Short Duration High-Level Exposure to Halon Substitutes: Potential Cardiovascular Effects

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The main outline of the cardiac sensitization protocols had been developed and explored within a time period of approximately three years in four basic publications. [Cardiac Arrhythmias and Aerosol **"Sniffing"**, Reinhardt et al Arch Environ Health 22, **265-279, (1971);** Halogenated Hydrocarbon-Induced Cardiac Arrhythmias Associated with Release of Endogenous Epinephrine, Mullin et al Amer. Ind. Hyg. Assoc. J. 33, **389-396, (1972);** The Pharmacoloaic Actions of Bromochlorodifluoromethane (BCF), Beck et al Toxicol. Appl. Pharmacol. 24, **20-29 (1973);** and Correlation of the cardiac sensitizing potential of halogenated hydrocarbons with their physicochemical properties, Clark, D.G. and Tinston D.J., Brit. J. Pharmacol 49, **355-357** (1973)]. Since the publication of these papers variations on the basic protocol have been made. Notably, changes were made to the protocol in the administration of epinephrine. Administration of the exogenous epinephrine is designed to achieve a threshold for epinephrine induced pacemaker activity in extra conduction tissue in the heart but the rationale for changes in the basic protocol are not always clear. Although the early papers raised concerns about the variability in the test

procedures, the risk assessment issues arising from the variability in the testing procedures have not been examined in a rigorous manner.

The purpose of this presentation is to show the variations in the testing protocols and to indicate how the EPA is using these varied protocols in its risk assessments of the halon alternatives.

In order for muscle tissue to contract, there must first occur a stimulus, usually in the form of an electric current generated by a nerve. Heart muscle also contracts following stimulus by an electric current but the current is not generated by a nerve. Instead, the heart muscle contracts, that is, beats, in a rhythmic manner orchestrated by an impulse generated intrinsically by certain specialized heart muscle tissue, the stimulus tissue. This stimulus tissue (generally noted as the conductive system) is distributed anatomically in a unique manner through the heart chambers. It is capable of carrying an electric current through the heart muscle in an organized manner. Different parts of the heart stimulus tissue generate an intrinsic impulse at different times. The stimulus tissue, which can become excited within the shortest time interval is the tissue in the right upper chamber, the right atrium, and thus this tissue is the normal pacemaker for the remaining parts of the heart. This self-excitatory tissue in the right atrium is called the SA node. Figures I and II illustrate the spread of the cardiac impulse (electric current) through the heart and indicate the normal delay in the speed of

travel of the impulses. This regular delay assures that sufficient time elapses for the heart chambers to fill with blood before the chambers contract and send blood through the vascular system.

All heart muscle tissue can act as its own pacemaker, however, and under certain circumstances contractions of the heart may occur prematurely, that is before the usual cardiac rhythm. This may be due to patches of heart muscle tissue emitting abnormal impulses, ectopic foci, perhaps from local lack of oxygen in the heart, due to drugs such as caffeine, or from emotional excitation including fright. Premature contractions can occur in the atria (the upper chambers) or in the ventricles (the lower chambers). Some premature ventricular contractions in normal persons may be due to lack of sleep, too much caffeine, smoking or even emotional excitement. Most premature ventricular contractions are not benign, and may be due to various stages of ischemia or other pathology (Guyton, Arthur C., in Textbook of Medical Physiology, 7th Ed. Philadelphia, Pa. W.B. Saunders Co. 1986). Figure III illustrates the effect of an ectopic focus, resulting in a strong contraction following the ectopic event. Figure IV (from Reinhardt et al) shows a series of electrocardiographs in the dog ranging from the normal pattern, to isolated abnormal beats, multiple ventricular beats and finally multiple ventricular beats preceding ventricular fibrillation.

What does epinephrine do to the heart? Epinephrine will cause the heart to beat faster, and with greater strength of contraction

resulting in greater cardiac output. Epinephrine can also cause isolated premature ventricular contractions in the normal healthy heart. The higher the dose of epinephrine, the greater the likelihood of premature contractions.

Cardiac sensitization is the ability of a compound to increase the reactivity of the mammalian heart to epinephrine, resulting in serious and sometimes fatal cardiac arrhythmias. This definition is generally accepted although in fact the action of the agent may be to inhibit the normal conduction tissue and thereby enable ectopic foci to exert pacemaker activity. Cardiac sensitization is evaluated by exposing animals to an agent by inhalation in the presence of epinephrine. The effects on the heart are noted by electrocardiography.

Some sources of variability in the quantitative determination of cardiac sensitization are a consequence of the experimental design; some sources of variability are a consequence of the animal model. Regarding the experimental design, for example, the criteria for adversity of effect is poorly defined.

The following criteria for cardiac sensitization are abstracted from a number of different studies:

1. The appearance of more **serious arrhythmias such as multifocal ventricular ectopic beats or ventricular fibrillation** [Brit. **J.** Pharmacol. **49, 255-257, (1973)].

2. **Ventricular tachycardia or ventricular fibrillation** resulting from the challenge injection of epinephrine. An increased number of ventricular beats was **not regarded** as evidence of cardiac sensitization since increased ventricular beats often occurred following the epinephrine injection in the absence of the 'sensitizing' agent. [Toxicol. Appl. Pharmacol. **24**, 20-29, **(1973)**].

3. Cardiac sensitization that resulted in ventricular tachycardia was characterized by an **increased heart rate** followed by **one or two** ventricular ectopic beats, then a **5 or 6 second run of multifocal ventricular ectopic beats** before the ECG returned to normal. The events leading to **ventricular fibrillation** were similar, but after 1 **or 2 seconds of ventricular tachycardia, ventricular fibrillation** occurred. [Human Toxicol. 1, **239-247**, (1982)3.

4. [An arrhythmia] **...** which was not observed following the control injection of epinephrine and which **...** [poses] a serious threat to life **(multiple consecutive ventricular beats)** or resulted in cardiac arrest **(ventricular fibrillation).** [Amer. Ind. Hyg. Assoc. J. **40**, 653-658, **(1979)**].

5.A **"...marked response"** recorded when an arrhythmia developed which was considered to pose a serious threat

to life (multiple consecutive ventricular beats) or which ended in cardiac arrest (ventricularfibrillation) [Arch. Environ. Health 22, 265-279, (1971)].

6. More than 3 premature ventricular contractions per 15 seconds (1992 unpublished study).

7. Ventricular fibrillation or more than 5 multifocal beats (Studies on the acute effects of a number of HFCs. *An* Abstract for a Presentation 1992, Collins, Tinston and Hardy).

Most investigators agree that an increased number of ectopic beats is not sufficient evidence of cardiac sensitization, since they can be produced by injection of epinephrine alone. Thus, the criteria for evaluating a positive response and the degree of adversity are poorly defined.

Additionally there are differences in epinephrine dose regimen as well as differences in the length of exposure time to the agent. Still other sources of variability can be found in the differing laboratory settings and procedures. Some laboratories utilize a face mask for exposures, others prefer whole body exposure. Some laboratories use indwelling catheters for delivery of epinephrine dose. Some use interval phlebotomy. There are sources of variability due to the required training of dogs (the preferred animal species) to accept ECG measurements and epinephrine dosing.

It is difficult to estimate the degree of difference in response which may result from such variations.

Finally, the small number of animals used, typically 6 to 12, usually acting as their own controls suggests that biological variability may be great.

The basic protocol for measurement of cardiac sensitization is conducted in the following manner. [See Figure V; Reinhardt et al (1971)]. Healthy male beagle dogs 2-3 years old are trained to maintain a standing position while lightly supported by a cloth sling, to wear a nose mask and accept venipuncture, and to become accustomed to ECG monitoring. At T_0, the ECG is started; at T_0 +2 min an epinephrine dose of 8ug/kg/1 ml saline, is delivered intravenously (IV) in 9 seconds; at T_0 + 7 min exposure to the test compound is started; at T_0 + 12 min a second challenge dose of epinephrine at 8ug/kg/1 ml saline is administered IV in 9 seconds; at T_0 + 17 min exposure to the test compound and the ECG recording are stopped; end of experiment.

Figures VI through X diagram other variants of the basic cardiac sensitization protocol. As can be seen from these illustrations, there are definite differences in the epinephrine dosing regimens. In some protocols an individual effective dose is established for each animal, whereas in other protocols the same dose of epinephrine is administered to each animal. This is likely the most significant variation in the epinephrine dose scheme. Table

I lists the main points related to the different epinephrine dosage regimens.

No systematic analysis has been published of the possible differences expected in measured endpoints if the same chemical were tested while varying the protocol, although Collins et al in "Studies on the acute toxicity of a number of hydrofluorocarbons", Toxicol. Lett. (supp 1992) p86 have done experimental work to find the most appropriate individual animal epinephrine dose. The degree of biological variability has not been established nor have other differences in each protocol design been standardized.

EPA is basing its risk assessment/risk management decisions for alternative total flooding agents upon previous decisions in the OSHA standards (CFR 1910.162) arising from the cardiac sensitization of Halon 1301, viz. if personnel can egress in less than 1 minute, a flooding system can be designed up to the Lowest Observed Adverse Effect Level (LOAEL) for cardiac sensitization; if personnel cannot egress in 1 minute, the system may only be designed to the No Observed Adverse Effect Level (NOAEL) for cardiac sensitization. Although EPA is still basing some decisions on cardiac sensitization studies utilizing early protocols, it is clear that the later protocols (see Figure IX) which vary the epinephrine dose to achieve maximum sensitivity are likely to yield more rigorous results.

Spread of Cardiac Impulse Through the Heart

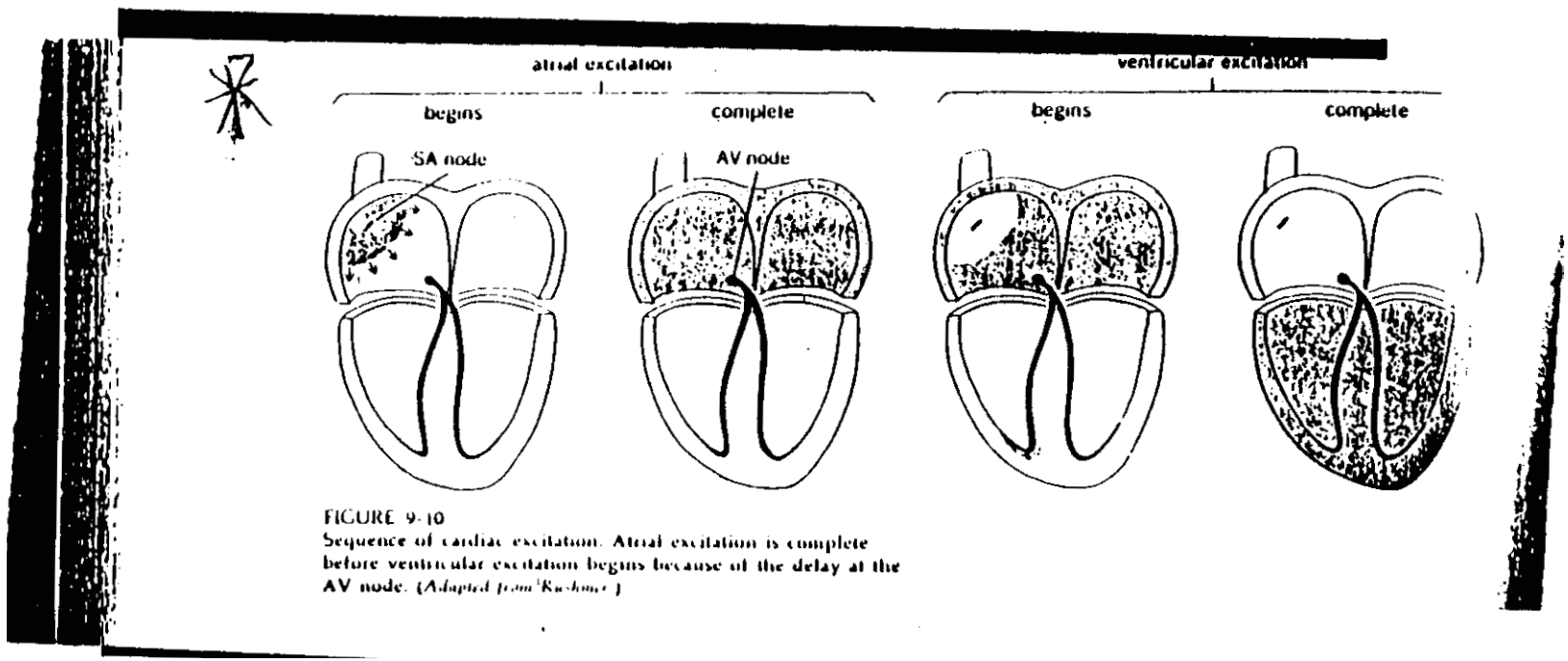


FIGURE 9-10
Sequence of cardiac excitation. Atrial excitation is complete before ventricular excitation begins because of the delay at the AV node. (*Adapted from* Kuchner.)

Figure I Vander, A. J., Sherman, J. H., and Luciano, D. S. Human Physiology: The Mechanisms of Body Function, Second Ed. New York, N. Y., Mcgrow-Hill Book Co. 1975

Spread of Cardiac Impulse Through the Heart

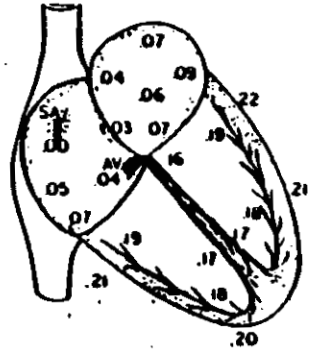
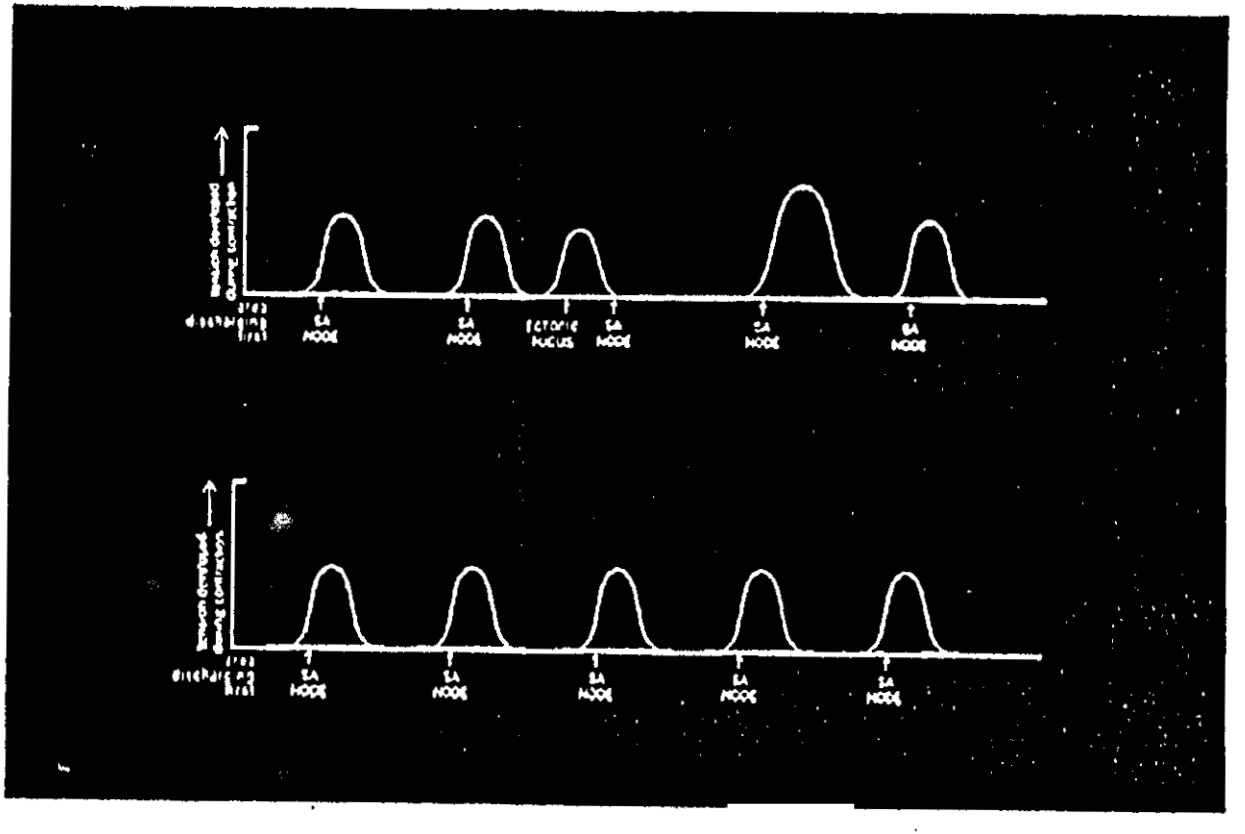


FIGURE 12-3 Transmission of the cardiac impulse through the heart, showing the time of appearance (in fractions of a second) of the impulse in different parts of the heart.

Figure II Guyton, A. C., Basic Human Physiology: Normal Function and Mechanisms of Disease, Philadelphia, PA, W. B. Saunders Co., 1971

ECG of Normal vs. Ectopic Heartbeat



(Vander, Sherman, and Luciano, 1975)

Figure III

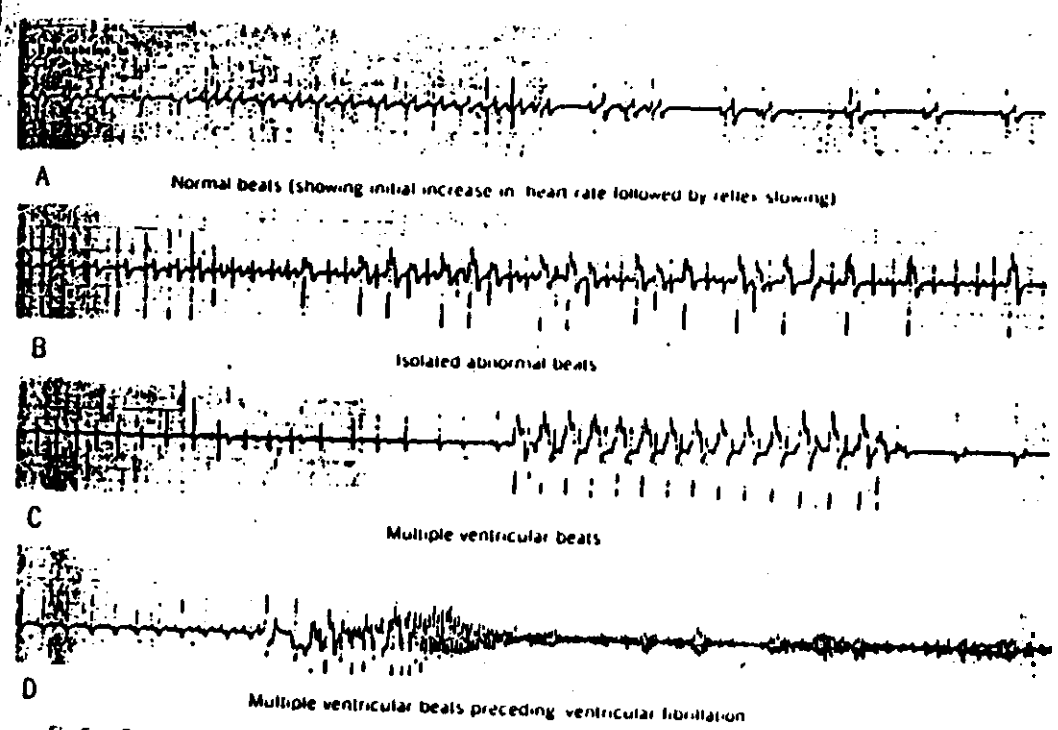
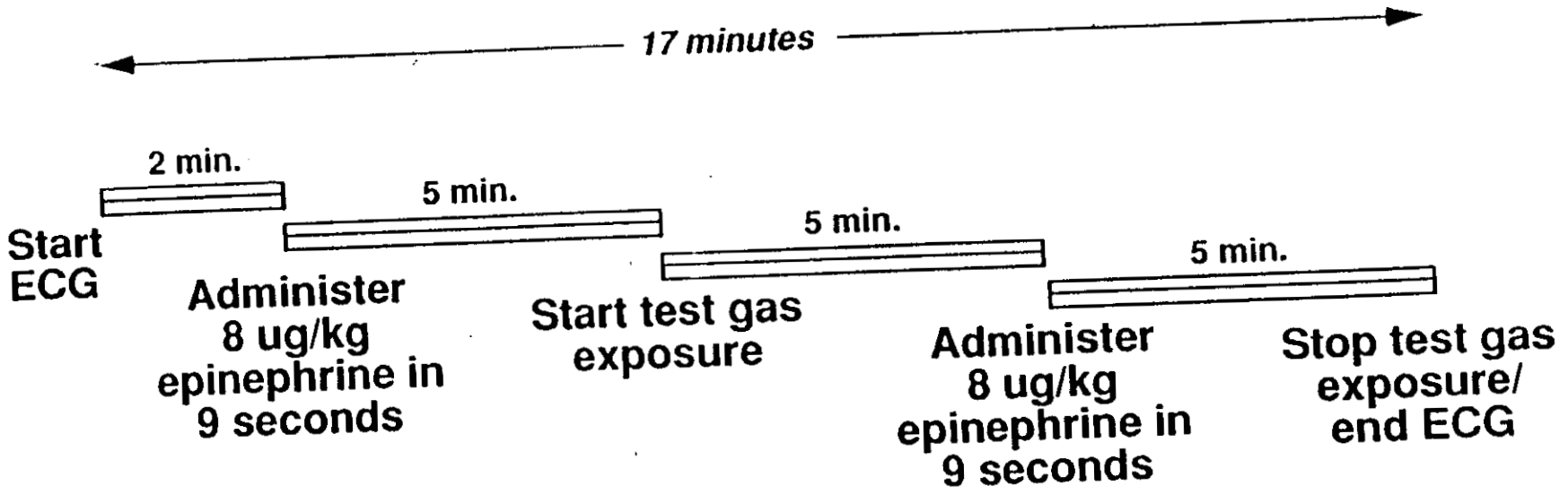


Fig 5.—Examples of electrocardiographic patterns recorded following challenge injection of epinephrine.

Figure IV

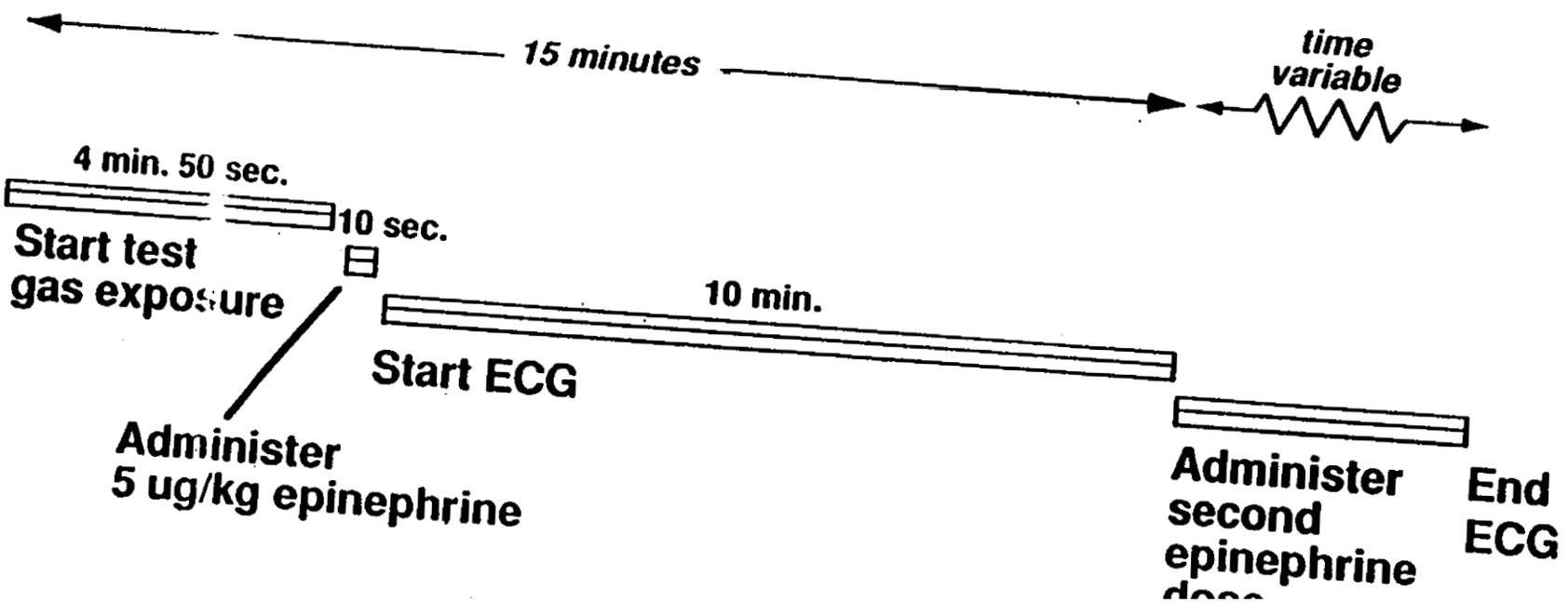
Basic Cardiac Sensitization Protocol



(Reinhardt et al., 1971)

Figure V

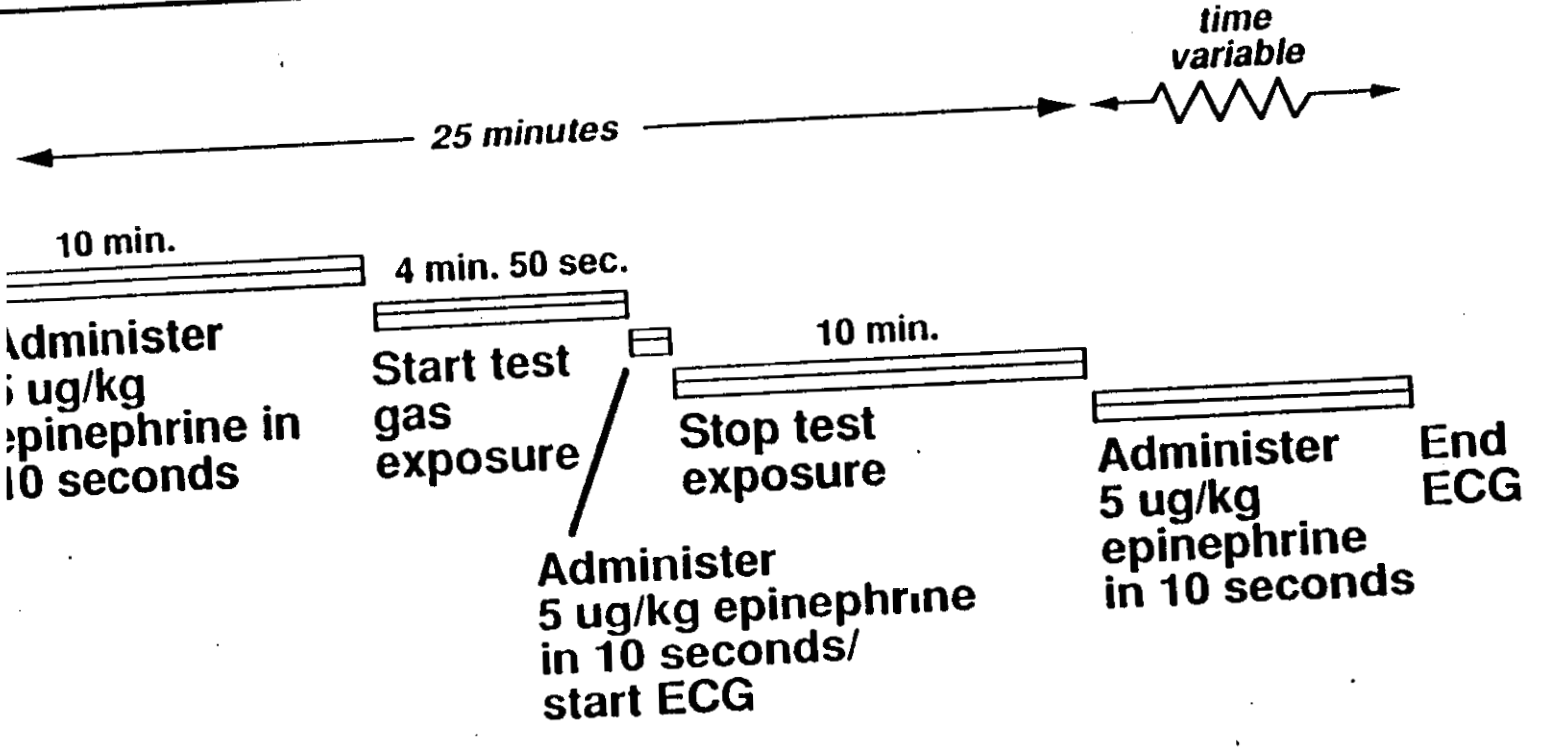
Variation of Cardiac Sensitization Protocol



(Clark and Tinston, 1973)

Figure VI

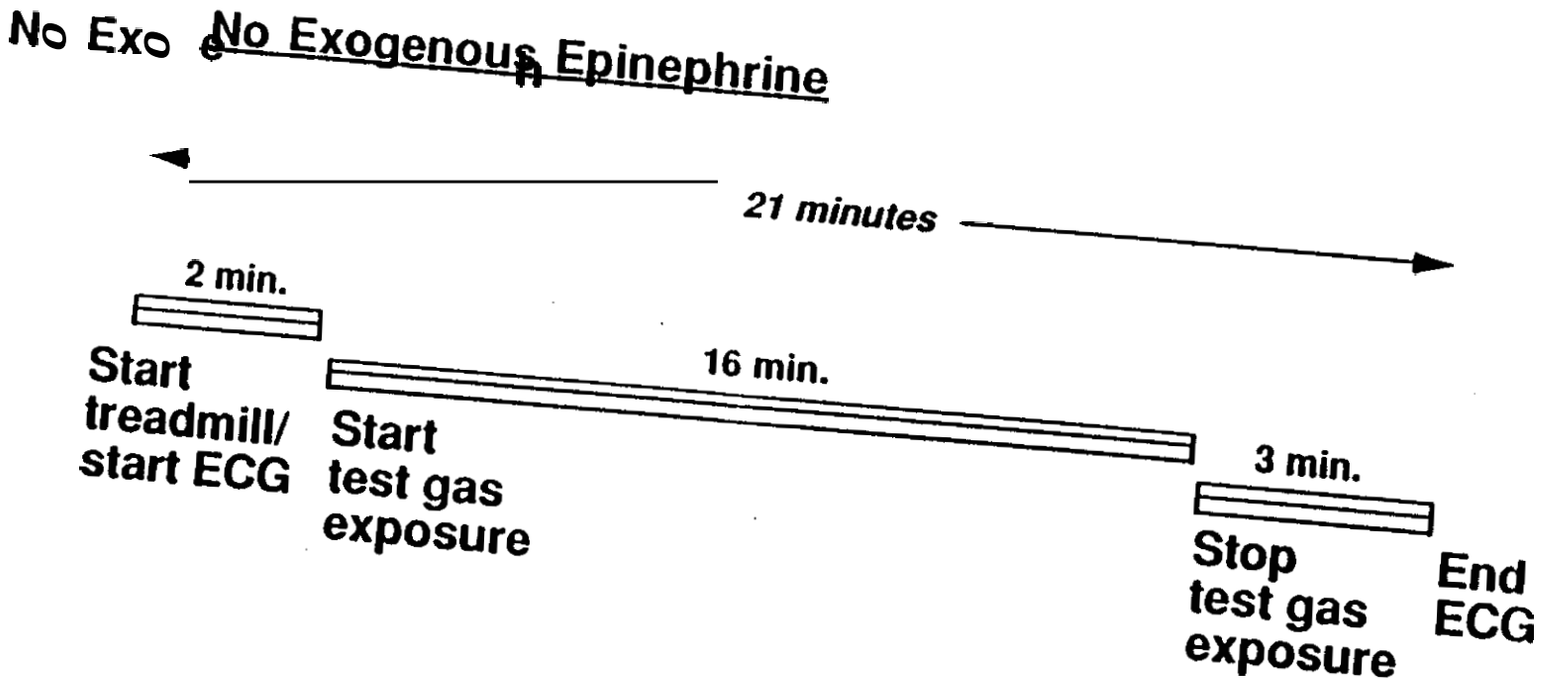
Variation of Cardiac Sensitization Protocol



(Beck, Clark, and Tinston, 1973)

Figure VII

Variation of Cardiac Sensitization Protocol



(Mullin et al., 1972)

Figu e VIII

Variation of Cardiac Sensitization Protocol

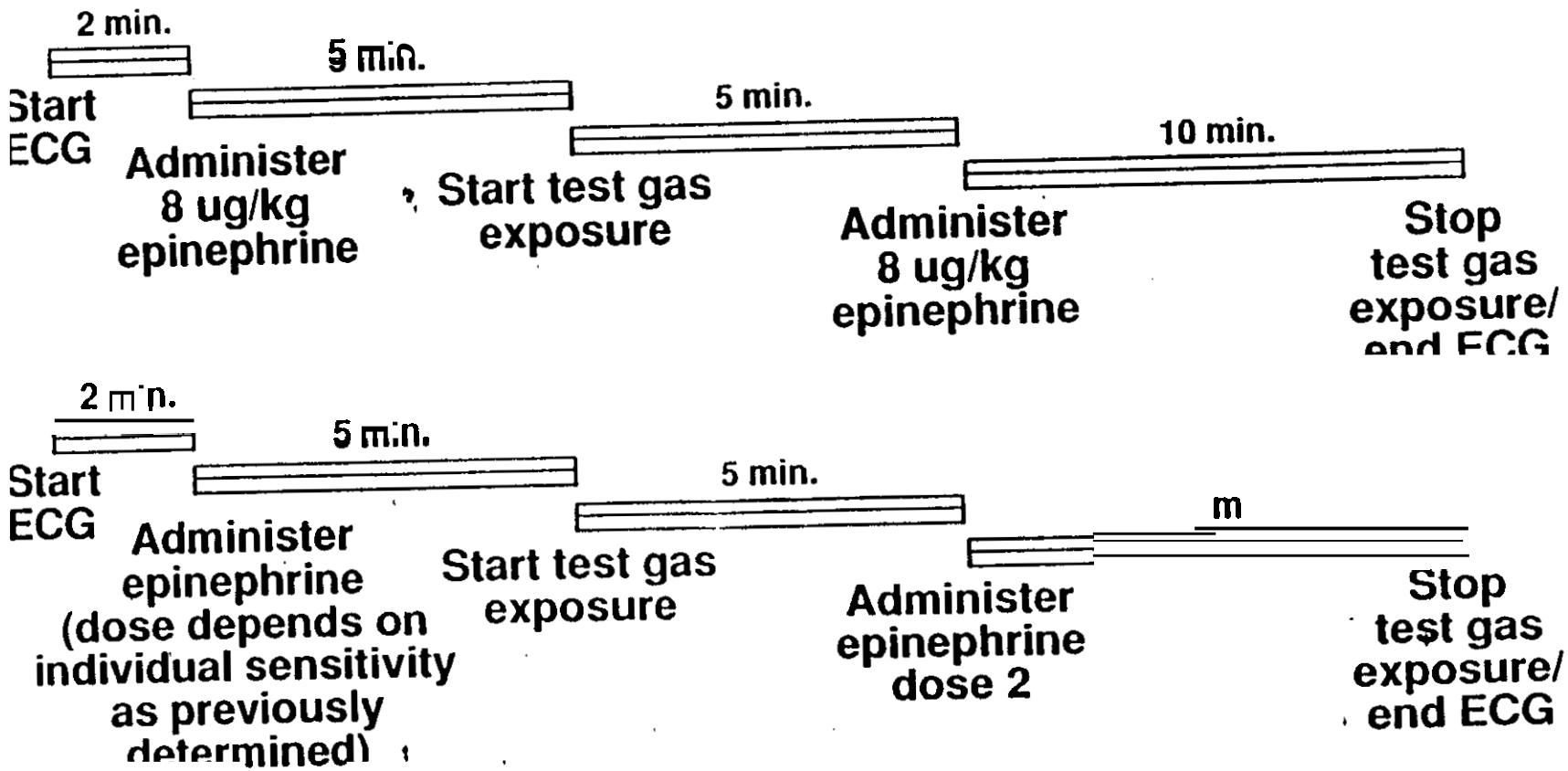
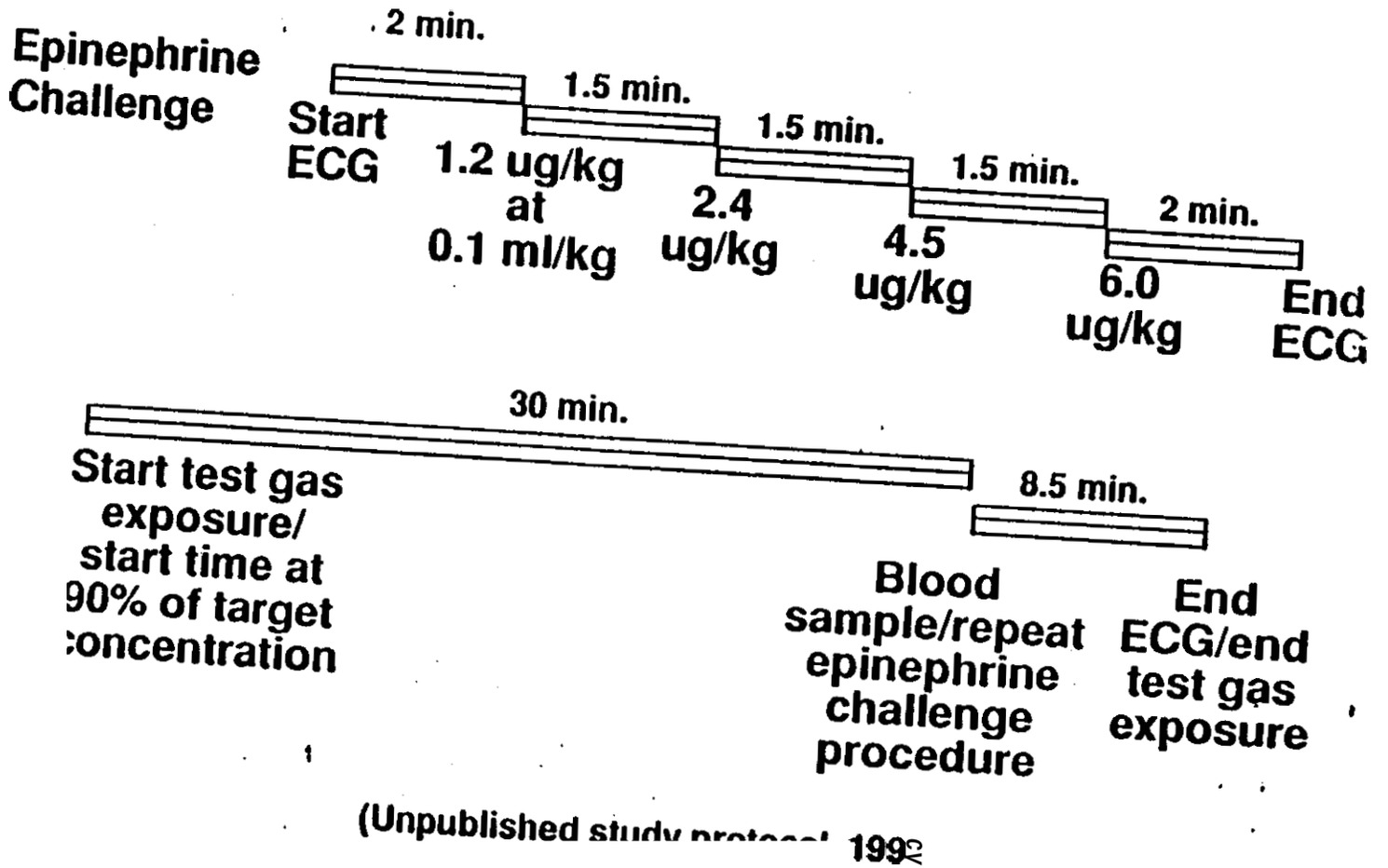


Figure IX (Collins, Tinston and Hardy, 1992)

Variation of Cardiac Sensitization Protocol



(Unpublished study protocol, 199[illegible]

Figure X

Differences in Epinephrine Dosing Regimens

- 2–65 ug/kg given over 10–40 seconds
- Same dose for all animals
  - 5 ug/kg in 0.1 ml/kg
  - 8 ug/kg in 1 ml (about 0.1 ml/kg) in 9 seconds
  - 10 ug/kg (volume and time not specified)
  - 14.4 ug/kg (volume and time not specified)
- Establish effective dose for individual animals