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The Ohio State University, Ph.D., 1970
Pharmacology

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SOME ASPECTS OF THE PHARMACOLOGY
OF BROMOTRFUOROMETHANE

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

* * * * * *

The Ohio State University
1970

Approved by
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Studies in Veterinary Toxicology, Professor T. E. Powers
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CHAPTER I

INTRODUCTION

An interest has developed in this country within recent years in methods of fire suppression within closed and open environments in and around air- and spacecraft. Conditions of extreme flammability are encountered with conventional fuels of air-breathing propulsion systems and within closed life support systems using breathing mixtures containing high concentrations of oxygen. The tragic results of fires involving such systems have been recorded with such incidents as the Apollo fire of early 1967 which claimed the lives of three astronauts, the fire some weeks later in an altitude chamber at the School of Aerospace Medicine, Brooks Air Force Base, Texas, and numerous aircraft accidents.

Transporting large volumes of water for fire suppression in air- and spacecraft is not practicable. The necessity for developing easily transportable, lightweight, efficient, chemical fire extinguishing systems became evident. Bromotrifluoromethane (CBrF₃) was tentatively selected as the chemical fire suppressing agent because of its effectiveness in rapidly stopping the oxidative process.
at a concentration of 7% (v/v) in air and 50% (v/v) in 100% oxygen fed fires (Botteri & Manheim, 1969). Preliminary investigations of the toxicity of CBrF$_3$ in laboratory animals suggested that the material was of relatively low toxicity compared to other halogenated hydrocarbons such as carbon tetrachloride.

CBrF$_3$ is a liquid which boils at -59° C. at one atmosphere and is stored as a liquid under pressure. CBrF$_3$ is slightly soluble in aqueous and most nonaqueous media. It is very soluble in chloroform and certain other halogenated hydrocarbons.

In the event of a fire CBrF$_3$ would be released into the immediate vicinity and almost instantly would vaporize. Long term skin exposures to the gas have not been performed but its relative chemical inertness and insolubility suggest that such exposures would be of little toxicological significance. Inhalation exposure, on the other hand, would be very likely, particularly in closed life-support systems. The inhalation of even such a relatively insoluble compound could be of profound toxicological importance because of the very large surface to volume ratio of the blood in the pulmonary circuit.

With these facts in mind this study was undertaken to investigate some of the more important aspects of the
pharmacology of the inhalation exposure of monkeys and dogs to CBrF₃. The results were intended to provide some assistance in evaluating the toxicological hazard to personnel accidentally exposed to the material.
CHAPTER II

LITERATURE REVIEW

Levy (1911) reported the sudden death of cats exposed to chloroform, a chemical congener of bromotri-fluoromethane (Figure 1). The immediate cause of death was attributed to cardiac arrest during ventricular fibrillation. Later during the same year he reported that the administration of 0.016 to 0.065 mg of adrenalin chloride (epinephrine) to cats exposed to chloroform resulted in premature ventricular contractions and bigeminy which culminated in ventricular fibrillation and cardiac arrest (Levy, 1911-12). Further investigation of the phenomenon revealed that electrical stimulation of the sympathetic nerves to the heart (the electrodes were placed at the right stellate ganglion) produced an effect similar to the injection of epinephrine in the cat exposed to chloroform (Levy, 1912-13). Levy continued his investigations and later reported that results similar to those achieved with epinephrine could be produced by treating cats exposed to chloroform with epinine (4-(2-methylaminoethyl)pyrocatechol) or tyramine. Aortic constriction also produced premature ventricular contractions and bi- and trigeminal rhythms but ventricular fibrillation was not reported (Levy, 1913-14).
FIGURE 1. Bromotrifluoromethane and related compounds.
Cl - C - H
Cl

CHLOROFORM

F - C - C - Br
F

FLUOTHANE

F - 1301

F - 113
Cyclopropane (Figure 1) was introduced as a gas anesthetic by Lucas and Henderson (1929). Seevers et al. (1934) conducted the first careful study into the cardiac irregularities which accompanied cyclopropane anesthesia. Atrioventricular blocks and premature ventricular contractions were observed to occur spontaneously in dogs exposed to 39 to 72% cyclopropane in air (lower cyclopropane concentrations) and in oxygen (higher cyclopropane concentrations). Meek (1941) confirmed the earlier findings of Levy concerning chloroform-epinephrine cardiac arrhythmias and extended his observations to cyclopropane. Multifocal ventricular rhythms which often proceeded to ventricular fibrillation frequently were seen as was ventricular tachycardia, a phenomenon not described in the case of chloroform.

Dresel and his co-workers (Dresel et al., 1960; Dresel and Sutter, 1961; Dresel et al., 1963) reported that the minimal cardiac arrhythmia in dogs anesthetized with cyclopropane and treated with epinephrine was a coupled, usually bigeminal, rhythm. The arrhythmia was reported to be elicited only in the simultaneous presence of a sympathomimetic amine and a minimal arterial blood pressure. If the rate of epinephrine infusion were increased the blood pressure threshold was lowered; if the rate of infusion were decreased the pressure threshold was raised. In the presence of a constant pressor amine infusion raising the blood pressure elicited the minimal response (bigeminy) which
progressed into a multifocal ventricular arrhythmia with rising blood pressure. In no case was any arrhythmia observed converted into ventricular fibrillation by the raising of blood pressure alone.

The explosive hazards associated with the use of ether and cyclopropane led investigators to seek new and nonflammable anesthetic agents. During the 1950's the British Committee on Nonexplosive Anesthetics introduced halothane (Figure 1) which rapidly was accepted as a safe and effective anesthetic (Raventos, 1956). Halothane is a partially halogenated derivative of ethylene which is a highly volatile liquid. Like chloroform and cyclopropane halothane is said to "sensitize" the heart to ectopic pacemaker formation and ventricular fibrillation in the presence of catecholamines.

Sensitization of the heart to the formation of ectopic pacemakers and ventricular fibrillation during the administration of pressor amines is not an exclusive property of inhalation anesthetics but rather a property common to a large number of halogen-substituted and unsubstituted hydrocarbons. Chenoweth (1945) demonstrated that methane (the parent compound of bromotrifluoromethane), butane, hexane, petroleum ether, heptane, gasoline, benzene, and other hydrocarbons possess this property.

Garb and Chenoweth (1948) used chloroform and benzene to sensitize the heart and produced ventricular
fibrillation with epinephrine or norepinephrine. They found that pre-treatment with 3 mg/kg of dibenamine (a dose insufficient to produce alpha-adrenergic blockade and thus an epinephrine reversal phenomenon) effectively protected sensitized animals from ventricular fibrillation.

Krantz et al. (1948) reported that cyclic and non-cyclic hydrocarbons sensitized the heart to epinephrine-induced arrhythmias. A singular exception to the rule was ethylene which produced no cardiac sensitization. Further studies with ethylene (Burgison et al., 1955) revealed that tetrafluoro- and difluoroethylene, like the parent compound, were incapable of sensitizing the heart, whereas partial chlorination of the fluoroethylenes produced mixed halocarbons which did sensitize the heart.

Clayton (1967) has reviewed fluorocarbon toxicity. The LC$_{100}$ in 15 minutes for rats for CBrF$_3$ was reported as 83% with air as the diluent. Such a value only underscores the low toxicity of CBrF$_3$ in rats. Since the mixture of CBrF$_3$ and air contained only 3.6% oxygen hypoxia must have played an important role in the death of the experimental animals. Rats, mice and guinea pigs were exposed to 50% CBrF$_3$ for 2 hr/da for 15 days, and rats and dogs were exposed to 2.3% CBrF$_3$ for 6 hr/da for 18 weeks without signs of toxicity.

Dogs exposed to 5% and 10% CBrF$_3$ for 5 minutes and then given 8 µg/kg of epinephrine intravenously responded
in 21% and 54% of the trials respectively, with abnormal rhythms, presumably ventricular premature beats (Stopps, personal communication).

Exposures of three adult male humans to atmospheres containing 1, 3, 5, 7, and 10% CBrF$_3$ in air at one atmosphere for periods of 3.5 minutes were performed (Haskell Laboratories, personal communication). No cardiac abnormalities were observed in the electrocardiograms of any of the subjects during the tests. The subjects' equilibrium and reaction time were estimated from a force platform and simple visual stimuli. The results from a small number of experiments suggested that equilibrium was slightly disturbed and reaction time slowed with increasing CBrF$_3$ concentration.

Monkeys, rats, guinea pigs, and rabbits were exposed for 2 hours to air, 10%, 15%, and 20% CBrF$_3$ (Haskell Laboratories, personal communication). SGPT, SGOT, sulfobromothalein excretion, and alkaline phosphatase determinations were performed on the monkeys before and after exposure. No differences were found which would distinguish the treated from the untreated monkeys. At the termination of the experiments gross necropsies were performed and a large selection of tissues were examined microscopically. Again no observations were made which would distinguish the treated from the untreated animals.
CHAPTER III

SHORT-TERM INHALATION EXPOSURE
TO BROMOTRIFLUOROMETHANE

Methods

Twelve beagle dogs (5 males, 7 females) 18-30 months of age which weighed 7.8 - 12.4 kg, 4 young adult female monkeys (Macaca mulatta) which weighed 4.6 - 6.1 kg, and 2 female baboons (Papio sp.) which weighed 10.0 and 12.5 kg were exposed to 10, 20, 30, 40, 50, 60, 70, or 80% bromotrifluoromethane (CBrF₃) in oxygen.

CBrF₃ and oxygen were introduced into large polyethylene bags (capacity: 300-400 liters) from steel cylinders under pressure. The gases were mixed in the required proportions by volume with the use of calibrated flowmeters, and no further effort was made to analyze the composition of the mixtures. Animals inhaled the gaseous mixtures from the bags and exhaled to the outside atmosphere by means of a one-way Fink valve.

Electrocardiograms (lead II) and arterial blood pressure recordings were made using a pressure transducer (8685, P234A, 0-75 mm Hg, Statham, Hato Rey, Puerto Rico) and direct writing oscillograph (Grass Model 5D Polygraph,
Grass Instrument Co., Quincy, Massachusetts). Cardiac output was determined by the dye-dilution technique using indocyanine green (Cardio Green, Hynson, Westcott & Dunning, Inc., Baltimore, Maryland) and a recording densitometer (Model 103IR Cuvette Densitometer, Gilford Instrument Laboratories, Inc., Oberlin, Ohio; Rectiriter, Rectilinear Recording Milliammeter, Texas Instruments, Inc., Houston, Texas). Arterial blood sampling and pressure reading were performed through a polyethylene catheter inserted through a femoral cutdown and advanced to the anterior abdominal level. Intravenous injections were performed through an 18-cm polyethylene catheter introduced through a cephalic vein. Arterial blood was withdrawn through the densitometer cuvette using a withdrawal pump (Infusion/Withdrawal Pump, Model 940, Harvard Apparatus Co., Inc., Dover, Massachusetts) at a rate of 24.7 ml/min.

Sodium pentobarbital (4% solution) was used in the experiments which required general anesthesia. Endotracheal catheters were inserted in anesthetized animals. For the experiments in which conscious animals were used, tracheotomies were performed and cannulas were inserted. This procedure obviated the necessity for fitting and training the animals with masks.

Conscious monkeys were restrained in a chair device (Lab-Care Division, Research Equipment Co., Bryan, Texas).
Dogs were restrained in a ventilated box of our own design in the normal ventrum-down posture of a reclining dog.

Five of the 12 dogs and all 4 monkeys were exposed to CBrF₃ while conscious. All the animals used were exposed to CBrF₃ while anesthetized. A majority of the exposures were to concentrations greater than 40% CBrF₃. At least 2 exposures at each concentration from 10 to 80% were performed on at least 5 dogs and 3 monkeys in both the conscious and anesthetized states. The baboons were exposed only to 50-80% CBrF₃. Because of the large number of exposures, particularly at the higher concentrations, it is not practical to tabulate all the exposure sequences. Table 1 illustrates the sequence of exposures of anesthetized dogs to 50% CBrF₃. Table 2 illustrates the sequence of exposures of conscious dogs to concentrations of CBrF₃ from 10 to 80%. Similar tables of values could be assembled representing experiments conducted on anesthetized dogs exposed to concentrations of CBrF₃ other than 50% and experiments conducted on monkeys.

Bromotrifluoromethane from two sources (Freon 1301, E. I. du Pont de Nemours & Co., Wilmington, Delaware Halon, Dow Chemical Co., Midland, Michigan) was used in this study. The respective supplies were analyzed for purity. Gas chromatography was used to separate the components, and infrared and mass spectrometry were used for the identification of separated materials. CBrF₃ from the second source
TABLE 1
EXAMPLES OF SEQUENCE OF EXPOSURES OF ANESTHETIZED DOGS TO 50% CBrF₃

<table>
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<td>6</td>
<td>2</td>
<td>7</td>
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<tr>
<td>C88</td>
<td>30</td>
<td>32</td>
<td>31</td>
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<td></td>
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<tr>
<td>B71</td>
<td>31</td>
<td>28</td>
<td>30</td>
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<td>L703</td>
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<td>L27</td>
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ᵃBreathing CBrF₃ in oxygen.
ᵇBreathing air.
ᶜNumbers in body of table represent minutes.
### TABLE 2

**EXAMPLES OF SEQUENCE OF EXPOSURES OF CONSCIOUS DOGS TO CBrF₃**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Cᵃ</th>
<th>Aᵇ</th>
<th>C</th>
<th>A</th>
<th>C</th>
<th>A</th>
<th>C</th>
<th>A</th>
<th>C</th>
<th>A</th>
<th>C</th>
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<tr>
<td>B85</td>
<td>3(80)ᶜ</td>
<td>78</td>
<td>5(70)</td>
<td>42</td>
<td>4(60)</td>
<td>98</td>
<td>8(50)</td>
<td>24 hr</td>
<td>21(40)</td>
<td>33</td>
<td>2.5(80)</td>
</tr>
<tr>
<td>B81</td>
<td>85(20)</td>
<td>60</td>
<td>21(30)</td>
<td>42</td>
<td>19(40)</td>
<td>24 hr</td>
<td>40(50)</td>
<td>56</td>
<td>8(60)</td>
<td>41</td>
<td>2.4(60)</td>
</tr>
<tr>
<td>C74</td>
<td>1.5(80)</td>
<td>45</td>
<td>6(80)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>B93</td>
<td>45(10)</td>
<td>84</td>
<td>60(20)</td>
<td>37</td>
<td>33(30)</td>
<td>71</td>
<td>24(40)</td>
<td>24 hr</td>
<td>53(50)</td>
<td>117</td>
<td>12(60)</td>
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<tr>
<td>B93 (cont.)</td>
<td>--</td>
<td>4</td>
<td>7(60)</td>
<td>62</td>
<td>7(50)</td>
<td>48</td>
<td>11(70)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

ᵃBreathing CBrF₃ in oxygen.

ᵇBreathing air.

ᶜNumbers in body of table represent minutes except when designated otherwise; numbers in parentheses represent concentration of CBrF₃.
contained about 4% fluoroform (CHF₃) and 1% CO₂ as contaminants. Other contaminants were present in trace amounts. The animal responses to inhalation of the CBrF₃ from the different sources were indistinguishable.

Results

Two dogs under pentobarbital anesthesia were exposed to an 80% mixture of CBrF₃ in O₂ for 35 and 40 minutes, respectively. The intravenous injection of 10 μg/kg of epinephrine resulted in ventricular fibrillation and cardiac arrest in both animals (Figure 2). A definite pressor response preceded by a few seconds the precipitous blood pressure drop associated with ventricular fibrillation. As a control, the dogs had received similar injections 24 hours earlier while breathing air which produced only the expected marked pressor response and resultant reflex vagal inhibition of heart rate.

The general response of the dog to nonlethal exposures of CBrF₃ consisted of a cardiovascular effect and a central nervous system effect. All the central nervous manifestations of CBrF₃ toxicity which were observed in the conscious dog were eliminated by the induction of Stage III Plane I pentobarbital anesthesia. At least some alteration of cardiovascular function was seen in all dogs in all experiments whether conscious or anesthetized when the dogs were exposed to 20-30% or greater CBrF₃ levels.
FIGURE 2. Electrocardiogram (lead II) and central arterial blood pressure recordings from 2 dogs exposed to bromotrifluoromethane immediately before and during the intravenous injection of 10 µg/kg of epinephrine which show the onset of ventricular fibrillation and subsequent cardiac arrest.
Dog C68 Breathing 80% CF₆₃
40 min, immediately prior to
epinephrine inj; Chart speed: 25 mm/sec

End of IV injection of 10 µg/kg
epinephrine

Dog C72 Breathing 80% CF₆₃ (+36 min)
Chart speed: 25 mm/sec

End of 10 µg/kg IV epinephrine inj
Electrocardiograms (from which heart rate was calculated) were obtained on all dogs. Arterial blood pressure and cardiac output were determined on anesthetized dogs only.

The heart rate was irregularly increased by 10-15% from resting rates of 67-100 beats/minute during the first several seconds of breathing 20-30% CBrF₃. The increase in heart rate was definite and regularly reproducible when the dogs were exposed to 40% or higher CBrF₃ concentrations. When arrhythmias appeared, which usually began at 15-120 seconds after starting the exposure to CBrF₃, a simple tachycardia became impossible to distinguish.

An increase in heart rate accompanied by some degree of hypotension was studied most extensively while the dogs were breathing the 50% or 80% mixture. The blood pressure fall varied from 20 to 60 mm Hg. The pulse pressure was decreased by 0-30 mm Hg from an average normal of 40-55 mm Hg.

The changes in heart rate, blood pressure, and pulse pressure reversed when an animal exposed to CBrF₃ was switched to room air. Recovery required approximately twice as long as development.

The most spectacular phenomenon observed was the bizarre alteration of the electrocardiogram (Figure 3). In some experiments as early as 15 seconds after beginning
FIGURE 3. Typical arrhythmias (ECG lead II) exhibited by nonanesthetized dog exposed to bromotrifluoromethane.
Dog B81 Exposed to 60% CBrF₃ for 3 Min
Period 105 sec after generalized tonic convulsion
Chart speed: 100 mm/sec

Dog B81 exposed to 40% CBrF₃ at respiratory rate +50/min for 12 min
Chart speed: 100 mm/min
CBrF₃ exposure, abnormalities began to appear which consisted of T-wave alterations, unifocal and multifocal ventricular arrhythmias, and bi- and trigeminy. In most experiments these ECG alterations appeared during the first minute of exposure to CBrF₃ and persisted until 2-4 minutes postexposure. The presence of these arrhythmias variously affected the central arterial pulse pressure. Some arrhythmias interfered with the dynamic efficiency of the heart and resulted in periodic rapid blood pressure fluctuations.

Some dogs apparently were less susceptible to the arrhythmic effects of CBrF₃ (cf. Chapter VI). Sometimes they developed the arrhythmias only at the higher concentrations or not at all. No particular consistency of this response could be ascertained. The typical arrhythmic response could, however, be elicited with the intravenous injection of 2-3 µg/kg epinephrine. The arrhythmia was always preceded by a somewhat diminished pressor response to the epinephrine and disappeared within a few seconds, depending on the amount of epinephrine given and the rate of injection.

Figure 4 illustrates the results of a typical experiment in which an anesthetized dog was allowed to respire a 50% mixture of CBrF₃. The most striking response to the CBrF₃ was the rapidly changing blood pressure. The
FIGURE 4. Results of an experiment in which a dog under pentobarbital anesthesia was exposed to 50% bromotrifluoromethane intermittently. During these periods hypotension was seen which was reversed immediately upon removal of the CBrF₃.
heart rate changed irregularly with cardiac output generally varying directly with heart rate. Peripheral vascular resistance was lowered during exposure to CBrF$_3$ (cf. Chapters IV, V). Determinations of cardiac output and calculation of total peripheral vascular resistance during the CBrF$_3$ exposures were performed after 2-3 minutes of exposure and at the end of 6-minute exposures. Exposure to 50% CBrF$_3$ did not affect pulse pressure during the relatively short periods of these experiments. Longer exposures produced a gradual decrease in pulse pressure over a period of 25-30 minutes. Pulse pressure decreased more rapidly when the gas mixture contained 80% CBrF$_3$.

Four of the 9 conscious dogs exposed to CBrF$_3$ had epileptiform convulsions of 10-30 second duration. The convulsions were characterized by generalized rigidity, apnea, and cyanosis of the tongue. The only apparent residual effects were an elevation in body temperature and fatigue which was associated with the muscular effort of the convulsion. Convulsions were precipitated with 2-5 successive exposures within a period of an hour. When exposed to 80% CBrF$_3$ the onset of the convulsions took place within 3-4 minutes. The length of exposure which was required to precipitate the convulsions was greater at lower concentrations of CBrF$_3$. Convulsions appeared after 12 minute exposure to CBrF$_3$, the lowest concentration which caused
convulsions after any length of exposure under 40 minutes. The dogs that did not develop convulsions showed the same general signs as those that convulsed. Dogs exposed to 20% or greater concentrations of CBrF₃ became visibly agitated within 1-2 minutes. The severity of the agitation increased with the concentrations of CBrF₃. The dogs looked about the room apprehensively. Within 1-3 minutes generalized muscular tremors (shivering) could be distinguished. Episodes of shivering lasted a few seconds and recurred every 5-20 seconds. General anesthesia induced by pentobarbital or thiamylal blocked all central nervous system signs.

The responses of the monkeys and baboons to CBrF₃ were very similar.

Two monkeys and both baboons were exposed to 80% CBrF₃ under pentobarbital anesthesia for 10 minutes or more. The intravenous injection of 10 μg/kg of epinephrine produced little significant alteration of the ECG (Figure 5). Although brief episodes of ventricular fibrillation were observed, no deaths occurred.

The general responses of the primates to CBrF₃ bore many similarities to those of the dog. The cardiovascular response was similar to that of the dog except that cardiac arrest could not be induced with large doses of epinephrine during exposure to the compound. The central nervous response was markedly different from that of the dog.
FIGURE 5. Monkey experiment similar to that represented in Figure 2. Unlike that of the dog the monkey heart did not fibrillate irreversibly when treated with bromotrifluoromethane and epinephrine.
Inj. 10 mg/kg epinephrine imedia te ly pre-injection

Chart speed: 50 mm/sec

Immediately post-injection

+30 sec

+1 min

+6 min

Monkey C67 Breathing 90% O2 F12

0 = Mg
The primates appeared to be more sensitive than the dog to the spontaneous formation of arrhythmias. All monkeys developed the same spectrum of ECG anomalies described for the dog. Arrhythmias beginning within 5-40 seconds of exposure were uniformly seen during exposures to 20-80% CBrF₃ and were not affected by the induction of general anesthesia with pentobarbital or thiamylal. Epinephrine (3-4 μg/kg) elicited about a one-half normal pressor response in monkeys exposed to 80% CBrF₃. Monkeys which had been exposed to 80% CBrF₃ earlier had normal pressor responses when returned to air.

Monkeys exposed to 80% CBrF₃ did not show a pressor response to electrical stimulation of the femoral nerve (5 Hz, 10 V, 2.5 msec). This stimulation was sufficient to produce a 20 mm Hg rise in blood pressure in the same monkey when breathing air. Heart blocks were observed in 2 monkeys exposed to 80% CBrF₃ (Figure 6). Electrical stimulation of the femoral nerve as described produced an alteration of the bizarre arrhythmia to incomplete or complete heart block. These episodes lasted 2-3 minutes; the ECG then would revert spontaneously to the bizarre form dominated by ectopic beats. This alternation of types of arrhythmia also occurred spontaneously on a few occasions.

In a few trials it was possible to determine changes in heart rate before the onset of the typical severe arrhythmias when monkeys were exposed to 20% - 80% CBrF₃.
FIGURE 6. Incomplete and complete heart block developed in a monkey exposed to 80% bromotrifluoromethane.
A 10-15% increase in heart rate was observed during the first minute of CBrF₃ exposure. When ventricular premature beats dominated, heart rate was difficult to determine accurately.

All 4 monkeys which were exposed to CBrF₃ while conscious exhibited signs of cortical depression in contrast to the agitation exhibited by dogs. Shivering was observed in monkeys exposed to CBrF₃. This was accompanied by tranquilization of the normally aggressive behavior of macaques. Their eyelids remained half-closed and they refused to bare the teeth or bite. They remained conscious, however, and showed enough interest in orange juice to swallow a few drops when offered.

Discussion

Over 50 years ago Levy (1911-1912) demonstrated that exposure to chloroform sensitized the heart to the arrhythmic effects of exogenous epinephrine and to sympathetic stimulation. A large number of hydrocarbons and halogenated hydrocarbons have been shown since to possess similar pharmacologic properties.

Meek (1941) cited evidence that cyclopropane increased cardiac output at low concentrations and decreased cardiac output at high concentrations. Cyclopropane caused an increase in dog heart rates, but the rhesus monkey, like man, reacted with a slowing of the heart which became
greater as the concentration of cyclopropane was increased. Cyclopropane and chloroform produced cardiac arrhythmias in man and dogs. Cyclopropane arrhythmias, in particular, were dominated by nonsinus rhythms, extrasystoles, and in some cases, ventricular fibrillation. The injection of 10 µg/kg of epinephrine during cyclopropane or chloroform anesthesia produced arrhythmias terminating in ventricular fibrillation and cardiac arrest. The course of development of the arrhythmias and the relationships between the anesthetic agents and epinephrine bore striking similarity to $\text{CBrF}_3$-epinephrine-induced arrhythmias.

Similar findings were published (Chenoweth, 1946; Garb and Chenoweth, 1948) which indicated that methane, butane, heptane, hexane, benzene, xylene, toluene, petroleum ether, gasoline, ethane, propane, and other hydrocarbons also possessed the pharmacologic property of sensitizing the heart to the effects of epinephrine and related synthetic amines. A singular exception in this series of compounds was ethylene, which did not sensitize the heart.

Krantz et al. (1948) reported that convulsive seizures accompanied the syndrome induced in the dog by $\text{cis-trans}$ butene-2 [sic] but that these were apparently unrelated to the arrhythmias since other compounds produced the myocardial sensitization without eliciting convulsions. The fact that excitation and convulsions accompanied the
cardiovascular effects of CBrF₃ on the dog, whereas the monkey became lethargic under similar conditions, supports a similar conclusion about bromotrifluoromethane.

Burgison et al. (1955) reported on the relationship of fluorination to the anesthetic property of hydrocarbons. Generally, progressive fluorination resulted in a reduction of anesthetic activity. Also, fluorination of ethylene did not alter its characteristic lack of ability to sensitize the myocardium. Partially chlorinated fluoroethylenes did possess this property, however. Bromotrifluoromethane falls into this general category, being partially fluorinated and partially brominated. It possesses no anesthetic property but does sensitize the myocardium.
CHAPTER IV

HYPOTENSION DURING BROMOTRIFLUOROMETHANE EXPOSURE

Methods

Eight beagle dogs of both sexes from 18 to 30 months of age weighing from 7.8 to 12.3 kg were used. The dogs were anesthetized by a single intravenous injection of 30 mg/kg of sodium pentobarbital. Endotracheal catheters were inserted and femoral venous and arterial cutdowns performed. Stage III, plane 1 anesthesia was maintained by the slow intravenous drip of a mixture of sodium pentobarbital (2500 mg/liter) and tubocurarine hydrochloride (60 mg/liter). The animals were ventilated mechanically at a rate of 150 ml/min/kg. The respiratory dead space of the mechanical system was 30 ml which was added to the required tidal volume.

Electrocardiograms were obtained from which heart rate was calculated. Arterial blood pressure was determined with the use of a pressure transducer (Statham). Cardiac output was determined by the indicator-dilution technic using indocyanine green (Guyton, 1966).

Total peripheral resistance (TPR) was calculated by dividing the mean blood pressure drop ($\Delta P$) across the
peripheral vascular bed by the cardiac output in ml/sec (Burton, 1965). Mean blood pressure was estimated from the central arterial blood pressure recordings according to the formula:

\[
\frac{(\text{Systolic pressure}) + (2 \times \text{diastolic pressure})}{3}
\]

Venous pressure was assumed to be 5 mm Hg and thus \( \Delta P \) was estimated to be equal to the mean arterial pressure minus 5 mm Hg. Since the calculation of TPR varies inversely with cardiac output values for TPR were multiplied by body weight to provide values for TPR-kg which afforded a basis for comparisons among different subjects.

Figure 7 illustrates the design of the experiment. The 10 dogs were divided into two groups of five. Five determinations of cardiac output were performed at 10 minute intervals during each period. The treatments of the series of "treated" dogs differed from the series of "untreated" dogs only during the second period during exposure to 70% CBrF3.

Comparisons of the mean values for mean arterial blood pressure, cardiac output, total peripheral resistance, heart rate, and stroke volume between the treated and untreated groups were made using a 2-way analysis of variance with interaction (Freund et al., 1960).
FIGURE 7. Design of experiment to determine if exposure to CBrF$_3$ alters peripheral vascular resistance. Five determinations of cardiac output were performed on each dog at 10-minute intervals during 3 consecutive exposure periods and the means for each period reported.
<table>
<thead>
<tr>
<th></th>
<th>PERIOD 1</th>
<th>PERIOD 2</th>
<th>PERIOD 3</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0-40 MIN</td>
<td>40-90 MIN</td>
<td>90-140 MIN</td>
</tr>
<tr>
<td>TREATED</td>
<td>AIR</td>
<td>70% CBrF₃</td>
<td>AIR</td>
</tr>
<tr>
<td>UNTREATED</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
</tr>
</tbody>
</table>

5 DOGS

5 DETERMINATIONS PER DOG PER PERIOD
Seven dogs from 6.5 - 9.0 kg and five monkeys from 2.0 - 5.5 kg were prepared for the measurement of left intraventricular pressure. They were anesthetized with sodium pentobarbital, intubated, and placed on a mechanical respirator. The heart was exposed by making a left parasternal incision through the costal cartilages from T2 to the xiphoid process. The pericardium was incised and a plastic catheter placed in the left ventricle through the left ventricular myocardium. The catheter was connected to a pressure transducer and the recordings made on a direct writing oscillograph.

Results

The effect of exposure to 70% CBrF₃ on mean arterial blood pressure is illustrated in Figure 8. The difference attributable to period was significant at the 5% level. The difference attributable to treatment was significant at the 1% level (Table 3).

The effect of exposure to 70% CBrF₃ on cardiac output is illustrated in Figure 9. The difference attributable to period and treatment were significant at the 1% level. The difference attributable to interaction was significant at the 5% level (Table 5).

The effect of exposure to 70% CBrF₃ on heart rate is illustrated in Figure 11. The difference attributable to treatment was significant at the 5% level (Table 6).
FIGURE 8. The effect of exposure to 70% CBrF₃ on mean arterial blood pressure. A difference attributable to period was significant at the 5% level. A difference attributable to treatment was significant at the 1% level.
MEAN BLOOD PRESSURE (mm hg)

UNTREATED

TREATED

PERIOD

105
110
115
120
125
130
<table>
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<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Significance</th>
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<td>1,774,873.63</td>
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<tr>
<td>Period</td>
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<td>3,242.82</td>
<td>1,621.41</td>
<td>4.794</td>
<td>5%</td>
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<tr>
<td>Treatment</td>
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<td>2,577.62</td>
<td>2,577.62</td>
<td>7.622</td>
<td>1%</td>
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<td>1,611.34</td>
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<td>338.19</td>
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FIGURE 9. The effect of exposure to 70% CBrF₃ on cardiac output. A difference attributable to period was significant at the 5% level.
### TABLE 4

**ANALYSIS OF VARIANCE OF THE EFFECT OF BREATHING 70% CBrF₃ ON CARDIAC OUTPUT**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Significance</th>
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<td>1.633382</td>
<td>0.81696100</td>
<td>3.401</td>
<td>5%</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.024568</td>
<td>0.02456800</td>
<td>0.102</td>
<td>NS</td>
</tr>
<tr>
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<td>0.32634900</td>
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<td>0.24013466</td>
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FIGURE 10. The effect of exposure to 70% CBrF₃ on peripheral resistance. Differences attributable to period and treatment were significant at the 1% level. A difference attributable to interaction was significant at the 5% level. The interaction effect was not interpreted as having biological significance since it was a mathematical result of the crossing of the respective curves.
TOTAL PERIPHERAL RESISTANCE

UNTREATED

TREATED

PERIOD

1

2

3
<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
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<th>Significance</th>
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<tr>
<td>Mean</td>
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<td></td>
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<td>Period</td>
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<td>1,787.15</td>
<td>893.5750</td>
<td>10.180</td>
<td>1%</td>
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<td>Treatment</td>
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<td>1,035.55</td>
<td>1,035.55</td>
<td>11.797</td>
<td>1%</td>
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<tr>
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<td>2</td>
<td>800.535</td>
<td>400.2675</td>
<td>4.560</td>
<td>5%</td>
</tr>
<tr>
<td>Error</td>
<td>114</td>
<td>10,006.755</td>
<td>87.7786</td>
<td></td>
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</tbody>
</table>
FIGURE 11. The effect of exposure to 70% CBrF$_3$ on heart rate. A difference attributable to treatment was significant at the 5% level.
## TABLE 6

ANALYSIS OF VARIANCE OF THE EFFECT OF BREATHING 70% CBrF$_3$
ON HEART RATE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Significance</th>
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<td>2,642,113.63</td>
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<tr>
<td>Period</td>
<td>2</td>
<td>511.12</td>
<td>255.56</td>
<td>0.642</td>
<td>NS</td>
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<tr>
<td>Treatment</td>
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<td>2,077.97</td>
<td>2,077.97</td>
<td>5.216</td>
<td>5%</td>
</tr>
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<td>Interaction</td>
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<td>2,325.09</td>
<td>1,162.5450</td>
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<tr>
<td>Error</td>
<td>114</td>
<td>45,412.17</td>
<td>398.3523</td>
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</tr>
</tbody>
</table>

NS = Not Significant
The effect of exposure to 70% CBrF₃ on stroke volume is illustrated in Figure 12. The differences attributable to period and interaction were significant at the 1% level. The difference attributable to treatment was significant at the 5% level (Table 7).

Figure 13 illustrates a progressive rise in left ventricular and diastolic pressure (bottom series of recordings) during exposure to 80% CBrF₃. The end diastolic pressure returned to pre-exposure values postexposure. As the end diastolic pressure rose the left ventricular systolic pressure (top series of recordings) fell markedly during exposure to CBrF₃.

Discussion

Blood pressure fell during exposure to CBrF₃. Cardiac output did not vary significantly between the treated and untreated groups. A decrease in blood pressure without a change in cardiac output indicated a fall in peripheral resistance. Cardiac output is the product of heart rate (HR) and stroke volume (SV). Since cardiac output did not change significantly as a result of the treatment during exposure to CBrF₃ and HR fell significantly, stroke volume apparently increased. The decrease in resistance to the outflow of blood from the left ventricle was sufficient, even in the presence of a decreased heart rate, to allow the stroke volume to rise sufficiently to maintain cardiac output.
FIGURE 12. The effect of exposure to 70% CBrF₃ on stroke volume. The differences attributable to period and interaction were significant at the 1% level. The difference attributable to treatment was significant at the 5% level. The interaction effect was not interpreted as having biological significance since it was a mathematical result of the crossing of the respective curves.
STROKE VOLUME (ml/kg)

TREATED

UNTREATED

PERIOD

1

2

3
<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Significance</th>
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</thead>
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<tr>
<td>Total</td>
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<td>125.0794</td>
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<td></td>
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<tr>
<td>Mean</td>
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<td>121.323630</td>
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<td></td>
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<tr>
<td>Period</td>
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<td>0.176435</td>
<td>0.08821750</td>
<td>30.430</td>
<td>1%</td>
</tr>
<tr>
<td>Treatment</td>
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<td>0.014450</td>
<td>0.014450</td>
<td>4.984</td>
<td>5%</td>
</tr>
<tr>
<td>Interaction</td>
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<td>0.13001450</td>
<td>44.848</td>
<td>1%</td>
</tr>
<tr>
<td>Error</td>
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</table>
FIGURE 13. Effect of CBrF₃ exposure on left ventricular blood pressure in the open-chested monkey. The top tracing shows the fall in systolic blood pressure during CBrF₃ exposure. The bottom tracing shows the same ventricular pressure curve amplified to show the rise in left ventricular and diastolic (LVED) pressure (systolic pressure is not shown in the bottom tracing).
during CBrF$_3$ exposure. If the blood pressure fall had been the result solely of impaired cardiac function without a concurrent relaxation of the resistance vessels the TPR would have been expected to rise sharply since the force tending to distend the arterioles would have been reduced which would have resulted in a decrease of diameter. This in turn would sharply raise resistance to the flow of blood (law of LaPlace, Burton, 1965).

The fall in HR observed in the anesthetized dogs in this experiment is in contrast to the elevation seen in conscious dogs in Chapter III. The elevation in HR was probably the result of increased sympathetic activity associated with CBrF$_3$-induced excitement. Since the excitement was blocked by anesthetization the sympathetic discharge and subsequent tachycardia were also prevented by anesthetization.

The experiments with open-chested animals indicated that exposure to CBrF$_3$ reduced the myocardial contractility and possibly the heart rate also. When blood pressure falls activation of the baroreceptor reflexes normally results in reduced vagal tone which has the effect of increasing both the heart rate and force of contraction. However, heart rate decreased significantly during the exposure of the intact dogs in the first experiment (Figure 11).

The rise in left ventricular end diastolic (LVED) pressure associated with exposure to CBrF$_3$ was apparently the
result of a combination of two factors: 1) decreased resistance vessel tone and 2) decreased myocardial contractility. The initial effect of CBrF₃ appeared to be a relaxation of the resistance vessels which caused the TPR to fall. The capacitance vessels distended in response to the flow of high pressure arterial blood into the venous system and this shift of blood caused the pressure fall in the arterial system. Because of the greater compliance of the venous system the venous pressure rises approximately 1 mm Hg for each fall of 24 mm Hg in the arterial side (Guyton, 1966). As the right arterial pressure rose in response to the filling of the venous system the heterometric auto-regulatory response of the normal heart would increase the cardiac output. The exposure of the heart to CBrF₃ resulted in a reduced myocardial contractility which interfered with its ability to compensate entirely for the increased venous return and blood was pooled in the venous system resulting in an elevated LVED pressure. A balance between the two factors of decreased TPR and myocardial contractility was established. The increased venous return was not entirely accommodated by the weakened heart which continued to pump against a reduced resistance with the result that the cardiac output remained the same and the LVED pressure rose (Guyton, 1966).
CHAPTER V

THE MECHANISM OF THE PERIPHERAL VASCULAR
RESISTANCE CHANGE DURING EXPOSURE OF
DOGS TO BROMOTRIFLUOROMETHANE

Methods

Thirteen beagle dogs of both sexes which weighed 9 to 11 kg were used. Approximately 1 week prior to experimentation the left lumbar sympathetic chain was excised from 5 dogs from L1 to L3. Approximately 1 week prior to experimentation left and right lumbar sympathetic chains were excised from another five dogs from L1 to L3. The remaining 2 dogs were untreated prior to experimentation.

The preparatory surgery was performed using thiamylal anesthesia and the experimental procedures were performed using alpha-chloralose (110 mg/kg initial dose with subsequent fractional doses as required, 9.09 mg/ml). All animals were restrained in the supine position and ventilated mechanically at a constant rate via endotracheal catheters. Mean arterial pressure was measured by means of a pressure transducer through a polyethylene catheter in a brachial artery. Left ventricular pressure, end diastolic pressure
and dp/dt were measured by means of a pressure transducer through a metal cannula introduced through a common carotid artery and advanced retrograde through the aortic semilunar valve into the left ventricle. All recordings were made on a direct-writing oscillograph. Femoral arterial blood flow was measured using an electromagnetic flowmeter.

The dogs with the left lumbar sympathectomy or the bilateral sympathectomy were exposed to 80% CBrF_3 in oxygen for 5-minute periods from 3 to 5 successive times at 10 to 20 minute intervals with the flowmeter sensor on either the left or right femoral artery. The flowmeter sensor was then moved to the other femoral artery and the exposure sequence was repeated.

Two of the intact dogs were exposed similarly to the above. The flowmeter sensor was placed around the left femoral artery for the first exposure sequence and then 5 mg/kg of phenoxybenzamine was infused over a 12 to 15-minute period. Thirty minutes after beginning the phenoxybenzamine infusion the exposure sequence was repeated with the flowmeter sensor remaining on the left femoral artery.

A second type of experiment was performed subsequent to the completion of the first using 5 beagle dogs of either sex which weighed 10.0 - 13.6 kg. The animals were anesthetized with 1.5 ml of Innovar-Vet (fentanyl 0.4 mg and droperidol 20 mg per ml) given subcutaneously followed in
30 minutes by an intravenous injection of 10 mg/kg of pentobarbital sodium. Subsequent fractional doses of pentobarbital sodium were administered as required throughout the course of the experiment to maintain light surgical anesthesia (Plane 1, Stage III).

Arterial blood pressure was measured through a polyethylene catheter inserted via a femoral artery as described for the brachial arterial recordings. Nictitating membrane tension was recorded using a force-displacement transducer.

Acetylcholine hydrochloride was dissolved in 0.9% NaCl and diluted to a final concentration of 10 μg/ml. Norepinephrine was similarly diluted to 10 μg/ml for injection.

Following anesthetization the right vagosympathetic trunk was transected and silver wire electrodes from a constant voltage stimulator were attached to the central and peripheral cut ends of the trunk. Ten-second trains of 5 v, 100 Hz, 1 msec, biphasic pulses were delivered to the central cut end and 5-second, 5-10 v, 100 Hz, 1 msec, biphasic pulses were delivered to the peripheral cut end at the appropriate times during the experiment.

The experiment was designed with a pre-exposure control period, an exposure period, and a postexposure period. The animals were exposed for 40-78 minutes to 80% CBrF₃ during the exposure period and to air at other times.
During the consecutive periods the following determinations were performed.

At intervals of 3 to 5 minutes 3 trains of electrical stimuli were applied first to the central and then to the peripheral cut ends of the vagosympathetic trunk. These were followed by 3 intravenous injections of 10 µg of acetylcholine and 3 injections of 10 µg of norepinephrine. Nictitating membrane tension was recorded during electrical stimulation of the central cut end of the vagosympathetic trunk and blood pressure was recorded during the other procedures. These procedures were performed prior to exposure, during exposure (beginning not before 10 minutes of exposure to the CBrF₃) and postexposure (beginning not before 30 minutes postexposure).

The CBrF₃-oxygen mixtures were prepared by flowing the respective gases through calibrated flow meters into a polyethylene mixing bag from which the mixture was introduced into the respiration pump intake.

Results

The results of the exposures of the dogs with the left lumbar sympathectomy are illustrated in Figures 14 and 15. Blood flow through the right femoral artery (intact sympathetic innervation) rose markedly during exposure to CBrF₃ and returned to pre-exposure values postexposure (Figure 14). Blood flow through the left femoral artery (denervated), on the other hand, rose only barely perceptibly
FIGURE 14. Dog H36. Left lumbar sympathectomy was performed 1 week prior to experimentation. A 5-minute exposure to 80% CBrF₃ resulted in a marked increase in flow through the right femoral artery.
FIGURE 15. Dog H36. Left lumbar sympathectomy was performed 1 week prior to experimentation. A 5-minute exposure to 80% CBrF₃ failed to elicit a significant increase in flow through the left femoral artery.
during CBrF$_3$ exposure (Figure 15). Both figures illustrate the negative inotropic effect of CBrF$_3$ as reflected in the decrease in $\frac{dp}{dt}$ and simultaneous increase in left ventricular end diastolic pressure.

The results of the exposures of the dogs with bilateral lumbar sympathectomy were similar to those illustrated in Figure 15. Femoral arterial blood flow through either femoral artery increased slightly or not at all during CBrF$_3$ exposure.

The results of the experiments on the intact dogs which were treated with phenoxybenzamine (alpha-adrenergic blockade) mimicked almost exactly the effects of sympathectomy (Figures 16 and 17).

Figure 18 illustrates the effect of exposure to 80% CBrF$_3$ on membrana nictitans tension (M.N. Tension) during constant electrical stimulation of the central cut end of the vagosympathetic trunk (sympathetic innervation of the m.n. via the superior cervical ganglion). Tension is given in arbitrary units. Tension was clearly reduced by 40 or more per cent during the exposure. The tension returned to pre-exposure values after 30 minutes postexposure.

Figure 19 illustrates the effect of exposure to CBrF$_3$ on vagal inhibition of the heart. Prior to exposure the stimulus was adjusted to just produce a brief cardiac arrest followed by escape from the vagal inhibition.
FIGURE 16. Dog H46. A 5-minute exposure to 80% CBrF₃ resulted in a marked increase in flow through the left femoral artery.
FIGURE 17. Dog H46. A 5-minute exposure to 80% CBrF₃ following alpha-adrenergic blockade with phenoxybenzamine failed to elicit a significant increase in flow through the left femoral artery.
FIGURE 18. Electrical stimuli were applied to the central cut end of the vagosympathetic trunks of 5 dogs before, during, and after exposure to 80% CBrF₃. Maximal tension developed in the nictitating membrane (M.N.), given in arbitrary units, was significantly lower during the CBrF₃ exposure.
FIGURE 19. Electrical stimuli were applied to the peripheral cut end of the vagosympathetic trunks of 5 dogs before, during, and after exposure to 80% CBrF₃. The degree of vagal inhibition was decreased during the CBrF₃ exposure.
The identical stimulus was subsequently applied during exposure and postexposure. Vagal inhibition was shown to be significantly reduced during the period of CBrF$_3$ exposure and to return to control values postexposure.

Figure 20 illustrates the decrease in responsiveness of the peripheral cholinergic vasodilator mechanism during CBrF$_3$ exposure.

Figure 21 illustrates the effect of CBrF$_3$ on the response to the intravenous injection of norepinephrine. The transitory rise in blood pressure is seen during both air-breathing and CBrF$_3$ exposure. The result is obscured somewhat in some cases by the simultaneous triggering of premature ventricular contractions by the hypertension in the presence of CBrF$_3$.

Discussion

The results clearly indicate that the decrease in peripheral vascular resistance (PVR) which was observed during exposure to CBrF$_3$ was mediated by a decrease in sympathetic vasomotor tone since the effect was almost completely abolished by sympathectomy or alpha-adrenergic blockade.

A comparison of mean flow and mean arterial pressure in Figures 14, 15, 16 and 17 indicates that the blood pressure fall during CBrF$_3$ exposure was primarily the result of the decrease in PVR. The regional flow rise seen in
FIGURE 20. Ten µg of acetylcholine was given in each of 3 intravenous injections before, during, and after exposure of 5 dogs to 80% CBrF₃. The peripheral cholinergic vasodilator response was significantly diminished during the CBrF₃ exposure.
PREEXPOSURE CONTROL

80 PCT BROMOTRIFLUOROMETHANE

POSTEXPOSURE

VAGAL STIMULATION
FIGURE 21. Ten μg of norepinephrine was given in each of 3 intravenous injections before, during, and after exposure of 5 dogs to 80% CBrF₃. The hypertensive response was not apparently affected by the CBrF₃ exposure. The result of the peripheral alpha-adrenergic receptor stimulation was obscured somewhat by the presence of ventricular premature beats triggered by the pressure elevation. These arrhythmias have been shown to be blood pressure-sensitive (cf. Chapter VI).
PREEXPOSURE CONTROL

80 PCT BROMOTRIFLUOROMETHANE

POSTEXPOSURE

CENTRAL SYMPATHETIC STIMULATION
Figure 16 was similarly abolished by chemical denervation but the hypotensive response was greatly reduced (Figure 17). The difference lay in the fact that sympathetic innervation to three-fourths of the circulatory bed remained intact following regional surgical denervation whereas it was 99% abolished by chemical denervation (alpha-adrenergic blockade).

Figures 14 and 16 also illustrate the temporal sequence of the alterations in myocardial contractility (MC) and PVR. As the PVR fell, flow rose rapidly (A). Simultaneously MC decreased more slowly (B). MC then fell more quickly as the PVR decrease approached its limit and flow momentarily declined (C). At this point an approximately steady state was reached except for a gradual increase in flow (D) attributable to the stress relaxation (delayed compliance, Guyton, 1966) of the vascular smooth muscle which had necessarily increased its fiber length during the passive relaxation of the resistance vessels which accompanied the decreased sympathetic vasomotor tone induced by the CBrF₃. The sequence was reversed postexposure. MC returned relatively quickly (E) before the return of vasomotor tone which resulted in a further increase in flow (F). Subsequently flow declined to pre-exposure levels (G) with the slower return of vasomotor tone. The observation that the CNS-mediated effect was the first to appear and the last to disappear can probably be explained in two ways. First, the
Basic mechanisms involved could be intrinsically more sensitive to the CBrF₃ effects in the CNS versus the myocardium. Second, the heptane:water partition coefficient of CBrF₃ is high (approximately 10) and so the CNS with its high lipid content could be expected to preferentially concentrate the CBrF₃ during exposure and gradually release it postexposure. This is a particularly attractive hypothesis regarding the relatively slow return of the vasomotor tone seen postexposure (cf. Chapter VIII).

In addition to the possibility that the central sympathetic outflow was decreased during exposure to CBrF₃ it was thought possible that an element of ganglionic blockade could also have contributed to the decreased peripheral vasoconstrictor tone. A significant decrease in nictitating membrane tension was shown to occur in Figure 18. This observation coupled with the observation that no appreciable alpha-adrenergic blocking occurred during CBrF₃ exposure (Figure 21) strongly suggests that a significant degree of adrenergic ganglionic blockade occurs during CBrF₃ exposure. This observation is given further credence by the observation that CBrF₃ decreases cardiac vagal inhibition at a constant level of preganglionic stimulation (Figure 19). This would suggest that an element of parasympathetic ganglionic blockade also occurs during CBrF₃ exposure. The observation that ganglionic blockade of both divisions of the autonomic
nervous system occurs would be consistent with the actions reported for the usual ganglionic blocking drugs such as hexamethonium (Kharkevich, 1967).

The final argument for the ganglionic blocking effect of CBrF₃ is illustrated in Figure 20. CBrF₃ apparently has an anticholinergic effect since the peripheral cholinergic vasodilator response to exogenous acetylcholine is diminished. Thus it would seem that the relatively weak autonomic ganglionic blockade seen during exposure to CBrF₃ is the result of either an interference with the acetylcholine-receptor interaction or perhaps of a decreased receptor sensitivity to acetylcholine.
CHAPTER VI

SPONTANEOUS CARDIAC ARRHYTHMIAS INDUCED
BY BROMOTRIFLUOROMETHANE

Methods

Eight monkeys (Macaca mulatta) from 2 to 4 years old and weighing 2.5 to 5.0 kg were used. A single group of 5 of the monkeys was used for 3 different experiments. Animals were anesthetized with intravenous 4% pentobarbital sodium. An initial dose of 30 mg/kg was given and subsequent small injections were given to maintain Stage III, Plane I anesthesia. Femoral arterial and venous catheters were inserted. Endotracheal catheters were inserted and connected to a respiratory pump (Harvard Apparatus Co., Dover, Massachusetts) and the monkeys were ventilated at 9 to 16 cpm. Arterial blood samples were obtained throughout the experiments at approximately 30-minute intervals and pH, PCO₂ and PO₂ determined (Blood Gas Analyzer Instrumentation Laboratories, Inc., Boston, Massachusetts).

During the first experiment (control) arterial blood pH was regulated between 7.35 and 7.45 with 100% O₂ at 10 respiratory cycles per minute by adjusting tidal volume. During the second experiment (acidosis) the arterial blood
pH was regulated between 7.10 and 7.30 with oxygen to which was added 0 to 5% CO₂. The animals were ventilated at 9 respiratory cycles per minute and a tidal volume of not less than 25 ml/kg. During the third experiment (alkalosis) the arterial blood pH was regulated between 7.50 and 7.80 with 100% O₂. The animals were ventilated at 12 to 16 respiratory cycles per minute and a tidal volume of about 35 ml/kg.

The animals were observed for 30 to 60 minutes in the control, acidotic, or alkalotic state and then exposed to 10, 20, 30, 40, 50, 60, 70, and 80% CBrF₃ in O₂ (or O₂ and CO₂) for 10-minute periods in succession. Electrocardiograms (Lead II) were recorded on a direct-writing electrocardiograph (Polygraph, Grass Instrument Co., Quincy, Massachusetts). Arrhythmias resulting from ventricular ectopic pacemaker formation appeared spontaneously during the first 5 minutes of exposure to 30% or greater concentrations of CBrF₃. When the arrhythmias appeared the arterial blood pressure which was monitored using a pressure transducer (Statham, Hato Rey, Puerto Rico) was lowered by bleeding from a vein. The blood pressure was lowered until the arrhythmias were abolished and then the blood was reinfused which returned the blood pressure to normal and caused the reappearance of the arrhythmias (Figure 22, lower tracing). Exsanguination and reinfusion were performed at each concentration of CBrF₃ from 30% to 80%. The successive exposures were performed twice on each animal during each experiment which provided
FIGURE 22. The occurrence of cardiac arrhythmias depended on the maintenance of a minimal blood pressure. Arrhythmias were triggered by raising the blood pressure by the intravenous infusion of epinephrine (upper tracing). Arrhythmias also were abolished and restored by the alteration of blood pressure by exsanguination and reinfusion (lower tracing).
4 arterial blood pressure values at which arrhythmias were abolished or reappeared for each animal, at each concentration, and in each acid-base state. Arterial blood pressure in most cases was not high enough to trigger arrhythmias spontaneously at CBrF₃ concentrations of 10% and 20%. During these exposures epinephrine was infused to raise the blood pressure to levels high enough to trigger arrhythmias (Figure 22, upper tracing).

At the conclusion of the preceding experiments the following experiments were performed using the entire group of 8 monkeys.

The abdominal cavities were opened in 4 monkeys and snares were passed around the thoracic aorta so that arterial blood pressure could be raised briefly by aortic constriction. These animals were exposed to 40% CBrF₃.

Two monkeys were prepared as described and found to have blood pressures too low to trigger arrhythmias spontaneously during exposure to 40% CBrF₃. The blood pressures were raised by expansion of the plasma volume with 6% dextran. In another experiment these monkeys were given alpha-adrenergic blocking doses of phenoxybenzamine (5 and 6 mg/kg, respectively) followed by epinephrine during exposure to CBrF₃.

Two monkeys were given 5 mg/kg of reserpine intraperitoneally. Approximately 24 hours later they were given 50 mg/kg of tyramine intravenously to test for catecholamine
depletion and then exposed to 70% CBrF₃. The reserpinized monkeys responded to the tyramine injections with a transitory rise in blood pressure of 5 torr.

**Results**

The results of the first 3 experiments are shown in Figure 23. Four determinations of the systolic blood pressure at which arrhythmias either appeared or were abolished were performed on each of five monkeys which provided 20 points at each CBrF₃ concentration (abscissa). In general the higher the CBrF₃ concentration the lower the systolic blood pressure necessary to trigger the arrhythmias (threshold pressure). The threshold pressure was significantly higher during alkalosis only at 10% and 20% CBrF₃. The threshold pressure was significantly lower during acidosis at 10% and 20% CBrF₃ and no significant differences were observed from 30% through 80% CBrF₃.

The data from the control experiment are presented in Figure 24. Linear, quadratic, and logarithmic relationships were fitted to the data. The fit of the equation with the highest multiple coefficient of correlation (0.85) led to the conclusion that the threshold blood pressure required to trigger arrhythmias (ordinate) varied as a function of the $\log_{10}$ of the CBrF₃ concentration to which the animals were exposed (abscissa).
FIGURE 23. The minimal blood pressure necessary to trigger arrhythmias varied inversely with the concentration of CBrF₃. Alkalosis elevated and acidosis lowered the blood pressure threshold during exposure to 10% and 20% CBrF₃ but was without significant effect at concentrations of CBrF₃ of 30% or greater. The vertical bars represent ±1 standard deviation. Since no statistically significant differences existed above 20% CBrF₃ the standard deviations are not shown.
FIGURE 24. The result of fitting linear, quadratic, and logarithmic equations to the control data suggested that the blood pressure threshold required to trigger arrhythmias during exposure to CBrF₃ varied inversely as a function of log₁₀ of the CBrF₃ concentration.
The upper tracing of Figure 25 illustrates how the arrhythmias were induced by raising the blood pressure by the expansion of the plasma volume with 6% dextran. The lower tracing of Figure 25 shows the development of arrhythmias by raising the blood pressure by aortic constriction. The arrhythmias were abolished following release of the aortic constriction.

The upper tracing in Figure 26 demonstrates the spontaneous formation and "self-abolition" of arrhythmias which can be observed in some individuals. This observation was made incidentally during the performance of another experiment. This monkey was exposed to 70% CBrF₃. The blood pressure began to fall as the result of a decrease in peripheral resistance and decrease in myocardial contractility. A further decrease in the blood pressure accompanied the onset of the arrhythmia. The progressively falling blood pressure finally fell below the threshold level necessary to maintain the arrhythmia resulting in the abolition of the arrhythmia.

One monkey was given 5 mg/kg of phenoxybenzamine intravenously, a dose which was shown in other monkeys to be sufficient to induce a high degree of alpha-adrenergic blockade. This monkey was exposed to 40% CBrF₃ which resulted in the appearance of spontaneous arrhythmias. The monkey was then bled until the arrhythmias were abolished and some of the blood reinfused slowly until the blood
FIGURE 25. The upper tracing illustrates the triggering of arrhythmias in a monkey exposed to 70% CBrF$_3$ by the expansion of plasma volume with 6% dextran. The lower tracing illustrates the triggering and abolition of arrhythmias during exposure to 70% CBrF$_3$ by constriction and release, respectively, of the thoracic aorta.
FIGURE 26. The upper tracing illustrates the phenomenon of the "self-abolition" of cardiac arrhythmias occurring spontaneously during exposure to 70% CBrF₃. Immediately after the beginning of the exposure (center) blood pressure began to fall as the result of a decrease in peripheral resistance and a decrease in myocardial contractility. The arrhythmia appeared and the blood pressure continued to fall until it fell below the threshold required to maintain the arrhythmias at which time the arrhythmia disappeared. The lower tracing illustrates the abolition of ventricular premature contractions in an animal which had received an alpha-adrenergic blocking dose of phenoxybenzamine. The administration of epinephrine caused the blood pressure to fall below the threshold required to maintain the arrhythmia.
pressure rose just high enough to cause the reappearance of the arrhythmias. At this point a single intravenous injection of 1 μg/kg of epinephrine was given which elicited the "epinephrine reversal" response. The lower tracing in Figure 26 illustrates the abolition of CBrF₃-induced ventricular premature contractions by eliciting the "epinephrine reversal" response. The lower tracing in Figure 26 illustrates the abolition of CBrF₃-induced ventricular premature contractions by eliciting the "epinephrine-reversal" phenomenon.

Figure 27 illustrates the results of an experiment similar to that illustrated in Figure 26. The monkey was given an alpha-adrenergic blocking dose of phenoxybenzamine (6 mg/kg) and exposed to CBrF₃. After the onset of the arrhythmia epinephrine was given. At the same time 6% dextran was infused to prevent the fall in blood pressure which otherwise would have accompanied the "epinephrine reversal." From this experiment it may be seen that epinephrine apparently lowered the blood pressure threshold required to trigger arrhythmias since the arrhythmia appeared following the injection of epinephrine in the absence of a pressor response.

Figure 28 illustrates another example of the "self-abolition" of arrhythmias illustrated in Figure 26. The monkey was exposed to 70% CBrF₃ and bled to a blood pressure
FIGURE 27. The monkey was given an alpha-adrenergic blocking dose of phenoxybenzamine and exposed to CBrF₃. Blood pressure was held just below the threshold required to maintain the arrhythmia. Epinephrine was injected (mark on left end of middle timing recording) and 6% dextran was infused at the same time to prevent a fall in the blood pressure. The result was a triggering of the arrhythmia by epinephrine in the absence of a pressure rise.
FIGURE 28. A monkey exposed to 70% CBrF$_3$ was exsanguinated until the blood pressure fell to just above the threshold required to maintain the arrhythmia. The arrhythmia in this instance was paroxysmal ventricular tachycardia. The tachycardia caused an additional fall in the blood pressure to a value below threshold at which time the arrhythmia disappeared. With the disappearance of the arrhythmia the blood pressure rose to a value above threshold thus triggering the arrhythmia again.
slightly above threshold. The arrhythmia which spontaneously
developed was paroxysmal ventricular tachycardia. The blood
pressure fall which accompanied each paroxysm was great
enough to abolish the arrhythmia. The blood pressure rose
again, however, following abolition of the arrhythmia which
caused another paroxysm thus establishing the peculiar pattern
of blood pressure oscillations illustrated. This observation
was made incidentally to the performance of another experiment.

The two reserpinized monkeys developed arrhythmias
spontaneously during exposure to 70% CBrF₃.

Discussion

Blood pressure sensitive arrhythmias were induced by
Dresel and co-workers (1960) by injecting dogs anesthetized
with 20% cyclopropane with small doses of epinephrine. Non-
sinus rhythms were readily converted to sinus rhythms by
lowering the blood pressure. However, the presence of
epinephrine or some other pressor amine was required to
trigger the arrhythmias. They showed later that a
constantly coupled bigeminy could be converted to a multi-
focal ventricular arrhythmia by elevating the blood pressure
well above the minimal threshold necessary to trigger the
bigeminy (1961).

Unlike cyclopropane, CBrF₃ did not require the
presence of significant levels of catecholamines to produce
arrhythmias. In Figure 26 (lower tracing) it was illustrated
that under certain conditions a CBrF₃-induced arrhythmia actually was abolished with epinephrine by the elicitation of the "epinephrine reversal" response. Also, arrhythmias appeared spontaneously in animals presumably depleted of endogenous catecholamines by reserpine providing the blood pressure was high enough. The presence of epinephrine was not, however, without effect on the arrhythmias. Figure 27 illustrates how the minimal threshold necessary to trigger arrhythmias was lowered at a constant concentration of CBrF₃.

It was reported in Chapter III that differences existed in individual susceptibility to the spontaneous formation of arrhythmias during exposure to CBrF₃. It now is apparent that this was related to the ability of the individual to maintain blood pressure during exposure to CBrF₃. A somewhat more hypertensive individual would be expected to develop arrhythmias more readily than a normo- or hypotensive individual. Another factor in the consideration of individual susceptibility would be related to the rate at which the initial CBrF₃-induced hypotension developed. After examining the self-abolition of arrhythmias illustrated in the top tracing in Figure 26 it is not difficult to imagine a situation to which the CBrF₃-induced hypotension occurring during the first few minutes of exposure would cause the blood pressure to drop below the threshold necessary to trigger the arrhythmia before enough time had
elapsed for the arrhythmia to appear spontaneously. The length of exposure required to induce arrhythmias with CBrF$_3$ in individuals with high enough blood pressure was about 0.1 to 0.5 minutes at the higher concentrations and 2.0 to 5.0 minutes at the lower concentrations.

The observations that acidosis lowered and alkalosis raised the blood pressure threshold necessary to trigger arrhythmias at any given concentration of CBrF$_3$ are consistent with the fact that carbon dioxide causes widespread activation of the sympathetic nervous system with an increase in the liberation of endogenous catecholamines (Goodman and Gilman, 1965). Since acid-base balance was manipulated via the respiratory system in these experiments, acidosis and alkalosis were predominantly "respiratory" and acidosis was accompanied by elevated blood P$_{CO_2}$ values. The high CO$_2$ levels in the blood would be expected to result in high circulating catecholamine levels which would lower the threshold required to trigger the arrhythmias.

The sensitivity of the arrhythmias to changes in blood pressure and to the level of circulating catecholamines could have been the result of variations in coronary perfusion and thus the amount of CBrF$_3$ actually delivered to the myocardium. Changes in arterial blood pressure could affect coronary perfusion and the alteration of circulating catecholamine levels does likewise independently of blood pressure alterations (Goodman and Gilman, 1965).
CHAPTER VII

ALTERATION OF THE ELECTROENCEPHALOGRAM DURING BROMOTRIFLUOROMETHANE EXPOSURE

Methods

Six beagle dogs 1 to 3 years old which weighed from 6 to 10 kg and 6 monkeys (Macaca mulatta) 2 to 4 years old which weighed from 2.5 to 5 kg were used. Preliminary operative procedures were performed following anesthetization by the intravenous injection of a single dose of 20 mg/kg thiamylal in 4% solution. Animals were immobilized during performance of the experiments by the intermittent intravenous infusion of tubocurarine hydrochloride (120 mg/liter). Cururized animals were ventilated mechanically. Blood pH was maintained between 7.35 and 7.45 by adjustment of the respiratory rate and minute volume.

Electroencephalographic (EEG) recording was performed using a 6-channel direct-writing oscillograph. A 6-lead, bipolar electrode pattern was used in both species (Caveness, 1962). Right and left electrodes were placed over the frontal, temporal, and occipital areas. One-fourth inch, 26 ga, stainless steel hypodermic needles were used as scalp electrodes.
The bell in a spring-wound alarm clock was used as an auditory stimulus and a 100 watt incandescent lamp was used as a photic stimulus.

Chemically pure bromotrifluoromethane and oxygen were transferred volumetrically from cylinders under pressure through calibrated flow meters into a polyethylene mixing bag from which the mixture was administered through the respiratory pump in the curarized animals. Halothane was vaporized quantitatively.

Experiment 1.--Each of 4 dogs and 4 monkeys was anesthetized with a single injection of thiamylal. Endotracheal catheters were placed. Polyethylene catheters were inserted into the femoral arteries from which blood samples were obtained for the measurement of pH, pCO₂, and pO₂. The scalp electrodes were placed. The infusion of tubocurarine was begun and the rate was gradually increased as the animal recovered from the effects of the thiamylal. Recovery from anesthetization was usually complete within 1 hour as indicated by the disappearance from the EEG of high-amplitude slow-wave activity characteristic of barbiturate anesthesia (Faulconer & Bickford, 1960) and the return of sensitivity of the EEG to activation by photic and auditory stimuli. Two types of experiments were performed on each of these animals. Activation of the EEG by photic and auditory stimuli was demonstrated during exposure to oxygen (control) and during exposure to 70% or 80% CBrF₃. The other part
of this experiment involved the recording of the EEG during the induction of the CBrF₃ effect. Recordings were made continuously during the first 6 minutes of exposure to 70% CBrF₃ and then at 2-minute intervals thereafter during a total exposure to 60 minutes.

Experiment 2.--The 12 animals were each anesthetized with thiamylal and prepared as in experiment 1. The animals were exposed during 3 successive 60-minute periods to 70% CBrF₃, 100% O₂, and 1% halothane. The sequence of administration was varied without apparent effect on the results. A 10-minute recovery period elapsed following cessation of the CBrF₃ exposure before beginning the next exposure and a 30-minute recovery period elapsed following cessation of the halothane exposure. EEG recordings were made at 10-minute intervals during the exposures.

Results

The results of the experiments conducted on the monkeys and dogs were qualitatively similar. The primary difference lay in the respective amplitudes of the brain wave recordings with those of the dogs averaging only about 30% of those of the monkeys. The figures represent the best examples of the phenomena which were observed in variable degree in all of the animals studied. The monkeys provided better examples than the dogs because the generally greater
amplitudes improved the contrasts observed among the various experimental states.

Experiment 1.--Figure 29 illustrates activation of the EEG detectable in recordings from all 6 electrode pairs following exposure of monkey E83 for 14 and 19 minutes, respectively, to 80% CBrF3. Activation coincided with presentation of the photic stimulus. This state, characterized by decreased amplitude and some degree of desynchronization, persisted for about 5 seconds and was followed by a return to the prestimulation state. Activation was again elicited when the stimulus was removed.

Figure 30 illustrates activation of the EEG of monkey E91 following exposure to 70% CBrF3 for 21 minutes. Activation coincided with presentation of the auditory stimulus. Unlike the photic stimulus the auditory stimulus elicited activation of the EEG which persisted throughout the duration of the presentation and for 6-8 seconds following removal of the stimulus.

Figures 31, 32, and 33 illustrate the progressive changes in the EEG of the monkey (E87) with time during exposure to 70% CBrF3. Increased amplitude and homolateral synchronization appeared during the second minute of exposure which became maximal during the third minute. The pre-exposure amplitude in the monkeys averaged between 25 and 50 μV and increased to a maximum of 150-175 μV during the
FIGURE 29. Monkey E83 was exposed to 10% CBrF₃ in O₂ for 19 minutes and presented with a photic stimulus through half-closed eyes in a darkened room. The heavy line indicates "light on." Recordings are continuous except for a 9-second interval cut out to compress the illustration.
FIGURE 30. Monkey E91 was exposed to 70% CBrF3 in O2 for 21 minutes and presented with an auditory stimulus. The heavy line indicates "stimulus on." Recordings are continuous except for a 5-second interval cut out to compress the illustration.
FIGURE 31. Monkey E87. This figure with Figures 32 and 33 forms a temporal continuum illustrating the induction of the effect of exposure to CBrF₃. Left—0 minutes, begin 70% CBrF₃; right—1 minute, 70% CBrF₃.
FIGURE 32. Monkey E87. This figure with Figures 31 and 33 forms a temporal continuum illustrating the induction of the effect of exposure to CBrF$_3$. Left--2 minutes, 70% CBrF$_3$; right--3 minutes, 70% CBrF$_3$. 
FIGURE 33. Monkey E87. This figure with Figures 31 and 32 forms a temporal continuum illustrating the induction of the effect of exposure to CBrF$_3$. Left--4 minutes, 70% CBrF$_3$; right--6 minutes, 70% CBrF$_3$. 
full CBrF$_3$ effect. Synchronization was primarily homolateral and more obvious on left side. Some synchronous components were detectable in recordings from 4 or more electrode pairs.

Experiment 2.—Figure 34 illustrates a comparison of the effect of 1% halothane (right) with that of 70% CBrF$_3$ (left).

The center recording represents air-breathing during a control period comparable to the respective exposure periods. Figure 34 represents the full effect of both compounds after 20 minutes exposure. The full effect was seen within 3 minutes of exposure (Figure 32) and hence, in this experiment in which recordings were made at 10-minute intervals for 60 minutes, all recordings from 10 to 60 minutes were essentially the same except that a gradual decrease of maximal amplitude during the CBrF$_3$ exposures was seen to occur with time. After 60 minutes, the maximal amplitude was 125-150 $\mu$V.

The EEG's obtained during CBrF$_3$ exposure were of greater amplitude and synchronization than those obtained during the control period and were dominated by waves in the range of 6-9 Hz. EEG's recorded during halothane exposure were characterized by a dominance of high amplitude slow waves in the 1-3 Hz (delta rhythm) range typical of sleep (Faulconer & Bickford, 1960). In two instances monkeys
FIGURE 34. Monkey E95. This animal was exposed to 70% CBrF₃ for a 60-minute period and to 1% halothane for a similar 60-minute period. During each exposure the EEG was recorded at 10-minute intervals. These recordings were made after 20-minute exposure. CBrF₃ is on the left, 1% halothane on the right, and a comparable air-breathing control period is illustrated in the center.
apparently fell asleep during the CBrF_3 exposure since a large amplitude slow wave pattern was superimposed on the 6-9 Hz dominant pattern. The difference between the animals in the halothane and CBrF_3 sleep states lay in their respective susceptibilities to arousal. Activation was readily elicited by sensory stimulation during the CBrF_3 sleep whereas it could not be elicited even by means of a painful stimulus (needle skin prick) during halothane exposure.

Discussion

The most striking electroencephalographic corollary to CBrF_3 exposure is the characteristic dominance of the non-activated EEG by 6-9 Hz waves. This, and other observations, suggests CBrF_3-induced functional changes reflected at the rhinencephalic level. The suggestion has been advanced that the theta wave is a non-propagated disturbance generated by the hippocampal pyramids (Green, 1960) and theta waves similar to those seen clearly in Lead LF-RF in Figure 2 have been recorded from the hippocampus by Kandel and Spencer (1961).

The opposite responses of the psychomotor trained (Carter, 1969) and untrained monkeys also suggest hippocampal involvement. Hunt et al. (1957) report that cats with hippocampal lesions do not retain conditioned avoidance
response to a buzzer but do retain the characteristic signs of emotional disturbance when the buzzer is presented during the tests. Ablation of the monkey hippocampus, on the other hand, renders the monkey nonreactive to the presence of a snake, a situation normally eliciting a fear behavior pattern (Gol et al., 1963). This correlates with the docility of the normally aggressive untrained rhesus during CBrF₃ exposure.

Tonic convulsions can be elicited in cats and rats via brainstem activation (Kreindler et al., 1958) and the hippocampus is known to have an inhibiting influence opposed to brainstem activating influences (Segundo & Galeano, 1960). Convulsions in dogs with epileptic predilections might be triggered as the result of hippocampal depression. The convulsions, on the other hand, may not be "convulsions" but rather a form of rigidity resulting from a depression of either or both the cerebral cortex and basal ganglia.

One observation on the behavior of conscious dogs during the first few seconds of exposure to CBrF₃ is that they look around the room apprehensively, a behavior pattern which might be interpreted as a form of orientation reflex, and since the hippocampus is referred to as an association center for incoming sensory signals (Guyton, 1966), such behavior might be construed to be hippocampal related.
CHAPTER VIII

BRAIN AND HEART ACCUMULATION OF BROMOTRIFLUOROMETHANE

Methods

One hundred seventy-six albino rats (Charles River) weighing 300-450 g were divided into 11 groups of 16 rats each. Both sexes were used indiscriminately. A 50-liter exposure chamber was fabricated from a corrugated paper box in the shape of a truncated pyramid and enclosed in polyethylene. CBrF₃ and O₂ were delivered from cylinders under pressure through flowmeters and introduced into the exposure chamber in a ratio of about 3:1. The gas delivery and sampling tubes were located in the chamber approximately 10 cm from the bottom and on opposite sides of the chamber. Intermittent chamber atmospheric samples were withdrawn through the sampling tube and the relative concentrations of CBrF₃ and O₂ determined. The chamber was purged prior to exposure with the CBrF₃-O₂ mixture until the CBrF₃ concentration exceeded 70%. Irrigation of the chamber was continuous and the CBrF₃ concentration was determined at 1-minute intervals throughout the exposure period.

Rats were killed by decapitation following a preliminary stunning by a blow to the head. One operator
quickly removed most of the brain tissue while another operator removed the heart. Tissues were quickly dipped in 0.9% NaCl solution transferred to glass containers of n-heptane and tightly capped. The tissues were in the capped containers within 30 seconds of removal of the rats from the exposure chamber.

Groups of 16 rats were selected at random from the population available for the study. Immediately prior to each exposure 1 rat was killed and the brain and heart removed to serve as the pre-exposure control. The 15 remaining rats were then placed in the chamber. Minute 0 of the experiment coincided with the placement of the rats in the exposure chamber. Individual rats were removed at minutes 1, 2, 3, 4, and 5 of the exposure period and the tissues obtained. At minute 5 the 10 remaining rats were removed from the chamber and placed in a ventilated box in room air. The tissues from the 10 rats were obtained at minutes 6, 7, 8, 9, 10, 15, 20, 30, 45, and 60. This procedure was repeated 10 times. During the 11th experiment intracardiac blood samples were obtained according to an identical sampling schedule. All of the other conditions of the previous 10 experiments were met during the 11th experiment.

The tissue analyses of CBrF$_3$ were performed using a gas chromatographic technic employing an electron capture detector (Kaplan, in preparation).
Comparisons of pairs of mean values were performed, where appropriate, using Student's t-test. A comparison of the mean values of the chamber concentrations of CBrF\textsubscript{3} was performed using a one-way analysis of variance (Freund et al., 1960).

Results

Figure 35 illustrates the results of the periodic analyses of the CBrF\textsubscript{3} mixture in the exposure chamber during 9 of the 10 exposures. These determinations were not available for one of the runs. The mean concentration rose significantly during the exposure periods and varied from 71\% to 76\% CBrF\textsubscript{3}. The broken line represents the CBrF\textsubscript{3} concentrations during the 11th experiment in which the blood concentration of CBrF\textsubscript{3} was determined. The concentrations in the 11th experiment were just outside the 95\% confidence interval during minutes 1-3 when compared with the chamber concentrations during the brain-heart experiments. There was no statistically significant difference between the concentrations during the 4th and 5th exposure minutes.

Figure 36 illustrates the results of the analyses of the tissue concentrations of CBrF\textsubscript{3}. The mean brain concentration of CBrF\textsubscript{3} reached 445 $\mu$g/gm of tissue after the first minute and rose at an average rate of 79 $\mu$g/gm/min to 760 $\mu$g/gm of tissue. The mean heart concentration reached
FIGURE 35. The concentration of CBrF$_3$ in the exposure chamber during 5-minute exposures of rats for the purpose of measuring the uptake of CBrF$_3$ by brain and heart tissue (n=9, mean ± S.D.).
FIGURE 36. Rat brain and heart concentrations of CBrF$_3$ during and after 5-minute exposures to 71-76% CBrF$_3$ in O$_2$ (n=10, mean ± S.D.). The broken line represents blood concentrations of CBrF$_3$ observed during an experiment in which the conditions were similar to those of the brain-heart experiments (n=1).
RAT BRAIN AND HEART CONCENTRATIONS OF BROMOTRIFLUOROMETHANE
DURING AND AFTER 5-MINUTE EXPOSURES

- BRAIN
- HEART
- BLOOD

MEAN ± S.D.

CBrF₃ EXPOSURE

MINUTES
350 µg/gm of tissue during the first minute and rose at an average rate of 35 µg/gm/min to 500 µg/gm of tissue. The brain concentrations of CBrF₃ were significantly higher than those of the heart at all times during the exposure from minute 1 through minute 5. The brain concentrations of CBrF₃ rose approximately twice as rapidly and to 50% higher levels than the heart during 5-minute exposures to 71-76% CBrF₃. The rates of decay of the respective tissue concentrations of CBrF₃ were comparable. One minute post-exposure the brain concentration was 210 µg/gm of tissue and the heart concentration was 100 µg/gm of tissue, a statistically significant difference. At 2 minutes post-exposure the respective concentrations were 95 and 68 µg/gm, a statistically significant difference (1% level). From 3 minutes postexposure the differences between the brain and heart concentrations of CBrF₃ were not significant. The blood concentrations closely paralleled the heart concentrations and they differed significantly from the brain concentrations. The tissue concentrations of CBrF₃ approached zero asymptotically postexposure. No significant differences in CBrF₃ concentration were seen among the three tissues beyond 2 minutes postexposure to the end of the experiment 55 minutes postexposure.
Discussion

Figure 14 illustrates the difference in the recovery times of the peripheral vasomotor tone and myocardial contractility in a dog following a 5-minute exposure to 80% CBrF₃. The index of contractility, dp/dt, returned to pre-exposure levels during the period 0.5 - 0.7 minutes postexposure (E). The blood flow through the right femoral artery rose from an already elevated level during this period (F). The blood flow then began to drop rapidly from 0.8 minutes postexposure and had returned to pre-exposure levels by 4 minutes postexposure.

The experiments reported in Chapter V proved that the flow change through the femoral artery was mediated by a change in vasomotor tone. Part of the observed change in vasomotor tone could have been the result of ganglionic blockade since this was strongly implied in Chapter V and has been reported for halothane which has pharmacologic properties similar to those of CBrF₃ (Dobkin & Su, 1966; Dundee, 1967). In addition to an element of ganglionic blockade, however, a decrease in central sympathetic outflow probably also occurs. A conclusion was drawn that the observed myocardial function returned earlier than the observed central nervous system function following a 5-minute exposure to 80% CBrF₃.

The heptane-water partition coefficient for CBrF₃ has been determined in our laboratory to be approximately
9.8. It is a nonpolar substance which readily crosses the blood-brain barrier. Because of its solubility in organic solvents CBrF₃ would be expected to be preferentially absorbed by the central nervous system because of its relatively high lipid content compared to that of the heart.

The observation that the brain does, indeed, accumulate a significantly higher rate than the heart may provide a partial explanation of the difference in functional recovery times. If one made the dubious assumption for the purpose of the discussion that the functional effects of a given amount of CBrF₃ were approximately equivalent for brain and heart tissue it would follow from these data that the postexposure effect would be expected to persist in the brain for about 2 minutes longer than the heart, which is the case.
CHAPTER IX

SUMMARY

Exposure of dogs and monkeys to 10-80% bromotrifluoromethane caused cardiovascular and central nervous system effects which increased in severity with increasing CBrF₃ concentration. An initial fall in mean arterial blood pressure of 10-20 mm Hg at the lower CBrF₃ concentrations and 40-60 mm Hg at the higher CBrF₃ concentrations was observed. Most animals developed spontaneous cardiac arrhythmias within 1-3 minutes of exposure to 40% or more CBrF₃. Arrhythmias could be produced in those animals not developing arrhythmias spontaneously by the intravenous injection of a pressor dose of epinephrine. Larger doses of epinephrine (5-10 µg/kg BW) caused ventricular fibrillation with cardiac arrest in dogs and, commonly, spontaneous defibrillation in monkeys. Epileptiform motor phenomena were seen in about 50% of the dogs exposed to 50-80% CBrF₃ while conscious. Conscious monkeys, on the other hand, became lethargic, and no convulsions were seen.

The subsequent investigations were designed to provide more information about the pharmacologic effects of exposure by inhalation to bromotrifluoromethane which
were suggested by the initial work. The mechanisms which contributed to the hemodynamic changes which were observed were of prime interest. Also of interest were the differences which existed in individual sensitivity to the spontaneous development of cardiac arrhythmias. Finally, some observations on the effects of exposure to CBrF$_3$ on the central nervous system were made.

The expanded series of experiments began with a study in which cardiac output and mean arterial blood pressure measurements were performed on anesthetized dogs during exposure to bromotrifluoromethane. In another set of experiments, left ventricular end diastolic pressure was recorded from anesthetized open-chested dogs and monkeys during exposure to CBrF$_3$. A significant decrease in peripheral vascular resistance (PVR) and myocardial contractility were shown to combine to produce a reversible hypotension during exposure to CBrF$_3$. Exposure to CBrF$_3$ apparently caused a relaxation of the resistance vessels which reduced the peripheral vascular resistance. Simultaneously the force of myocardial contraction decreased with the net effect that blood shifted from the arterial, or resistance, vessels to the venous, or capacitance, vessels. The increased venous distension eventually led to an increased venous return which was not accommodated by the weakened heart. Thus, no change in cardiac output
accompanied the fall in peripheral resistance and the left ventricular end diastolic pressure rose.

The next phase of the investigation was designed to provide more information on the change in peripheral vascular resistance. Arterial blood flow, left ventricular dp/dt, pressure, and end diastolic pressure, and arterial blood pressure were monitored in dogs before and after regional sympathetic neurectomy or alpha-adrenergic blockade during exposure to CBrF₃.

Exposure to CBrF₃ caused a marked increase in regional blood flow which was abolished by either sympathetic neurectomy or alpha-adrenergic blockade. This suggested that the decrease in PVR was the result of a decrease in sympathetic vasoconstrictor tone. A decrease in vasoconstrictor tone could be mediated through a decreased central sympathetic outflow, ganglionic blockade, alpha-adrenergic blockade, or altered presso- or chemoreceptor activity. The next experiment was designed to determine if ganglionic or alpha-adrenergic blockade contributed to the hypotensive effect of CBrF₃ exposure.

The vagosympathetic trunks of 5 dogs were severed. The central cut ends were stimulated electrically and the tension developed by the nictitating membrane was measured. The central arterial blood pressure was monitored during electrical stimulation of the peripheral cut end by which
means the degree of vagal inhibition of the heart could be ascertained. The blood pressure responses to the intravenous injection of acetylcholine and norepinephrine were likewise recorded. During exposure to 80% CBrF₃ nictitating membrane tension was significantly diminished but the response to norepinephrine was not. Vagal inhibition and the peripheral cholinergic vasodilator response were markedly diminished during the CBrF₃ exposures and it was concluded that CBrF₃ did not alter the response to adrenergic transmitter but did have mild anticholinergic and ganglionic-blocking effects. The ganglionic-blocking effect was assumed to be sufficient to account for the decreased peripheral sympathetic vasoconstrictor tone. The possibility was not excluded, however, that some degree of decrease of central sympathetic outflow also occurred.

The observations which were made in Chapter VI and information which is known about the effects of chemically related hydrocarbons and halogenated hydrocarbons suggest that most of the central nervous system effects of this class of compounds can be considered depressant. Since a considerable degree of central nervous system function depression was observed during exposure to CBrF₃ it is not unlikely that some decrease in sympathetic outflow under the control of the lower brain centers occurs. The element of ganglionic blockade is not unexpected since this effect has
been reported for halothane, a chemical congener of CBrF\(_3\), which has many pharmacologic properties similar to those of CBrF\(_3\). Finally, a significant degree of alpha-adrenergic blockade was not expected in view of the fact that the chemical structures of the class of compounds to which CBrF\(_3\) belongs differs markedly from the structures of the compounds which are known to possess any significant alpha-adrenergic blocking property. An alteration in presso- or chemoreceptor activity represents a distinct possibility as a mode of action of CBrF\(_3\). The studies conducted to this point, however, do not provide a basis for an evaluation of any possible contribution which this potential effect might have on the hemodynamic response to exposure to CBrF\(_3\).

The temporal sequence of the PVR and myocardial contractility changes was established. During the induction of the CBrF\(_3\) effect the PVR decreased first, followed by the dp/dt. During the recovery phase, postexposure, myocardial contractility increased first followed by PVR.

Some of the pharmacologic properties of CBrF\(_3\) bear a similarity to those of the anesthetic cyclopropane. The early work performed by Dresel and co-workers (1960, 1961) demonstrated that both a minimal blood pressure and a high level of circulating catecholamines were necessary to maintain ventricular ectopic cardiac rhythms. The next experiments were designed to test the hypothesis that CBrF\(_3\)
also required these conditions for the production of cardiac arrhythmias.

Monkeys were prepared in a manner which allowed control of the blood pH, $P_{\text{CO}_2}$, and $P_{\text{O}_2}$, and blood volume by which means the blood pressure could be mechanically regulated. Cardiac arrhythmias spontaneously appearing in monkeys exposed to CBrF$_3$ were found to require a minimal blood pressure threshold for their production. The blood pressure threshold varied as an inverse function of the log$_{10}$ of the CBrF$_3$ concentration to which monkeys in normal acid-base balance were exposed. Acidosis decreased the threshold and alkalosis increased the threshold at concentrations of 10% and 20% CBrF$_3$ but were without effect at 30% or greater concentrations.

Epinephrine was found to decrease the blood pressure threshold required to trigger arrhythmias but was not necessary for their production since arrhythmias appeared spontaneously in monkeys in which the endogenous stores of catecholamines had been depleted by pretreatment with large doses of reserpine.

The conclusion was drawn that the differences in individual susceptibility to the spontaneous formation of arrhythmias during exposure to CBrF$_3$ were the result of differences in individual ability to maintain blood pressure during the exposure.
Preliminary considerations of the central nervous system effects of CBrF$_3$ were based on the few behavioral observations mentioned earlier and changes in the electroencephalogram observed during exposure. Conscious mechanically restrained dogs and monkeys were exposed to 80% CBrF$_3$ for periods ranging from 10 to 60 minutes and their outward behavior observed. Conscious dogs and monkeys restrained with tubocurarine were exposed to 70% or 80% CBrF$_3$ and the EEG's recorded. Such recordings were made during the induction of the CBrF$_3$ effect and during sensory stimulation. EEG's recorded during CBrF$_3$ exposure were compared with corresponding air-breathing controls and animals exposed to 1% trifluorobromochloromethane (halothane, a chemical congener of CBrF$_3$ with some similar pharmacologic properties).

The most significant findings were 1) dominance of the EEG by 6-9 Hz waves beginning 2-3 minutes after beginning exposure to CBrF$_3$, and 2) a nearly normal susceptibility of the EEG to activation by auditory and photic stimuli during exposure to CBrF$_3$. One line of reasoning which was explored led to the suggestion that CBrF$_3$ acted at the rhinencephalic level. No electrographic correlates to the motor phenomena observed in dogs could be elicited in susceptible dogs which led to the suggestion that rather than representing convulsions the motor disturbances could be a form of
rigidity resulting from a decrease of inhibition of spinal reflexes.

The final aspect of the investigation of the pharmacologic properties of CBrF₃ reported herein dealt with the accumulation by brain and heart tissue of CBrF₃ during brief exposures. It was noted earlier that following a 5-minute exposure of dogs to 80% CBrF₃ a return of normal myocardial contractility preceded by about 2-3 minutes the return of vasomotor tone. This was expected to be a function, in part, of a difference in uptake and washout of CBrF₃ based on the fact that the solubility in heptane of CBrF₃ exceeds that of water by 10 times. Because of this partition coefficient CBrF₃ was expected to be more soluble in nervous tissue with its high lipid content than in myocardial tissue with its relatively low lipid content.

Ten groups of rats were exposed to CBrF₃ for 5 minutes each and sampled serially throughout the exposure and postexposure periods. Brains and hearts were collected and analyzed for their content of CBrF₃. The brain was shown to accumulate about 50% more CBrF₃ per gram of tissue and do so at a greater rate than the heart. The rates of washout were, however, comparable, the brain requiring about 5 minutes longer than the heart to reach very low concentrations of CBrF₃ because of the higher concentration at the beginning of the washout. If one were to make the questionable
assumption (for the sake of discussion) that the brain and heart tissues had equivalent susceptibilities to the effects of equal concentrations of CBrF$_3$ the greater concentration of CBrF$_3$ which persisted for a longer time postexposure in the brain as compared to the heart would be consistent with the observation that the recovery of the possibly partially CNS-mediated vasomotor tone trails by about 2-3 minutes the recovery of myocardial contractility.

A brief diagrammatic summary of CBrF$_3$ effects is presented in Figure 37. Exposure to CBrF$_3$ introduces the chemical into the pulmonary arterial blood and from there it flows through the left heart (H) and aorta (A). Part of the aortic blood is diverted through the coronary circulation resulting in the perfusion of the myocardium with CBrF$_3$. If the amount of CBrF$_3$ delivered to the myocardium is sufficient (coronary perfusion rate x CBrF$_3$ conc) ectopic pacemaker formation results. The decreased dynamic efficiency of the heart which results from the arrhythmia and a negative inotropic effect (decreased myocardial contractility) of the CBrF$_3$ cause the blood pressure (BP) to fall. As the remainder of the body is perfused with CBrF$_3$ the mechanisms controlling the caliber of the vascular resistance vessels (R) are affected with the result that the resistance to the flow of blood from the arterial system is decreased which contributes to a further decrease in blood pressure.
FIGURE 37. Summary of some of the pharmacologic effects of exposure to CBrF$_3$ (see text for explanation).
NEGATIVE INOTROPIC EFFECT

HIGH PRESSURE SMALL VOLUME
LOW PRESSURE HIGH VOLUME

ECTOPIC FOCI FORMATION
NEGATIVE FEEDBACK LOOP

LVEDP
VR
BP

EEG

SC

I?
Inhibition of vasomotor tone may take place at the lower brain level (vasomotor center, VC), at the ganglionic level (sympathetic chain, SC), or at the myoneural junction (alpha-adrenergic receptor, alpha). CBrF₃ has been shown to induce a limited degree of ganglionic-blockade. When the resistance to flow from a high pressure system to a low pressure system is decreased the flow is increased down the pressure gradient with the result that the pressure in the high pressure side falls and the pressure in the low pressure side rises. Ordinarily a normal heart responds to the increased filling pressure (venous return, VR) with an increased output, but the heart, temporarily impaired by the presence of CBrF₃, is unable to accommodate the increased venous return and a new steady state is established which is characterized by an elevated pressure (left ventricular end diastolic pressure (LVEDP)). An interesting negative feedback loop exists in relation to the ectopic pacemaker formation. Since the ectopic pacemaker formation is blood-pressure dependent the blood pressure fall during CBrF₃ is frequently sufficient to result in a spontaneous abolition of the arrhythmias after they have developed.

The electroencephalogram (EEG) contains elements generated at all levels of the brain (B). The waking electrocorticogram is characterized by relatively higher frequencies and lack of synchronization whereas electrograms
of the activity of deeper structures are often characterized by lower frequencies and a greater degree of synchronization. The EEG recorded with surface electrodes is primarily an electrocorticogram which is changed in its characteristics with varying cortical influences such as depression with chemicals and assumes more of the characteristics of the electrogram recorded at other levels. Some levels of the brain (i.e., reticular activating system) are more sensitive than others (i.e., lower brain stem) to the effects of most chemical depressants. The alteration in the character of the EEG during exposure to CBrF₃ is characteristic of classes of compounds that depress the reticular activating system thus allowing slower, synchronized rhythmic activity to dominate the electrogram. The depression is not of a degree sufficient to induce unconsciousness, however, since stimulation (S) via any of the three modalities (tactile, visual, auditory) causes activation of the EEG as in the untreated individual.
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