Journal of Anesthesia History 2 (2016) 85–88

Contents lists available at ScienceDirect

Journal of Anesthesia History

journal homepage: www.anesthesiahistoryjournal.org

Review

Noteworthy Chemistry of Chloroform

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ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form 14 April 2016
Accepted 19 April 2016

Keywords:
Claude Bernard
Robert M. Glover
James Tayloe Gwathmey
Methionine synthase
Phosgene
James Young Simpson
John Snow
Horace Wells
Vitamin B12

ABSTRACT

Inhaled chloroform anesthesia was introduced in 1847. Soon thereafter, the chemical reactivity of aerobically heated chloroform permitted John Snow and Claude Bernard to do seminal experiments in the assay of drug levels and drug metabolism. However, it was not widely appreciated until a clinical mishap in 1899 that thermal decomposition generated significant levels of toxic phosgene from air-polluting quantities of chloroform in poorly ventilated operating rooms that were illuminated by flames. Phosgene is also generated metabolically from chloroform. A clue appeared in the 1950s when subanesthetic traces of inhaled chloroform proved accidentally lethal to strains of male mice spontaneously expressing high levels of chloroform-metabolizing enzymes. Furthermore, in microbial experiments of 1967, the reactive chloroform molecule was inadvertently discovered to selectively inactivate vitamin B12-dependent enzymes. Chloroform can also activate enzymes. As a solvent, it was serendipitously found in 1903 to activate what is now known as plasminogen to plasmin.

Introduction

Chloroform is an artificial molecule that was unknown until the 1830s. James Young Simpson (1811-1870), a Scottish obstetrician, introduced inhaled chloroform anesthesia in 1847. Structurally useful atomic weights were not established until 1858, when Italian chemist Stanislao Cannizzaro (1826-1910) argued convincingly that the atomic weight of carbon is 12 g/mol and not 6 as had been widely supposed. Because of the earlier value, Simpson thought that chloroform has C_2HCl_3 as its composition, whereas the molecule actually has but 1 carbon atom.[1] Theoretical understanding of molecular structure and reactivity was seriously impeded during the times during which the numbers of carbon atoms in any organic molecules were not correctly known.[2] Even afterward, the chemistry of chloroform had surprising and noteworthy historical features. Examples to be discussed here include the accidental discovery in 1899 that flames used for illumination of operating areas tended to release poisonous gas from air-polluting levels of chloroform. That gas, phosgene, was eventually found to be a metabolic product of chloroform. The deleterious metabolism became a landmark in pharmacogenetics in the 1950s when certain strains of laboratory mice were accidentally found to be exquisitely sensitive to lethal harm from metabolism of laboratory-polluting levels of chloroform. Furthermore, a microbiology experiment accidentally revealed in 1967 that chloroform reacts with vitamin B12.

Assay of Chloroform

Both chloroform and sulphuric ether were synthesized from ethanol,[3] but chloroform is relatively nonflammable with respect to the other two molecules. Although chloroform does not burst into flames, it is unstable at flame temperatures, and its thermal decomposition produces hydrochloric acid (HCl). The volatility and instability of chloroform permitted assay of the drug in biological fluids. An English physician, scientist, epidemiologist, and anesthetist to the Queen, John Snow (1813-1858) performed seminal experiments in this regard.

For instance, Snow inhaled chloroform and then identified the molecule in his exhaled breath by blowing through a flame-heated glass tube.[4] He found that the gas exiting the heated tube was strongly acidic with respect to colored pH indicators. Furthermore, the HCl-containing gas would precipitate insoluble silver chloride salt from solutions of silver nitrate. The weight of silver chloride was a quantitative indication of the weight of exhaled chloroform.

This silver nitrate assay was used by French physiologist and pharmacologist Claude Bernard (1813-1878) and others to look for chloroform in blood and urine. Bernard was a pioneer in elucidating molecular mechanisms of bioactive molecules, including curare,

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http://dx.doi.org/10.1016/j.janh.2016.04.008
2352-4529/

carbon monoxide, and inhaled anesthetics.[5] In Lecture 2 of his 1875 book *On Anesthetics*, Bernard explained that air was passed through warmed blood or urine samples, then through a flame-heated glass tube, and finally through silver nitrate solution (Figure 1).[5] Interestingly, urine was negative for chloroform after brief anesthetics. Although blood was fully expected to contain inhaled chloroform, Bernard felt it was important to actually demonstrate so. He explained:

> *It is a general principle of physiology that when any substance acts on the organism it has to act through the blood. Nevertheless this principle has been challenged for chloroform and ether. It has been contended that these anesthetics do not have to enter the blood to produce unconsciousness. Dr. Faure is of this opinion and hypothesizes that the anesthetic acts on the vagus nerve endings where it produces a kind of asphyxial stimulation.*[5]

Bernard added that Faure had done some rabbit experiments in which gas leaking around a tracheal tube had given the misimpression.

In Lecture 10, Bernard discussed the use of the assay system to determine whether the hypnotic drug chloral hydrate, CCl_3-$CH(OH)_2$, acts through its conversion to chloroform, CCl_3-H, in the body.[5] The assay results were equivocal because chloral hydrate is slightly volatile from warm blood and decomposes at flame heat. However, the experiments foretold the now-routine science of the chemical analysis of drug levels and drug metabolism.

Phosgene

Even though chemists including Snow were aware that chloroform decomposes at flame temperatures, clinicians learned the hard way that chloroform anesthesia was dangerous in the vicinity of lit candles and other flames. Unlike sulfuric ether, chloroform does not support fire, but it does liberate toxic fumes. Near flames, chloroform was dangerous to everyone in the operating room or maybe in the field shelter.[6] In 1899, an event occurred near Boston, MA.[7] Two physicians and a nurse were attending a forceps obstetrical delivery with the aid of chloroform in a small room lit by three gas flames. The three, but not the anesthetized patient, experienced a "choking, stinging, irritating sensation in the throat and chest, resulting in incessant coughing and gasping for breath." After inferring that there was a problem with the chloroform, they subsequently ventilated the room and switched to ether. Kenelm Winslow of Newton, MA, was the reporting physician. He wrote:

> *A recent experience seems worthy of notice, not on account of its extreme rarity but rather because a similar incident might befall any practitioner, and because I believe that most well-informed physicians are unaware of the reason for, or possibility of, such an occurrence.*[7]

He admitted that, at the time of the event, he was "included in the ignorant majority." The problem of toxic fumes arising from chloroform near flames was not clinically appreciated in the earliest decades of chloroform anesthesia. Winslow found a few prior reports, but the first was dated 1889.

Aerobically, a particularly noxious thermal decomposition product is phosgene, Cl-CO-Cl, a potent substance that was applied in gas warfare in the 20th century (Figure 2).[8] Phosgene is a so-called acid anhydride and releases hydrochloric acid on contact with moisture. However, it is not merely an acid-generating compound. It is also capable of covalently modifying proteins and other biomolecules. For instance, two amino groups ($R\text{-}NH_2$) can become cross-linked as a ureido group (R-NH-CO-NH-R).[9,10]

Metabolism-Dependent Toxicity and Pharmacogenetics

Phosgene is also a metabolic product of inhaled chloroform, and liver and kidney damage can ensue from its production. Anesthetic doses of chloroform readily elicit liver injury in diverse laboratory animals.[11,12] However, the males of some strains of mice are exquisitely sensitive to air pollution levels of chloroform. The phenomenon was independently discovered in several laboratories in which mice were accidentally exposed to faint fumes.[13–15] Males, but not females, succumbed to chloroform-induced renal failure, and testosterone proved necessary for the effect.[16,17] The major mechanism involves the renal conversion of chloroform to phosgene in a reaction catalyzed by a testosterone-induced cytochrome P-450 isozyme.[18,19] Along with succinylcholine,[20] then, chloroform provided a dramatic example of pharmacogenetics in drug action and safety.

Antivitamin Action

In 1967, American microbiologist Thomas Bauchop[21] was studying methane biosynthesis in flasks containing microbe-rich juice

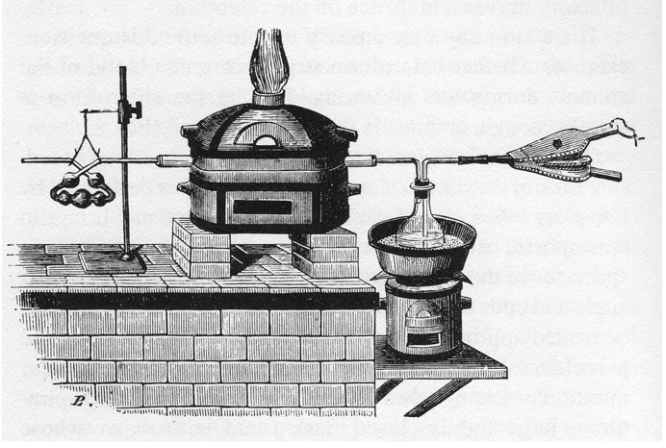

Fig. 1. Apparatus for the detection of chloroform.[5] A bellows on the right propels air through the sample in the warmed flask. The air extracts any volatile chloroform and carries it through a flame-heated length of tubing. Chloroform breaks down and releases HCl and Cl-CO-Cl in the hot tube. Those molecules release chloride ions when they are bubbled through the five-chambered tubing on the left. The multichambered tube contains a solution of $Ag(NO_3)_2$ in water. Chloride ions precipitate out of solution in the form of solid $AgCl_2$. The silver chloride is collected, and its weight corresponds to that of detected chloroform.