behaved like mice isolated at 30 days. King (1957a) suggests that the investigatory period preceding behavior is prolonged among the isolated C57Bl/6 because they have not seen another mouse since weaning at 20 days. In contrast those mice which have had the opportunity to investigate other mice through a wire screen for only 10 days following weaning acquired sufficient experience with each other to react aggressively in a fighting situation. Bauer (1956) found that C57Bl/10 and Balb/C strains isolated from 30 days did not differ from mice that lived with one female from 30 days of age when they were tested at ages from 70 to 134 days. The similarity
in results probably depend on the lack of previous agonistic experience in both situations since neither the lone male nor the male with the female will fight. Kahn (1954) finds that C57Bl/10 mice raised in isolation from 21 days of age were more defensive, aggressive and less investigatory than those reared to maturity with siblings by mother. Although one would expect the C57Bl/10 raised in isolation from 21 days to be less aggressive than the non-isolates, the presence of the mother may have an inhibitory effect, or since male siblings are present the mouse tested has already experienced non-fighting behavior with males. Fredericson (1949) isolated animals at 21 days and found that if they were brought together for a short period daily all the animals fought vigorously by 36 days of age.

It has often been observed that litter-mates which are raised together may live peacefully in the same box even at an advanced age, and Scott (1946) has found considerable difficulty in training these mice to fight. However, it is also well known that a considerable amount of fighting takes place in stock mice that are caged together. This quality may vary considerably in different strains (Calhoun, 1950a). It may also be a result of social disorganization (Calhoun, 1950b) which reflects a vague territoriality. In a well organized society of mice or rats little fighting occurs but when strange animals are introduced into such a colony considerable fighting ensues. For instance, in the case of a predatory mouse, Onychomys leucogaster, it was found that when mice were returned to their own cages between "training-to-fight" episodes there was no fighting within the group. Mice that were housed separately during training and returned to their cages at the end of the training period participated in considerable fighting which reached destructive proportions (Clark, 1962).
It thus seems that the use of isolation in testing aggressive behavior in the laboratory achieves first of all the creation of constant conditions for the testing of behavior so that the contact of the animals can be observed and controlled. Second, isolation may establish territorial rights over the area since aggressive tests are usually conducted in the same cages in which the mice are housed. Third, isolation may prevent the normal adaptation that occurs in mice that are raised together, and create a situation which is similar to that of a disorganized society where considerable fighting takes place. This effect would then be dependent to some extent on the normal social adaptability of each species or strain which has been shown to differ. Fourth, if isolation takes place before 32-36 days of age, it may have an inhibiting effect on aggressive behavior since no fighting is observed before this time. This has been shown to be true for C57Bl/10 but not for Balb/C strain (King, 1957a). Fifth, isolation may create internal change that is not related to above factors; this change may be strain specific. Christian (1956) has reported a change in weight of adrenal glands, thymus and seminal vesicles in mice after periods of isolation.

Pain

Another major factor which influences fighting behavior in mice is pain (Scott, 1946; Scott and Fredericson, 1951). When mice are pinched on the tail with forceps from the day of birth they develop a habit of biting the forceps. This occurs approximately at the time of opening of the eyes (about 2 weeks of age) and long before spontaneous aggressive behavior takes place in the male; it occurs in both male and female. It also seems that the vigorous grooming and investigation that takes
place between males may accidentally cause slight pain and incite fighting behavior.

O'Kelly and Steckle (1939), Daniel (1943), and Ulrich and Azrin (1962) have obtained fighting behavior in mice and rats when electric shock was applied. Ulrich and Azrin (1962) report that the fighting induced in this way is different in appearance from normal fighting, and that it is of short duration. This fighting takes place regardless of sex, strain, previous experience with each other, or the number of animals present when shocked. Similar responses were seen in strains of rats whether they proved more or less aggressive in other tests. With this testing procedure rats will attack other small animals such as hamsters or guinea pigs; attack will occur whether or not the partner participates. Hamsters also respond to shock by fighting, while guinea pigs do not. Tedeschi et al. (1959) used this method in male mice and obtained fighting in 65% of mice tested. The stereotypic position of these mice is similar to the stereotypic position in rats. It is not known whether or not the response would be similar in females, in other strains, and under different environmental conditions, but from the work of Ulrich and Azrin (1962) similar responses could be expected.

Pain is thus a strong stimulus to behavior of an aggressive type in both males and females. It is possible that shock stimulus is strong enough to overcome both high and low thresholds to aggressive behavior and may eliminate some of the difference in response in different strains and species. If the electroshock stimulus for fighting is strong and constant any differences observed between animals would be apt to reflect aggressiveness that is independent of the normal sensory
stimulus threshold to fighting. On the other hand any differences in threshold might represent differences in necessary sensory input before fighting occurs.

**Experience and Other Factors**

Infantile experience is a factor which influences aggressive behavior. Mice that are severely defeated at immature age will be less aggressive as adults (Kahn, 1951). The effect of isolation has already been discussed. Levine (1959) has demonstrated that handling of C57Bl/10 mice in infancy resulted in increased aggressiveness especially during the first fight trials. These data are consistent with previous data obtained using rats and dogs (Fisher, 1956).

Other factors that might affect fighting behavior are weight and physical well being. Uhrich (1940) studied the effect of weight upon winning and concluded that if a mouse is 5 gms. heavier than his partner it has a tendency to win a fight. In his studies he found that relative age was of no consequence if weight differences were not too great. Weight apparently has no effect upon initiation of a fight.

Beeman and Allee (1945) studied the effect of thiamine deficiency on social dominance and found that only when the state of general debilitation had been reached was there some change in social status, and that this occurred only with mice intermediate in the social hierarchy. Vogel (1950) found the X irradiation did not cause a change in dominance-subordination pattern. Shortly before death all signs of fighting ceased. It seems then that once the dominance-subordination pattern is firmly established it is very difficult to change it.

All of the discussion thus far except where stated deals with fighting between male mice. Competition for food is
another type of aggressive behavior in mice. It differs from spontaneous fighting since it can be elicited in both male and female (Fredericson, 1952a; Fredericson and Birbaum, 1954). Actual fighting occurs, however, only when the mice have been previously deprived of food and when only one piece of loose food is available. In the two strains studied, the C57B1/10 actively fought for possession of the food pellet while the Balb/C mice did not fight but ate the food together (Fredericson 1951, 1952a). The C57B1/10 mice that had learned competitive fighting did not fight but exhibited possessive behavior, when ample food was present, which consisted in taking possession of food, carrying it around the cage, hunching over it and quickly running away with it when other mice approached (Fredericson, 1952b). When the Balb/C strain was placed in a competitive situation with the C57B1/10, fighting occurred, but when the Balb/C fighters were placed again with mice of their own strain, they reverted to their original behavior. C57B1/10 when raised by Balb/C mothers retained their competitive behavior and challenged their foster parents for food which caused Balb/C parents to adopt C57B1/10 fighting pattern. Balb/C mice raised by C57B1/10 parents retained their non-competitive behavior. Competitive fighting is thus seen in both male and female; it is also strain specific but can emerge in a strain that is not competitive when tested with a competitive strain.

B. METHODS

Mice were tested for aggressive behavior by a modification of the method of Fredericson (1949). Metal cages containing two 4½" X 6" X 4" compartments, separated by a partition, were used for isolating the mice. Mice were tested at various times after the initiation of isolation. During the test the partit-
ion was removed and mice were exposed to each other. The time between lifting of partition (fighting latency) and first fight was recorded. The mice were exposed to each other daily for a period of five minutes or for five seconds following fighting. C57Bl/6, C57Bl/10, and Balb/C mice were used.

C. INTRODUCTORY EXPERIMENTS

The original purpose in the study of aggression was to perfect a method to study the effect of ataractic agents as well as to further investigate the complex problem of aggression.

**Experiment I**

Thirty-four mice of C57Bl/6 strain were isolated when 56 days old and tested four weeks later. Ten mice from the group gave consistent fighting behavior. Their average fighting latency is recorded on Graph I and is consistent with similar results obtained by King (1954, 1957a, 1957b; King and Mavromatis 1956), Kahn (1951), Fredericson (1949; Fredericson et al., 1955b). The remaining mice developed a dominance-subordination pattern or passive behavior. The mice who fought exhibited the preliminary behavior described by Scott and Fredericson (1951), which consists of pilo-erection (which is not always easy to determine since a poorly groomed animal may give a similar appearance), investigation, grooming, sexual mounting, chasing, tail rattling and a mincing gate. The animal sometimes pushes the shavings excitedly with its front paws or nibbles vociferously on pellets. As the latency of attack decreases, preliminary behavior decreases and disappears and the animal fights efficiently and vigorously.
**Experiment II**

Sixteen Balb/C mice age 35-42 days were isolated for six weeks. Ten mice showed consistent fighting behavior (Graph II). Less preliminary behavior before fighting was observed in the Balb/C strain when compared with the C57Bl/6 strain (see Experiment I). The fighting latency of the Balb/C stabilized at the fourth trial while that of the C57Bl/6 stabilized at the ninth trial. The percent of Balb/C fighters that either attacked or fought during the first trial is 56% which is comparable to 59% for the C57Bl/6 mice in Experiment I. However, when first seven trials are considered, 63% of Balb/C maintained fighting behavior compared to 35.3% of C57Bl/6 for the first seven trials. Although these tests can be compared only with caution, they indicated that the C57Bl/6 mice are not as stable in their fighting behavior while the Balb/C achieve stable behavior more rapidly.

**Experiment III**

The effect of administration of saline by oral, intraperitoneal, intramuscular, and subcutaneous routes of administration were tested. All routes of administration interfered with the fighting latency and a change from fighting to a dominance-subordination pattern could sometimes be observed. The oral administration had the greatest effect (Graph III). Subcutaneous had a smaller effect (Graph IV). Intramuscular and intraperitoneal injections had practically no effect when given one hour before testing. There was evidence that the mice that were most affected by injections would have the greatest response after the drug (Table I). However in Experiment VI (Graph VI), an intraperitoneal injection caused an opposite effect, that is a decrease in latency. In this procedure the mice remained together for five minutes whether or not
attacking developed. During the sixth trial, which is the one preceding the injection, the latency to attacking increased because the subordinate mice became more passive therefore less stimulating to the dominant mice which became slower and less vigorous. What probably occurred here is that the injection caused slight discomfort or pain which lowered the threshold to attacking; this did not occur (Graph VIII) where the passive mice were more vigorous and the attacking latency was much shorter.

**Experiment IV**

In an attempt to improve fighting behavior 20 mice of Balb/C strain age 9-12 weeks, isolated for 30 days were trained by a method described by Scott and Fredericson (1951). The experimental mice were trained to attack other mice that were helplessly dangled by their tails. Four mice were presented at each trial; every other mouse was released in the cage for a short period. The experimental mice learned to attack vigorously the helpless mice. At the end of a six day training period the trained mice were combined and exposed to each other. The fighting latency increased with each trial and dominance-subordination patterns developed. At the end of five trials only one pair of mice was fighting. It was felt that this method of training was not successful for obtaining a large number of fighting mice with consistent behavior. Perhaps if a longer training period were used it might be possible to obtain more vigorous fighters.

It was possible by selection of the better fighters from the previous groups to obtain a small number of vigorous and consistent fighters. Drugs were tested on these groups. External and internal controls were used.
Experiment V

A dose of 2 mg./kg. chlorpromazine given intramuscularly one hour before testing time completely prevented fighting behavior in Balb/C mice. The next day the fighting behavior of the mice was normal (Graph V). 1 mg./kg. given to three pairs of fighters also completely disrupted fighting. The effect of the same dose on 3 pairs of C57Bl/6 was not significantly different from the effect of control. Sedation was observed in all Balb/C mice and in 2 pairs of C57Bl/6. Motor activity was markedly reduced by doses of chlorpromazine causing changes in fighting latency. This effect was similar for C57Bl/10 and Balb/C mice, and Table II shows combined data.

Experiment VI

In order to avoid some of the difficulties encountered in the fighting latency test, such as the change in aggressive behavior during the test, a dominance-subordination pattern was allowed to develop. Attack latency instead of fighting latency was recorded as well as the number and the duration of attacks. The mice were exposed to each other for a full five minutes for each trial instead of being separated five seconds after attack. The end point of attack latency is characterized by a burst of activity, biting, and chasing on the part of the dominant mouse and attempts to escape accompanied by squealing on the part of the passive mouse.

Fifty-six mice of C57Bl/6 strain isolated for 32 days, age 67 to 88 days, were tested for a total of eight daily consecutive trials. One hour before the seventh trial meprobamate 150 mg./kg. was given intraperitoneally to half the dominant mice and physiological saline was given to the remaining dominant mice. Graphs VI, VII, and Table III show attack latency,
attack duration, and number of attacks respectively. All three parameters were affected; the attack latency and duration and the number of attacks were decreased. Motor activity of mice receiving this dose was somewhat greater than that of controls when measured in activity cages (Table II). The recording of number and duration of attacks seemed to add little knowledge beyond what could be obtained by measurement of latency while it introduces considerable variation in testing for latency. However, Knight (1963) found that acetophenazine prolonged the duration of fighting and the length of time from onset of first fighting to submission, while the latency was prolonged.

Experiment VII

The following test was designed to achieve more uniform response with the attack latency test. The total time of exposure was cut down to 120 seconds or 5 seconds after attack. The separation of animals 5 seconds after attack produced more consistent results. Only those mice were used whose attack latencies fell within a 60 second period for three consecutive tests before injection of meprobamate (Graph VIII). The increase in control levels of attack latency in Experiments VI and VII at the eighth and ninth trial is due to the subordinate mouse becoming so passive that the dominant mouse became slower and less vigorous in attack. However, in all the attacking experiments the dominant-subordination pattern did not change. Even when no attack took place the dominant mouse took the initiative in terms of nosing, smelling and grooming behavior in its relation to the subordinate mouse. Meprobamate increased the attack latency five-fold. This compares to a three-fold increase in Experiment VI.
D. COMPARISON OF TWO STRAINS

At the end of this testing the emphasis was changed from the study of the anti-aggressive effects of drugs and centered on comparison of differences between strains. When it was observed that the common laboratory mouse SC-I that had been isolated demonstrated aggressive behavior that seemed more vigorous than the strains studied it was chosen along with the C57Bl/6 for a comparative study.

The best testing procedure under the circumstances called for a fairly short term experiment where no other person except the experimenter would care for and handle the mice. Although this method does not control early experience it would give an adequate measure of the aggressiveness of each strain of mice developed within its own social structure. Two groups of mice of each strain SC-I and C57Bl/6 raised without coming in contact with each other, were isolated so that one from each group occupied the two compartments of the same cage, insuring that the fighter had had no previous experience with its partner. In order to see whether certain procedures might change or influence aggressive behavior variations were introduced in the test, such as prolonging the period of isolation and increasing the variety of fighting partners.

The total period of exposure was ten seconds after initiation of attack or 30 minutes. The latency to attack or fight and the percentage of mice fighting or attacking during one minute and 30 minute periods were recorded.

Inter-strain and intra-strain aggressiveness was tested. For the intra-strain test, 25 pairs of each strain were used. Testing took place after 4, 21, and 312 hours of isolation. After 25-27 days the mice were retested and a round robin schedule was used. Each mouse was tested four times during
the same day. In each experiment each mouse was paired with a different partner.

For the inter-strain test, the ten best fighters of each strain were selected and matched on the basis of their fighting or attacking latency (ten tests). The number of fighters and attackers was determined for each strain.

The mice were 11-12 weeks old. The SC-I mice averaged 25.0 gms. and the C57Bl/6 averaged 24.4 gms. at the beginning of the test and 24.0 and 23.3 gms. respectively, at the end of the test period. Any two mice that were fought together did not vary more than 4 gms. between themselves except in the round-robin test where the difference was sometimes as high as 6 gms.

E. RESULTS

In intra-strain tests of aggression (Table IV and Table V) the fighting and attacking latency decreased with each succeeding test and the frequency of fighting tended to increase. This is similar to results obtained in introductory experiments and to those obtained by King (1957) and others and represents learning phenomena associated with test situation. The fighting or attacking latency is shorter for the SC-I than for the C57Bl/6 strains. The number of SC-I mice fighting was greater than that of C57Bl/6 whether a 1 minute or 30 minutes period was employed.

The first trial is more representative of the amount of aggressive behavior characteristic in both groups of mice since it is the period least affected by manipulations such as isolation and learning. This was also the trial that differed most in both latency and frequency measurements. The effect of the longer isolation period and the round robin schedule does not seem to have affected the aggressive behavior of either strain.
to any extent greater than can be accounted for by the learning involved in four more testing episodes. The change in fighting partners may have introduced more variation than would have occurred if the same partners were tested together.

In the inter-strain test, where the ten best fighters of both strains were matched and fought against each other, eight SC-I mice attacked or fought and only three C57Bl/6 mice were attackers or fighters.

F. CONCLUSIONS

The preliminary experiments demonstrated many of the problems involved in measuring aggressive behavior. The end point of fighting is the most reproducible end point when care is taken to end the fight immediately after its inception. The number of mice in which this behavior is maintained varies considerably and is small when compared to the total population tested. Changes can be brought about by chance injuries, injections, and bring about a dominance-subordination relationship which is qualitatively different. On the other hand, the end-point of attack latency is more variable in latency response but almost no changes occur in the basic relationship. The variations in the attack latency can be decreased by selecting animals whose latencies are short and by also separating animals after first attack. This increases accuracy but decreases total information obtained such as duration and frequency of attacks. Although in our tests, this added information did not seem pertinent, Knight et al. (1963) have shown that acetophenazine could differentially affect latency and duration of aggressive behavior. The effect of the dangling method (Experiment IV) was not very successful in producing fighters whose behavior was consistent. A much longer period of train-
ing would be necessary in a very tedious training procedure. Both chlorpromazine and meprobamate decreased aggressive latency. The effective doses of chlorpromazine that were used decreased motor activity while those of meprobamate had no effect or slightly increased motor activity. There was an indication that Balb/C mice were more sensitive to chlorpromazine in this testing situation than C57Bl/6. This difference was probably non-specific since it was also indicated in the motor activity measurement.

In the comparison of two strains, the SC-I mice were more aggressive than the C57Bl/6 mice in both the inter-strain and intra-strain experiments under all variations introduced and for all parameters measured. In addition, during work with these SC-I mice, death could often be attributed to fighting. SC-I mice caged together for long periods of time often had scarred backs as the results of severe fighting. During the same period, no fighting and no scarring of backs was observed in the C57Bl/6 strain.

The C57Bl/10 and C57Bl/6 strains will be discussed together; they both originated from a strain which was inbred since 1921. The C57Bl/6 were separated from the C57Bl/10 in 1937 and are known to differ mainly in percentage of eye defects which are greater in the C57Bl/10 strain. The two strains are similar in behavior (King, private communication). The C57Bl mice are labile in behavior (Arnold et al., 1959; Calhoun, 1950b) and adjust to new situations as they arise. In their "normal" environment in which they are raised together they tend to be pacific (Calhoun et al., 1950a) but changes in environment bring rapid changes in behavior as demonstrated by: their competitive fighting for food; the effect of a female or sexual experience on aggressive behavior, and the effect upon later
aggression of isolation at a critical period of 25 to 30 days of age.

The Balb/C mice on the other hand do not change their aggressive behavior in a competitive situation or as the result of early isolation, the presence of a female or sexual experience. The Balb/C strain is more aggressive than C57Bl in an isolated testing situation and in home cages. The Balb/C resemble SC-I in response to handling when compared to C57Bl/6 which were more reactive and squealed and squirmed more than the other two strains when handled. Both the Balb/C and SC-I strains were more aggressive than C57Bl/6 in present experiments.

The establishment of a dominance-subordination pattern did not differ in quality or quantity in the three strains tested in the isolated situation. However, the establishment of a dominance-subordination pattern can vary with strain and circumstances. In a normal environment the DBA strain forms a rigid hierarchy while the C57Bl do not (Calhoun, 1950b). However, in the situation described by Allee and Ginsburg (1941) where a small number of strange male mice are brought together, the C57Bl/10 do form a hierarchy based on aggressiveness. In some groups, one mouse is dominant and all others are submissive while in other groups one mouse may be submissive towards some mice and dominant toward others. It is easier to condition a dominant mouse to be submissive than vice-versa. Dominance is established by learning. A mouse that has been allowed to win easily will dominate a mouse that has previously been subjected to defeat. Between two naive mice the heavier mouse will have a tendency to win a fight and gain dominance. Once a dominance-subordination relationship is firmly established it is very difficult to change.
Daily handling of mice in infancy increased aggression in adulthood in C57Bl/10 mice (Levine, 1959). Handling due to injection just prior to testing increases fighting latency (Graph III and IV) and tend to create a dominance-subordination relationship between fighting pairs. On the other hand an injection produced the opposite effect, a decrease in latency, when the attack latency had been prolonged due to inactivity of the subordinate mouse. A relationship seemed to exist between the prolonging of latency due to saline and that which is due to chlorpromazine.
Average fighting latency for 5 pairs of C57BL/6 mice. 
Ordinate: Fighting latency in seconds; abscissa: Daily trials.
Average fighting latency for 5 pairs of Balb/C mice.
Ordinate: Fighting latency in seconds; abscissa: Daily trials.
Oral administration of saline. Ordinate: Fighting latency in seconds; abscissa: Daily trials. Saline was given one hour before sixth trial.
Subcutaneous administration of saline. Ordinate: Fighting latency in seconds; abscissa: Daily trials. Saline was given one hour before fifth trial.
Effect of 2 mg./kg. chlorpromazine given intramuscularly one hour before the fourth trial in Balb/C mice. Ordinate: Fighting latency in seconds; abscissa: Daily trials.
Effect of meprobamate on attack latency of 56 C57Bl/6 mice. Meprobamate 150 mg./kg. was given intraperitoneally one hour before the seventh trial to half the attacking mice. The other half received saline. Ordinate: Attack latency in seconds; abscissa: Daily trials.
Effect of meprobamate on attack duration of 56 C57Bl/6 mice. Meprobamate was given intraperitoneally one hour before the seventh trial to half the attacking mice. The other half received saline. Ordinate: Attack latency in seconds; abscissa: Daily trials.
Effect of meprobamate on attack latency of 32 C57Bl/6 mice. Meprobamate was given intraperitoneally one hour before the seventh trial to half the attacking mice. The other half received saline. Ordinate: Attack latency in seconds; abscissa: Daily trials.
**TABLE I**

Fighting Latency 
After Control Injection of Saline, s.c., 
and After Chlorpromazine, 0.5 mg./kg., s.c. 

Five Pairs of C57Bl/6 Mice.

<table>
<thead>
<tr>
<th>CAGE NO.</th>
<th>CONTROLS</th>
<th>AFTER INJ. of SALINE</th>
<th>AFTER INJ. of CPZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4</td>
<td>5 6</td>
<td>7 8 9</td>
</tr>
<tr>
<td></td>
<td>---------</td>
<td>---------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>10</td>
<td>7 6 6 7</td>
<td>7 6</td>
<td>38 11 5</td>
</tr>
<tr>
<td>13</td>
<td>5 6 7 7</td>
<td>9 6</td>
<td>12 6 6</td>
</tr>
<tr>
<td>14</td>
<td>7 6 21 10</td>
<td>38 11</td>
<td>47 122 55</td>
</tr>
<tr>
<td>17</td>
<td>15 9 7 8</td>
<td>19 30</td>
<td>77 58 262</td>
</tr>
<tr>
<td>23</td>
<td>8 6 7 6</td>
<td>16 7</td>
<td>25 8 7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>42 33 48 38</td>
<td>89 60</td>
<td>199 205 335</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>8.4 6.6 9.6 7.6</td>
<td>17.8 12.0</td>
<td>39.8 41.0 67.0</td>
</tr>
</tbody>
</table>
TABLE II
Effect of Chlorpromazine and Meprobamate Tested in Bastian Cages

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1Chlorpromazine (I.M.)</td>
<td>6</td>
<td>0.5</td>
<td>36.8</td>
<td>11-61</td>
<td>0.9% saline</td>
<td>6</td>
<td>32.3</td>
</tr>
<tr>
<td>1Chlorpromazine (I.M.)</td>
<td>6</td>
<td>1.0</td>
<td>15.0</td>
<td>3-38</td>
<td>0.9% saline</td>
<td>6</td>
<td>49.0</td>
</tr>
<tr>
<td>1Chlorpromazine (I.M.)</td>
<td>6</td>
<td>2.0</td>
<td>0.0</td>
<td>----</td>
<td>----</td>
<td>-</td>
<td>---</td>
</tr>
<tr>
<td>2Meprobamate (I.F.)</td>
<td>6</td>
<td>150.0</td>
<td>34.5</td>
<td>7-75</td>
<td>0.9% saline</td>
<td>5</td>
<td>19.0</td>
</tr>
<tr>
<td>3Meprobamate (I.F.)</td>
<td>8</td>
<td>150.0</td>
<td>45.7</td>
<td>15-94</td>
<td>0.9% saline</td>
<td>8</td>
<td>41.7</td>
</tr>
</tbody>
</table>

1Combined C57Bl/10 and Balb/C

2C57Bl/10

3C57Bl/6
TABLE III

Effect of Meprobamate, 150 mg./kg. i.p., on Number of Attacks in C57Bl/6 Mice.

<table>
<thead>
<tr>
<th>TRIALS</th>
<th>Meprobamate Group 13 Pairs</th>
<th>Control Group 12 Pairs</th>
<th>Difference in Average No. of Attackers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average No. of Attacks</td>
<td>Range</td>
<td>Average No. of Attacks</td>
</tr>
<tr>
<td>4 Control</td>
<td>3.7</td>
<td>1-8</td>
<td>3.7</td>
</tr>
<tr>
<td>5 Control</td>
<td>4.1</td>
<td>0-7</td>
<td>3.4</td>
</tr>
<tr>
<td>6 Control</td>
<td>4.3</td>
<td>0-11</td>
<td>4.6</td>
</tr>
<tr>
<td>7 After Inj.</td>
<td>2.2</td>
<td>0-8</td>
<td>4.3</td>
</tr>
<tr>
<td>8 After Inj.</td>
<td>3.7</td>
<td>0-10</td>
<td>3.1</td>
</tr>
</tbody>
</table>
**TABLE IV**

Fighting Latency and Fighting Frequency

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>TEST NO.</th>
<th>ISOLATION HOURS</th>
<th>LATENCY AVERAGE</th>
<th>GENERAL FREQUENCY %</th>
<th>FREQUENCY WITHIN FIGHTERS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57Bl/6 (Black)</td>
<td>1</td>
<td>4</td>
<td>17'30&quot;</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>SC-I (White)</td>
<td></td>
<td></td>
<td>6'50&quot;</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
<td>21</td>
<td>9'48&quot;</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
<td>3'36&quot;</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>Black</td>
<td>3</td>
<td>312</td>
<td>4'03&quot;</td>
<td>44</td>
<td>4</td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
<td>4'17&quot;</td>
<td>55</td>
<td>32</td>
</tr>
</tbody>
</table>

* These mice fought within one minute.
### TABLE V

Round Robin Fighting Test  
Isolation - 27 Days

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>TEST NO.</th>
<th>LATENCY AVERAGE</th>
<th>FREQUENCY %</th>
<th>FREQUENCY WITHIN* FIGHTERS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>1</td>
<td>4'12&quot;</td>
<td>53</td>
<td>16</td>
</tr>
<tr>
<td>White</td>
<td>1</td>
<td>2'31&quot;</td>
<td>63</td>
<td>48</td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
<td>2'02&quot;</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>White</td>
<td>2</td>
<td>0'24&quot;</td>
<td>54</td>
<td>87</td>
</tr>
<tr>
<td>Black</td>
<td>3</td>
<td>1'26&quot;</td>
<td>46</td>
<td>55</td>
</tr>
<tr>
<td>White</td>
<td>3</td>
<td>1'32&quot;</td>
<td>56</td>
<td>79</td>
</tr>
<tr>
<td>Black</td>
<td>4</td>
<td>3'11&quot;</td>
<td>31</td>
<td>60</td>
</tr>
<tr>
<td>White</td>
<td>4</td>
<td>1'30&quot;</td>
<td>40</td>
<td>66</td>
</tr>
</tbody>
</table>

* These mice fought within one minute.
CHAPTER II

ESCAPE AND AVOIDANCE BEHAVIOR AND DRUG RESPONSES

A. INTRODUCTION

The observation of the two strains of mice C57Bl/6 and SC-I demonstrated a difference in reactivity when the mice were picked up, held, injected, etc. The C57Bl/6 mice squirmed, squealed and reacted more than the SC-I mice. These behavioral characteristics suggested that the strains were different in escape and avoidance behavior. This was tested in the climbing screen apparatus described in the methods. The effect of chlorpromazine, phenobarbital sodium and reserpine (Sandril) upon the responses of the mice in this procedure were also studied.

B. LITERATURE SURVEY

An animal can respond to a noxious stimulus by movement toward stimulus which takes the form of aggressive behavior; no movement at all - freezing behavior; or movement away from noxious stimulus - avoidance or escape behavior.

Such terms as wildness and timidity have been used to describe movement away from stimulus. Yerkes (1913, 1916) studied these qualities in rats and Coburn (1922) studied them in mice. Their findings indicated that these characteristics were inherited. Coburn's experiment indicated that wildness was dominant over tameness.

Bagg (1920), Tolman (1924) and Sadovnikova Koltzova (1926) all contributed evidence for the inheritance of these characteristics. Dawson (1932) in a study involving thousands of mice established that wildness is dominant over tameness.
Segregation was obtained when the F₁ generation were mated interspecifically and with the tame stock. The association of the young with the mother did not influence this behavior. King and Mavromatis (1956) showed differences in acquiring avoidance conditioning in C57Bl/10 and Balb/C mice. Collins (1964) in a study of inbred strains of mice and their hybrids found that good performance in an avoidance conditioning situation was dominant over poor performance and that females of each strain were superior to males.

Levine (1956) found that, in rats, early handling increases the ability to learn a conditioned avoidance task. King (1958) found that rats with septal lesions acquire avoidance response more rapidly than normal animals. No differences appeared in the rate of acquiring an avoidance response between rats with lesions in the amygdaloid nucleus and normal rats.

Willingham (1956) found a positive correlation in mice between avoidance and escape behavior.

Besides inherited differences in avoidance, experimental factors play a part. Wolf et al. (1962) demonstrated that the closer the testing situation is to the natural habitat of the mice the better is the performance. Peromyscus who lived in semi-arboreal habitat performed better in avoidance situation in which the escape area was a pole compared to a situation in which the escape area was a flat surface.

Generally the more rapidly acquired the avoidance response the better the performance and the more difficult it is to extinguish it (Ader and Clink, 1957; Wolf et al., 1962; Denenberg and Bell, 1959). This holds true when drugs are used to impair avoidance response. The effect of chlorpromazine and pentobarbital have been studied in mice by Wolf et al. (1962). The performance of Peromyscus maniculatus gracilis was impaired to
a lesser extent in an avoidance situation in which the mice performed well compared to the avoidance situation in which the performance was poorer.

This supports the work of Kornetsky and Dawson (1961) who find that in rats the better learned the procedure the greater the resistance to drugs. Kornetsky (1961) also demonstrated that tolerance to drugs develop in avoidance situation with increased learning but no change occurs in motor responses. Irwin (1961) also reports tolerance development in rats to avoidance suppressant action of chlorpromazine within two weeks after chronic daily administration; no tolerance developed to their locomotor suppressant action.

Irwin (1963) postulates that this decreased response to drugs in an avoidance situation is due to the arousal provoking quality of the experimental situation. This is presented as a primary or fundamental factor affecting behavior and the response to drugs regardless of what the particular experimental contingencies may be. For example, in the avoidance situation the initial dose of chlorpromazine reduced the responses of an animal to stimuli which results in the animal receiving a greater number of shocks, which causes an increase in the arousal provoking quality of the test situation. Eventually the threshold to stimuli is decreased and the animal becomes resistant to the drug. In the measurement of motor activity the test situation does not change and tolerance does not develop. In order to support this concept Irwin demonstrated that increasing the intensity of the conditioned or the unconditioned stimulus resulted in rapid development of tolerance. This tolerance development was greater in females than in males at higher shock intensities. This was in keeping with the fact that the performance of the females in the avoidance situation was bet-
ter than that of the males. Similar resistance to drugs can be obtained by merely increasing the background noise level of the experimental room or by administration of an amphetamine-like agent (Irwin, 1963).

Chlorpromazine is particularly good in demonstrating this since one of its main actions is to raise the threshold to sensory stimulus (auditory, Shurtleff et al., 1962); it also alters perception (Kornetsky and Humphries, 1958). The order of impairment of performance is related to strength of stimulus (Wolf et al., 1962; Maffi, 1959). Verhave et al. (1958) have demonstrated that the secondary conditioned response is affected first, followed by the effect on the primary conditioned response, and then on the unconditioned response. Phenobarbital does not demonstrate this as well since it produces greater motor impairment and has a narrow dose range producing depressant effect (Brown, 1959; Miller, 1957). Cook and Weidley (1957) found that reserpine blocked the conditioned response but makes no statement concerning the development of tolerance.

The relationship within one strain of mice between rapidity of learning, slowness of extinguishing and resistance to drug is well supported but there is evidence that this may not hold when comparing mice of different strains. King and Mavromatis (1956) in a study including two strains of mice, C57Bl/10 and Balb/C mice found that Balb/C mice exhibited a slower rate of conditioning than the C57Bl/10 although they extinguished at a significantly slower rate and needed fewer relearning trials. Another factor, beside changes in arousal, must operate in this situation.

C. METHODS

Escape and avoidance behavior was measured in a climbing
screen apparatus developed in this laboratory. This apparatus also measured climbing (motor) activity. It consisted of a wooden board covered with a fine mesh screen measuring 3" X 12", inclined at a 35° angle and enclosed by a 5" high siding. Compartments 5" X 3" were built at the bottom and at the top of the screen. The floor of the bottom compartment consisted of a shocking grid. Photo-electric cells which activated timers were placed at the top and the bottom of the inclined screen. A sliding door was removed between the bottom compartment and the screen 5 seconds after the mouse was placed in the compartments. A 10 second shock was applied to the grid 5 seconds after the door was removed. Mice were removed from the grid if they did not leave it within 30 seconds after being placed upon it; they were removed from the screen if the climbing time exceeded 30 seconds. The current applied measured approximately 0.1 m. amp. The length of time (avoidance latency) necessary for the mice to leave the grid as well as per cent of trials in which the animals were shocked (shock frequency) were the two parameters used to measure avoidance. The length of time that the mice spent on the inclined screen was a measure of climbing motor activity.

In Part I the experiments differed in the following manner: In the first experiment the screen was used without the compartments and without the shock grid. The mice were placed on the bottom of the screen and the climbing time measured. The only stimulus was handling by the experimenter. In experiments II and III a compartment was present at the top of the screen. In experiment III shock was used.

In Part II shock was used in all experiments except the first one where the test situation was the same as Part I, Experiment II.

The mice were 10-12 weeks old in all the experiments except
those where drugs were used. In these experiments the SC-I mice averaged 25.3 gms. and the C57Bl/6 24.9 gms.

In the drug experiments because of the need for trained animals the mice were from 4 to 6 months old. In this situation the CF-I averaged 28.7 gms. while the C57Bl/6 averaged 25.6 gms.

Drugs

Chlorpromazine hydrochloride and phenobarbital sodium were dissolved in saline. Reserpine (Sandril) was diluted in distilled water. These drugs were administered intraperitoneally (0.01 ml./gm. of mouse weight). Saline was used in control animals.

Avoidance and Climbing Time

Data were analysed for significance by means of the unpaired t-test. Shock frequency was analysed by calculation of exact probabilities since cell frequencies were sometimes small (Batson, 1960).

D. RESULTS

Part I, Escape, Avoidance, and Climbing Behavior in the Climbing Screen Apparatus.

Experiment I

When the mice were tested on the climbing screen without shock a marked difference in climbing for the two strains was observed. The climbing time of the SC-I mice increased gradually while that of the C57Bl/6 remained constant for 4 trials before an increase occurred (Graph I).

Experiment II
When a compartment was added at the top of the climbing screen (without shock) the climbing behavior of both strains improved but again the SC-I mice climbed more slowly (Graph II). The climbing of the C57Bl/6 mice remained constant for six trials before the climbing time increased, while the SC-I climbing time remained constant for 4 trials before a rapid increase took place.

In both these tests the SC-I mice explored while climbing; the C57Bl/6 climbed more rapidly and directly. In both these tests the handling of the experimenter contributed a stimulus from which the mice attempted to escape and which contributed to the total climbing behavior.

Experiment III

In order to provide a constant stimulus in lieu of variable stimulus resulting from handling, the shocking grid was introduced in a closed compartment at the bottom of the screen. This allowed separation of escape behavior from avoidance behavior and climbing activity. In this experiment the mice were tested during four consecutive days. During the first day no shock was given. The mice were placed in the bottom compartment for five seconds, then the door between the compartment and the climbing screen was removed. This situation was analogous to that in Experiment II except that the mouse was not handled immediately before testing. The difference in behavior between the strains was maintained both when length of time spent on the grid and climbing time were compared.

During the remainder of the experiment shock was used. Both the climbing time and time in compartment (avoidance latency) decreased. The differences between the two strains decreased. On the second day shock was administered during 92% of trials indicating that avoidance of shock had not yet been
learned. During the third day shock frequency was 65% for SC-I and 36% for C57Bl/6. During the fourth day shock frequency for SC-I and for C57Bl/6 mice was 35.6% and 8.8% respectively, which approaches the control values achieved in most of the experiments.

At this time, the values for shock frequency, avoidance latency, and climbing time were less for the C57Bl/6 mice and from 1.4 to 2.4 seconds for SC-I mice. The climbing time ranged from 1.9 to 6.5 for SC-I mice and from 1.1 to 2.1 for C57Bl/6 mice. The difference for each pair of results was significant (P < 0.05 - 0.001 level in the T-test). During the same control period C57Bl/6 and SC-I mice were shocked in 0.0 to 5.0% and in 17.9 to 35.5% of trials respectively (see Tables III, IV, V). The control values for both strains were exceptionally high in one experiment (Table I); although the results were in the same direction as in the other experiments, the difference was not significant. These results may have occurred because the room temperature reached 90°F on that day. (Room temperature usually varied between 72°F and 80°F.)

The escape time\(^1\) was examined in mice who remained on grid longer than five seconds and therefore received shock. This was tested in 15 mice, 225 trials/strain during three days. The shock frequency for SC-I mice was 37.7% and 7.1% for C57Bl/6. Although the shock frequency was very different, the average escape times were approximately the same (0.7 and 0.5 seconds for SC-I mice and C57Bl/6 mice respectively).

\(^1\)The escape time is defined as the time elapsing between reception of shock and departure from grid and is a response to shock while avoidance latency is the time elapsing from opening of partition to departure from grid and is a function of learning.
Conclusions

C57Bl/6 mice had a higher level of motor activity than SC-I when measured in the climbing screen apparatus whether or not shock was used. The degree of difference between strains diminished with increased stimulus; the least motivated situations gave the largest differences (Graph I; Graph III, Day 1) while the most motivated ones (Graph III, Days 2, 3, 4) had the smallest differences between strains. When considered in terms of escape behavior, a mild stimulus such as handling by experimenter (Graph I) indicates a difference between strains exists but the stronger stimulus shock obliterates this difference. If a jet of air is directed at the mice in the climbing apparatus, the C57Bl/6 will rapidly run up the inclined screen while the SC-I will turn around and face the air. This is a further indication that C57Bl/6 has a stronger escape behavior.

C57Bl/6 mice were also more avoiding than SC-I as indicated by both avoidance latency and shock frequency. This supports Willingham's (1956) finding that escape and avoidance behavior are correlated and also supports King's finding that a mouse learns best in a situation in which it is naturally predisposed. Krushinskii (1960) has also found this to be true in dogs. Dogs bred for aggressive and timid characteristics learned rapidly where these characteristics fitted the test situation and poorly otherwise. Other behavioral traits observed during this testing procedure included a greater propensity towards investigative and freezing behavior by the SC-I mice than by the C57Bl/6 mice.

Part II. The Effect of Chlorpromazine Hydrochloride, Reserpine and Phenobarbital Sodium in the Climbing Screen Apparatus.
Chlorpromazine and phenobarbitol were administered one-half hour before testing. Tests were carried out every one-half hour. Reserpine was given one hour before testing and tests were carried out every hour.

**Chlorpromazine Hydrochloride**

The effect of chlorpromazine on mice was measured by means of the climbing screen apparatus with and without the application of the grid shock.

**Experiment I**

The mice were tested in climbing apparatus without use of shock. The test situation was the same as in experiment II, Graph II. Naive mice were used for each test. The cumulative results are expressed in Table I; Graph IV. The average data for each trial for control and for the 8 mg./kg. dose are shown in Graph V.

The lowest doses of chlorpromazine which significantly increased the climbing time were 4 and 8 mg./kg. respectively for C57Bl/6 and SC-I mice (P < 0.05). Although in control experiments C57Bl/6 mice climbed faster than SC-I mice, this trend was reversed by administration of the 4, 8 and 10 mg./kg. doses of chlorpromazine.

**Experiment II**

The effect of chlorpromazine on climbing time was essentially similar when grid shock was employed. The controls for this experiment had not reached the usual base level of performance so that the results may have been affected by improvement of performance.

Saline was administered on the first day of the experiment
while chlorpromazine was given on subsequent days: Both strains received 4 mg. on the second day and 6 mg. on the third day; C57Bl/6 received 5 mg. and SC-I 8 mg. on the fourth day.

The two strains responded differently. The climbing time, avoidance latency, and shock frequency of the SC-I mice decreased at the 4 and 6 mg./kg. doses while a significant increase in all three parameters occurred at the 8 mg./kg. dose. The climbing time and avoidance latency of the C57Bl/6 mice increased while the frequency of shock decreased at the 4 mg. dose. The 6 mg. dose resulted in a pronounced effect on all three parameters.

When the 5 mg. dose was given to C57Bl/6 mice on the day following the 6 mg./kg. dose a noticeable increase in response occurred only with climbing time. The decreased avoidance latency and frequency of shock indicated that further learning took place probably because of the high shock level used on the preceding day. Differences in climbing time between SC-I and C57Bl/6 mice were significant ($P < 0.001$) for all doses tested (Table I). Differences in avoidance latency and shock frequency for the two strains were significant at the 6 mg. dose ($P < 0.001$). At this dose the data is most comparable since the mice were probably not influenced by the preceding day's drug or experience. The C57Bl/6 mice showed a pronounced increase in all parameters while the SC-I mice showed a decrease.

A single dose of chlorpromazine 6 mg./kg. was employed when the two strains were compared overnight in Bastian Activity Cages. The effect of chlorpromazine was about eleven times greater for the C57Bl/6 when compared to SC-I mice (Table II).

**Phenobarbital Sodium**

For testing the action of phenobarbital sodium, the
climbing apparatus with shock procedure was employed. Three doses of phenobarbital were used (Graph IV, Table III). At 75 mg./kg. climbing time, avoidance latency and shock frequency of C57Bl/6 mice increased but only climbing time increased significantly (P < 0.05). All three parameters mentioned were significantly increased at the 100 and 150 mg./kg. dose (P < 0.001).

In the case of SC-I mice 100 mg./kg. dose showed a significant increase of avoidance latency only (P < 0.05) while the 150 mg./kg. dose significantly increased all parameters (P < 0.001). The avoidance latency was decreased with the 75 mg./kg. dose however the difference was not significant (P < 0.1). The effect of phenobarbital, 100 and 150 mg./kg., was not as great in SC-I mice as in C57Bl/6 mice.

Reserpine

Reserpine was tested in three separate experiments. In Experiment I, three doses of reserpine were given 0.625, 1.25 and 2.5 mg./kg.; 5 mice per dose per strain were used. These mice were given 75 or 100 mg./kg. of phenobarbital 39 days previously. It is presumed that this previous experience had no effect on this experiment.

In Experiment II, the same mice were tested 13 days later with 2.5 mg./kg. Four of the treated mice of each strain had received previously the dose of 2.5 mg. and two the dose of 1.25 mg./kg. The controls had received previously either of the two lower doses.

In Experiment III, control mice of experiment II received 1.25 mg./kg. of reserpine while the rest served as controls. Thus, 4 of the experimental mice had received, 24 days previously, 0.625 mg./kg. of reserpine; one had received 1.25 mg./kg. The control had received 2.5 mg./kg. eleven days
previously.

Under the conditions of Experiment I reserpine had a greater effect on the SC-I than on C57Bl/6 mice (Graph VI, Table IV). In SC-I mice there was a gradual increase in avoidance latency, climbing time, and in shock frequency with increasing doses.

In the case of the C57Bl/6 mice the lowest dose had no effect; the intermediate dose had no effect on climbing latency but had an effect on avoidance latency on the first day (increased 1.4 to 2.6 seconds) and had only a small effect on shock frequency (increased from 5% to 18.2%). The large dose increased significantly climbing time and shock frequency but not avoidance latency. The responses in all parameters measured was greater in SC-I mice than C57Bl/6 at all doses.

1.25 and 2.5 mg./kg. respectively were employed in Experiments II and III. Both doses of reserpine proved effective in the two strains and increased climbing time, avoidance latency, and shock frequency (Table V, Graphs VII, VIII). These responses were much more pronounced than in Experiment I which indicated that there was a cumulative drug effect. C57Bl/6 strain was affected to a greater extent than the SC-I strain which is the reverse of the results obtained in Experiment I. At the 1.25 mg./kg. dose, the avoidance and climbing latency of C57Bl/6 was about double that of SC-I mice. In fact, the dose of 1.25 mg./kg. was maximally effective in C57Bl/6 mice but not in SC-I mice. The response of the SC-I mice to either dose was less than that of the C57Bl/6 mice except for similar climbing responses on the first day. Also the duration of the effect of both doses of reserpine was greater in C57Bl/6 strain than SC-I strain.

At the 1.25 mg./kg. dose, the length of time required for
the SC-I mice to leave the grid only slightly exceeded the five second level which indicated that the mice were not avoiding but were escaping immediately upon receiving shock. The avoidance latency of the C57Bl/6 mice reached as high as 10 seconds on the second day after drug administration, indicating considerable impairment of escape behavior.

Both strains appeared more sedated in experiment II than III. In both experiments ataxia was observed in the C57Bl/6 while climbing; there was also in both strains a peculiar elongation of the limbs; tremor and lacrimation also occurred; ptosis was present to a greater degree in C57Bl/6 than in SC-I mice.

Several of the controls and treated SC-I remained in the middle of the screen in a fixated running position. One of the C57Bl/6 mice turned around on the grid and tried escaping by the outside door in a manner similar to that sometimes seen when a naive mouse first received a shock.

Some of the mice from Experiment I and II, tested on the climbing screen, were also tested in the Bastian cages. In addition, the dose of 1.25 mg./kg. was tested on four mice per strain. All mice were tested for a period of 17 hours overnight (Table V).

Four mice of each strain that had received 2.5 mg./kg. of reserpine in Experiment I were tested on the evening before the fourth day of trials on the climbing screen. Two control mice and two mice that had received 2.5 mg./kg. in Experiment II were tested after the first, second, third and fourth evenings following injections. The four mice per strain that received 1.25 mg./kg. were tested during three consecutive nights. Considerable variations was found in the results. The activity of the mice receiving 2.5 mg./kg. in Experiment I was much greater than those receiving the same amount in Experiment II.
which is in keeping with the climbing screen data. Although the results are variable, the indication is that the SC-I mice are affected to a lesser extent and recover more rapidly than C57Bl/6.

E. CONCLUSIONS

The hypothesis that the difference in reactivity of the SC-I and C57Bl/6 mice indicated that the two strains would vary from each other in escape and avoidance was confirmed. It was also found that these strains differed in climbing motor activity (see next chapter). The C57Bl/6 mice avoided more, had the lowest frequency of shock, and the highest level of motor activity.

The C57Bl/6 mice were more affected by chlorpromazine, phenobarbital sodium and large doses of reserpine than SC-I mice. The reverse was true for lower doses of reserpine.

The expectation that the drugs used would differentially affect the climbing time when compared to avoidance behavior did not occur uniformly. Chlorpromazine would be expected to do this to a greater extent than phenobarbital since it has been demonstrated that chlorpromazine impairs performance in relation to strength of stimulus while phenobarbital produces greater motor impairment and has a narrow dose range which produces a depressant effect. Phenobarbital and the first experiment with reserpine did not differentially affect climbing and avoidance behavior. Chlorpromazine on the other hand increased climbing time at the 5 mg. dose which simultaneously decreased avoidance behavior in C57Bl/6 mice. This difference did not occur in the case of the SC-I mice.

In the last two experiments where a pronounced effect was observed with reserpine, the climbing time of the SC-I mice re-
turned towards normal before the avoidance behavior. It appears that the climbing time of C57Bl/6 can be disrupted more rapidly than its avoidance behavior and that the opposite may be true for the SC-I mice.

In both the chlorpromazine and phenobarbital experiments, there was a decrease in response of the SC-I mice instead of the increase seen in the response of the C57Bl/6 mice. This could be due to increased learning but could also be due to improved performance because of decreased "anxiety" or "fear." Both Knight et al. (1963) and Clark (1962) report that phenothiazine derivatives can improve a test performance at low doses while it impairs performance at higher doses.

The differential response of the two strains of mice to chlorpromazine is not specific for the climbing screen test situation since a similar difference occurs when the mice are tested in the Bastian cages. The activity of C57Bl/6 was reduced to a greater extent than that of the SC-I mice. This was also true when reserpine was given. These last results were quite variable. There was also an indication in this test that SC-I recovered from reserpine more rapidly than C57Bl/6.

When the two strains were compared, no relation was found between sensitivity to drug, poor acquisition of response and motor activity as found by Irwin (1961) in individual animals. On the contrary a correlation existed between strain sensitivity to drug, good acquisition of avoidance responses and high motor activity.

The effect seen with all three depressant drugs may be analogous to the situation reported in humans where sensitivity to depressant drugs are related to personality types. Shagass (1959) reported that depressive, introverted individuals exhibited high thresholds to pentothal while active and hysterical
people exhibited low pentothal thresholds.

When considering the mechanism of action of reserpine, there are several possibilities that would explain the different responses obtained with this drug in the two strains of mice. Reserpine acts pharmacologically by depleting monoamines from the nervous system and the adrenals although there is no correlation between low levels of depletion and depression of behavior; a behavioral change occurs when the remaining small amount is also depleted (Haggendal and Lindquist, 1964). Hillarp (1960) has shown that in the adrenal medullary granules the amines occur in a small labile and a large inert fraction. Monoamines are primarily incorporated into the labile fraction (Carlsson et al., 1963). Changes of the monoamines in the labile fraction is likely to be more responsible for behavioral changes than changes in the stable fraction. Experiments on adrenal medullary granules (Lundborg, 1963; Carlsson et al., 1963) and on adrenergic nerve functions (Anden et al., 1964) show that functional changes caused by reserpine are correlated to the ability of the tissues to take up monoamines and that reserpine acts by blocking the incorporation of monoamines into the labile fraction. If the two strains studied varied in the total labile fraction or in the uptake of monoamines then a difference in level and duration of response could be expected. Maas (1963) has shown that Balb/C and C57Bl/10 differ in the total level of serotonin and in their ability to bind serotonin.
Climbing time of C57Bl/6 and SC-I mice. "No shock" procedure. Nine mice per strain, six consecutive trials. Average climbing time in seconds.
Climbing time of C57Bl/6 and SC-I mice. "No shock" procedure. Compartment present at top of climbing screen. Five mice per strain, seven consecutive trials. Average climbing time in seconds.
Climbing time and avoidance latency of C57Bl/6 and SC-I mice. "No shock" was used on Day 1. Shock was used on days 2, 3, and 4. Fifteen mice per strain, four consecutive days. Average climbing time and avoidance latency in seconds.
Effect of chlorpromazine and phenobarbital on climbing time of C57Bl/6 and SC-1 mice. Ordinate: Climbing time in seconds; abscissa: Dose in mg./kg. Drugs administered intraperitoneally. All points represent average climbing times. Grid shock was employed in the case of phenobarbital experiments.
Effect of chlorpromazine 8 mg./kg. and saline on climbing time of C57Bl/6 and SC-I. Ordinate: Climbing time in seconds; abscissa: Number of trials. "No shock" procedure. All points represent average climbing time. Four mice per strain received chlorpromazine. Nine mice per strain received saline.
Effect of reserpine on climbing time and avoidance latency of C57Bl/6 and SC-I mice. Ordinate: Climbing time and avoidance latency in seconds; abscissa: Number of days. Each point represents average daily trials for five mice.
Effect of reserpine 1.25 mg./kg. on climbing time and avoidance latency of C57Bl/6 and SC-I mice. Ordinate: Time in seconds; abscissa: Number of days. Each point represents average daily trials for five mice.
Effect of reserpine 2.5 mg./kg. on climbing time and avoidance latency of C57Bl/6 and SC-I mice. Ordinate: Time in seconds; abscissa: Number of days. Each point represents average daily trials for six mice.
TABLE I
Effects of Chlorpromazine Climbing Test

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose mg./kg. i.p.</th>
<th>No. of Animals</th>
<th>No. of Tests</th>
<th>Average Climbing Time (secs.)</th>
<th>Average Avoidance Latency (secs.)</th>
<th>Shock Frequency in Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-I</td>
<td>Saline</td>
<td>5</td>
<td>60</td>
<td>7.0</td>
<td>3.2</td>
<td>67.0</td>
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<td>4</td>
<td>5</td>
<td>60</td>
<td>4.0</td>
<td>2.0</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>30</td>
<td>2.2</td>
<td>1.5</td>
<td>46.6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>45</td>
<td>7.8</td>
<td>3.9</td>
<td>75.5</td>
</tr>
<tr>
<td>C57B1/6</td>
<td>Saline</td>
<td>9</td>
<td>36</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>16</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>16</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4</td>
<td>16</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>16</td>
<td>21.2</td>
<td></td>
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</tr>
<tr>
<td>SC-I</td>
<td>Saline</td>
<td>9</td>
<td>36</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>16</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>16</td>
<td>11.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4</td>
<td>16</td>
<td>23.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>16</td>
<td>29.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Climbing procedure, trained mice

Climbing procedure, "no-shock" results
TABLE II

Effects of Chlorpromazine Measured in Bastian Activity Cages

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose mg./kg. i.p.</th>
<th>No. of Animals</th>
<th>Average Activity (Digital Counter Readings)</th>
<th>Range of Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-I</td>
<td>6</td>
<td>3</td>
<td>239.7</td>
<td>103-574</td>
</tr>
<tr>
<td>SC-I</td>
<td>6</td>
<td>16</td>
<td>815.3</td>
<td>16-3835</td>
</tr>
<tr>
<td>C57B1/6</td>
<td>--</td>
<td>16</td>
<td>1375.1</td>
<td>504-4048</td>
</tr>
<tr>
<td>C57B1/6</td>
<td>6</td>
<td>4</td>
<td>26.8</td>
<td>6-74</td>
</tr>
</tbody>
</table>
### TABLE III

**Effects of Phenobarbital Climbing Test**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose mg./kg.</th>
<th>No. of Animals</th>
<th>No. of Tests</th>
<th>Average Climbing Time (secs.)</th>
<th>Average Avoidance Latency (secs.)</th>
<th>Shock Frequency in Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-I</td>
<td>Control</td>
<td>20</td>
<td>100</td>
<td>4.2</td>
<td>1.4</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>10</td>
<td>50</td>
<td>3.4</td>
<td>0.8</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>50</td>
<td>3.3</td>
<td>2.2</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>(1/2 hr.)</td>
<td>10</td>
<td>10</td>
<td>4.2</td>
<td>3.2</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>10</td>
<td>50</td>
<td>21.1</td>
<td>6.2</td>
<td>84.0</td>
</tr>
<tr>
<td>C57Bl/6</td>
<td>Control</td>
<td>18</td>
<td>90</td>
<td>1.6</td>
<td>0.6</td>
<td>4.4</td>
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<tr>
<td></td>
<td>75</td>
<td>10</td>
<td>50</td>
<td>2.1</td>
<td>0.8</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>50</td>
<td>6.4</td>
<td>2.1</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>(1/2 hr.)</td>
<td>10</td>
<td>10</td>
<td>11.0</td>
<td>5.5</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>8</td>
<td>40</td>
<td>29.3</td>
<td>9.6</td>
<td>97.5</td>
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### TABLE IV

Reserpine

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose mg./kg.</th>
<th>No. of Animals</th>
<th>No. of Tests</th>
<th>Average Climbing Time (secs.)</th>
<th>Average Avoidance Latency (secs.)</th>
<th>Shock Frequency in Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-I</td>
<td>Saline Control</td>
<td>15</td>
<td>45</td>
<td>1.9</td>
<td>2.4</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>0.625</td>
<td>5</td>
<td>55</td>
<td>2.3</td>
<td>3.5</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>1.250</td>
<td>5</td>
<td>55</td>
<td>6.5</td>
<td>3.6</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>2.500</td>
<td>5</td>
<td>55</td>
<td>10.8</td>
<td>4.8</td>
<td>81.8</td>
</tr>
<tr>
<td>C57Bl/6</td>
<td>Saline Control</td>
<td>15</td>
<td>45</td>
<td>1.1</td>
<td>1.1</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>0.625</td>
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<td>55</td>
<td>1.2</td>
<td>0.8</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>1.250</td>
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<td>55</td>
<td>1.1</td>
<td>1.5</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td>2.500</td>
<td>5</td>
<td>55</td>
<td>3.1</td>
<td>1.6</td>
<td>23.7</td>
</tr>
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</table>
### TABLE V

**Reserpine, 2.5 mg./kg.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of Animals</th>
<th>No. of Tests</th>
<th>Average Climbing Time (secs.)</th>
<th>Average Avoidance Latency (secs.)</th>
<th>Shock Frequency in Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SC-I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline Control</td>
<td>6</td>
<td>126</td>
<td>2.7</td>
<td>2.2</td>
<td>30.2</td>
</tr>
<tr>
<td>Drug Group</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. control period</td>
<td>6</td>
<td>48</td>
<td>4.5</td>
<td>2.2</td>
<td>25.0</td>
</tr>
<tr>
<td>b. drug period</td>
<td>6</td>
<td>77</td>
<td>16.7</td>
<td>5.6</td>
<td>75.3</td>
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<tr>
<td><strong>C57BL/6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline Control</td>
<td>6</td>
<td>126</td>
<td>1.6</td>
<td>0.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Drug group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. control period</td>
<td>6</td>
<td>48</td>
<td>2.1</td>
<td>0.7</td>
<td>2.1</td>
</tr>
<tr>
<td>b. drug period</td>
<td>6</td>
<td>78</td>
<td>23.1</td>
<td>7.5</td>
<td>81.0</td>
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</table>

**Reserpine, 1.25 mg./kg.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of Animals</th>
<th>No. of Tests</th>
<th>Average Climbing Time (secs.)</th>
<th>Average Avoidance Latency (secs.)</th>
<th>Shock Frequency in Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SC-I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline Control</td>
<td>5</td>
<td>84</td>
<td>6.5</td>
<td>1.5</td>
<td>17.9</td>
</tr>
<tr>
<td>Drug group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. control period</td>
<td>5</td>
<td>20</td>
<td>2.1</td>
<td>2.1</td>
<td>30.0</td>
</tr>
<tr>
<td>b. drug period</td>
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<td>80</td>
<td>11.0</td>
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<td>86.3</td>
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<td><strong>C57BL/6</strong></td>
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<td>Saline Control</td>
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<td>92</td>
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<td>Drug group</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>a. control period</td>
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<td>20</td>
<td>1.4</td>
<td>1.0</td>
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</table>
## TABLE VI

Effects of Reserpine
Measured in Bastian Cages

<table>
<thead>
<tr>
<th>Reserpine mg./kg.</th>
<th>No. of Animals</th>
<th>No. of Trials</th>
<th>C57B1/6</th>
<th>SC-I</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ave.</td>
<td>Range</td>
<td>Ave.</td>
<td>Range</td>
</tr>
<tr>
<td>Experiment I - 2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th night</td>
<td>4</td>
<td>4</td>
<td>106.5</td>
<td>38-178</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>809.5</td>
<td>15-3304</td>
</tr>
</tbody>
</table>

| Experiment II - 2.5| Saline Control | 2    | 8    | 737.1 | 383-1732 |
|                    | 1st night      | 2    | 2    | 4.0   | 4-4 |
|                    | 2nd night       | 2    | 2    | 7.5   | 5-10 |
|                    | 3rd night       | 2    | 2    | 0.5   | 0-1 |
|                    | 4th night       | 2    | 2    | 0.0   | 0-0 |

| Experiment II - 1.25| 1st night | 4    | 4    | 47.5  | 39-57 |
|                    | 2nd night | 4    | 4    | 433.0 | 242-750 |
|                    | 3rd night | 4    | 4    | 572.7 | 281-869 |
|                    |           |      |      | 613-3580 |
CHAPTER III

MOTOR ACTIVITY

A. INTRODUCTION AND LITERATURE SURVEY

Motor activity measurements have been used since 1898 to measure both inherent differences in mice and rats, and to detect response of depressant and stimulant drugs.

Many types of cages have been used. Stewart (1898) and Slonaker (1907) used revolving cages which operated clockwork recording mechanisms developed from old alarm clocks. Jiggle cages which were suspended on a spring were made to record directly onto a moving kymograph or to record through a tambour system (Abreu et al., 1946). Schulte et al. (1941) caused the cages to operate a counting devise whereas Waterman (1947) used an electrode which dipped into an electrolyte controlling the speed at which a revolution counter was driven by an electric motor.

However, all moving cages have inherent sources of error due to the fact that the cage itself is in motion, a factor which in itself modifies spontaneous activity since the animals are continually using compensatory righting reflexes (Campbell and McLean, 1948). Inertia is also an inherent factor in a moving cage and consequent inaccuracies of recording are to be expected.

More recently Cobbin et al. (1955) have reported on a non-moving cage, measurement of activity being derived from changes in the capacitance of a tuned circuit, brought about by the movements of the animals themselves. Dews (1953) had described an activity cage which records optically.¹ Rundquist (1933)

¹Similar cages are in present use in our laboratory.
studied inheritance of spontaneous activity in rats and found that differences in activity were largely inherent and that activity was dominant over inactivity. Some anticholinergic drugs (Harris, 1961) as well as adrenergic drugs (Greenblatt and Osterberg, 1961) increase spontaneous activity. The effect of central sympathomimetic agents such as amphetamine and its analogs (Tainter, 1930; Lands, 1949), and related piperidine stimulants is well known (Cook and Weidley, 1957).

B. METHODS

Motor activity was measured in three types of apparatus. The mice were 11-12 weeks old. The SC-I mice weighed an average 25.3 gms. and the C57Bl/6 24.9 gms. in all the motor activity tests.

Jiggle Cages

The cages recorded vertical and horizontal movement including scratching and shaking. Mice were placed individually in plastic containers which were connected to Statham force transducers; the motion was recorded on a Grass 5A polygraph at a paper speed of 30 mm./second. Resonance in the system was reduced by means of vanes extending from the bottom of the container and immersed in motor oil. Daytime activity was recorded for a period of 20 minutes.

For analysis of data fifty pen excursions were chosen at random from each record and the sum of these amplitudes in mm. was taken as a measure of motor activity.
Bastian Cages (Bastian, 1957)

These cages, constructed on a teeter-totter principle are activated when the mouse moves from one side of the cage to the other. A veder counter records the number of crosses. Mice were left in cages overnight and the total count recorded at the end of a 17 hour period.

Four mice of each strain were tested during four separate nights. Since the cages varied in sensitivity of movement both strains were distributed among the cages so that an equal number of mice from each strain were tested in all cages.

Climbing Screen Apparatus

This apparatus described in Chapter II measured climbing motor activity as well as avoidance. Climbing activity was measured with and without shock stimulus. Measurement took place during the day. Data were analysed by means of Students unpaired T-test (Batson, 1960).

C. RESULTS

In all motor activity tests C57Bl/6 mice were more active than SC-I mice (Table I). In jiggle cages, which recorded all movements of the mice, the C57Bl/6 strain was 29% more active than the SC-I strains (P < 0.1). In the Bastian cages, the data for each test was similar so the results were combined for analysis. The C57Bl/6 mice were 69% more active than SC-I mice (P < 0.1).

The climbing motor activity is discussed in Chapter II. In this situation the C57Bl/6 strain was 264% more active in "shock" procedure and 200% more active in "no shock" procedure than the SC-I strain (P < 0.001).
The difference in activity existed between the two strains whether the activity was measured during the day in the climbing apparatus, in jiggle cages, or overnight in Bastian cages.

Motor activity was highest in the C57Bl/6 mice, the strain that also showed greater escape and avoidance behavior as well as drug sensitivity while the lower activity occurred in the CF-I strain that was also more aggressive.

Since serotonin and nor-epinephrine in the brain are believed to be related to a decrease in activity such as occurs with reserpine tranquilization (Sulser and Brodie, 1960), one can postulate that the normal level of motor activity in animals may also be correlated with these substances. Irwin (1961b) has found a positive correlation between brain levels of serotonin and norepinephrine, and motor activity after injection of monoamine oxidase inhibitors. Smith and Dews (1962) found that 5-hydroxytryptophan which is a precursor of serotonin did not increase motor activity in rats. However, Joyce and Mrosovsky (1964) found that although total motor activity was reduced after administration of 5-HTP, the number of active periods were increased, indicating that the rat was awake but that its motor ability was reduced. (Hind-leg movement was impaired at high doses.)

Since the two strains of mice tested differ in their sensitivity to chlorpromazine and reserpine drugs that block and deplete monoamines the difference in motor activity observed could be due to a difference in these substances in the central nervous system.
### TABLE I

Motor Activity

#### Jiggle Cages

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of Mice</th>
<th>Average mm.</th>
<th>Range</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-I</td>
<td>8</td>
<td>626.5</td>
<td>416-916</td>
<td>100</td>
</tr>
<tr>
<td>C57Bl/6</td>
<td>8</td>
<td>808.0</td>
<td>552-1180</td>
<td>129</td>
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#### Bastian Cages

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<tr>
<th>Strain</th>
<th>No. of Mice</th>
<th>Average Counts</th>
<th>Range</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-I</td>
<td>16</td>
<td>815</td>
<td>16-3835</td>
<td>100</td>
</tr>
<tr>
<td>C57Bl/6</td>
<td>16</td>
<td>1375</td>
<td>504-4048</td>
<td>169</td>
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</tbody>
</table>

#### Climbing Screen

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of Mice</th>
<th>No. of Tests</th>
<th>Ave. Climbing Time (secs.)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-I</td>
<td>31</td>
<td>275</td>
<td>3.7</td>
<td>100</td>
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<tr>
<td>C57Bl/6</td>
<td>31</td>
<td>283</td>
<td>1.4</td>
<td>264</td>
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<tr>
<td>&quot;Shock&quot;</td>
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<td></td>
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<tr>
<td>SC-I</td>
<td>9</td>
<td>36</td>
<td>3.2</td>
<td>100</td>
</tr>
<tr>
<td>C57Bl/6</td>
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<td>1.6</td>
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</tr>
<tr>
<td>&quot;No Shock&quot;</td>
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</tbody>
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CHAPTER IV

MEASUREMENT OF BRAIN BIOAMINES AND ACETYLCHOLINE

PART I. BIOAMINES

A. SEROTONIN AND NOR EpINEPHRINE

1. Introduction and Literature Survey

The physiological role of serotonin (5-hydroxytryptamine; 5HT) is currently the subject of widespread investigation. Page (1954) and Erspamer (1954) have discussed its possible functions which are based on its contractile effect on smooth muscle and include control of vascular tone, regulation of urinary excretion of water and hemostatic action. With regard to its possible central action, it has been suggested that the central effects of reserpine are mediated through the change of serotonin from a bound to a free form (Shore et al., 1955a; Pletscher et al., 1955; 1956) and that lysergic acid diethyl amide (LSD) acts by suppressing central actions of serotonin (Gaddum, 1953; Wooley and Shaw, 1954; Shore et al., 1955b). These findings have lead to the postulate that serotonin may function in the brain as a neurohumoral agent (Brodie et al., 1955; Brodie and Shore, 1957). Critical analysis of postulated functions of serotonin was published by Erspamer (1961).

In the course of the studies on the effect of reserpine in the body it was found necessary to determine serotonin in brain tissue. Amin et al. (1954) and Twarog and Page (1953) have determined serotonin in brain using bioassay procedures.

The development of the spectrophotofluorometer described by Bowman et al. (1955) made it possible to chemically identi-
fy and assay serotonin in the brain. This instrument is capable of activating compounds and measuring their emitted fluorescence continuously from 250 to 650 μm. It uses a xenon lamp as a continuous light source with one quartz monochromator to select the activating light and another to select the emitted fluorescence. A photomultiplier scanning device coupled to an oscillograph or automatic recorder produces records of the spectra which can be used for identification and quantitative assay.

Udenfriend et al. (1955a) have applied this instrument to the estimation of serotonin in blood platelets by a procedure involving its extraction into butanol, its return to an aqueous phase buffered to pH 4, and measurement of the fluorescence at 330 μm resulting from its activation at 290 μm. This method was found to be inadequate for the assay of serotonin in brain because other materials in this organ, also extracted by butanol, interfered in the fluorescence assay. The finding that serotonin fluoresces at 550 μm in 3 N HCl (Udenfriend et al., 1955b) has made it possible to make positive identification of serotonin in mammalian brain and to accurately assay it without interference from other normally occurring material. There is good correlation between the fluorescent method and bioassay as measured by clam heart assay (Twarog and Page, 1953).

Norepinephrine was first discovered in the brain by von Buler (1946) and Holtz (1950) who used a bioassay procedure. Vogt (1954) established also by means of bioassay, that norepinephrine in dog brain is unevenly distributed, with high levels being found in the hypothalamus. Vogt concluded that at least a portion of brain norepinephrine is present in sites other than vasomotor nerves. The work of Brodie and Shore (1957) implicated norepinephrine in regulation of the central sympathetic nervous system.
Because of the increased interest in brain norepinephrine, Shore and Olin (1958) developed a physicochemical method for isolation and identification of norepinephrine in the brain. Other methods include Ellman's (1958) which identifies epinephrine and related compounds by using paper chromatography and Falck's (1962; Falck and Owman, 1965) which identifies cellular localization of monoamines.

As a result of investigation of serotonin and norepinephrine in the brain, a controversy arose as to the roles of these two monoamines. In 1957, Brodie and Shore showed that reserpine impairs serotonin storage and produces sedation; he proposed that these two events were causally related. This view seemed plausible as many effects of reserpine including decreased motor activity, lowered reactivity to external stimuli and increased parasympathetic outflow, are elicited by electrical stimulation of areas in the limbic system (Kaada et al., 1954) and diencephalon (Hess, 1954) where serotonin is heavily concentrated (Paasonen et al., 1957; Kaada et al., 1954). Reserpine was postulated to stimulate a neuronal network modulated by 5HT, which ties in central parasympathetic activities with patterns of behavior for conservation and protection of the organism. The discovery of Carlsson and Hillarp (1956) and Holzbauer and Vogt (1957) that reserpine also blocks storage of norepinephrine led to the view that the control effects of reserpine are caused by a deficiency of catecholamines at active sites. In support of this Carlsson et al. (1957) demonstrated that dopamine counteracts the central actions of reserpine and suggested that dopamine substitutes for the loss of norepinephrine. Brodie and his associates proposed the theory that the effect of reserpine was due to the uncontrolled release of 5HT at active sites. Since reserpine impairs pro-
cesses that store amines, synthesis continues unabated but lacking a mechanism for storage, amine release is deprived of all control and the newly-formed molecules flow unobstructed on to active sites. The amount present on active sites would depend on the balance between rates of synthesis and disappearance. Support for this hypothesis was fully discussed at the First International Pharmacology Meeting (Costa et al., 1962). Dr. Carlsson's present concept is that norepinephrine, serotonin as well as dopamine are excitatory transmitters and that the monoamines storing granules serve as a store for monoamines as well as facilitates the transfer of monoamines from the site of synthesis to the site of liberation into the synaptic cleft and thus is necessary for transmission (Carlsson et al., 1964).

The significance of the store of monoamines to behavior is not understood but Carlsson (1964) suggests that storage may be of importance in emergency conditions.

Most of the studies investigating a causal relationship between monoamines and behavior have involved changes in the brain amines due to drugs such as reserpine benzquinamide and a-methyl-m-tyrosine. These studies have not been well correlated with concomitant behavioral work. There is no consistent behavioral relationship between different levels of monoamines induced by drugs; also these studies do not deal with physiologically normal levels.

If an excess of serotonin, or a deficit of serotonin and other monoamines are responsible for central activities resulting in a sedated state, then a difference in these amines may exist in the SC-I and C57Bl/6 strains of mice since they differ in behavioral characteristics such as motor activity and avoidance. Since the higher activity of C57Bl/6 mice
reflects a continuous situation, these mice may need a greater storage of monoamines especially in the mobile pool.

2. Methods for Determination of Serotonin and Norepinephrine

Ten brains per strain were pooled for each of three determinations of total serotonin and also norepinephrine. The brains were frozen on dry ice and kept frozen until time of analyses. Fifteen pooled hearts were analysed once for comparison. The method of Bogdanski et al., (1956) was used for the determination of serotonin and the method of Shore and Olin (1958) was used for the determination of norepinephrine. One part of the tissue was homogenized in two parts of 0.1 N HCl using a motor driven glass homogenizer and the following methods were used.

The mice used were 11-12 weeks old. The SC-I averaged 25.0 gms. and the C57Bl/6 24.9 gms. in weight at the time of decapitation.

Determination of Serotonin

In this procedure serotonin was isolated from biological material at pH 10 by extraction into butanol. The extraction was augmented by saturating the aqueous phase with sodium chloride. The butanol phase was washed twice with salt saturated borate buffer, pH 10, to remove interfering material. The compound was then returned to an aqueous phase by the addition of heptane and shaking with 0.1 N HCl. The aqueous solution was adjusted with HCl to a normality of 3, activated in the spectrophotofluorometer at 295 mu and the resulting fluorescence was measured at 550 mu.

An aliquot of homogenate containing 0.5 to 5 micrograms of serotonin was transferred to a 60 ml. glass-stoppered bottle and adjusted to approximately pH 10 by the addition of an-
hydrous sodium carbonate. Five ml. of borate buffer pH 10 was added. The homogenate was diluted with water to a volume of 15 ml. and then 5 gm. of NaCl and 20 ml. of n-butanol\(^1\) was added\(^2\). The suspension was shaken for 15 minutes and centrifuged. The fluid was decanted into another bottle. The aqueous layer was removed by aspiration and the butanol phase was washed twice by shaking with equal volumes of borate buffer. Fifteen ml. of the butanol phase was transferred to another bottle containing 30 ml. of heptane\(^2\) and 3 ml. of 0.1 N HCl. The contents were shaken, centrifuged and the supernatant solvent removed. One ml. of the acid layer was added to 0.3 ml. of concentrated HCl in a quartz cuvette. The solution was activated at 295 mu in the spectrophotofluorometer and the resultant fluorescence measured at 550 mu.

Because of the distribution of serotonin between salt-saturated buffer and butanol, with the volumes used, only about 95% was extracted into the organic solvent. Since three equilibrations of butanol with salt-saturated buffer was employed, about 15% of the serotonin was lost. Standards were prepared by carrying known amounts of serotonin through the

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\(^1\) To 94.2 gm. of boric acid dissolved in 3 liters of water, 165 ml. of 10 N NaOH was added. This was saturated with n-butanol and sodium chloride by adding these substances in excess and shaking. The fluid was decanted from the excess salt and the excess n-butanol was removed by aspiration.

\(^2\) Solvents are purified by successive washings with 1 N NaOH, 1 N HCl, and two washings with water.
entire extraction procedure. A small blank equivalent consisting of 0.1 ug. of serotonin was present in the reagents. Approximately 0.1 ug. of serotonin could be detected in the tissue but 0.3 ug. were needed for accurate assay.

**Determination of Norepinephrine**

Norepinephrine is poorly extracted into butanol but the extraction is markedly facilitated by saturation of the aqueous phase with sodium chloride. With ten volumes of butanol to one of salt-saturated aqueous phase about 65% of the amines are extracted. This partition ratio is independent of catechol amine concentration. The amines are extracted from acid solution since they are unstable in alkaline solution. The catechol amines are extracted back into an aqueous phase by lowering the solubility of the substances in butanol by the addition of heptane and shaking with a small volume of 0.01 N HCl.

**Procedure**

Three ml. samples of the homogenate were transferred into a 100 ml. glass-stoppered bottle containing 3 to 4 gm. of solid NaCl and 30 ml. of butanol. After shaking for one hour on a shaking apparatus, the fluid was centrifuged and a 20 ml. aliquot of the butanol was transferred to another 100 ml. bottle containing 2.5 ml. of 0.01 N HCl and 35 ml. of heptane. The mixture was shaken for 5 minutes and was then centrifuged. The aqueous phase was analysed fluoremetrically for catechol amines.

1 The pH requirements for extraction of norepinephrine are not critical; the same partition ratio was obtained when the pH of the homogenate was varied from 2.5 to 5.5.
as described below.

Standards were prepared by carrying known amounts of norepinephrine through the procedure along with the samples. Tissue-free solutions of norepinephrine were shaken for only a few minutes, however, as extraction occurred rapidly under these conditions and partial loss of norepinephrine occurred following prolonged shaking in the absence of tissue components, standards were prepared by adding a known amount of norepinephrine to a separate aliquot of tissue homogenate and the added norepinephrine was carried through the extraction procedure.

**Fluorometric Assay**

After extraction from tissues, the catechol amines are converted to highly fluorescent trihydroxyindoles by a modification of the oxidation method of Lund (1950). Oxidation with iodine and removal of excess reagent with thiosulfate was found to be satisfactory (Udenfriend and Wyngaarden, 1956).

To estimate the catechol amines in the acid extract, a 1.5 ml. sample of the extract containing 0.02 to 0.3 mg./ml. was transferred to a test tube. Half a ml. of pH 5 buffer was added followed by 0.1 ml. of iodine reagent. After six minutes the excess iodine was destroyed by the addition of 0.2 ml. of thiosulfate solution. The contents of the tubes were thoroughly mixed after each addition. One ml. of the alkaline ascorbate solution was then added and 45 minutes were allowed to elapse. The solution was activated at 500 μm and the resulting fluorescence read at 520 μm in a spectrofluorometer.

As noted by Lund (1950) the fluorescence of the product derived from norepinephrine was decreased when oxidation was carried out at a low pH while the fluorescence derived from epinephrine was affected only slightly. After oxidation at
pH 5, the products of both amines showed high fluorescence, but with substitution of the pH 3 for the pH 5 buffer, the fluorescence exhibited after oxidation of norepinephrine was negligible compared to that of epinephrine. This difference in oxidation at the two pH's served as a convenient means of differential assay of the two catechol amines. To correct for non-catechol amine fluorescence, the blank fluorescence was measured in a sample of tissue extract omitting the oxidation step by reversing the order of addition of iodine and thiosulfate reagents.  

3. Results and Conclusions

Norepinephrine and serotonin levels in brain of C57Bl/6 mice were higher than those of SC-I mice in all determinations. The brain content of serotonin averaged 0.61 ug./gm. for C57Bl/6 mice and 0.54 ug./gm. for SC-I mice. The difference was not significant (P<.1). The difference between strains of brain norepinephrine was somewhat greater; the C57Bl/6 averaged 0.60 ug./gm. while the SC-I averaged 0.42 ug./gm. (P<.05) A difference in the opposite direction was found with regard to the heart norepinephrine (Table I).

Although the differences were small, they were consistently in the same direction. The differences would probably have been larger if the cortex and cerebellum had been removed. Maas (1962, 1963) found that the differences in serotonin was greater when these parts had been removed in C57Bl/10 and Balb/C mice.

1 The bioamine assay was carried out in the laboratory of Chemical Pharmacology, National Institutes of Health. We want to express our thanks to Dr. Costa.
Since C57Bl/6 mice have a higher level of motor activity as well as escape and avoidance behavior compared to SC-1 mice it is possible that the higher level of monoamines in the C57Bl/6 mice represents greater storage capacity, greater synthesis, and/or decreased metabolism of the monoamines; one of these factors, or a combination of all three could be necessary to maintain the greater activity of C57Bl/6 mice. The fact that the small difference between the two strains studied may have physiological significance is supported by our results obtained with reserpine tested in the climbing screen apparatus. In the first experiment where the responses of both strains of mice were small the C57Bl/6 strain was affected less than the SC-1 strain indicating that the monoamines were depleted to a greater extent in SC-1. In the subsequent experiments the results were very pronounced but the SC-1 mice returned to normal sooner than the C57Bl/6 mice. It is likely that in this second situation the depletion of monoamines was pronounced if not complete in both strains but that a longer period of time was required to restore function in the C57Bl/6 mice whose brains normally contained more monoamines. Also the daily testing in the climbing screen apparatus is stressful and this may have accentuated the difference in duration of response to reserpine. Sulser and Brodie (1960) have shown that the effect of reserpine on rats kept at 4°C indicates that at least serotonin is not depleted as rapidly as it is in animals kept at room temperature. This could be due to the effect of a stressful situation induced by cold which increases the amount of serotonin present in brain by increasing its transmission, synthesis and/or storage. Liberson's (Liberson et al., 1964) work on the effect of hypnosis on guinea pigs indicates that in a prolonged stressful situation at least serotonin is depleted. Also Welch and Welch (1964)
showed that seven days after the intramuscular injections into mice of labeled 3,4-dihydroxyphenylalanine (DOPA), a precursor of the monoamine, 3,4-dihydroxyphenylethylamine (Dopamine) and norepinephrine, resulted in twice the amount of radioactivity in brains of grouped animals when compared to isolated animals. This result was attributed to the increased stress caused by the presence of other animals.

The fact that both serotonin and norepinephrine are higher in animals that are more active tends to support Carlsson's hypothesis of the roles of monoamines as excitatory transmitters and that the deficiency of these same amines results in a sedated state. However these interpretations are too simple when one considers the recent work of Utena (1965) in Japan. Utena found that amphetamine and chlorpromazine chronically injected into the rats changed the monoamine contents of brain tissue but that these changes were not uniform, since increases were found in certain areas whereas no changes or decreases were found in other areas.

The study of the effect of drugs on much more discreet brain sections for a prolonged period of time and with concomitant behavioral studies are needed to elucidate relationships between brain monoamine and behavior.
<table>
<thead>
<tr>
<th></th>
<th>C 57 Bl/6</th>
<th>SCI</th>
</tr>
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<tbody>
<tr>
<td>Brain serotonin, microg/mg</td>
<td>0.60</td>
<td>0.54</td>
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<tr>
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<td>0.72</td>
<td>0.60</td>
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<td>0.57</td>
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<td>Brain nor-epinephrine, microg/mg</td>
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<td>Heart nor-epinephrine, microg/mg</td>
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<td>0.72</td>
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CHAPTER IV

MEASUREMENT OF BRAIN BIOAMINES AND ACETYLCHOLINE

PART II. ACETYLCHOLINE

A. INTRODUCTION AND LITERATURE SURVEY

It has been suggested that the cholineacetylase–acetylcholine–cholinesterase system plays an important role in synaptic transmission in the central nervous system (Loewi, 1945; Feldberg, 1945b; Feldberg and Vogt, 1948; Koelle, 1955; Patton, 1958). Nachmansohn (1939) demonstrated that the concentration of cholinesterase although constant in identical areas of the same species, varies considerably in different areas of the same species and in the same area of different species. He also reported that in comparison with the caudate and lentiform nuclei, the cortical areas of the human brain exhibited low cholinesterase activity. Some spinal reflexes are depressed (knee jerk) others are enhanced (flexors) by the cholinesterase inhibitor eserine. The spinal cord is stimulated to motor discharge when perfused with acetylcholine (Bulbring and Burn, 1941). Acetylcholine injected into the spinal circulation reduces the latency and increases the amplitude of polysynaptic reflexes and eserine prolongs the spinal effects of acetylcholine (Burn, 1945). The only spinal cord synapses proved to be cholinergic are those on the Renshaw cells which are activated by motor axon collaterals (Eccles, 1961).

The response measured from a Renshaw cell is potentiated by eserine and blocked by d-tubocurarine and the cell is sti-
mulated by acetylcholine and by nicotine (Curtis et al., 1957). The cerebral cortex of mammals is excited by acetylcholine especially after eserine.

High amplitude, spikelike brain waves are set up and these may be associated with clonic motor activity. The level of acetylcholine in an anesthetized cortex is three times that in cortex taken during a convulsion (Richter and Crossland, 1949). Fluid exuding from active cortex, but not from anesthetized contains considerable acetylcholine. Dendritic post-synaptic potentials in the cortex elicited by thalamic or cortical stimulation are eliminated by d-tubocurarine but the cortical cell bodies are still excitable (Grundfest, 1959).

Randic et al. (1964) studied individual neurons in the cortex and the effects of acetylcholine applied by iontophoresis. Out of 415 units examined 24.5% were directly excited by acetylcholine; 5.5% of neurons tested were depressed by acetylcholine; also 5% of medullary neurons tested were reported depressed by Salmoiraghi and Steiner (1963). In both the hypothalamus (Bloom et al., 1963) and olfactory bulb (von Baumgarten, 1963) the proportion of cells depressed by acetylcholine is about 18% of the total number examined. This effect was reversed by atropine. Eccles (1961) has postulated that in general, inhibition in the central nervous system is mediated through interneurons. This theory is based on the studies of the Renshaw cell system in the spinal cord. Responses of optic cortex to impulses coming in transcallosal fibers from the opposite cortex are enhanced by acetylcholine or by diisopropyl-fluorophosphate but blocked by d-tubocurarine (Marrazzi, 1953).

Anesthetics will raise the level of acetylcholine in brain (Crossland, 1955b) while changes during electroconvulsions show a rapid fall of acetylcholine so that cerebral contents are
halved in animals put into liquid air 2 seconds after current was applied to head. Convulsion began after brain had recovered initial acetylcholine (Richter and Crossland, 1949). Focal tissue removed from patients with epilepsy are found to be normal in initial bound acetylcholine and free acetylcholine. However they have a low production of bound acetylcholine. Tissue from alumina or methionine-treated animals was also low in its formation of bound acetylcholine (McIlwain, 1955).

Because acetylcholine is very labile the choice method of extraction involves freezing of the whole animal in liquid air or oxygen. Crossland et al. (1955b) compared the levels of acetylcholine in brains of rats in which the whole animal was frozen with those that were frozen immediately after decapitation and found that the values were somewhat less in the latter situation. Brains that were frozen immediately after decapitation did not differ in acetylcholine levels from brains that were allowed to stand for three minutes before freezing. However, McIlwain (1955) reports that in some investigations the opposite is to be found.

The differences found in acetylcholine may be due to two possibilities:

1. Immersion in liquid gas provides a strong stimulus to the exposed cord and this may cause the brain to release and destroy the acetylcholine before the brain is frozen.

2. Act of decapitation provides a stimulus which causes loss of acetylcholine but in the excised brain resynthesis occurs rapidly aided by oxygen and air. Tower and Elliot (1952) reported that the presence of oxygen decreased the loss of acetylcholine with time in chunks of brains by aiding synthesis which replaced acetylcholine which was being lost.
The acetylcholine content of brain refers mainly to bound acetylcholine since the free substance could be present in normal brain in only minute amounts.

Elliot and Henderson (1951) showed that freezing of a brain suspension caused the release of bound acetylcholine.

Elliot et al. (1950) showed that acetylcholine content of brain excised from anesthetized animals is increased but were not able to show decrease during drug-induced hyperactivity unless acetylcholine had been elevated by anesthesia.

Richter and Grossland (1949) were able to show a decrease in brain acetylcholine content in convulsing or excited animals by freezing the whole animal. Resynthesis of acetylcholine can occur after excision since freezing the whole animal discloses a decrease of acetylcholine in the brain of insulin treated animals which is not detectable if animal is not frozen.

The relationship between behavior and the cholinergic system has been studied by many workers. Sackler et al. (1952) reported a decrease in alcohol preference in schizophrenics given acetylcholine. Diethelm et al. (1945, 1950) and Fleetwood (1955) have studied blood fractions in humans exhibiting anxiety, tension and resentment. They identified the tension substance as having properties similar to acetylcholine. Fleetwood (1955) reports that ingestion of alcohol reduced the tension substance in the blood; the effects being greater in alcoholics than in normals. Cholinergic stimulation of the ventral amygdalina increased water intake and reduced food consumption. Atropine produced opposite effects. Gamma-aminobutyric acid gave the same response as cholinergic stimulation and hydroxylamine gave the same response as atropine. The responses were not duplicated by control substances (Grossman, 1964).
Bennett et al. (1960) studied strain differences in acetylcholine concentration in rat brain. He compared Tryon animals which were bred for maze brightness and maze dullness and Roderick animals which had been selectively bred for high and low levels of brain cholinesterase. He found that in the Tryon "bright" rats, the high cholinesterase levels paralleled the high acetylcholine levels while in the Tryon "dull" rats the low cholinesterase levels paralleled the low acetylcholine levels. However in the Roderick animals there was no significant difference in the brain acetylcholine between the high cholinesterase and the low cholinesterase strains. Bennett concluded that in order to understand the behavioral effect of acetylcholine the relationship between the synthesis of acetylcholine by choline acetylase and its hydrolysis by cholinesterase as well as the level of acetylcholine must be studied in the same preparation.

Myers (1964) found that muscarinic cholinergic blocking drugs disrupt the acquisition but not the retention of a conditioned avoidance response. The action was probably on the central nervous system since peripherally acting drugs like methyl atropine were ineffective. Since cholinergic blocking drugs have amnesic properties in humans (Migdal and Frumin, 1963; Ostfeld et al., 1960) Myers felt that muscarinic cholinergic systems might be implicated in the process of recent memory and this would explain why the drugs block acquisition but not retention of a conditioned avoidance response. (However this may not be a specific effect since Wolf (1962), and Kornetsky and Dawson (1961) have shown that in individual rats, the better learned the procedure the greater the resistance to drugs.) If this were true then a critical site of action might be the medical temporal structures especially the hippocampus.
which appear to be intimately associated with the process of recent memory (Scoville and Milner, 1957).

Since there is a high concentration of endogenous acetylcholine in the hippocampus (Feldberg and Vogt, 1948; Gerbitzoff, 1959; Hebb and Silver, 1956) cholinergic blocking drugs should suppress activity emanating from this site.

If the cholinergic system is implicated in recent memory the disruption in it caused by the cholinergic blocking drugs must be severe since Tapp (1964) found no difference in the acquisition of a lever pressing "reward" response by the two strains of Tryon rats who differed in brain cholinesterase and acetylcholine. However, Tapp (1964) found that the Tryon "bright" rats showed more suppression of a conditioned emotional response than Tryon "dull" rats.

B. METHODS

The level of acetylcholine in C57Bl/6 and SC-I mice was studied. Because of the difficulty of excising the brain from the whole frozen animal and since comparable values of both strains were required rather than absolute values, the brain was frozen immediately following decapitation and excision.

The mice used were 10-14 weeks old. The SC-I averaged 25.9 gms. and the C57Bl/6 averaged 25.0 gms. in body weight at the time of decapitation.

Acetylcholine Extraction

For each experiment, the brains of two animals of each strain were pooled. Each mouse was decapitated and the brain excised and immediately placed in liquid oxygen. The total time from decapitation to freezing did not exceed 45 seconds. The two brains were placed in a chilled solution (−12°C.)
containing 0.2% glacial acetic acid in 95% alcohol. Amount of solution used is 0.5 ml./100 mg. of tissue. Tissue was homogenized in a motor driven glass conical tissue grinder for a period of 10 minutes. By this time the temperature in the tube reached approximately 0°C. The homogenate was centrifuged (2 min.-70G) and the supernatant was decanted into an evaporating dish. The residue was washed twice with 1 ml. of 0.5% glacial acetic acid in 70% alcohol and then centrifuged. The wash was added to the supernatant. Five ml. of distilled water was added and the extract concentrated to 4 ml. or less at 38-42°C. Distilled water was added so that the final extract contained 1 or 2 ml. for each 100 mg. of tissue. The extract was used the same day in Experiments I, II, III, but stored in a freezer overnight for Experiment IV. A blank was prepared by the Feldberg method (Feldberg, 1945a) in which the extract was freed from acetylcholine by adding 1/10 volume of NaOH boiling for 1 minute and then neutralizing with N HCl. The pH of the solutions was 4.3.

**Bioassay**

The brain extracts of the C57Bl/6 and 3C-1 mice were assayed by their effect on the cat blood pressure. A spinal preparation was used and ether anesthesia was administered during surgery. The spinal cord was severed at C-1 and both vagi were cut. Blood pressure was measured in the carotid artery by means of a pressure transducer and a Grass polygraph. The femoral vein was cannulated and used for all injections and for a slow intravenous drip of saline which was administered throughout the experiment. An injection of 0.5 ml. of test solution was immediately followed by 0.5 ml. of saline which was administered throughout the experiment.
In Experiments I, II, and III the following procedure was used. A dose-response curve was obtained with acetylcholine chloride; an appropriate dose of acetylcholine was chosen from this curve and used for comparison with the extracts. The assay consisted of five injections each of the acetylcholine solution and the two extracts administered alternately every three minutes. Acetylcholine was added to both extracts treated by the Feldberg method and tested in two experiments. Pyrilamine maleate (1 mg./kg.), an antihistaminic agent, was employed in two experiments. Potentiation with physostigmine was attempted in one experiment and blockage by atropine in all three experiments. In one additional experiment a dose-response curve was obtained for each extract and for acetylcholine. A Feldberg blank was also used here and potentiation with physostigmine and blockage with atropine was also attempted.

In the first three experiments per cent drop in blood pressure was plotted against doses of acetylcholine and a dose-response curve was drawn. The control injections of acetylcholine were averaged and the value plotted on the same graph. A line was drawn through this point parallel to the curve in order to correct for changes in the sensitivity of the preparation. The per cent drop in blood pressure caused by the extract was plotted on this line and the amount present in the extract calculated.

C. RESULTS

The amount of acetylcholine present in the brains of C57Bl/6 and SC-1 mice did not differ substantially. The acetylcholine content of whole brain assayed in three experiments averaged 0.79 ug. for C57Bl/6 and 0.85 ug. for SC-1 strains; C57Bl/6 averaged 1.83 ug./gm. and SC-1 averaged 2.10 ug./gm. of brain
tissue (Table I).

The drop in blood pressure for each experiment obtained with a comparable aliquot of both extracts overlapped in range responses; the shift in responses followed the changes in sensitivity of the preparation (Graphs I, II, III). Extracts treated by the Feldberg method in the amounts injected had no effect. When acetylcholine was added to the Feldberg treated extracts, the response was in the same range as that of an equivalent amount of acetylcholine. Physostigmine had no significant effect on the amplitude of the blood pressure caused by acetylcholine and by both extracts but it increased the duration of response for both acetylcholine and the brain extracts. Atropine blocked the fall in blood pressure caused by acetylcholine and the extracts but a small rise in blood pressure was unmasked in the extracts. One half ml. of extract caused a rise in Experiment I of 7.5% in SC-I mice and 3.2% in C57Bl/6 mice; in Experiment II, a rise of 1.0% in SC-I and 2.9% in C57Bl/6; in Experiment III, no change occurred in SC-I mice and a rise of 1.3% occurred in C57Bl/6 mice. One ml. of extract caused a rise in Experiment I of 12.2% in SC-I and 16.6% in C57Bl/6; in Experiment III, a rise of 3.7% for C57Bl/6 and a rise of 8.4% for SC-I occurred. Since ½ ml. of extract was used for the experiments, the effect of the unknown pressure substance probably did not interfere with the fall in blood pressure; the greatest pressure responses occurred in Experiment I in which the acetylcholine content assayed highest.

Pyrilamine maleate did not cause a change in responses.

In Experiment III, where a dose-response curve was obtained the curves were roughly parallel although additional points should have been obtained for the SC-I extract (Graph IV). Two additional experiments were carried out which for various
reasons were not completed but which gave essentially the same responses for both extracts.

D. CONCLUSIONS

There is no significant difference in the acetylcholine content of whole brain of C57Bl/6 and SC-I mice measured by the cat blood pressure method.

Little is known concerning the cholinergic transmission in the central nervous system and its relation to behavior. What little has been studied is controversial. Krech et al. (1960, 1962a, 1962b, 1963) have found good correlations between changes in cholinesterase activity and learning behavior but as Bennett (1960) points out, this does not prove that there are differences in acetylcholine. Cholinesterase also appears early in ontogeny sometimes in the unfertilized and in the blastula of many species. This cholinesterase could not be associated with transmission of nerve impulses (Karczmar, 1963).

Our data do not point to a relationship between acetylcholine levels and learning since C57Bl/6 mice learned shock avoidance better than SC-I in the climbing screen apparatus. Although not significant the level of acetylcholine was slightly greater in the SC-I than in the C57Bl/6 strain.
Effect of acetylcholine and brain extracts of C57B1/6 and SC-I mice on cat blood pressure. [ ] = range of responses.
Ordinate: % drop in blood pressure; abscissa: ug. acetylcholine.
Effect of acetylcholine and brain extracts of C57Bl/6 and SC-I mice on cat blood pressure. \( \bar{I} \) = range of responses. Ordinate: % drop in blood pressure; abscissa: ug. acetylcholine.
Effect of acetylcholine and brain extracts of C57Bl/6 and SC-I mice on cat blood pressure. $\overline{\text{I}}$ = range of responses. Ordinate: % drop in blood pressure; abscissa: ug. acetylcholine.
Dose response curves of brain extracts of C57Bl/6 and SC-I mice compared to dose response curve of acetylcholine. Ordinate: % drop in blood pressure; abscissa: Top line — µg. acetylcholine, bottom line — ml. of brain extract.
**TABLE I**

Acetylcholine Content in Whole Brain

<table>
<thead>
<tr>
<th>Experiment</th>
<th>microgram/brain</th>
<th>microgram/gram</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C57Bl/6</td>
<td>SC-I</td>
</tr>
<tr>
<td>Experiment I</td>
<td>1.23</td>
<td>1.29</td>
</tr>
<tr>
<td>Experiment II</td>
<td>0.68</td>
<td>0.74</td>
</tr>
<tr>
<td>Experiment III</td>
<td>0.46</td>
<td>0.57</td>
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</table>
CHAPTER V

ELECTROSHOCK AND PENTYLENETETRAZOL SEIZURES

A. INTRODUCTION AND LITERATURE SURVEY

Electroshock and metrazol (pentylenetetrazol) induced seizures have been used to differentiate between different aspects of electrical activity in the brain and to differentiate among anticonvulsant drugs. Electroshock and metrazol seizures have been repeatedly compared and there is disagreement as to whether or not the two tests are qualitatively as well as quantitatively different.

There are several important differences between maximal electroshock seizure (MES) and maximal metrazol seizure (MMS). A maximal seizure is achieved when muscular discharge can no longer be increased by increase in stimulus; the major feature of this seizure is a tonic convulsion characterized by initial flexion and subsequent extension of the hindlegs. The MMS pattern is clonic-tonic in mice whereas the MES pattern is tonic-clonic. This is also true in man (Finkelman et al., 1938). Relaxation characteristic of early postictal depression supervenes after the tonic component of the MMS whereas a long period of clonus follows the tonic component of the MES. The absence of terminal clonus accounts for the shorter total duration of the MMS. Recurrent seizures are never seen after supramaximal electroshock whereas they occur in about half of the mice injected with metrazol (38 mg./kg.). The mortality rate from MMS is higher than that from MES. The high mortality following MMS is probably due to a combination of factors which include marked depression of vital medullary centers by the persisting high con-
centration of circulating metrazol and hypoxia and exhaustion resulting from the initial and recurrent seizures (Goodmann et al., 1953). The initial clonus of the MMS is probably due to the early selective stimulation of the motor cortex during the period (several seconds) necessary for the intravenously injected metrazol to reach a concentration capable of causing the areas of high frequency repetitive discharges and the appropriate seizure spread required for the maximal (tonic) seizure (Goodman et al., 1953). The requirements for a maximal convolution obtain almost instantaneously after supramaximal electrical stimulation of brief duration and hence the MMS pattern is tonic from the start. Supramaximal stimulation is used to insure maximal seizure. If a sufficiently high concentration of metrazol could be delivered to the brain all at once it is quite likely that the resulting seizure would be tonic from the start. The absence of terminal clonus in the MMS is more difficult to explain. Although the anatomical pathways for clonic seizures are complex (Rosenblueth et al., 1942) it is usually considered that clonic activity is initiated in the cortex (Penfield and Erickson, 1941). Since this is so, postictal refractoriness occurring in the cortex may be more intense after the tonic component of the MMS since it is not followed by clonic seizures compared to that of the MMS which is followed by clonic seizures. The cerebral activity evoked by metrazol disappears exponentially with an average half-life of about 34 minutes (Toman and Goodman, 1948), a value in very good agreement with the chemical data of Tatum and Kozelka (1941). Recurrent seizures after large intravenous doses of metrazol is explained by the relationship between the persisting concentration of drug and the degree of refractoriness of the cerebral cortex. Even though the concentration of metrazol remains high, the
absolute refractoriness which follows the tonic seizure discharge temporarily prevents any further response to the persisting chemical stimulus, and motor quiescence and relaxation supervene. As neuronal recovery progresses into the relative refractory period a second seizure may occur. This is usually clonic because the brain is still incapable of sustaining a maximal tonic discharge. Increased refractoriness again ensues stopping the clonus relationship between the falling concentration of metrazol and the level of postictal refractoriness of the brain (Goodman et al., 1953). The quantitative and temporal aspects of this relationship have been studied in detail (Woodbury, 1952). Recurrent seizures do not follow maximal convolution induced by a brief supramaximal electrical stimulus because the external exciting agent does not persist. During the postictal recovery period the normal balance between facilitatory and inhibitory function is restored.

Mitchell and Keasling (1960) concluded from their experiments that MMS and MES are qualitatively different. They measured the effects of anticonvulsant drugs on maximal electroshock seizure, maximal metrazol seizure, and the metrazol threshold test (MET). These drugs can be separated into at least two groups by the ratios ED50MES/ED50MMS and also by the ratios ED50MES/ED50MET but not by the ratios ED50MET/ED50MMS. This indicates the similarity of the metrazol threshold and maximal metrazol seizure test and lack of similarity between the electroshock test and the metrazol tests.

In addition to this Mitchell and Keasling (1960) separated mice into two groups on the basis of their response to electroshock (presence or absence of tonic extension). The number responding to the same stimulus 24 to 48 hours later were the same. If however, metrazol followed electroshock, or electro-
shock followed metrazol, the mice no longer remained in the same groups. In other words mice that were sensitive to seizures induced by electroshock were not equally sensitive to seizures induced by metrazol. Jenney and Pfeiffer (1956–1958), in their studies of convulsant and anticonvulsant drugs agree that the two convulsant stimuli yield different information.

The most important and constant feature of the maximal seizure, the tonic hindleg extensor component, its character and duration is the same whether metrazol or electroshock is employed. Goodman et al. (1953) suggest that for this component the underlying neurophysiological mechanisms of excitation and recovery are constant and are independent of the initiating stimulus.

The latency period following very strong shocks have been studied in rabbits (Toman and Goodman, 1948), humans (Jeans and Toman, 1956), and mice (Toman et al., 1954). In all these instances there is an initial limiting latency of approximately one second below which latency cannot normally go regardless of shock strength. This limiting latency is regarded as a manifestation of a true inhibitory process initiated by the shock and masked by the overwhelming excitatory character of the full seizure but still continuing even into the postictal phase. Latency phase should be regarded as a period during which the already predetermined seizure is held in abeyance by the faster build up of the inhibitory process. In mice, a midbrain center may hold the medullary extensor facilitatory centers in abeyance (Toman et al., 1957). Lorente de No (1947) demonstrated that inhibitory field effects accompany any travelling nerve impulse.

The effect of metrazol and other convulsant agents have been interpreted to mean that a convulsant agent operates by
inhibiting processes normally operating to end a period of activity. In intact animals a number of convulsive agents greatly increases the frequency of discharge from the pyramidal cells of the motor area of the cerebral cortex (Adrian and Moruzzi, 1939). The impulses began at times related to the spontaneous changes of electrical potential in the cortex or in relation to an applied electrical or sensory impulse. It is this increased frequency of discharge synchronized with spontaneous activity that suggests that the drugs inhibit inhibitory processes (McIlwain, 1955).

Toman and Everett (1958) studied the effect of drugs upon latency to electroshock and demonstrated that amphetamine, epinephrine, norepinephrine, and shaking cause an increase in electroshock latency while reserpine causes a decrease in latency. Reserpine and chlorpromazine are able to abolish the latency increase due to stress (shaking).

Among the tranquilizers only reserpine decreases latency but increases in latency is seen with many drugs and is therefore non-specific.

The relationship of susceptibility to convulsion and latency to seizure have been studied in several strains of inbred mice using audiogenic stimulus (Fuller and Williams, 1951). At least in this situation strains with high convulsive risks have short latencies to convulsions and vice versa. However, death following a convulsion is determined by a different set of genes and different physiological factors than those which determine convulsion risk. Hybrids of A albino and DBA/2 strains have a high convulsion risk and a relatively low death risk while the hybrids of A x C57Bl have a low convulsive risk and a high death rate after convulsion.
B. METHODS

1. Electroshock Latency

The method of Toman and Everett (1958) was used for measuring electroshock latency. Electroshock seizures were produced by means of a Grass stimulator delivering 1 msec. pulses at 140V. and 100/sec. frequency for 0.3 sec. through corneal electrodes. Latency from onset of shock to initiation of the tonic phase of the seizure was measured by stopwatch; two experiments were performed. Ten to twelve mice per strain per experiment were used. In the second experiment the duration of extensor tonus was recorded and also no artificial respiration was used so that the number of fatalities could be recorded. This data was analysed by means of Student's unpaired T-test. In addition, three Peromyscus maniculatus bairdii mice were tested.

2. Pentylentetrazol Responses

Pentylentetrazol (metrazol), 1.5% solution was infused into the tail vein by means of a motor-driven syringe at the rate of 0.28 ml. per minute. The needle connected to the syringe by means of polyethylene tubing was placed in the vein. The test mouse was allowed to run free under an inverted clear plastic cage before the infusion began. Latencies of clonic convulsions, tonic convulsions, and of death were measured in seconds by means of a stopwatch (Bastian and Krause, 1959).

C. RESULTS

1. Electroshock Latency
The latency of extensor tonus response to electroshock of C57Bl/6 mice was 33% longer in the first experiment and 60% longer in the second experiment than that of the SC-I strain (Table I). The difference in latency between the strains was highly significant in both tests ($P > 0.001$). The length of extensor tonus averaged 14.4 seconds for C57Bl/6 and 18.1 seconds for SC-I strains, which was not significant. The three Peromyscus mice tested did not convulse when subjected to electroshock of duration and intensity found effective in the SC-I and C57Bl/6 strains. When the shock duration was increased from one to ten milliseconds, these mice responded with slight clonic jerks. In Experiment II, as a result of convulsions no death occurred among the SC-I while three out of 10 C57Bl/6 mice died.

2. Pentylenetetrazol Responses

In these experiments, the duration of pentylenetetrazol infusion prior to convulsions and death was measured. In two separate experiments, no differences were found between the two strains with regard to the onset of clonic convulsions and of extensor tonus (Table II).

However, C57Bl/6 mice died sooner than SC-I mice; i.e., the lethal dose of pentylenetetrazol was lower in C57Bl/6 strain. The average time to death in two experiments were 57.0 and 52.6 seconds for C57Bl/6, and 81.2 and 78.1 seconds for SC-I mice. However, the difference between strains was not statistically significant, when analyzed by Student's unpaired T-test. The ranges of death latencies for C57Bl/6 mice were small (48-70 seconds) compared to the ranges of the latencies for the SC-I (43-122 seconds). In both experiments all of the C57Bl/6 mice died between 17-26 seconds after tonic extensor tonus. The SC-
I mice fell into two groups: Eleven of the mice died 14 to 24 seconds after extensor tonus, and seven -- 46 to 119 seconds after extensor tonus; one mouse did not undergo extensor tonus but died in 104 seconds, which falls in the time range of the second group.

All the mice of both strains that died within 26 seconds following extensor tonus died of respiratory failure due to the seizure since none ever respired again. The later deaths were probably due to other causes (Bastian and Krause, 1959). If exact probabilities are calculated treating the data as occurrence or non-occurrence of death during the first time period, a significant difference is found between the two strains ($P = 0.0015$).

D. CONCLUSIONS

There is a highly significant difference in latency response to electroshock seizure between SC-I and C57Bl/6 mice. It is unlikely that the longer latency of C57Bl/6 mice can be attributed to greater stress placed on this strain by the experimental situation even though increased stress does lengthen the latency response (Toman and Everett, 1958), since stress as measured by Toman and Everett also abolishes fatalities and decreases tonic seizures. In the electroshock procedure all animals had a full tonic seizure and three fatalities occurred in the C57Bl/6 strain that had the longer latencies compared to no fatalities in the SC-I strain.

Since susceptibility to seizure correlated with latency of response in strains of mice subjected to an audiogenic stimulus (Fuller and Williams, 1951) it is probable that the difference in latencies to electroshock also reflect a difference in sus-
ceptibility to convulsions. The lack of response in Peromyscus may simply mean that these mice are low on a susceptibility to seizure scale while C57Bl/6 would be intermediate and SC-I would be high on this same scale.

However, convulsive seizures induced by metrazol did not differentiate between the two strains. These results confirm the assumption referred to in the literature, that a qualitative difference exists between MES and MMS procedures.

A real difference exists in the three groups of mice studied. Since metrazol may act by inhibiting normal inhibitory processes, these inhibitory mechanisms are probably the same in SC-I and C57Bl/6 strains. The difference in electroshock seizure latencies may be due to a difference in an inhibitory process initiated by the shock. The balance between the excitatory and inhibitory processes may determine the length of latency and the occurrence or non-occurrence of seizure.

Death due to convulsions also differed between strains. Both electroshock and metrazol had greater lethality for C57Bl/6 than for SC-I strains. All C57Bl/6 deaths were due to respiratory failure while the SC-I deaths due to metrazol fell in two categories: Death due to respiratory failure occurred within 24 seconds after extensor tonus; death due to other causes occurred later. (Mice dying in the second period recovered initial respiration but recurrent convulsions occurred and death was probably the result of hypoxia and exhaustion. The mice were not allowed to recover since metrazol was infused until death occurred.) The difference here may lie in the different capacity of the respiratory centers to return to normal.
### TABLE I

**Electroshock Latency**

<table>
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<tr>
<th>Strain</th>
<th>Wt.</th>
<th>Number Animals</th>
<th>Ave. Lat. Sec.</th>
<th>Range</th>
<th>% Difference</th>
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<tbody>
<tr>
<td>SC-I</td>
<td>25</td>
<td>10</td>
<td>1.73</td>
<td>1.5-1.8 (one 2.6)</td>
<td>100</td>
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<tr>
<td>C57B1/6</td>
<td>24.4</td>
<td>12</td>
<td>2.3</td>
<td>1.9-2.8</td>
<td>133</td>
</tr>
<tr>
<td>Peromyscus</td>
<td>18</td>
<td>3</td>
<td>---</td>
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#### Experiment II

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<th>Strain</th>
<th>Wt.</th>
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<th>Ave. Lat. Sec.</th>
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<th>% Difference</th>
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<tr>
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<td>10</td>
<td>2.1</td>
<td>1.8-2.3</td>
<td>100</td>
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<td>10</td>
<td>3.3</td>
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TABLE II

Pentylenetetrazol Convulsions

Experiment I

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<tr>
<td>C57Bl/6</td>
<td>10</td>
<td>23.3 21-25</td>
<td>35.1 23-48</td>
<td>36.7 28-53</td>
<td>57.0 48-70</td>
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<tr>
<td>SC-1</td>
<td>10</td>
<td>22.8 20-24</td>
<td>32.0 18-48</td>
<td>35.2 21-61</td>
<td>81.2 45-122</td>
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Experiment II

<table>
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<td>C57Bl/6</td>
<td>9</td>
<td>24.4 22-26</td>
<td>27.5 24-32</td>
<td>31.8 28-36</td>
<td>52.6 47-57</td>
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<tr>
<td>SC-1</td>
<td>9</td>
<td>24.9 22-27</td>
<td>28.7 16-39</td>
<td>32.8 22-47</td>
<td>78.1 43-141</td>
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</table>
CHAPTER VI

SUMMARY AND CONCLUSIONS

The C57Bl/6 strain had a higher level of motor activity than the SC-I strain whether spontaneous activity was measured in Bastian cages and jiggle cages or motivated activity was measured in the climbing screen apparatus. The C57Bl/6 mice were more reactive to handling and performed better in both escape and avoiding situations than the SC-I mice. The SC-I mice demonstrated more exploratory, aggressive and "freezing" behavior. The C57Bl/6 mice had a longer electroshock latency but did not differ in convulsive response to metrazol infusion. The C57Bl/6 mice were more susceptible to convulsive death than the SC-I mice. In general C57Bl/6 mice were more sensitive to chlorpromazine, reserpine, and phenobarbital than SC-I mice. C57Bl/6 mice exhibited a slightly higher level of brain serotonin and norepinephrine than SC-I while SC-I had a slightly higher brain level of acetylcholine than C57Bl/6.

With regard to the differences in drug sensitivity between the strains, C57Bl/6 mice were more sensitive than SC-I mice to depressant agents and to pentylentetrazol. Sensitivity to stimulants seems to parallel that to depressant agents in humans and in animals. For instance Kornetsky and Evarts (1957), showed that individual rats characterized by high pentobarbital threshold exhibited also high amphetamine threshold. In humans Shagass (1960) found that tolerance to excitant drugs is high in individuals who exhibit high tolerance to sedative agents.

The drug sensitivity in these mice correlated with high motor activity. This correlation seems to be analogous to
under anesthesia that differences between strains could be found. Furthermore, since the cholinergic system has been implicated in recent memory and in acquisition of avoidance response (Myers et al., 1964; Migdal and Prumin, 1963; Ostfeld et al., 1960) (discussed in Chapter II) one might expect that the difference in performance of the two strains of mice tested in the avoidance situation might be indicative of a difference in the cholinergic system of these mice. This is not indicated by our results.

The results were as expected that the strain with the higher levels of brain amine had also a longer electroshock latency, higher motor activity and were more sensitive to depressant and stimulant drugs while the expectation of differences in the cholinergic system was not found.

Behavioral aggressiveness seemed to be inversely correlated with the parameters already discussed. The more aggressive SC-I mice had short electroshock latency and exhibited low motor activity as well as low sensitivity to agents studied at present. In fact, continuous correlation between electroshock and aggressiveness can be established for three mice strains studied at present; the more aggressive, less-avoiding SC-I strain exhibited shorter, while less aggressive, more avoiding C57Bl/6 mice exhibited longer electroshock latency; and finally, Peromyscus which were described as unaggressive and highly avoiding (King, 1957b; Terman, 1959) were found most resistant to electroshock. It is of interest that a highly aggressive mouse strain, Onychomys leucogaster, is highly resistant to chlorpromazine (Clark, 1962). Finally, if any way anger and aggressive behavior can be related in humans, it is an interesting fact that Shagass (1960) reports that anger in humans produced marked increase in pentothal threshold.
It has been shown that differences in the behavior of several strains of mice can be correlated with differential responses to centrally-acting agents and with neurophysiological and biochemical characteristics. The mechanisms underlying these correlations are only speculative at present.

Since environmental conditions were kept constant for each experiment, differences between strains were presumed to be genetically determined and true for the male sex since the female was not studied.

The circumstances that bring about aggression are very complex and the releaser in this particular situation is the presence of another male. The limited environment probably aggravates agonistic behavior that would be solved in a less limited place by withdrawal of one of the mice.

The SC-I mice probably have a greater propensity to form immediately a hierarchal relationship when compared to C57B1/6; this situation could be analogous to that studied by Fuller (1951) when comparing DBA/2 with C57B1/6 litters raised together from birth. Confrontation for DBA/2 mice resulted in immediate challenge and rapid formation of a hierarchy while C57B1/6 mice shared to a greater extent space and possessions. This former type of confrontation is also very prevalent in a disorganized society (Calhoun, 1950b). Both environment and heredity can bring about the aggressive confrontation. One could speculate that when the difference is genetically determined the threshold of the "fight center" in the brain is lower and more easily activated by the input of visual, olfactory and tactile stimuli. Pain would also increase the stimulus input. The fighting that takes place in a more specialized situation such as competition for the possession of a single loose food pellet after food deprivation observed for C57B1/6 mice (Fredericson, 1951; 1952a;
1952b) probably involves complex cortical pathways. Monoamines are probably not important in the transmission of impulses in these pathways since the brain level of the more aggressive strain, SC-I, is lower than in the less aggressive strain, C57Bl/6.

Since SC-I strain has been observed to eat more and gain more weight and are less active than C57Bl/6, one would suspect that the difference between these two strains would involve the areas of the ventromedial nucleus, the dorsomedial nucleus and the lateral parts of the hypothalamus since these areas are known to be involved in these functions. Lesions in the ventromedial nucleus of the cat often produce a marked increase in appetite (Anand, 1951; Ingram, 1952), savageness, and decrease in activity (Ingram, 1952). Also stimulation in the hypothalamic area of the cat can result in attack which may then change into escape (Hess, 1957). A multirepresentational system exists in the cat which when activated leads to escape (Ursin, 1960; Hess, 1957) also shows that the escape areas concentrated in the lateral hypothalamus are interspersed with areas of motor activity and that frequently stimulation which begins by increasing motor activity results in flight.

Since attack is stronger in SC-I while escape is stronger in C57Bl/6 one can postulate that the neuronal balance of the hypothalamic area of the C57Bl/6 strain tends towards high activity in the motor activity and escape areas and may be mediated through monoamines.

The fact that the C57Bl/6 strain has a slightly higher brain level of monoamines, while at the same time it is more sensitive to drugs affecting monoamines, and responds to electroshock when compared to the SC-I strain in a manner consistent with a higher level of monoamines in this strain indicates
that the difference in level of monoamines in these two strains may be important.

The difference in acquisition of avoidance response in the two strains studied could also involve areas closely connected to the hypothalamus: The septal area and the cingulate gyrus. Rats with septal lesions acquire an avoidance response more rapidly than rats with lesions in the amygdala and normal rats (King, 1958) while removal of the cingulate cortex impaired avoidance conditioning in the cat (McCleary, 1961).

All the differences observed between SC-I and C57Bl/6 strains could be accounted for by differences in neuronal balance of the hypothalamus and related limbic structures.
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APPROVAL SHEET

The dissertation submitted by Priscilla C. Bourgault has been read and approved by five members of the faculty of the Graduate School.

The final copies have been examined by the director of the Dissertation and the signature which appears below verifies the fact that any 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.

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Signature of Adviser