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Amanita muscaria (fly agaric): from a shamanistic hallucinogen to the search for acetylcholine

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Abstract

The mushroom *Amanita muscaria* (fly agaric) is widely distributed throughout continental Europe and the UK. Its common name suggests that it had been used to kill flies, until superseded by arsenic. The bioactive compounds occurring in the mushroom remained a mystery for long periods of time, but eventually four hallucinogens were isolated from the fungus: muscarine, muscimol, muscazone and ibotenic acid.

The shamans of Eastern Siberia used the mushroom as an inebriant and a hallucinogen. In 1912, Henry Dale suggested that muscarine (or a closely related substance) was the transmitter at the parasympathetic nerve endings, where it would produce lacrimation, salivation, sweating, bronchoconstriction and increased intestinal motility. He and Otto Loewi eventually isolated the transmitter and showed that it was not muscarine but acetylcholine. The receptor is now known variously as cholinergic or muscarinic. From this basic knowledge, drugs such as pilocarpine (cholinergic) and ipratropium (anticholinergic) have been shown to be of value in glaucoma and diseases of the lungs, respectively.

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Introduction

Amanita muscaria is probably the most easily recognised mushroom in the British Isles with its scarlet cap spotted with conical white fleecy scales.[1] The cap is about 20 cm across, at first hemispherical, then convex and finally flat. The gills are crowded and the stipe grows to a maximum of 23 cm (with a bulbous base encased in a small volva). It favours an acidic environment, particularly in birch forests, and is most prevalent in late summer or autumn. The *Amanita* genus comprises many species of which two others, *Amanita Phalloides* (the death cap) and *Amanita pantherina* (the panther cap[2]), deserve mention.

The Siberian shamans

The Eastern part of Siberia was isolated from the rest of Russia for millennia. In the early 19th century, when it began to be explored by travellers, a Stone Age culture that depended totally on reindeer for its economy (their hides, meat and milk) was found. Their 'medicine men' were known as shamans (Figure 1) and their religious rites were based significantly on the use of *A. muscaria*, which they gathered from the local birch forests.[3] The use of this hallucinogenic fungus is based on the mythical tale of the Big Raven, as recorded by the Swedish-American ethnologist Waldemar Jochelson, who lived with the tribes in the early part of the 20th century. His version of the tale reads as follows:

> Existence spat on the earth and where it fell, a fungus appeared. The Big Raven ate the fungus and began to feel gay and then started to dance. The bird then developed special muscular power and lifted a bag that contained a very heavy whale. He took the whale out to sea and released it. The spirit of the mushroom (Wapag) was able to show him a vision of the whale swimming in the ocean (returning to its brothers and sisters). Big Raven then said: 'Let the fungus remain on earth and let my children see what it will show them'.[3]

Other explorers studied the Koryak tribe of Kamchatka and found that they obeyed Big Raven's instruction to eat the fungus. A typical hallucinatory experience evolved. First the participant fell into a drunken stupor. When he awoke, a period of frenetic activity ensued with accompanying auditory hallucinations and changes in colour vision. The active hallucinogen passed into the urine which they still valued for its psychedelic activity. The local reindeer would often follow the drunken individual around and if he relieved himself in

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Figure 1 Siberian shaman under the influence of muscarine. Note the ritual drumming and the head dress of reindeer antlers. The reindeer and the birch tree were the basis of the tribe's economy

the snow, the reindeer would eat it and become similarly intoxicated. Reindeers in this state can easily be roped and killed. Death from eating fly agaric is virtually unknown, in contrast to another *Amanita* (*A. phalloide*s, the death cap). Reindeer meat, if taken shortly after slaughter, can on occasion affect the consumer. Where fly agaric was scarce or absent, the Koryaks would barter one reindeer for one mushroom (thus further supporting the reindeer economy). The natives would often carry the dried mushrooms around in little boxes made of birch wood bound in reindeer leather. The birch (*Betula*) has a mycorrhizal relationship with the fungus and their leather was made of reindeer hide; both these materials being highly symbolic in the ritual significance of the fly agaric. Finally, a thriving trade in the mushroom was developed by the Kamchatka merchants who traded it with many other communities around the Gulf of Pershina.

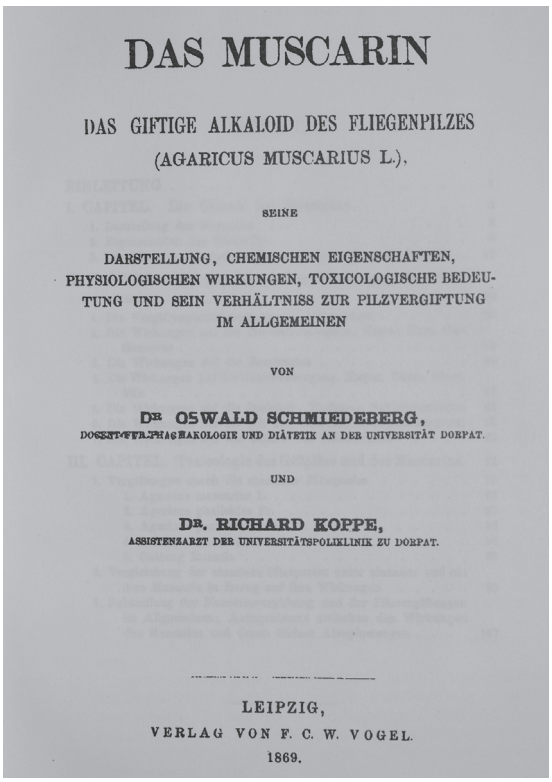
Oswald Schmiedeberg and muscarine

Oswald Schmiedeberg[4] (1838–1921) (Figure 2a) was one of the great pharmacologists of his generation and his new institute at Strasbourg played host to many young research workers (from all points of the compass). His laboratory produced a series of important publications including the synthesis of urea and hippuric acid in the liver (the latter by conjugation of benzoic acid with glycine). The first cardiac glycoside, digitoxin, was isolated in his institute in 1876. However, his main work, which has resonated to the present day, concerns muscarine, one of the alkaloids present in *A. muscaria* (although at very low concentration). Together with his colleague Richard Kloppe, he published the excellent monograph *Das Muscarin: Das Giftige Alkaloid des FliegenPilzes (Agaricus muscarius L.)* in 1869 (Figure 2b).

Briefly, he and his co-worker found that if the vagus nerve to the heart was stimulated electrically, the heart slowed and this effect could be blocked by atropine (the alkaloid

Figure 2a Oswald Schmiedeberg, together with Buchheim, one of the co-founders of German pharmacology, isolated muscarine from *Amanita muscaria* and studied its pharmacological effects

Figure 2b Frontispiece of *Das Muscarin: Das Giftige Alkaloid des FliegenPilzes (Agaricus muscarius L.)* by Schmiedeberg and Koppe


DAS MUSCARIN

DAS GIFTIGE ALKALOID DES FLIEGENPILZES
(AGARICUS MUSCARIUS L.).

SEINE

DARSTELLUNG, CHEMISCHEN EIGENSCHAFTEN, PHYSIOLOGISCHEN WIRKUNGEN, TOXICOLOGISCHE BEDEUTUNG UND SEIN VERHÄLTNISS ZUR PILZVERGIFTUNG IM ALLGEMEINEN

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LEIPZIG,
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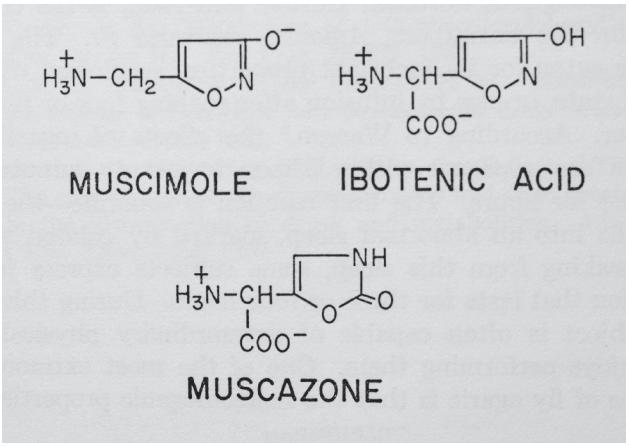
1869.

extracted from *Atropa belladonna*). Muscarine produced the same effect as electrical stimulation and they reasoned that stimulation of the vagus nerve released a substance that mimicked muscarine (the Vagusstoff). Thus an electrical stimulus seemed to release a chemical transmitter. Could that chemical transmitter be muscarine? This simple explanation proved false and the mysterious 'Vagusstoff' would keep its identity secret until the work of Henry Dale

Figure 3 Three of the most hallucinogenic alkaloids found in *Amanita muscaria*



and Otto Loewi in the 1920s and 1930s.

The chemical constitution of *Amanita muscaria*

It turns out that muscarine is only a minor chemical component of the fly agaric, perhaps 1–3% by weight.[5] Schmiedeberg and Kloppe had been very fortunate to isolate this alkaloid with its marked parasympathetic effect. The other active alkaloids in *A. muscaria* were not isolated until the 1960s; they are ibotenic acid, muscazole and muscazone (Figure 3). All three appear to act as gamma-aminobutyric acid receptor agonists and induce stupor followed by frenzy (by affecting the central nervous system). Ibotenic acid appears to be the principal alkaloid (concentrations of 0.3–1.0 g per kg of dry weight) and it is decarboxylated in the body (removal of the COOH group) to form muscimole (which is readily passed into the urine). This observation would explain clearly the ancient observation of the Koryak tribe in Siberia: that the urine of devotees of the rite still contained an active compound, which was hallucinogenic. The different *Amanitas* contain varying amounts of hallucinogenic activity at changing seasons of the year. This could be a consequence of fluctuations in the ibotenic/muscimole ratio.

With regard to other causes for muscarine poisoning, it should be pointed out that another member of the *Amanita* family, *A. pantherina* (the panther cap), contains a far higher concentration of muscarine than *A. muscaria*, but little ibotenic acid or muscazone. Consequently, the panther cap produces a pure 'muscarinic' syndrome with few effects on the central nervous system. Other genera of mushroom also tend to produce a pure 'muscarinic' syndrome when an overdose is taken. These include *Inocybe* and *Clitocybe*. It should be emphasised that ingestion of *A. muscaria* rarely causes death and this is also true of *A. pantherina* (and the genera *Inocybe* and *Clitocybe*). Nevertheless, they produce a short lived, but dramatic, constellation of symptoms, notably vomiting and diarrhoea, salivation, bronchoconstriction and bradycardia. Fortunately, the administration of intravenous saline (to correct the fluid loss from the gut) and treatment with atropine (a blocker of acetylcholine at the peripheral muscarinic receptor) will rapidly control the situation and lead to a full recovery.

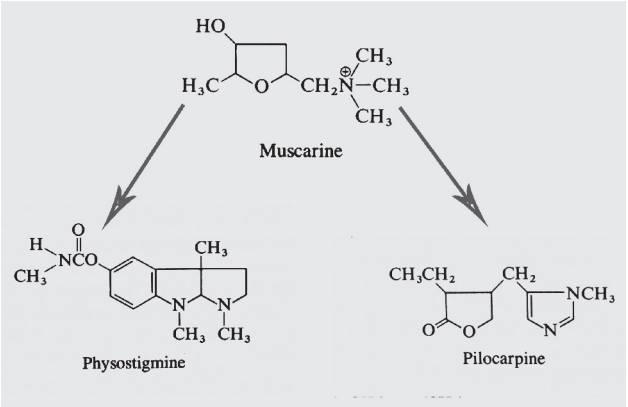
Figure 4 *Pilocarpus jaborandi* (the slobbering herb). A powerful parasympathetic agonist that produces classic effects in humans, such as bradycardia, bronchoconstriction, salivation and increased peristalsis. Between 1880 and 1920 it was the principal treatment of cardiac failure as a result of its powerful property of markedly increasing sweating

Pilocarpine: a muscarine-like alkaloid derived from the jaborandi shrub

Jaborandi,[6] (Figure 4) known to the Guarani Indians as the 'slobbering drug' (that is causing excessive salivation), first came to the attention of Europeans in 1570 when Gabriel Soares de Souza, a Portuguese explorer, noted that the leaves of the shrub were being used by the natives to treat mouth ulcers. In the 1630s two scientists from the Dutch West Indian Company observed that many Indian tribes used the leaf for a variety of conditions, ranging from gonorrhoea to epilepsy to diphtheria. The indigenous tribes regarded the plant with great satisfaction, as a pound of sweat could be secreted within 10 minutes of its administration (a desirable action to remove 'toxins' from the body). In 1873, Symphonio Contino, a Brazilian doctor seeking an extra degree in medicine, went to Paris and took with him samples of the Jaborandi leaves. French physicians found that the leaves 'increased the sweating and salivation enormously, together with, a much less degree secretion from the mucous membranes of the nose, the bronchial tubes, the stomach and the intestines'.

A 'Jaborandi Rush' then developed in which botanic explorers hurried to South America to try and identify the plant scientifically and examine its distribution. They found that the

Figure 5 Three muscarinic agonists: two direct (muscarine and pilocarpine) and one indirect (physostigmine)



chief site for the Jaborandi shrub was the state of Maranhão, in north-east Brazil, abutting the Atlantic Ocean. The plant is a small shrub, 3–7.5 metres tall, and grows as an individual or in stands. There are many species of *Pilocarpus* (the name derives from the Greek *pilo* 'felt hat' and *carpus* 'flower'). These include *P. microphyllus*, *P. jaborandi* and *P. trachylopus*. In 1875, its most active alkaloid, pilocarpine, was isolated and subjected to detailed investigation.

Pilocarpine[7] is an imidazole alkaloid and the leaves of the Jaborandi contain 0.5% of this substance per total dry weight (Figure 5). Later, the demand for the alkaloid would be so massive, for the treatment of oedema in cardiac failure, that the pharmaceutical company Merck set up a large plantation in São Luís, the capital of Maranhão state, Brazil. In the early studies on pilocarpine in man, a novel effect soon became apparent. If the alkaloid was instilled into the conjuctival sac, pupillary constriction (meiosis) was regularly observed. Also, the pupillary accommodation reflex was abolished and the intraocular pressure fell. This last observation was made possible by the concomitant development of the aplanar tonometer, the first instrument to measure intraocular pressure accurately. This was a pivotal observation and led rapidly to the successful use of the alkaloid in the treatment of glaucoma (raised intraocular pressure).

Physostigmine from the Calabar bean

In the 1860s, a novel alkaloid, physostigmine, was isolated from the Calabar bean[8] (*Physostigma venenosum*) (Figure 6). This alkaloid also had the properties of constricting the pupil. The bean had a similar effect to that of pilocarpine in that it also lowered intraocular pressure. This seemed to have an additive effect, together with pilocarpine, on intraocular pressure. This combination became the standard treatment for glaucoma until the 1960s when β-adrenergic blocker drugs, such as timolol, began to supplant them. Pilocarpine is still listed in the British National Formulary and can be used when the more modern drugs for the treatment of glaucoma, such as beta-blockers and prostaglandins, have failed.[9] Its main use now is in the treatment of xerostomia (dry mouth), a troublesome condition which often occurs after radiotherapy to the tongue and mouth in the treatment of squamous (and other) carcinomas. This beneficial effect is produced by its marked stimulation of salivation.[9]

Figure 6 Calabar Bean (*Physostigma venenosum*), the source of the alkaloid physostigmine which, by its inhibition of cholinesterase, permitted the isolation of acetylcholine, an endogenous neurotransmitter

The early investigators noted that the actions of the three alkaloids – muscarine, pilocarpine and physostigmine – could be reversed by the belladonna alkaloid, atropine (and this latter tropane could be useful in overdose of the muscarine-like drugs). These observations were tantalising: the three alkaloids obviously worked on a common mechanism, but what was it? This conundrum would not be solved until the 1930s and would require the combined efforts of Henry Dale and Otto Loewi (Figures 7a,b). They would jointly receive the Nobel Prize in 1936 for the discovery of acetylcholine.

The search for the elusive parasympathetic transmitter succeeds: acetylcholine is isolated

In 1912, Henry Dale, director of The Wellcome Laboratory for Medical Research in London, was a puzzled man. In his review of the parasympathetic nervous system, he adumbrated that there were two divisions of the autonomic nervous system: the sympathetic and the parasympathetic. The sympathetic resembled the actions produced in the body by the then recently discovered adrenaline (a catecholamine). These effects also mimicked those produced by nicotine (the alkaloid found in *Nicotiana*) and he therefore called them 'nicotinic'. The parasympathetic transmitter remained a complete mystery and as its actions resembled those produced by muscarine, he called them 'muscarinic'. He called the unknown transmitter the 'true' muscarine. He

Figure 7a Sir Henry Dale (1875–1968), widely known as the father of British pharmacology, pioneer in his work on ergot, histamine and in particular acetylcholine. He and Otto Loewi were jointly awarded the Nobel Prize for medicine in 1936 for the isolation and characterisation of acetylcholine

Figure 7b Professor Otto Loewi (1873–1961), Graz, Austria, carried out fundamental studies on the 'Vagusstoff' which proved to be acetylcholine. As a Jew, he had to flee Austria in 1938 (after the Anschluss)

suspected that this transmitter was an ester of choline, perhaps the acetyl derivative, but this was pure conjecture. The key to unlock this door was provided separately by Dale and Loewi when, 20 years later, they discovered that physostigmine preserved the parasympathetic transmitter from destruction. As a result, when this enigmatic compound was recovered and analysed, it was indeed acetylcholine. The last piece of the jigsaw clicked into position. Minute amounts of acetylcholine were released at the receptor and acted there. It was then rapidly inactivated by a specific esterase enzyme, acetylcholinesterase (Figure 8). The action of the esterase was inhibited by physostigmine which was thus classified as an anticholinesterase. This action of physostigmine allowed acetylcholine to accumulate in the heart (and the sympathetic ganglion) and thereby it could be more easily detected. On the other hand, muscarine and pilocarpine were not anticholinesterases, but instead acted directly on the parasympathetic receptor to produce the classic actions of bradycardia, pupil constriction and intestinal contraction. Atropine (and hyoscine) blocked the actions of acetylcholine and became known as anticholinergic compounds.[10,11,12]

As Lee has described,[10] this led to the development, at St Alfege's Hospital in London, of physostigmine as a new treatment for myasthenia gravis, a serious disorder of muscle giving rise to weakness and eventually to fatal paralysis. However, perhaps the greatest advance in therapy has come with the discovery of the 'muscarinic receptors' in the bronchioles and lung tissue. When these are activated inappropriately, this can result in bronchial asthma, or aggravate chronic obstructive pulmonary disease (COPD).

The use of antimuscarinic drugs in lung disease

Muscarinic blockade is one of the most ancient treatments for asthma. For example, in the ancient Egyptian Ebers Papyrus (several thousand years old), a traditional treatment was to place a distillate of henbane (*Hyoscyamus niger*) onto heated bricks.[13] The patient would then inhale hyoscine that came off in the smoke, leading to dilation of the bronchi. Later in the 19th century, atropine, a related alkaloid (derived from *Atropa belladonna*), would become the treatment of choice for asthma. However, atropine is not the perfect drug for this condition as the side effects are severe and potentially lethal. They include dry mouth, urinary retention, blurring of vision and dangerous acceleration of the heart. Also, atropine crosses the blood–brain barrier producing undesirable effects such as mania and hallucinations in the pregnant woman. The World Health Organization still lists atropine as an essential drug, but there are now very few indications for its use. There was therefore a need to develop a drug that would be less well absorbed and would have limited serious toxic effects. This led to the production of new antimuscarinic (anticholinergic) medicines for the treatment of asthma (and COPD): ipratropium and tiotropium (Figure 9).

Ipratropium is a synthetic quaternary ammonium compound.[14] As it is highly charged, this limits its systemic bioavailability. Ipratropium came into prominent use for COPD in the 1980s; probably because the adrenergic bronchodilators (such as salbutamol and salmeterol) tended to lose their efficacy when used for long periods. It has become apparent that there are at least five subtypes of muscarine receptors in the body (M1 to M5). Three of these (M1–M3) occur in the bronchioles and lung tissue. Unfortunately, ipratropium blocks all three muscarinic receptors with equal affinity. Blockade of the M2 receptors can, paradoxically, potentiate vagally induced bronchoconstriction. As a result, attention turned to finding a muscarine blocker that would be specific (or relatively specific) for the M3 receptors.

Tiotropium bromide is the first anticholinergic to be an effective treatment of poorly controlled asthma. The drug is functionally selective for the M3 receptor and this effect is likely to be attributed to the two thiophene rings that are present in the molecule. In several clinical trials in asthma and COPD,[14,15] tiotropium proved more effective on inhalation than ipratropium. Another definite advantage of tiotropium is its long half-life, necessitating only one dose of inhalant per day. Tiotropium also has additional effects in respiratory

Figure 8 The formation and destruction of acetylcholine (the parasympathetic agonist). Physostigmine inhibits acetyl cholinesterase and therefore acetylcholine accumulates and can be measured accurately

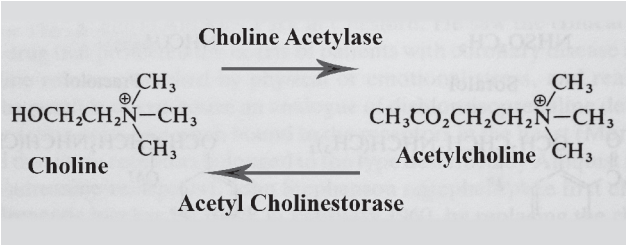
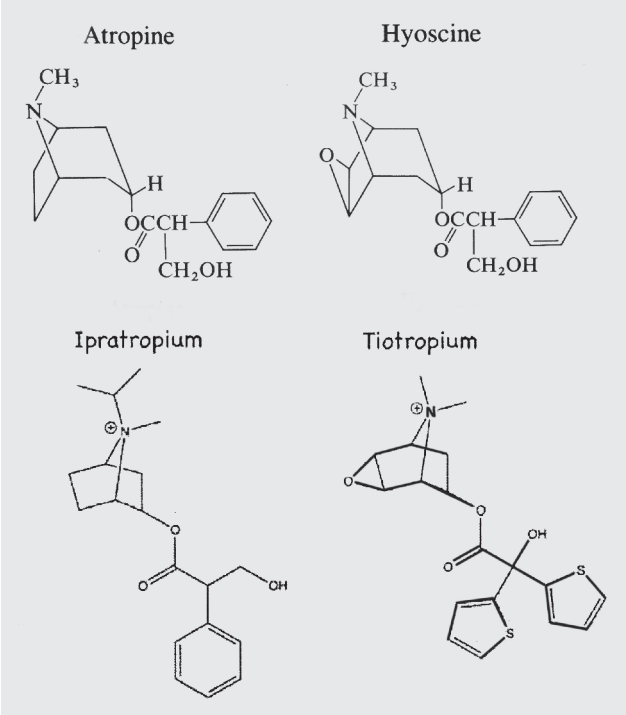


Figure 9 Four drugs which block the muscarinic receptors (M1 to M3); two ancient (atropine and hyoscine) and two modern (ipratropium and tiotropium)



disease which are not mediated by direct action on smooth muscle. These include inhibition of neutrophil migration into the lung, eosinophil deactivation and the prevention of lung remodelling (seen in chronic asthma and COPD).

Several other active selective M3 antagonists are under development and it remains to be seen whether any of those will challenge tiotropium as an effective drug for the long term treatment of lung disease. Certainly, combination inhalant therapy with a β2-adrenergic agonist (salmeterol) , a steroid (such as beclamethasone) and an M3 antagonist (based on tiotropium) seems to offer a better prospect for the future treatment of asthma (combined with the recent development of drugs acting against the eosinophil leucocyte).

Conclusion

The history of *Amanita muscaria* and its contribution to modern medicine is barely credible: from the shamanistic cults of Eastern Siberia, to the pharmacology laboratories of Europe in the 19th century and then onto Dale's serendipitous suggestion that acetylcholine was the muscarine-like compound (released at the parasympathetic nerve endings). This culminated in the development of useful drugs (such as pilocarpine and tiotropium). The whole process seems unlikely in the extreme. Yet again, it illustrates the critical sequence: identify the plant (or the fungus in this case), isolate the active principle (or principles) and then determine whether they have useful therapeutic activity in human disease.

A suitable conclusion to this article is a quotation from the Friar's speech in Shakespeare's *Romeo and Juliet*, Act II, Scene 2, verses 15 to 18:

> 'O mickle is the powerful grace that has
> In plants, herbs and their true qualities
> For naught so vile that on earth do live
> But to the earth some special good doth give'

This speech sums up exactly the history of *Amanita muscaria*. On the one hand, it was regarded by the Russian authorities as a dangerous, devilish and indeed deadly fungus. It was anathematised by the Orthodox Church. On the other hand, some benefit would accrue when the active substance muscarine would prove to be a vital key in unlocking the parasympathetic puzzle and in time to the isolation of acetylcholine. Useful compounds like pilocarpine, physostigmine and tiotropium would eventually emerge to contribute to our knowledge (and treatment) of glaucoma, asthma and COPD.

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