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Comparison of Brain and Blood Levels of Methoxyflurane to Sleep-Time in C57BL and BALB/C Mice

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COMPARISON OF BRAIN AND BLOOD LEVELS OF METHOXYFLURANE TO SLEEP-TIME IN C57BL AND BALB/C MICE

By

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DEDICATION

To my mother and brother for their financial assistance and moral support.

LIFE

The author, Kurt Douglas Woss, is the son of Aloysius Woss and Garnet (Lubka) Woss. He was born October 14, 1950, in Michigan City, Indiana.

His elementary education was obtained in the public schools of Michigan City, Indiana, and secondary education at Issac C. Elston Senior High School, Michigan City, Indiana, where he was graduated in 1969.

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CHAPTER I

INTRODUCTION

The mechanism of action of anesthetics is still unknown in spite of extensive studies in this area. Anesthetics act in the central nervous system (C.N.S.), by depressing transmission of nerve impulses. There is general agreement that anesthetics act nonspecifically and in a physical manner (Goth, 1974; Mullins, 1971). Although anesthetics exert their effects on all areas of the nerve cell membrane, the synaptic areas are the most sensitive (Larrabee and Posternak, 1952; Karis, 1967). It is not known to what part of the membrane (lipid or protein) that anesthetics bind. This will be easier to answer when membrane architecture and its' role in impulse transmission, is known beyond hypothetical models. An important region in the brain associated with the maintenance of consciousness, is the reticular formation. Early studies isolated this region as the target of anesthetic action, producing unconsciousness (Moruzzi, 1949; Davis, 1958; Mori et al., 1968), but a theory that anesthesia is caused by depression of many regions, is rapidly gaining support (Darbinjam et al., 1971; Crawford, 1970; Clark and Rosner, 1973).

One approach in studying the manner in which anesthetics act is to study these drugs in different strains of mice.

Different sleep-times between strains of mice have been seen frequently, following administration of the C.N.S. depressants alcohol and pentobarbital (Jay, 1955; McClearn, 1962; Kakihana et al., 1966; Randall and Lester, 1974). Several studies indicate that sleep-time with anesthetics is linearly correlated with brain concentration (Jori et al., 1970; Wollman and Smith, 1975), and any difference in sleep duration is usually accompanied by different metabolic rates of the anesthetic agent (Noordhoek, 1968; Belknap et al., 1972). Some investigators though, have discovered sleep-time differences in mouse strains (BALB and C57BL) that could only be explained by each strain's inborn sensitivity (genetic susceptibility) to the anesthetic (Kakihana et al., 1966; Randall and Lester, 1974). From this work, the question arose whether or not BALB and C57BL mice would demonstrate contrasting sensitivities to a volatile anesthetic; and if so, would the results be similar to previous studies with alcohol, or barbiturate depressants? The following study is intended to answer these questions. Sleep-times of C57BL and BALB/C strains of mice have been determined using methoxyflurane, a volatile anesthetic. Brain and blood levels of methoxyflurane have been measured and correlated with sleep-time to determine strain sensitivity.

CHAPTER II

REVIEW OF LITERATURE

Strain Differences

Central nervous system sensitivities to C.N.S. depressants have been studied by observing sleep-time, a behavioral response. Mice have most frequently served as the test animal of choice. Generally, sleep-time response between different strains of mice seem to correspond to brain levels and metabolic rates of the anesthetic agent (Tabakoff and Erwin, 1971; Noordhoek, 1968). These results conform to accepted concepts of anesthetic action - however, some investigations have shown results to the contrary (Introduction). Ethyl alcohol has induced a 3-fold sleep-time difference between mice strains of BALB/C and C57BL. The C57BL strain was found to have shorter sleep-times accompanied with greater brain and blood levels (Kakihana *et al.*, 1966). The C57BL strain is known to possess a higher liver alcohol dehydrogenase level than the BALB/C strain (Schlesinger, 1966), but *in vivo* rates of alcohol metabolism are the same (Kakihana, 1966). Randall and Lester (1974), studied central nervous system sensitivities to pentobarbital and ethyl alcohol, with mice strains BALB/CJ and C57BL/6J. They found that ethyl alcohol caused a 2-fold difference in sleep-time - with the BALB/CJ strain sleeping longer. These differences

are not attributed to the greater alcohol dehydrogenase levels found in C57BL's (Kakihana, 1966). An opposite effect was seen with pentobarbital. The sleep-times were equal but the BALB/CJ strain had greater barbiturate brain and blood levels upon awakening - rendering it less sensitive.

Characteristics Of General Anesthetics

The physicochemical aspects of the action of anesthetics have been studied and there is general agreement that no chemical reaction is responsible for anesthesia (Bennett, 1969; Mullins, 1971).

Three theories commonly explain the physical actions of anesthetics. These theories involve the action of anesthetic agent with lipid, protein, and water structure. Pauling, 1961; Miller, 1961, separately claimed that inhalation anesthetics form hydrated microcrystals within the central nervous system. This involves a structural arrangement of the molecules of the anesthetic with water molecules. Pauling stated that the resultant microcrystals ("clathrates") forming in the synaptic regions of the brain, interfere with the neural network, causing a decreased energy of electric oscillations. Miller's account is more specific as he stated that the microcrystals ("icebergs") cause narcosis by lowering conductance, through stiffening or plugging up of the nerve cell membrane. Both theories are weak in that the microcrystals are not stable at either body temperature or at atmospheric pressure. Further, such anesthetics as ethyl

ether, halothane, and methoxyflurane, do not form microcrystals (Nunn, 1972). Pauling defends the theory by noting that "clathrates" are stabilized by charged side chains of proteins or C.N.S. solutes.

The potency of inhalation anesthetics is proportional to their lipid solubility. This relation is thought to be much closer than a capacity of these agents to function as modifiers of water structure (Eger et al., 1969). Studies with the noble gases demonstrate an excellent correlation between lipid solubility and narcotic potency (Bennett, 1969). A wide variety of inhalation anesthetics also show excellent correlations between their potency and their oil/gas coefficients (Eger et al., 1969). However, alkaloids and inorganic ions do not comply with the theory and many fat soluble substances have no depressant action or actually produce convulsions (Krantz et al., 1958). The difficulty with the lipid solubility/anesthetic potency correlation is that it offers no real explanation for the mechanism of anesthetic action.

Experiments with cell membranes support a membrane protein site of action. Nitrous oxide and chloroform have both been found to increase the lateral pressure of lipoprotein films (Clements,1962). He speculates that similar actions may be seen with nerve cell membranes during anesthesia.

Burgen (1971) demonstrated with red blood cells that there is strong binding of anesthetic to membrane protein, and that the concentration of anesthetic in membrane protein appears to exceed the concentration in membrane lipid.

Seeman and Roth (1972) demonstrated that clinical concentrations of anesthetics protected (8.0 %) red blood cells against hypotonic hemolysis. This protection is thought to be due to a 0.4% expansion of the cell membrane (Seeman et al., 1969). Other studies have shown that this 0.4% expansion is associated with a 0.02% or less, increase in volume of the anesthetic in the membrane (Seeman, 1972; Seeman and Roth, 1972). Since membrane expansion is roughly 20 times the occupying volume of the drug in the membrane phase, it has been suggested that extensive conformational changes in membrane proteins may be involved (Seeman, 1972).

Mullins (1971) and Nunn (1972) have both postulated theories which state that ion pores in the nerve cell membrane, are affected during anesthesia. Mullins reasoned that anesthetics may directly block ion pores (channels) in the membrane. His theory states that the size of the anesthetic molecule is an important parameter in the blocking of the membrane pore. Decane is lipid soluble but it is not an anesthetic. Mullins would explain this by saying that decane is too large to fit into the membrane pore. The molecular attraction of anesthetic in the membrane pore is thought to be the result of electrostatic attraction of dipols (Van der Waal's intramolecular force). Nunn's theory is slightly different. He theorized that anesthetics produce effects by interaction with proteins in the nerve cell membrane. He feels that this interaction can produce conformational changes sufficient to alter the property of proteins.

This action could interfere with nerve cell membrane pores. Nunn's literature review points out that anesthetics are highly selective for different proteins and that this selectivity accounts for different actions of anesthetics. He notes that trichloroethylene, nitrous oxide, and methoxyflurane are analgesic in subanesthetic doses, while halothane is not. Nunn's theory accounts for this difference of action as due to unique binding sites (proteins) of these anesthetics.

Nerve cell membranes are thought to be altered by anesthetics, in some way, to affect depolarization by impeding transmembrane fluxes of ions. Anderson and Amaranath (1973) took a comprehensive look at experimental evidence of anesthetic effects on transport across cell membranes. They concluded that anesthetics uniformly affect transmembrane fluxes, and that all agents producing general anesthesia, including the charged forms of local anesthetics, act thru a single basic mechanism of action, a nonspecific physical effect in the membrane. In their conclusion they say, "it is theoretically possible that anesthetics affect transmembrane fluxes by modification or conformational changes in membrane proteins."

Gergis et al., 1972 measured acetylcholine concentrations released at synaptic junctions during anesthesia. They found that although the anesthetics depressed nerve transmission, the acetylcholine volumes remained unchanged when compared to the period just before anesthetic application. This work discourages earlier theories that synaptic vesicle membranes are

affected.

Research has shown that the synaptic junction is the most sensitive area of the neuron to anesthetics. Using platinum electrodes, Larrabee and Posternak (1952) studied the effects of sodium pentobarbital, on synaptic transmission and axon conduction. They found that the anesthetic had a greater depression effect on synaptic transmission than on axon conduction. Karis (1967) supported these conclusions with work he did with neuromuscular responses to blocking concentrations of di-ethyl ether.

In 1947, studies of lesions in the reticular formation of the cat by Lindsley, Bonden, and Magoun, showed a persistent sleep pattern on the electroencephalogram which could not be activated by peripheral stimuli. Many studies have emerged from this work to demonstrate that the reticular formation is the most important brain region depressed during general anesthesia.

Stimulation of the reticular formation system in sleeping cats, resulted in a change in the electroencephalogram from the sleeping pattern to the awake pattern (Moruzzi, 1949). Ether and pentobarbital selectively depressed the multiple unit activity of the reticular formation, and stopped electroencephalographic arousal produced by reticular stimulation (French, 1953). Later studies with animals have shown that commonly used general anesthetic agents exert their initial effect in the reticular activating system, before acting upon other areas of the brain (Davis, 1957; Davis, 1958). Mori, Winters, and Spooner

(1968) experimented with cats using di-ethyl ether, halothane, and pentobarbital to study multiple unit activity with auditory evoked responses. They found that the spontaneous activity in the reticular formation decreased progressively with increased doses of anesthetic.

Traditionally, the brain stem reticular formation has been accepted as the main target of anesthetic action causing unconsciousness - however, research is mounting to support a multi-site theory of action. Ether anesthesia abolishes the impulse resulting from an auditory stimulus somewhere in its route thru the reticular formation, but does not block the direct pathway via the lateral leminscus (French et al., 1953). Similarly, nitrous oxide, ethylene, and cyclopropane primarily decrease responses evoked from the reticular activating system (tested by stimulation of the radial nerve), but these agents also reduce the direct response in the thalamus by up to 50 percent (Davis et al., 1957). The effects of thialbarbital and di-ethyl ether on the reticular formation and cerebral cortex were studied in the cat. Both anesthetics had different depressant effects on the brain. Thialbarbital caused marked depression on cortical activity. Inhalation of ether depressed the cerebral cortex but it also showed early depression of synaptic input to the reticular formation - however, the functional state of the reticular formation was not altered (Darbinjam et al., 1971). Cortical neuron sensitivity to acetylcholine, and an amino acid excitant (DL-homocysteic acid), was studied in cats with barbiturate and inhalation anesthetic agents.

Barbiturates were found to depress this sensitivity to cortical neurons in doses less than used in surgical anesthesia. Halothane at 1.5 percent depressed excitability, but methoxyflurane was found not to alter chemical excitability of cortical neurons - even at anesthetic levels. These findings indicate that neurons in the cerebral cortex differ in their susceptibility (sensitivity) to different anesthetic agents (Crawford, 1970). Clark and Rosner (1973) discovered that in man the electroencephalographic effects of methoxyflurane, halothane, and chloroform are similar to each other, but are distinctly different from those of ether, nitrous oxide, and cyclopropane. This work suggests that even similar anesthetics (inhalation anesthetics) may have different regional sites of action. Randall and Lester (1974) found different C.N.S. sensitivities of two strains of mice (C57BL/6J and BALB/CJ) to alcohol and pentobarbital. Sensitivities to the two drugs were determined by making comparisons of sleep-time with brain and blood anesthetic concentrations. The differences of C.N.S. sensitivity could not be attributed to any metabolic differences of the drugs between the two strains. The results of these studies suggest that anesthetics act at more than one region in the central nervous system.

Quastel and Wheatly (1932) postulated that anesthesia results from depression of cerebral respiration. However, excessive anesthetic concentrations often are required to inhibit respiration in isolated tissue preparations (Quastel, 1965). It has been suggested that the overall brain oxygen consumption might fail to reveal profound inhibition in vital local areas.

In support of this notion a small number of cells in fish retina called "controller cells" respond within seconds to anoxia or hypercapnia caused by alcohols and several volatile anesthetics (Negishi and Svaetichin, 1966). If "controller cells" exist in the central nervous system, and they modify the activity of other cells, then anesthesia might inhibit the metabolism and action of these "controller cells" without affecting the total brain metabolism.

In vitro studies of brain carbohydrate metabolism suggest that anesthetics inhibit electron transport between cytochrome oxidase and a flavoprotein concerned with the oxidation of reduced nicotine adenine dinucleotide (NADH) (Quastel, 1965). Since oxidation of NADH allows phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), it follows that anesthetics might inhibit oxidative phosphorylation. However, suppression of ATP formation may result from lowered utilization of ATP. Since ATP inhibits its own synthesis, lowered utilization would discourage further formation, and suppression of formation therefore would not necessarily prove a direct effect of the anesthetic. Also, the in vivo concentration of brain ATP is unchanged during anesthesia, which suggest that this is not the cause of anesthesia (Michenfelder et al., 1970).

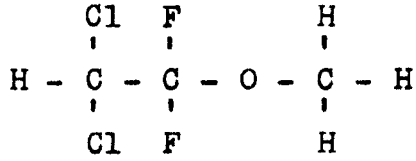
If anesthetics act by inhibiting metabolism, then a likely site of action is the mitochondria. Major functions of mitochondria include those of energy transfer and storage. As noted previously, cerebral ATP levels do not decrease as a result of anesthesia (Michenfelder et al., 1970).

All the enzymatic theories of anesthesia leave one question unanswered - that is, are effects observed the cause or result of anesthesia? In other words, do enzymatic changes cause inhibition of synaptic transmission, or are they themselves caused by the general depression of the nervous system?

Characteristics Of Methoxyflurane

Physical Properties:

Methoxyflurane (2,2-dichloro-1,1-diflouroethyl methyl ether) was first synthesized in 1958, and it was first used clinically in 1960, by Artusio *et al*. Methoxyflurane has the structural formula:



Physical constants of methoxyflurane (Abbott Laboratories) are:

Molecular weight	164.97
Boiling point at 760 mm. Hg	104.65° C.
Partition coefficient at 37° C.	
water/gas	4.5
blood/gas	10.2
oil/gas	825.0
Vapor pressure	20 mm. Hg
Flash points	
in air	62.8° C.
in oxygen (closed system)	32.8° C.
in nitrous oxide 50% with 50% oxygen	28.2° C.
Lower limits of flammability of vapor concentrations	
in air	7.0 %
in oxygen	5.4 %
in N_2O 50%	4.6 %

Methoxyflurane is the first clinically useful nonexplosive ether to be found. Many of its' pharmacologic properties are similar to those of other halogenated hydrocarbons.

Pharmacology:

Knox and colleagues (1962), using gas chromatography, determined the vapor concentrations necessary to induce and maintain anesthesia in humans. During induction an excess of one percent is required for surgical anesthesia. An inhaled concentration of 0.2 to 0.5 percent is needed for anesthesia maintenance.

During anesthesia, adipose tissues continue to absorb methoxyflurane. In subcutaneous fat the level of methoxyflurane (3.4 mg./g.) plateaued after five hours of administration at 1.0%. In the postanesthetic period, the brain was found to have the second highest tissue concentration - second only to the suprarenal gland. This information was obtained using dogs. Deep levels of anesthesia were associated with methoxyflurane levels of 0.51 milligram per milliliter of venous blood and 0.72 milligram per milliliter was found in venous blood when the dogs had regained consciousness. No brain levels were measured at this time (Chenoweth et al., 1962).

Methoxyflurane is mostly eliminated by the lungs. Species differences occur however in the amount that is metabolized. Van Dyke et al., (1964) detected that rats exhaled 85-90 percent of methoxyflurane within 30 hours after injection. Labeled chloride in concentrations of 1.1 percent were found in urine within 48 hours after anesthetic injection. They reported that the majority of rats yielded a 1-2 percent conversion of methoxyflurane. Degradation of 20 to 50 percent are reported to occur

in man (Cohen, 1971; Halsey, et al., 1971).

The respiratory center of the central nervous system is greatly affected by methoxyflurane. Respiratory depression is directly related to the depth of anesthesia. Respiratory paralysis is easily produced and for this reason methoxyflurane is commonly administered thru an endotracheal tube to control vital respiration (Price, H.L., 1975).

Methoxyflurane creates a blood pressure fall that is proportional to the depth of anesthesia. This hypotension is accompanied by bradycardia, reduced cardiac output, and reduced contractile force of the heart (Wood-Smith et al., 1973; Price, 1975).

At relatively light planes of anesthesia, methoxyflurane provided substantial analgesia and muscular relaxation. Analgesia may even persist after consciousness has returned. Skeletal muscular relaxation is profound at deep levels of anesthesia, but so are the circulatory affects; for this reason, neuromuscular blocking drugs are usually used to achieve muscle relaxation (Price, 1975).

Methoxyflurane is not believed to have any specific toxic effects on the liver - however, a few cases of liver necrosis have been seen after anesthesia (Wood-Smith et al., 1973).

Renal dysfunction associated with methoxyflurane is rare but high output renal failure has been observed. This syndrome is characterized by: dilute urine, dehydration, and azotemia (Mazze et al., 1971).

CHAPTER III

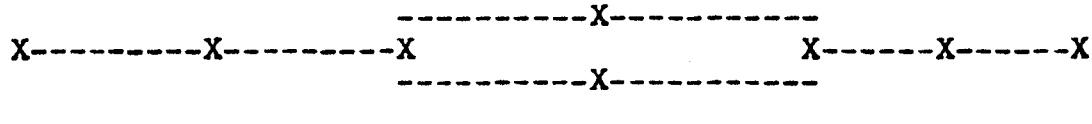
METHODS

The animals for this study consisted of 21 male BALB/C and 21 male C57BL mice, 10-13 weeks of age, weighing 22-30 grams. The mice were maintained on Purina Lab Chow and tap water ad libitum. They were kept in an artifically lighted vivarium for ten days before each test began. All experiments were started at 1:00 p.m. (± 30 minutes), to avoid circadian variation (Speciale and Friedman, 1971).

This study consisted of three experiments. In the first experiment, pain-pressure and righting reflex recoveries were recorded and were compared with brain and blood methoxyflurane extractions taken immediately after righting reflex recovery. The second experiment was concerned with righting reflex recovery only. In experiment three, the brain and blood methoxyflurane extractions were performed immediately after the mice were taken out of the anesthesia flask. A measurement of fall-time was also recorded (see experiment III for definition).

Experiment I. Part A

Ten pairs of mice were anesthesized separately in two 500 millliliter Erlenmeyer flasks, using a semi-closed system. A flow chart of this system is diagramed on the following page.



The purpose of the water trap is to keep out environmental air.

One mouse from each strain was placed in the anesthesia flasks. The flasks were alternated so that each strain had the same flask every other test. Each mouse was subjected to methoxyflurane via a vaporizer for 35 minutes. Initially, the vaporizer was set at 1.5 percent, with an oxygen flow rate of 4.0 liters per minute, for 25 minutes. Anesthesia was maintained with the vaporizer set at 0.5 percent, with an oxygen flow rate of 1.0 liter per minute, for 10 minutes.

Experiment I. Part B

At the end of the 10 minute anesthesia maintenance period, the mice were taken out of the anesthesia flask and were placed on their backs. Pain-pressure responses and righting reflex recovery were then recorded as measurements of sleeptime. The time lapse, from placing the mice on their backs until they responded to pain (by pinching their tails) was recorded as pain-pressure recovery. The response took the form of limb or abdominal movement caused by the pinch. The tails were pinched half way up at six second intervals. After the pain-pressure response was elicited, the pinching was continued (at thirty second intervals) until their righting reflex was recovered. Righting reflex recovery was measured as the time lapse from placing the mice on their backs until they

rolled over twice within one minute.

Experiment I. Part C

Preparation for assaying the concentrations of anesthetic in brain and blood were initiated immediately after each mouse had regained its' righting reflex. The mice were sacrificed by cervical dislocation. This was achieved by sharply pulling the tail while pressing a probe against the posterior portion of the first few cervical vertebrae. Blood samples of approximately 20 microliters were collected in heparized glass capillary tubes. This was done by exposing and removing the heart, and by plunging the capillary tube in and out of the pooled blood in the mediastium. The tubes were sealed at both ends with clay. Three samples were taken simultaneously for each strain. The brains were then removed and were placed in 3.0 milliliter glass vials which contained 1.5 milliliters of extracting solution and 7 glass beads. The vials were topped with aluminum foil. A rubber septum was placed on top of the foil and was pressed down the vial 1/8 inch to complete the sealing. The brain weight was then recorded. This was determined by subtracting the weight of the vial (extracting sol., glass beads, foil, septum) before and after the addition of the brain. The brains were then broken up on a Vortex mixer for 12 minutes. Further mixing and anesthetic extraction was performed with a mechanical mixer for 10 minutes. The homogenized brains were then centrifuged for 15 minutes, at 3000 r.p.m. The centrifuge separated the homogenate into a cellular and a clear layer.

The methoxyflurane was localized in the clear layer at the top of the vial. The clear layer was then transfered by a glass syringe into a 1.0 milliliter glass vial. The vial was sealed with a teflon lined twist-cap. Both blood and brain extractions were then ready to be assayed by gas chromatography.

Experiment II. Part A

Eleven pairs of mice were anesthesized under a 10.5 liter Bell jar. A flow chart of this system is diagramed below.

X---------X----------X---------X---------X---------X

Oxygen : Vaporizer : Holding tank : Bell jar : Water trap : To Vent

The Bell jar was divided by a hollow plywood partition which separated the space into equal volumes. The Bell jar sat on a 2'x2' plywood base which was covered with a towel. The methoxyflurane entered the jar via glass tubes which passed thru the base and were located between the partition. Five mice from each strain were placed on opposite sides of the plywood wall. The mice were subjected to methoxyflurane for 45 minutes. During the first 8 minutes the vaporizer was set at 1.5 percent with an oxygen flow rate of 5.0 liters per minute. The oxygen flow was then cut to 1.0 liter per minute - with the vaporizer remaining at 1.5 percent, for 22 minutes. Anesthesia was maintained at 0.5 percent, with an oxygen flow rate of 1.0 liter per minute, for 15 minutes.

Experiment II. Part B

At the end of the 15 minute anesthesia maintenance period,

the mice were taken out of the Bell jar and were placed on their backs. The time lapse from placing the mice on their backs until they rolled over twice within one minute, was recorded as righting reflex recovery.

Experiment III. Part A

The mice that were tested in Exp. II were given a ten day rest period, prior to being used in Exp. III. The administration of methoxyflurane (technique and apparatus) was the same as that used in experiment I.

Experiment III. Part B

A measurement of fall-time was made for each strain. Fall-time is a measurement of time lapse from when the anesthetic is turned on, until the mouse falls and can not offer resistance to being positioned on its' back. The mice were positioned on their backs (rolled over) by gently shaking the anesthesia flask.

Experiment III. Part C

The extraction of methoxyflurane from blood and brain was executed as soon as the mice were taken out of the anesthesia flasks. A blood sample (45.7 ul.) approximately half of one heparized capillary tube, was collected from the mediastium in the same manner as stated in Experiment I. Part C. The blood was then transfered to a three millliliter glass vial which was precooled in an ice bath, and contained 78.0 ul. of extracting solution (see materials). The blood was then mixed in the extracting solution thru vigorous stirring with a capillary tube.

The blood mixture was left in the ice bath so that the anesthetic extraction of the brain could be started with no further delay. The brain anesthetic extractions were handled in the same manner as described in Experiment I. Part C. After the brains were sealed in the glass vial, attention was again directed to the blood extraction. One capillary tube for each strain was filled with the blood mixture and was sealed at both ends with clay. The tubes were then centrifuged in a microcapillary centrifuge for 8 minutes. A cellular, plasma, and clear layer (extraction sol.) resulted, with the methoxyflurane captured in the clear layer. The clear layer was separated from the other two layers by breaking and sealing the capillary tube at the plasma/extracting solution border. The blood anesthetic extraction was now complete. The brains were then weighed, homogenized, and centrifuged as described in Experiment I. Part C. The extracted methoxyflurane was transfered via a glass syringe into a 1.0 milliliter glass vial, and was capped with a teflon lined twist-cap.

This method of anesthetic extraction from brain and blood is adapted from those used by Wolfson et al., 1966; Cervenko, 1968. Modification came in the choice of toluene as the anesthetic solvent. Prior studies (Chenoweth et al., 1962; Allott et al., 1971) used carbon disulphide as the solvent. Carbon disulphide is not an ideal solvent because of toxicity, effects on the chromatograph base line, and plugging effects on the chromatograph column and detector. In addition, other solvents which were tried, such as heptane and carbon tetrachloride, both

elute from the column at the same time as methoxyflurane, thus precluding their use. Toluene was found to be a good solvent in that it separated from methoxyflurane cleanly, it is relatively nontoxic, and it has no clogging effect on the chromatograph. The only drawback in using toluene is that it takes a relatively long time (15 minutes) for it to clear thru the column.

Experiment I. & III. Measurement of methoxyflurane in brain and blood.

Gas chromatography is a commonly used method for the measurement of inhalation anesthetics. The chromatograph is comprised of an analytical column, a detector, and a recorder. The sample to be analyzed is injected into a heated port where it is volatilized. It is then carried by an inert gas (nitrogen) thru the column to the detector. The column contains different substances which adsorb the various volatilized components for different lengths of time. Multiple components thus reach the detector separately. The detector sends a signal to the recorder, for each component, varying in intensity, according to the quantity present. The recorder displays the signal in the form of a peak. The area under this peak gives an accurate indication of the quantity present. When the peaks are sharply defined with a narrow base, it is acceptable to use the height of the peak for quantity determination (Burchfield and Storrs, 1962).

The analyses were performed on a Hewlett/Packard model

5750-B gas chromatograph (G.C.) - equipped with a hydrogen flame detector. The column consisted of a 6 foot by 1/8 inch coiled brass tube, and was packed with 5% SE 30 on 60-80 Chromosorb W. Settings for the brain and blood samples for experiment I were as follows: Brain: column temperature 100° C., carrier gas flow (N_2 - 39 p.s.i. at 20 ml./min.) at setting 1.0, range 10, attenuation 16, paper speed 0.5 inch a minute; Blood: column temperature 90° C., carrier gas flow (N_2 - 39 p.s.i. at 30 ml./min.) at setting 1.5, range 10, attenuation 16, paper speed 0.5 inch a minute. Oxygen (30 p.s.i.) was burned with the hydrogen (10 p.s.i.) during all assays. The G.C. settings for the brain and blood assays in experiment III were the same as the brain settings for experiment I.

Dose response and internal standard techniques were employed to determine the anesthetic concentrations. The dose response method was used for the blood assays. In experiment I, the gas chromatograph was calibrated by injecting different dilutions of known amounts of methoxyflurane disolved in water. A response to 40 and 20 milligram percent dilutions was attained before and after each blood assay (fig. I). In experiment III, dose responses were attained with a 10 milligram percent solution (see materials). The calibrating solutions were made up fresh every three days. Blood anesthetic concentrations were determined by comparing the unknown peak heights to the known (mg./ml.) peak heights.

The internal standard technique (detailed by Burchfield et al., 1962) was employed for determining the brain quantities

of anesthetic. This technique was used in both experiments (I & III). Toluene, (extracting solvent) di-ethyl ether, (internal standard) and a precisely known amount of methoxyflurane (10 mg. %), comprised the calibrating solution (see materials for amounts). The calibrating solution was made up fresh every three days. The calibrating solution functioned by displaying peak ratios of methoxyflurane to di-ethyl ether, with a known amount of methoxyflurane (fig. 2). The unknown methoxyflurane is thus determined by dividing the known ratio by the unknown ratio and by multiplying the quotient by the known milligram quantity of methoxyflurane in the calibrating solution. This yields the total milligram amount extracted. This number is then multiplied by the ratio 1000 mg./brain wt. This will give the amount in mg. of methoxyflurane extracted, contained in 1.0 gram of brain. This formula is represented below:

$$\frac{\text{Ratio from cal. sol.}}{\text{Ratio from unknown}} \times \text{Mg. amount of anesthetic in the cal. sol.} \times \frac{1000 \text{ mg.}}{\text{mg. brain wt.}}$$

Blood samples were assayed by breaking the capillary tubes, drawing the blood (Exp. I) or extracting solution (Exp. III) with a 1.0 microliter syringe, and injecting it directly into the gas chromatograph. Three injections of 0.5 microliter amounts were assayed for each blood and brain assay. The mean value of the three injections was recorded as the quantity of anesthetic present.

Statistical analysis

Comparisons of the sleep-time evaluations, and blood and brain anesthetic concentrations between the two strains (BALB/C

and C57BL) were made by using analysis of variance.

CHAPTER IV

RESULTS

The results of experiment I are presented in tables I - VII (pages 27 - 31). Significant sleep-time differences were found between BALB/C and C57BL strains of mice. With both measurements of sleep duration the C57BL mice slept longer than the BALB/C mice - pain-pressure ($F = 12.2$, $df = 1/18$, $P < .005$) and righting reflex ($F = 11.1$, $df = 1/18$, $P < .005$). When comparing the mean differences between the two sleep-times, it was found that pain-pressure (53%) showed a 18% greater difference than the righting reflex evaluation (35%). This may indicate that the pain-pressure test is a more sensitive test, when measuring recovery, than the righting reflex method. Despite these sleep-time differences, no significant contrast was detected in either blood or brain anesthetic concentrations, extracted immediately after righting reflex recovery. A comment should be made about the reliability of the blood data. The procedure of injecting blood samples straight into the chromatograph is not a good one, as it yields variable results between injections. The average standard deviation, of the three injections, in all the mice, was 0.12 milligram. This is fairly high when considering that the mean of the whole test was 0.24 milligram. Even with the great variability, the data was still

in agreement with blood methoxyflurane recovery levels reported with dogs (Chenoweth et al., 1962). No report of such variability was made in this publication. Another unfavorable characteristic of the procedure was that the syringe and G.C. injection port both frequently needed to be cleared of dried blood. Because of these liabilities, a different procedure was used for blood extractions in experiment III.

The results of experiment II are displayed in tables VIII - X (pages 32 - 34). The sleep-times in experiment II were in the same direction as those found in experiment I. The C57BL's slept 44% longer than the BALB/C's. Because of the greater length of anesthetic time, both strains slept almost twice as long as in experiment I.

The results of experiment III are found in tables XI — XVI (pages 35 - 40). No fall-time differences were detected between the two strains. No anesthetic (blood or brain) differences, extracted at the greatest depth of anesthesia, were seen either. These results indicate that the mice received equal amounts of methoxyflurane which was absorbed and distributed at similar rates. The procedure for blood extraction and analysis in experiment III were far superior to those used in experiment I (see figures III and IV). The average deviation, between the three injections, of all the mice, was 0.04 milligram. This is very acceptable when considering that the mean for the whole study was 1.50 milligrams. Also, the problem of dried blood in the syringe and injection port was eliminated, which decreased the time and labor of assaying the samples.

TABLES

TABLE I. Sleep-time duration beginning at the end of the anesthesia maintenance period.

SLEEP-TIME

PAIN-PRESSURE (in minutes)		RIGHTING REFLEX (in minutes)	
C57BL	BALB/C	C57BL	BALB/C
5.1	5.0	21.4	20.9
8.5	2.5	46.8	15.1
4.0	2.5	19.3	12.3
9.0	7.0	25.5	24.0
6.7	2.9	22.3	15.7
16.4	4.3	32.4	22.8
10.7	5.5	28.8	18.2
11.4	3.5	28.6	12.0
7.0	4.0	23.0	16.7
12.0	5.8	28.0	23.2

TABLE II. Anesthetic concentration extracted at the time of righting reflex recovery.

ANESTHETIC CONCENTRATION

BLOOD (mg./1.0 ml.)		BRAIN (mg./1.0 g.)	
C57BL	BALB/C	C57BL	BALB/C
.20	.21	.28	.28
.12	.15	.12	.20
.21	.20	.22	.26
.24	.30	.22	.24
//	.34	.25	.24
.23	.29	.17	.26
.19	.30	.27	.25
.31	.42	.21	.30
.13	.30	.15	.19
.28	.23	.20	.15

// Technical problem

TABLE III. Analysis of Variance Table, applied to sleep-time duration beginning at the end of the anesthesia maintenance period.

PAIN-PRESSURE

Variance Source	DF	SS	MS	F	PROBABILITY
Between	1	114.24	114.24	12.19	.001 > P > .005
Within	18	168.73	9.37		

RIGHTING REFLEX

Variance Source	DF	SS	MS	F	PROBABILITY
Between	1	453.15	453.15	11.10	.001 > P > .005
Within	18	733.60	40.75		

TABLE IV. Analysis of Variance Table, applied to anesthetic concentration extracted at the time of righting reflex recovery.

BLOOD ASSAY

Variance Source	DF	SS	MS	F	PROBABILITY
Between	1	500.10	500.10	6.4	$.05 > P > .10$
Within	17	1324.70	77.90		

BRAIN ASSAY

Variance Source	DF	SS	MS	F	PROBABILITY
Between	1	17.10	17.10	1.6	$.10 > P > .25$
Within	18	198.70	11.00		

TABLE V. Statistics applied to sleep-time duration beginning at the end of the anesthesia maintenance period.

SLEEP-TIME	PAIN-PRESSURE (in minutes)		RIGHTING REFLEX (in minutes)	
	C57BL	BALB/C	C57BL	BALB/C
Range	4.0-16.4	2.5-7.0	19.3-46.8	12.0-24.0
Mean	9.1	4.3	27.6	18.1
Standard Deviation	3.7	1.5	7.9	4.5
Alpha Error	.001 > P > .005		.001 > P > .005	

TABLE VI. Statistics applied to anesthetic concentration extracted at the time of righting reflex recovery.

ANESTHETIC CONCENTRATION	BLOOD ASSAY (mg./1.0 ml.)		BRAIN ASSAY (mg./1.0 g.)	
	C57BL	BALB/C	C57BL	BALB/C
Range	.12-.31	.15-.42	.17-.28	.15-.30
Mean	.21	.27	.19	.22
Standard Deviation	.06	.08	.05	.05
Alpha Error	.05 > P > .10		.10 > P > .25	

TABLE VII. Animal weight, brain weight, and methoxyflurane levels extracted per whole brain.

	Animal weight (in grams)		Brain weight (in milligrams)		Anesthetic extracted (in milligrams)	
	C57BL	BALB/C	C57BL	BALB/C	C57BL	BALB/C
	24.0	30.0	.324	.379	.091	.106
	24.0	29.0	.270	.264	.032	.053
	22.0	28.0	.220	.229	.048	.059
	22.0	30.0	.250	.250	.055	.060
	25.0	27.0	.230	.250	.057	.060
	22.0	27.0	.273	.250	.046	.065
	22.0	26.0	.210	.247	.057	.062
	23.0	27.0	.270	.225	.057	.068
	22.0	28.0	.376	.393	.056	.075
	22.0	29.0	.287	.395	.057	.059
Mean	23.0	28.0	.271	.288	.056	.067

TABLE VIII. Sleep-time duration beginning at the end of the anesthesia maintenance period.

SLEEP-TIME	
RIGHTING REFLEX (in minutes)	
C57BL	BALB/C
14.5	16.8
50.8	18.3
65.8	38.7
67.2	38.4
74.5	60.0
19.0	12.9
31.6	24.3
44.2	28.3
65.3	31.0
66.3	33.8
82.3	45.3

TABLE IX. Analysis of Variance Table, applied to sleep-time duration taken at the end of righting reflex recovery.

RIGHTING REFLEX

Variance Source	DF	SS	MS	F	PROBABILITY
Between	1	2480.62	2480.62	6.99	$.005 > P > .025$
Within	20	7098.59	354.93		

TABLE X. Statistics applied to sleep-time duration taken at the end of righting reflex recovery.

SLEEP-TIME	RIGHTING REFLEX (in minutes)	
	C57BL	BALB/C
Range	14.5-82.3	12.9-60.0
Mean	52.9	31.6
Standard Deviation	22.8	13.8
Alpha Error	$.005 > P > .025$	

TABLE XI. Fall-time during the induction period, and anesthetic concentration at the end of the anesthesia maintenance period.

FALL-TIME		ANESTHETIC CONCENTRATION			
ROLL-OVER (in minutes)		BLOOD (mg./1.0 ml.)		BRAIN (mg./1.0 g.)	
C57BL	BALB/C	C57BL	BALB/C	C57BL	BALB/C
1.5	1.7	1.28	1.53	.40	.54
1.4	1.4	1.39	0.98	.46	.34
1.2	2.1	2.50	1.05	.72	.36
1.7	1.4	1.13	1.08	.38	.27
1.4	1.9	2.37	1.92	.61	.62
1.4	1.1	1.89	2.56	.53	.55
1.3	1.7	1.55	1.39	.57	.52
1.3	1.3	1.76	0.81	.53	.57
1.4	1.7	1.39	0.98	.45	.28
1.3	1.2	1.49	1.29	.59	.41
1.4	1.4	1.37	1.21	.55	.38

TABLE XII. Analysis of Variance Table, applied to fall-time during the anesthesia induction period.

FALL-TIME

Variance Source	DF	SS	MS	F	PROBABILITY
Between	1	0.11	0.11	3.79	$.05 > P > .10$
Within	20	0.58	0.03		

TABLE XIII. Statistics applied to fall-time during the anesthesia induction period.

FALL-TIME	ROLL-OVER (in minutes)	
	C57BL	BALB/C
Range	1.2-1.7	1.1-2.1
Mean	1.4	1.5
Standard Deviation	0.13	0.31
Alpha Error	$.05 \geq P \geq .10$	

TABLE XIV. Analysis of Variance Table, applied to anesthetic concentration extracted at the end of the anesthesia maintenance period.

BLOOD ASSAY

Variance Source	DF	SS	MS	F	PROBABILITY
Between	1	0.50	0.50	2.20	.10 > P > .25
Within	20	4.54	0.23		

BRAIN ASSAY

Variance Source	DF	SS	MS	F	PROBABILITY
Between	1	4.12	4.12	3.27	.05 > P > .10
Within	20	25.19	1.26		

TABLE XV. Statistics applied to anesthetic concentration extracted at the end of the anesthesia maintenance period.

ANESTHETIC CONCENTRATION	BLOOD ASSAY (mg./1.0 ml.)		BRAIN ASSAY (mg./1.0 g.)	
	C57BL	BALB/C	C57BL	BALB/C
Range	1.13-2.50	0.81-2.56	0.38-0.72	0.27-0.62
Mean	1.65	1.35	0.53	0.44
Standard Deviation	0.44	0.50	0.10	0.12
Alpha Error	.10 ≥ P ≥ .25		.05 ≥ P ≥ .10	

TABLE XVI. Animal weight, brain weight, and methoxyflurane levels extracted per whole brain.

	Animal weight (in grams)		Brain weight (in milligrams)		Anesthetic extracted (in milligrams)	
	C57BL	BALB/C	C57BL	BALB/C	C57BL	BALB/C
	25.0	23.0	.289	.333	.116	.180
	25.0	25.0	.288	.311	.133	.106
	23.0	24.0	.200	.277	.142	.099
	23.0	26.0	.227	.316	.086	.085
	25.0	27.0	.236	.262	.144	.162
	24.0	25.0	.228	.276	.121	.152
	24.0	24.0	.239	.267	.136	.139
	25.0	24.0	.241	.221	.127	.126
	25.0	24.0	.330	.367	.149	.103
	25.0	24.0	.287	.327	.170	.134
	25.0	24.0	.304	.379	.167	.144
Mean	24.0	25.0	.261	.303	.136	.130

CHAPTER V

DISCUSSION

The results of this study support the findings of Kakihana et al., 1966, and Randall and Lester (1974), that genotype is an important determinant of response to C.N.S. depressants in mice. The findings of the present study, that C57BL and BALB/C strains awaken at different times, with similar brain and blood anesthetic concentrations, can be explained by two concepts. The first concept deals with physiological differences such as respiration, circulation, neurochemical substances, and metabolism. These factors have general effects on the anesthetic process but are separate from the local actions of anesthetics on the central nervous system. The second concept deals specifically with brain affinity and sensitivity.

Respiration and blood flow are two parameters which affect the rate of elimination of gaseous anesthetics. Any naturally occuring (at rest) differences in respiration and heart rates, between the two strains, could account for the results observed. Respiration was measured with one pair of mice. The C57BL mouse was observed to breath slightly faster (185 breaths/min.) than the BALB/C mouse (160 breaths/min.). Blizard and Welty (1971) have previously found that C57BL's have faster heart rates than the BALB/C strain ($P < .01$). These observations are contrary

to the findings of the present study. It is acknowledged though, that no measurements were made of blood or respiratory volumes. There is no direct evidence that methoxyflurane affected circulation, respiration, or heart rate differently between the two strains. However, one could speculate that the sleep duration differences between C57BL and BALB/C mice may be attributed to such factors, either local or general (Altura, 1971).

Serotonin, a brain biogenic amine (neurochemical) associated with sleep (Mitler et al., 1973) has been found (in brain stem sections) in lesser quantities in C57BL/10J than BALB/C mice (Maas et al., 1962; Sudak and Maas, 1964). The actions of serotonin are not known, but these concentration differences may directly or indirectly be responsible for the results of the present study.

A known metabolite of methoxyflurane is fluoride (Holaday et al., 1970). Toxic doses of fluoride cause brain excitability just prior to respiratory paralysis (Arena, 1970). Studies with rats have shown that only 1-2% of inhaled methoxyflurane is metabolized in 48 hours (Van Dyke et al., 1964). Although the possibility of metabolic differences between the strains can not be ignored, it should be stressed that brain and respiratory effects of fluoride are seen with toxic doses. In view that reversable anesthetic levels of methoxyflurane were used, and that the length of time that the mice were subjected to methoxyflurane was short (35 min.), it does not seem possible that

increased fluoride levels could account for any differential affects.

If methoxyflurane acts nonspecifically, then it is expected that the strain that sleeps longer (C57BL) should possess less anesthetic in the brain. As noted earlier, no intra strain (blood or brain) methoxyflurane differences were observed at the time of righting reflex recovery, even though significant contrast in sleep duration were recorded. Thus, the difference in methoxyflurane levels is either not great enough to be significant or factors such as brain affinity or sensitivity may explain these results.

Such an affinity concept can be related to methoxyfluranes' lipid solubility. The potency of an anesthetic, with few exceptions, is related to its' lipid solubility (Meyer, 1899; Overton, 1901; Bennett, 1969). Methoxyflurane with its' high lipid solubility, is present in large amounts, in the nerve cell membrane, at the end of the anesthesia maintenance period. Thus methoxyflurane is available in large enough quantities to saturate the membrane binding sites. It seems reasonable to assume that species differences in membrane lipid and/or protein composition (membrane architecture) could cause significant variations in the binding affinity of methoxyflurane - holding the anesthetic for a longer time in C57BL than BALB/C mice. Anesthetics do bind to proteins and change their conformation (Wishia, 1962; Wetlaufer, and Lovrien, 1964; Wetlaufer and Balasubramnian, 1966). If protein is the main membrane unit disturbed by anesthetics, then differences in type and number of binding proteins,

could account for the sensitivity differences seen in this study. It should be noted though, that such a supposition could also be applied to any enzymatic theories of anesthesia (Quastel and Weatly, 1932; Negishi and Svaetichin, 1966; Quastel, 1965).

This lack of correlation between anesthetic concentration and sleep duration, may also be accounted for by affinity or sensitivity of anesthetics to C.N.S. cardiovascular or respiratory centers. With inhalation anesthetics the rates of change of anesthetic tension in brain tissue, needed to produce anesthesia, is affected by respiratory volume and cardiac output (Wollman and Smith, 1975). Methoxyflurane at fairly low concentrations, affects respiration and cardiac output (CHAPTER II). For this reason, C57BL and BALB/C mice might have different cardiopulmonary sensitivities to methoxyflurane. Such a phenomenon could significantly affect the strains rate of elimination of the anesthetic; causing different sleep durations with similar brain and blood anesthetic levels at wake time. Other than affinity, variations in the number of neurons or connections in the central nervous system, could also account for any differences in cardiopulmonary sensitivities.

Previous studies with alcohol (McClearn, 1962; Kakihana et al., 1966) have demonstrated that BALB/C mice are more sensitive to alcohol than C57BL mice. Randall and Lester (1974) found that C57BL/6J mice had greater sensitivity to pentobarbital than to alcohol, the reverse being true for the BALB/CJ mice. They concluded that the depressant affect of alcohol is thus

not generalizable and that their results support the notion that different brain sites are involved. The results of the present study, gives some indication that methoxyflurane, a volatile anesthetic, acts at similar brain sites as pentobarbital, a barbiturate anesthetic.

No fall-time (loss of righting reflex) differences were observed in the present study. It should be noted though, that the anesthetic was administered at a relatively high concentration (1.5%), which resulted in short fall-times ($\bar{X}$ - 1.45 min.), and that no brain anesthetic levels were taken at this time. A test comparing fall-time and anesthetic levels, by introducing the anesthetic at lower concentrations, seems important for future study.

Also indicated, is a study comparing C57BL and BALB/C's respiration and heart rates during anesthesia.

Continued study should include work with membrane composition. An initial step in this direction would be a study comparing C57BL and BALB/C's brain lipid and protein make-up.

Future research should also include an experiment of brain binding capacity. Such an experiment could by devised by: homogenizing brains from each strain in a dialysis bag, subject the brains to equal amounts of methoxyflurane, and placing the bags in a water bath. Comparisons could then be made of the quantity of methoxyflurane still bound in the brain, for each strain, for different lengths of time.

It is intended that these suggested experiments will give some insight as to the reasons for the results seen in the

present study. It is acknowledged though, that critical differences in nerve cell membrane structures, may be so subtle, that present technology is not available to measure significant differences in either brain anesthetic level, or membrane composition.

CHAPTER VI

SUMMARY AND CONCLUSION

Two groups consisting of 21 each, C57BL and BALB/C strains of mice, were anesthesized with methoxyflurane and oxygen. Two evaluations of sleep-time (pain-pressure and righting reflex) were compared with brain and blood anesthetic levels. The anesthetic levels were assayed on a gas chromatograph and were extracted at the beginning and the end of righting reflex recovery.

The results of this study can be summarized as follows:

1. Both strains of mice lost consciousness (fall-time) at the same time (experiment III).
2. No differences occured in blood and brain anesthetic concentrations, between strains, when measurements were made at the greatest depth of anesthesia achieved (experiment III).
3. C57BL mice slept longer than BALB/C mice, when either pain pressure, or righting reflex evaluations of recovery were used (experiment I and II).
4. Pain-pressure appears to be more sensitive than righting reflex in evaluations of anesthetic recovery (experiment I).
5. Both strains of mice awakened with equal blood and brain anesthetic concentrations (experiment I).

The findings in this study support that genotype is an important determinant of response to C.N.S. depressants in mice. C57BL and BALB/C strains have demonstrated different sensitivities to the volatile anesthetic, methoxyflurane. These

contrasting sensitivities may be due to differences in "methoxyflurane-membrane" affinities between the two strain.

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APPENDIX

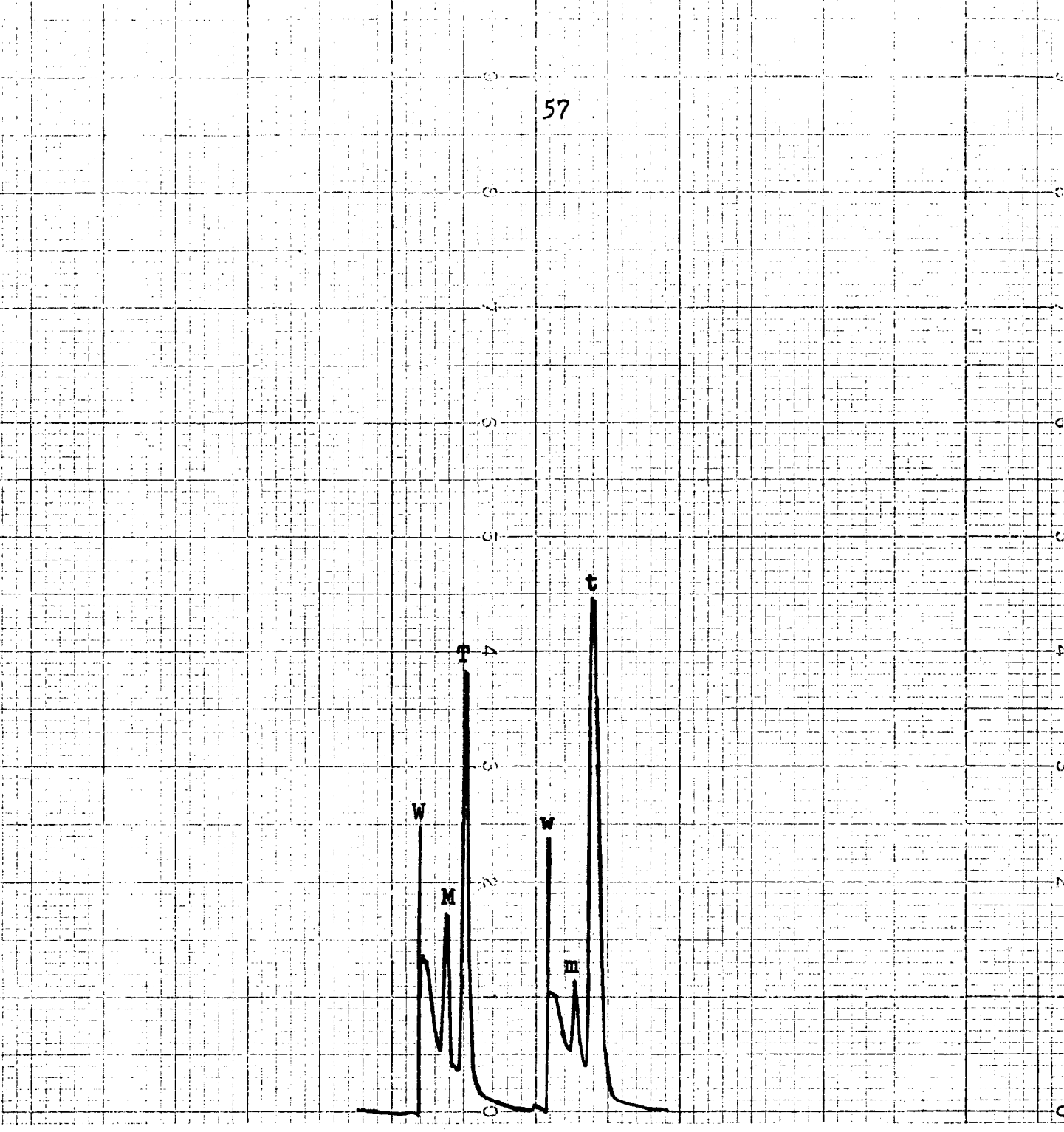

Figure I

Chromatogram of dose response solutions of methoxyflurane (M & m) in water (W & w), and column residue (toluene) (T & t) from brain calibrating solution. Methoxyflurane concentrations are 40 milligram percent (M) and 20 milligram percent (m). G.C. settings: column temperature 90° C., nitrogen flow setting 1.5, range 10, attenuation 16, paper speed 0.5 inch per minute. Sample size 0.5 microliter.

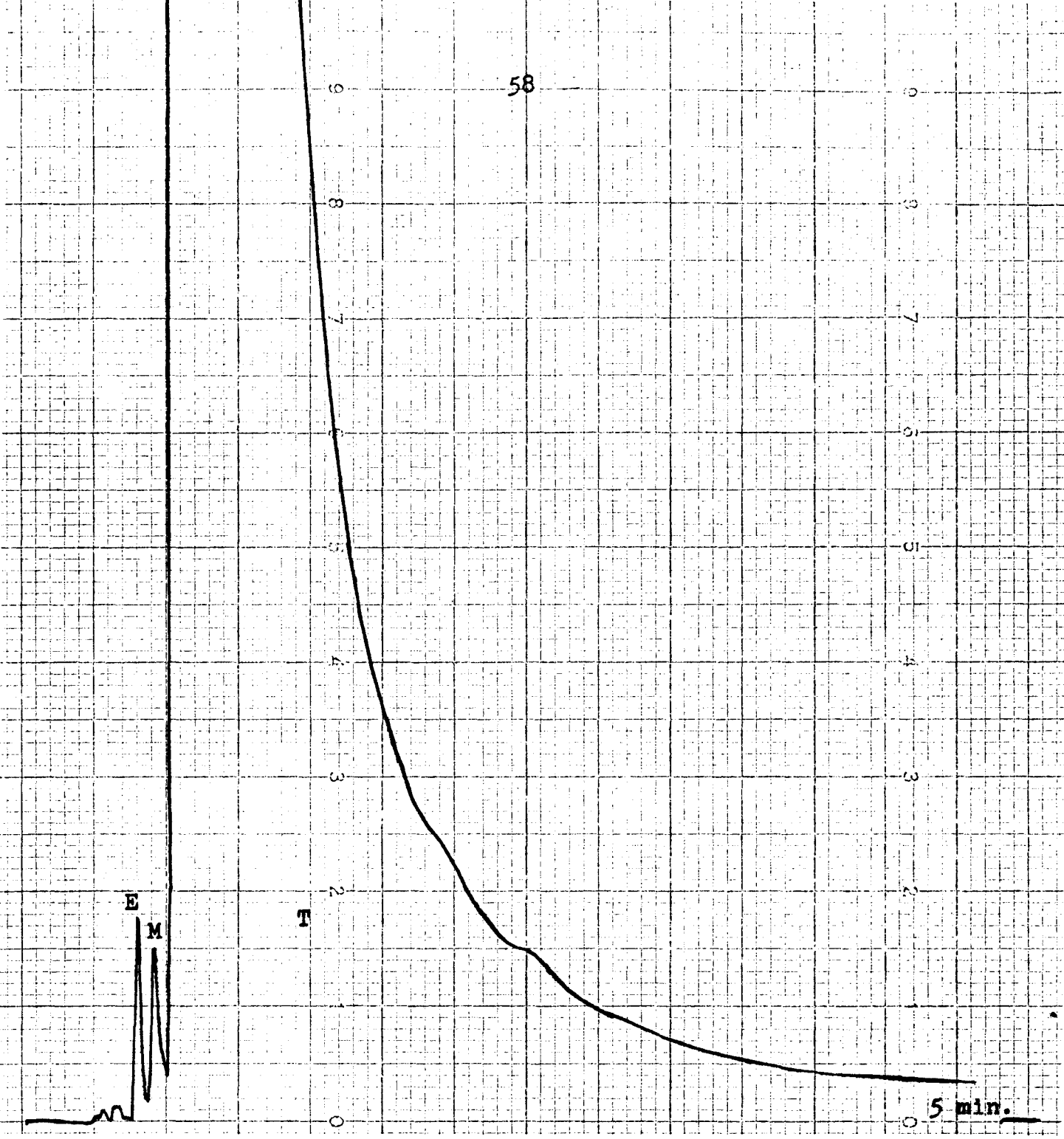

Figure II

Chromatogram of calibrating solution of methoxyflurane (M) and di-ethyl ether (E) (internal standard) in toluene(T). Methoxyflurane concentration equals 8.0 milligram percent. G.C. settings: column temperature 100° C., nitrogen flow setting 1.0, range 10, attenuation 16, paper speed 0.5 inch per minute. Sample size 0.5 microliter (approximately).

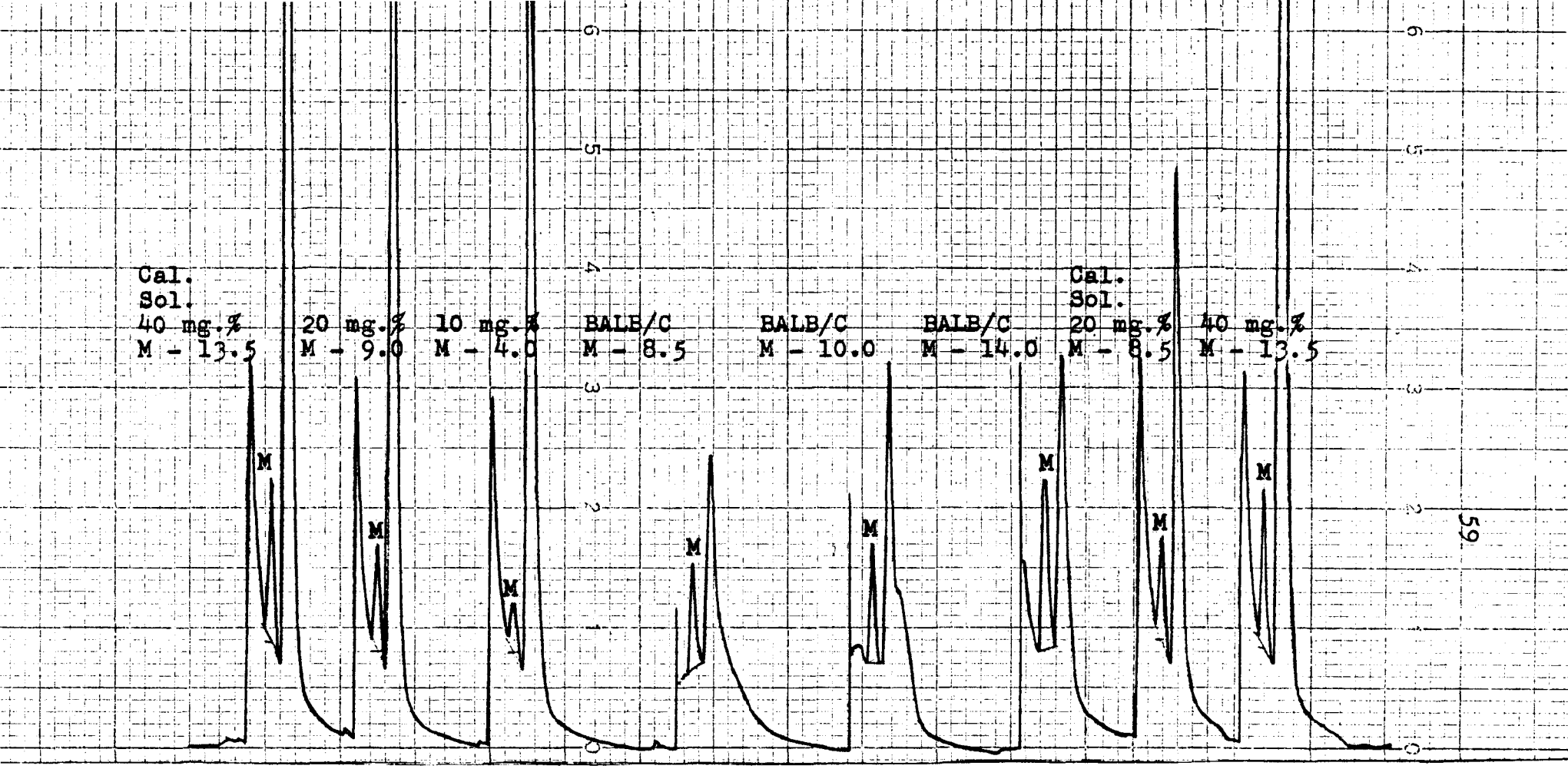

Figure III

Chromatogram of blood data from experiment I. Note the variances in the methoxyflurane (M) peak heights (8.5, 10.0, 14.0) between the injections. G.C. settings: column temperature 90° C., nitrogen flow setting 1.5, range 10, attenuation 16, paper speed 0.5 inch per minute. Sample size 0.5 microliter.

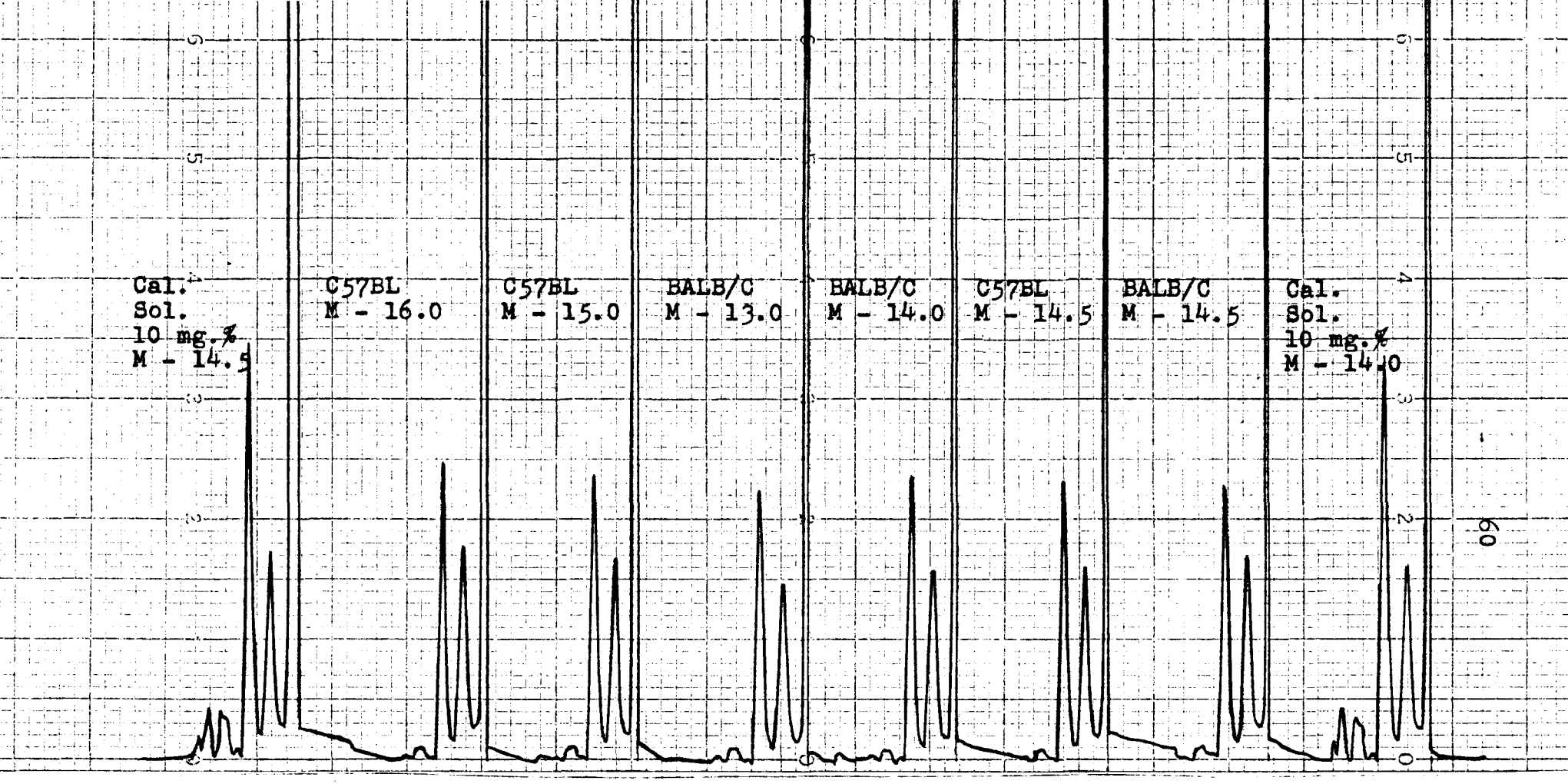

Figure IV

Chromatogram of blood data from experiment III. Note the uniformity in the methoxyflurane (M) peak heights between the injections of each strain. G.C. settings: column temperature 100° C., nitrogen flow setting 1.0, range 10, attenuation 16, paper speed 0.5 inch per minute. Sample size 0.5 microliter.

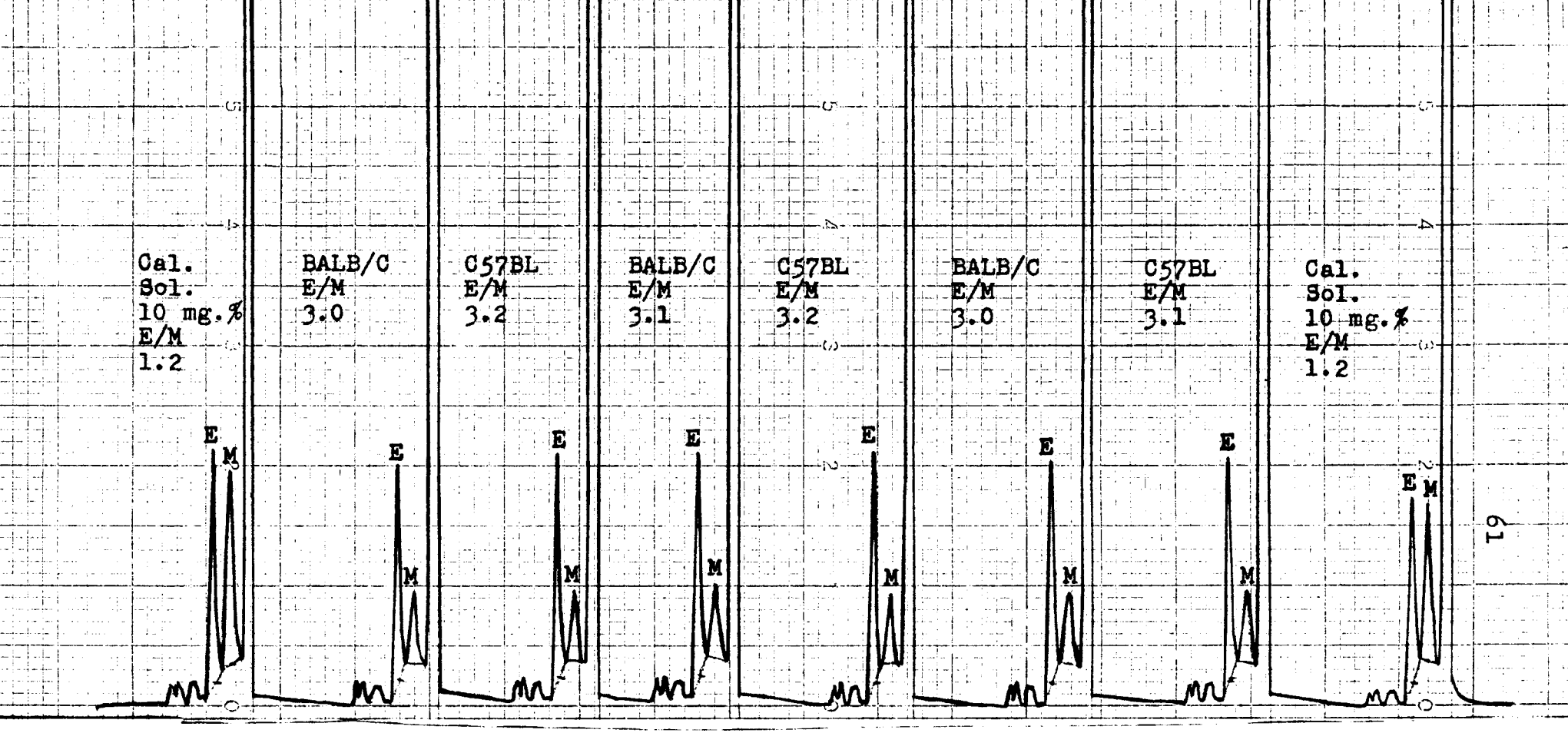

Figure V

Chromatogram representing brain anesthetic extractions of experiments I and III. Note the uniformity in the di-ethyl ether (E)/ methoxyflurane (M) ratio between the injections of each strain. G.C. settings: column temperature 100° C., nitrogen flow setting 1.0, range 10, attenuation 16, paper speed 0.5 inch per minute. Sample size 0.5 microliter (approx.).

Materials:

This study utilized the following materials:

42 male mice (10-13-week-old, 22-30 grams)
21 C57BL
21 BALB/C
Methoxyflurane (375 ml.)
Oxygen (8 E-tanks)
Eight 3-milliliter glass vials
Four 1-milliliter glass vials
One Erlenmeyer flask - 250 ml. (holding tank)
Three Erlenmeyer flasks - 500 ml.
Bell jar - 10.4 liters
2' x 2' by 3/4" plywood (base)
Three no. 7 & one no. 5 rubber stoppers
Seven glass tubes - 6" each
Plastic tubing 12'
Heparized capillary tubes (150)
Seal-ease clay sealer - 1 tray
Aluminum foil - 2 sq. feet
Four G.C. septums
Glass beads

Extraction solution:
Toluene - 200 ml.
Di-ethyl ether - 1.6 microliter (ul.)
Calibrating solution:
Brain (Exp. I & III)
Extracting solution - 25 ml.
Methoxyflurane - 1.75 ul., or 2.45 mg. (10 mg. percent)
Blood (Exp. I)
Distilled water 10ml., methoxyflurane 2.8 ul. (40 mg. %)
Distilled water 10ml., methoxyflurane 1.4 ul. (20 mg. %)
Blood (Exp. III)
Extracting solution - 25 ml.
Methoxyflurane - 1.75 ul. or 2.45 mg. (10 mg. %)

This study utilized the following equipment:

Oxygen liter-flow gas regulator
Vaporizer, Pentec I (1.5 % maximum output)
Disection kit
Centrifuge (3000 r.p.m.)
Vortex mixer
Mechanical mixer
One 1-microliter glass syringe (Hamilton Company)
Gas Chromatograph (Hewlett-Packard Series 5750B)
Gas Chromatograph columns (LABCO INC., Connecticut)
Electric timer
Micro-capillary centrifuge (I.E.C. model MB)

APPROVAL SHEET

The thesis submitted by Kurt Douglas Woss has been read and approved by the following Committee:

Dr. Priscilla C. Bourgault, Chairperson
Assistant Professor, Physiology/Pharmacology, Loyola Dental School

Dr. Silas N. Glisson
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Dr. Douglas C. Bowman
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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with references to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

March 4, 1977 Priscilla C. Bourgault

Date Director's Signature