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POSTMORTEM ABOUT AMYGDALIN

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ABSTRACT

Amygdalin (from Ancient Greek: ἀμυγδαλή *amygdalē* "almond") is a naturally occurring chemical compound found in many plants, most notably in the seeds (kernels) of apricots, bitter almonds, apples, peaches, cherries, and plums. Amygdalin is classified as a cyanogenic glycoside because each amygdalin molecule includes a nitrile group, which can be released as the toxic cyanide anion by the action of a beta-glucosidase. Eating amygdalin will cause it to release cyanide in the human body, and may lead to cyanide poisoning. Since the early 1950s, both amygdalin and a chemical derivative named *Laetrile* have been promoted as alternative cancer treatments, often under the misnomer vitamin B_{17} (neither amygdalin nor Laetrile is a vitamin). Scientific study has found them to be clinically ineffective in treating cancer, as well as potentially toxic or lethal when taken by mouth due to cyanide poisoning. The promotion of Laetrile to treat cancer has been described in the medical literature as a canonical example of quackery, and as "the slickest, most sophisticated, and certainly the most remunerative cancer quack promotion in medical history".

KEYWORDS: Amygdalin, Toxicity, Cyanide Toxicity Effect.

Chemistry

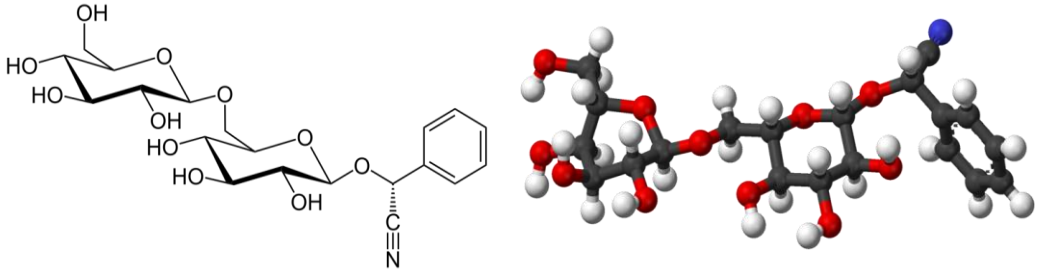


Figure-1: Amygdalin.

Amygdalin is a cyanogenic glycoside derived from the aromatic amino acid phenylalanine. Amygdalin and prunasin are common among plants of the family Rosaceae, particularly the genus *Prunus*, Poaceae (grasses), Fabaceae (legumes), and in other food plants, including flaxseed and manioc. Within these plants, amygdalin and the enzymes necessary to hydrolyze it are stored in separate locations so that they will mix in response to tissue damage. This provides a natural defense system.[1]

Amygdalin is contained in stone fruit kernels, such as almonds, apricot (14 g/kg), peach (6.8 g/kg), and plum (4–17.5 g/kg depending on variety), and also in the seeds of the apple (3 g/kg). Benzaldehyde released from amygdalin provides a bitter flavor. Because of a difference in a recessive gene called *Sweet kernal [Sk]*, less amygdalin is present in nonbitter (or sweet) almond than bitter almond. In one study, bitter almond amygdalin concentrations ranged from 33 to 54 g/kg depending on variety; semibitter varieties averaged 1 g/kg and sweet varieties averaged 0.063 g/kg with

significant variability based on variety and growing region.

For one method of isolating amygdalin, the stones are removed from the fruit and cracked to obtain the kernels, which are dried in the sun or in ovens. The kernels are boiled in ethanol; on evaporation of the solution and the addition of diethyl ether, amygdalin is precipitated as minute white crystals. Natural amygdalin has the (*R*)-configuration at the chiral phenyl center. Under mild basic conditions, this stereogenic center isomerizes; the (*S*)-epimer is called neoamygdalin. Although the synthesized version of amygdalin is the (*R*)-epimer, the stereogenic center attached to the nitrile and phenyl groups easily epimerizes if the manufacturer does not store the compound correctly. Amygdalin is hydrolysed by intestinal β-glucosidase (emulsin) and amygdalin beta-glucosidase (amygdalase) to give gentiobiose and L-mandelonitrile. Gentiobiose is further hydrolyzed to give glucose, whereas mandelonitrile (the cyanohydrin of benzaldehyde) decomposes to give benzaldehyde and hydrogen cyanide. Hydrogen cyanide in sufficient quantities (allowable daily intake: ~0.6 mg) causes cyanide poisoning which has a fatal oral dose range of 0.6–1.5 mg/kg of body weight.

Drug administration

Drug overdose

Amygdalin yields hydrogen cyanide after ingestion and when ingested in sufficient quantities *Prunus* species cause cyanide poisoning. For instance, a total consumption of about 48 apricot kernels produced forceful vomiting, headache, flushing, heavy sweating, dizziness, and faintness before vomiting was induced in the emergency room, whereafter the symptoms rapidly subsided. In another case accidental poisoning was fatal.[2]

A 67-year-old woman with lymphoma presented with a neuromyopathy following treatment with laetrile. She had high blood and urinary thiocyanate and cyanide concentrations. Sural nerve biopsy specimen showed a mixed pattern of demyelination and axonal degeneration, the latter being prominent. Gastrocnemius muscle biopsy specimen showed a mixed pattern of denervation and myopathy with type II atrophy.

Besides the risk that a large dose can lead to acute cyanide poisoning, there is also the question whether continued ingestion of cyanogenic pits or kernels can cause chronic intoxication.

Adverse reactions

Amygdalin was first isolated in 1830. In 1845, it was used to treat cancer in Russia, and again in the 1920s in the United States, but it was considered too poisonous. In the 1950s, a reportedly nontoxic, synthetic form was patented for use as a meat preservative and later marketed as Laetrile for cancer treatment. Amygdalin, also referred to as laetrile or vitamin B_{17}, was popularized as a cancer cure. However, a clinical trial of amygdalin carried out in 1982 found that "no substantive benefit was observed in terms of cure" and more than 2 of the 178 patients suffered from cyanide toxicity. Amygdalin is sometimes confused with laetrile; however, amygdalin and laetrile are different chemical compounds. Laetrile, which was patented in the United States, is a semisynthetic molecule sharing part of the amygdalin structure, whereas the "laetrile" made in Mexico is usually amygdalin, the natural product obtained from crushed apricot kernels, or neo-amygdalin. Excess consumption of apricot kernels (to produce more than 1 mg/l cyanide in blood) may cause poisoning. The fatal dose of hydrogen cyanide has been reported to be 0.5 mg/g. A systematic review in 2006 concluded that the claim that Laetrile has beneficial effects for cancer patients is not supported by data from controlled clinical trials. Due to lack of evidence, laetrile has not been approved by the U.S. Food and Drug Administration.

Toxicological Aspects of Ingredients Used in Non-alcoholic Beverages

Amygdalin

Amygdalin (d-mandelonitrile-β-d-gentiobioside) is one of the cyanogenic glycosides which naturally formed in plants. Cyanogenic glycosides are presented > 2500 species in the plant kingdom. They were located in vacuoles in plant cells. When cell disruption occurs due to a mechanic process, the cyanogenic glycosides get in contact with β-glucosidases and α-hydroxynitrile lyases, which are endogenous enzymes, ending up the delivery of hydrogen cyanide. While cyanogenic glycosides act as crucial compounds against animals those only consumes plants as a chemical protector. They are toxicant and poisoner, which induce several symptoms such as anxiety, headache, dizziness, and confusion for humans.

Amygdalin is the most abundant cyanogenic glycosides which found in the seeds and kernels of some fruits, that is, apricot, almond, apple, cherry, plum, lemon, peach, and nectarine. Some of the seeds of mentioned fruit are not eaten directly as a food. However, fruits with high amount of cyanogenic glycosides containing seeds also have cyanogenic glycosides in the fruit flesh.[3]

Acute cyanide infection has been identified after swallowing the apricot kernels and almonds. When amygdalin contained seeds eaten hydrogen cyanide dispense at acidic stomach media. Decreasing the cyanide amount in foods, many processing procedures that is, crushing, soaking, fermentation, and drying applied. Even apple seeds are not eaten by people, apple juice is commonly generated from whole apples without separating the seeds. This may cause break into pieces of seeds along juice processing and contaminate the juice.

Apricot seeds have higher amygdalin content and comparatively easy deliver hydrogen cyanide than apple and peach. Amygdalin concentration of apricot varies between 0.1 and 4.1 mg/g as well as on the growing area

and bitterness of seeds. Amygdalin is not toxic alone but after ingested it is hydrolyzed by β-glycosidase to obtain one molecule hydrogen cyanide, two molecules glucose, and one molecule benzaldehyde.

There are three stages occur during enzymatic degradation of amygdalin. At the first stage, amygdalin lyase enzyme contributes to divide amygdalin into prunasin and glucose. After this split, prunasin is hydrolyzed to mandelonitrile by prunasin lyase. The final part is pulling apart mandelonitrile to benzaldehyde and hydrogen cyanide by hydroxynitrile lyase.

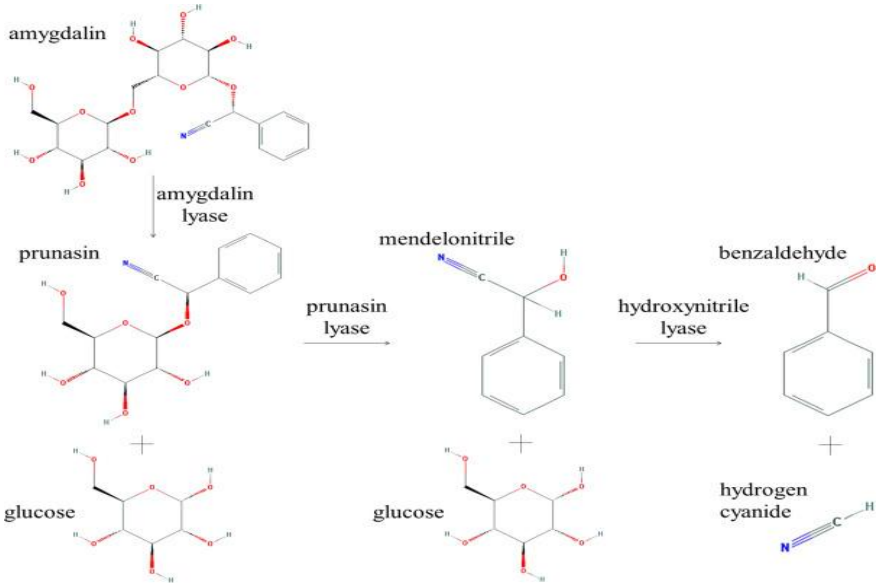


Figure-2: The enzymatic hydrolysis of amygdalin.

Enzyme-assisted degradation of amygdalin in plant foods occurs within 30 min to 6 h according to the level of maceration of the food sample. These enzymes optimally work at 20–40°C and can be deactivated at higher temperature. The stated average toxic dose of cyanide in humans has differed between 39.2 and 106.4 mg for 70 kg/person.

Because it is sensitive to processes, there were no quantified amygdalin content found in food and processed foods (Bolarinwa et al., 2014). Voldřich and Kyzlink (1992) identified that stewed fruits had 3–4 mg/kg and canned stone fruits had 4 mg/kg hydrogen cyanide equivalents which are lower than acute toxicity level. Toydemir et al. (2013) investigated the industrialized sour cherry nectar processing effects on phenolic compounds by comparing metabolites of fruits and nectars. According to their results, amygdalin was the sole component represented higher in the fruit, compared to the juices. Amygdalin may have been removed due to discard of cherry seeds. Even this compound broke down during process by heat treatments, products processed by novel technologies requires detailed investigation for possible amygdalin content.[4]

Biocompatibility, Surface Engineering, and Delivery of Drugs, Genes and Other Molecules

Amygdalin-based self-assembled prodrugs

Amygdalin, an anticancer agent, belongs to cyanogenic glycoside family. D-Amygdalin, [*O*-β-glucopyranosyl-(1→6)-β-d-glucopyranosyloxy] benzeneacetonitrile (Figure 8), is a by-product of the processing of apricots, almonds, peaches, and cherries, and its rigid sugar backbone is rich in directional hydroxyl groups. The primary hydroxyl group on amygdalin was selected as a site to generate amphiphilic prodrugs by tethering natural fatty acids with an enzyme-cleavable ester linker. Interestingly, the extremely regioselective esterification ability of lipase has been utilized as a tool to synthesize amygdalin-prodrug amphiphiles (Amy-Prodrugs) in quantitative yields (Figure 8). Amy-Prodrugs underwent self-assembly to form nanofiber-rich nanoarchitectures in a wide range of solvents including aqueous solutions, and both polar and nonpolar organic solvents at extremely low MGC values (0.05–0.2%, w/v) revealed the presence of helical fibers and sheet-like structures. Furthermore, to evaluate the ability of Amy-Prodrug gels to deliver multiple drugs simultaneously, akin to acetaminophen prodrugs, curcumin has also been encapsulated within the Amy-Prodrug-based hydrogels. The ester-based Amy-Prodrug amphiphiles were susceptible to lipase, causing an esterase-mediated enzymatic degradation, and therefore, a detailed investigation was carried out to achieve controlled drug delivery. The inherent hydrophobic domains within the self-assembled fibers were utilized for enhanced encapsulation, as in this manner, hydrogels have the potential to increase the bioavailability of water-insoluble drugs. Interestingly, in the absence of esterase enzyme, Amy-Prodrug-based hydrogels

exhibited unprecedented temporal stability, with no drug leakage occurring under physiological conditions (PBS, pH 7.4, and 37 °C). In addition, we demonstrated controlled gel degradation, allowing drug release through modulation of either enzyme concentration and/or incubation temperature. Interestingly, Amy-Prodrug-based hydrogels also did not exhibit any detectable burst release profile, akin to hydrogels made from Apn-Prodrugs.

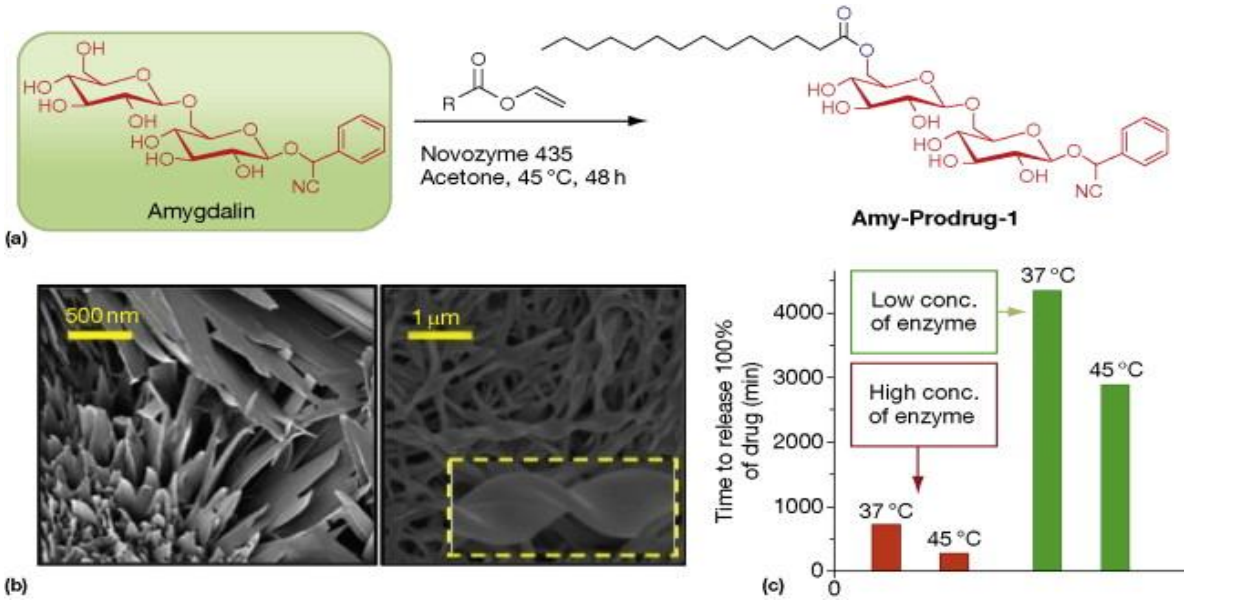


Figure-3: (a) Enzyme-mediated regioselective synthesis of amygdalin-based amphiphilic prodrug, Amy-Prodrug-1, which could form both hydro- and organogels at lower concentration. (b) Scanning electron microscopy images of organogel (left) and hydrogel (right), which show sheet-like and helical fibers, respectively. (c) Effect of enzyme concentration (10 and 100 U ml−1) and incubation temperature on gel degradation/encapsulated drug release. By modulating temperature or enzyme concentration, the drug release kinetics from the networks are modulated.

As discussed earlier, it is equally important to understand the by-products formed after enzyme-mediated prodrug-based hydrogel degradation. Conventional thin-layer chromatography (TLC) was performed to characterize the components formed after gel degradation; tetradecanoic acid was shown by ^{1}H NMR spectroscopy. Subsequently, further TLC analysis of the solution demonstrated the presence of amygdalin and curcumin. Taken together, the formation of amygdalin and fatty acid suggests that prodrug degradation occurred as expected at the ester bond of amygdalin prodrugs by lipase (esterase) enzyme.

Prunus Species

Toxicokinetics

Cyanogenic glycosides contain amygdalin. Amygdalin is erratically absorbed from most of the gastrointestinal tract but is effectively absorbed from the duodenum. Amygdalin is not toxic until it is metabolized by the enzyme emulsin to hydrocyanic acid. This metabolism may occur slowly and result in delayed clinical toxicity. Emulsin is found within the seeds of the *Prunus* species and in certain bacteria found within human intestinal flora. The presence of amygdalin in the seed kernels is not harmful unless the seed is crushed (masticated) and moistened, allowing release of emulsin. Amygdalin may result in cyanide toxicity in humans. Cyanide is converted to thiocyanate by an enzymatic reaction catalyzed by rhodanese. Thiocyanate is renally excreted.[5]

Apricot

Antinociceptive activity

The intramuscular injection of amygdalin in apricot significantly reduced the formalin-induced tonic pain in both early and late phases. During the late phase, amygdalin did reduce the formalin-induced pain in a dose-dependent manner in a dose range less than 1 mg/kg. Molecular analysis targeting c-Fos and inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), also showed a significant effect of amygdalin, which matched the results of the behavioral pain analysis. Amygdalin is effective at alleviating inflammatory pain and it can be used as an analgesic with antinociceptive and antiinflammatory activities.

Toxic Neuropathies

Amygdalin Neuropathy

The cyanogenic glycoside, amygdalin, is found in several plant sources, particularly in the seeds of apples, pears, and members of the *Prunus* species (apricots, plums, peaches, etc.). Amygdalin is converted into hydrogen cyanide after ingestion and may induce cyanide toxicity. Reported neurological complications of cyanide toxicity include peripheral nerve demyelination, optic

neuropathy, deafness, and Parkinsonism. Because these fruit seeds are uncommon in Western diets, clinical cyanide toxicity is rarely seen. The increased popularity of herbal medicines, however, may change this.

Recently, two cases were reported of a subacute polyneuropathy in young, otherwise healthy individuals who took no medications other than daily herbal "supplements"—apricot kernels in one and "taoren," or peach seeds, in the other. Both noted gradual, slowly progressive sensory loss and mild weakness involving the distal extremities symmetrically several weeks after seed ingestion began. Burning dysesthesias were reported by one. Deep tendon reflexes were diffusely reduced. Electrophysiological studies revealed a mixed sensorimotor polyneuropathy with diffusely reduced amplitudes. Conduction velocities were normal, but distal motor latencies were prolonged and the terminal latency index was reduced in one. Laboratory workup for other causes of peripheral neuropathy was normal except for reduced vitamin B_{12} levels in one. This latter finding was considered unrelated because no concomitant evidence of subacute combined degeneration or macrocytosis was present. Symptoms gradually improved after discontinuation of herbal supplements without residual neurological sequelae.

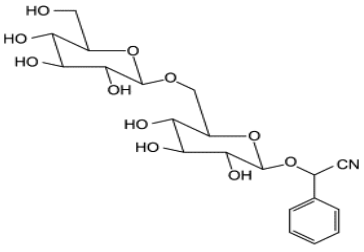
Toxicology and Human Environments

Prunus species

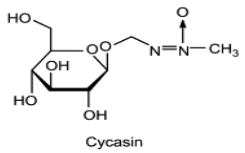
Members of the genus *Prunus* contain amygdalin, a cyanogenic diglucoside, d-mandelonitrile-beta-d-gentiobioside, usually in the kernels of the pits. Prunasin is the hydrolysis product of d-mandelonitrile-beta-d-glucoside. Prunus species include *P. armeniaca* (apricot), *P. dulcis* (bitter almond), *P. persica* (peach), *P. serotina* (black or wild cherry), and *P. virginiana v. melanocarpa* (choke cherry). Amygdalin becomes dangerous when hydrolyzed by emulsin in the crushed seed or by some human gut microorganisms to yield cyanide. Laetrile, a purported cancer cure, is largely amygdalin. Amygdalin [(6-*O*-β-d-glucopyranosyl-β-d-glucopyranosyl)oxy]benzenacetonitrile is a cyanogenic glycoside occurring in seeds, principally in bitter almonds and peach and apricot pits. It has been promoted as a treatment for cancer, but controlled clinical trials have repeatedly failed to confirm such claims. Its toxic actions are those that can be ascribed to cyanide as the cyanide released on hydrolysis inhibits cellular respiration by binding to the trivalent iron of cytochrome oxidase in mitochondria, blocking oxygen utilization and resulting in cytotoxic hypoxia.[6]

Cyanide Toxicity and its Treatment

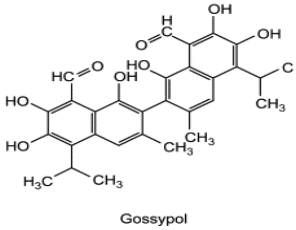
Cyanide is a metabolic product of amygdalin (Laetrile®) that was introduced as an anti-neoplastic agent in the 1950s and was responsible for several cyanide poisoning cases. Intestinal beta-d-glucosidase digests the amygdalin, releasing HCN. Also, iatrogenic exposure to cyanide may result after the use of sodium nitroprusside, an anti-hypertensive agent and succinonitrile, an anti-depressant. Sodium nitroprusside is used medicinally as Nipride®, and its intravenous infusion is used to lower blood pressure in hypertensive emergencies. This application of sodium nitroprusside occasionally causes classical cyanide toxicity. Death caused by mercuric cyanide or mercuric oxycyanide poisoning was reported because of possible ingestion of an antiseptic or a hair lotion commercialized in France.



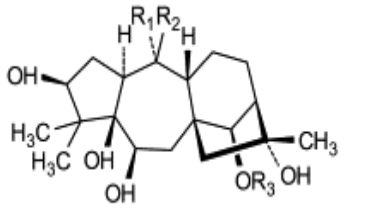
Amygdalin



Cycasin



Gossypol



	R_1	R_2	R_3
Grayantoxin I	OH	CH_3	$COCH_3$
Grayantoxin III	OH	CH_3	H
Grayantoxin II	CH_2	CH_2	H

Figure-4: Amygdalin, Cycasin, Gassypol, Grayantoxin I, Grayantoxin II, Grayantoxin III.

Laetrile

Laetrile (patented 1961) is a simpler semisynthetic derivative of amygdalin. Laetrile is synthesized from amygdalin by hydrolysis. The usual preferred commercial source is from apricot kernels (*Prunus armeniaca*). The name is derived from the separate words "laevorotatory" and "mandelonitrile". Laevorotatory describes the stereochemistry of the molecule, while mandelonitrile refers to the portion of the molecule from which cyanide is released by decomposition. A 500 mg laetrile tablet may contain between 2.5 and 25 mg of hydrogen cyanide.

Like amygdalin, laetrile is hydrolyzed in the duodenum (alkaline) and in the intestine (enzymatically) to D-glucuronic acid and L-mandelonitrile; the latter hydrolyzes to benzaldehyde and hydrogen cyanide, that in sufficient quantities causes cyanide poisoning.[7]

Claims for laetrile were based on three different hypotheses: The first hypothesis proposed that cancerous cells contained copious beta-glucosidases, which release HCN from laetrile via hydrolysis. Normal cells were reportedly unaffected, because they contained low concentrations of beta-glucosidases and high concentrations of rhodanese, which converts HCN to the less toxic thiocyanate. Later, however, it was shown that both cancerous and normal cells contain only trace amounts of beta-glucosidases and similar amounts of rhodanese.

The second proposed that, after ingestion, amygdalin was hydrolyzed to mandelonitrile, transported intact to the liver and converted to a beta-glucuronide complex, which was then carried to the cancerous cells, hydrolyzed by beta-glucuronidases to release mandelonitrile and then HCN. Mandelonitrile, however, dissociates to benzaldehyde and hydrogen cyanide, and cannot be stabilized by glycosylation.

Finally, the third asserted that laetrile is the discovered vitamin B-17, and further suggests that cancer is a result of "B-17 deficiency". It postulated that regular dietary administration of this form of laetrile would, therefore, actually prevent all incidences of cancer. There is no evidence supporting this conjecture in the form of a physiologic process, nutritional requirement, or identification of any deficiency syndrome. The term "vitamin B-17" is not recognized by Committee on Nomenclature of the American Institute of Nutrition Vitamins. Ernst T. Krebs (not to be confused with Hans Adolf Krebs, the discoverer of the citric acid cycle) branded laetrile as a vitamin in order to have it classified as a nutritional supplement rather than as a pharmaceutical.

CONCLUSION

Advocates for laetrile assert that there is a conspiracy between the US Food and Drug Administration, the pharmaceutical industry and the medical community, including the American Medical Association and the American Cancer Society, to exploit the American people, and especially cancer patients.

Advocates of the use of laetrile have also changed the rationale for its use, first as a treatment of cancer, then as a vitamin, then as part of a "holistic" nutritional regimen, or as treatment for cancer pain, among others, none of which have any significant evidence supporting its use.

Despite the lack of evidence for its use, laetrile developed a significant following due to its wide promotion as a "pain-free" treatment of cancer as an alternative to surgery and chemotherapy that have significant side effects. The use of laetrile led to a number of deaths. The FDA and AMA crackdown, begun in the 1970s, effectively escalated prices on the black market, played into the conspiracy narrative and enabled unscrupulous profiteers to foster multimillion-dollar smuggling empires.

Some American cancer patients have traveled to Mexico for treatment with the substance, for example at the Oasis of Hope Hospital in Tijuana. The actor Steve McQueen died in Mexico following surgery to remove a stomach tumor, having previously undergone extended treatment for pleural mesothelioma (a cancer associated with asbestos exposure) under the care of William D. Kelley, a de-licensed dentist and orthodontist who claimed to have devised a cancer treatment involving pancreatic enzymes, 50 daily vitamins and minerals, frequent body shampoos, enemas, and a specific diet as well as laetrile.

Laetrile advocates in the United States include Dean Burk, a former chief chemist of the National Cancer Institute cytochemistry laboratory, and national arm wrestling champion Jason Vale, who falsely claimed that his kidney and pancreatic cancers were cured by eating apricot seeds. Vale was convicted in 2004 for, among other things, fraudulently marketing laetrile as a cancer cure. The court also found that Vale had made at least $500,000 from his fraudulent sales of laetrile.

In the 1970s, court cases in several states challenged the FDA's authority to restrict access to what they claimed are potentially lifesaving drugs. More than twenty states passed laws making the use of Laetrile legal. After the unanimous Supreme Court ruling in *United States v. Rutherford* which established that interstate transport of the compound was illegal, usage fell off dramatically. The US Food and Drug Administration continue to seek jail sentences for vendors marketing laetrile for cancer treatment, calling it a "highly toxic product that has not shown any effect on treating cancer."

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