

# A brief history of biological weapons programmes and the use of animal pathogens as biological warfare agents

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## Summary

Any one of thousands of different microorganisms that affect the health and safety of the world's populations of humans, animals and plants could potentially be weaponised; that is undergo research and development whose aim is to create species or strains that could serve as deadly payloads for spray systems, bombs, rockets or missiles. However, many historical studies of warfare have made it clear that only a few species of bacteria and viruses have been weaponised. As is made clear in this paper, of the pathogens weaponised in the 20th century by Japan, the Union of Soviet Socialist Republics (USSR), the United States and Iraq, most were zoonotic pathogens. If a nation or terrorist group were to acquire biological weapons in the future, it is most likely that their payload would be a zoonotic pathogen.

## Keywords

Biological warfare – Biological weapon – Bioterrorism – Iraq – Japan – Union of Soviet Socialist Republics – United States – Weaponisation – Zoonotic pathogen.

## Introduction

This paper does not address biological warfare (BW) methods used in ancient times, such as catapulting corpses or sick people infected with *Yersinia pestis* into besieged cities or enemy encampments, because these methods are unlikely to be used in current or future conflicts. Instead, this paper addresses biological weapons pathogens that have either been used in modern times, or were developed to the point of usage but not deployed, but nevertheless might be used in the future. Four national BW programmes and the pathogens they weaponised are pertinent to this issue – those of Japan, the United States (US), Iraq and the Union of Soviet Socialist Republics (USSR). Particular attention is paid to the USSR because the legacy of its BW programme continues in today's Russian Federation. Accordingly, the four sections that follow are dedicated to each of these countries and the pathogens that they weaponised. The author concludes with an assessment of those pathogens that are most likely to be acquired and, perhaps, used in the future.

## Japan's biological warfare programme

In the 1930s, the Japanese Imperial Supreme War Command ordered the establishment of the Kwantung Army Epidemic Prevention and Water Supply Department, whose code name was Unit 731 and which was staffed with BW specialists drawn from the Imperial Japanese Army. This unit was commanded by a military physician, Major Shiro Ishii, who was particularly interested in plague (1). In 1936, Unit 731 relocated to the Pingfan district in occupied Manchuria, which was located approximately 24 km south-east of Manchuria's largest city, Harbin. When the unit reached its full strength in 1940, it comprised eight divisions that employed an estimated 3,000 personnel. In addition to Unit 731, several other Japanese units deployed throughout occupied China were involved in developing biological weapons. For example, in 1936, Unit 100 was established near Hsinking and was led by veterinarian Yujiro Wakamatsu. Its responsibility was to develop weapons against animals. Unit Ei 1644 (Tama Unit), established in

1939 under the cover name 'Anti-Epidemic Water Supply Unit' and headed by medical doctor Masuda Tomosada, was located in Nanking. Like Unit 731, it developed weapons against humans.

After the Soviet Union entered the war against Japan in August 1945, its army quickly overran Manchuria and in the process captured ten of Unit 731's servicemen and two from Unit 100. The 12 were charged with developing, manufacturing and using 'bacteriological weapons' and were tried for these war crimes in Khabarovsk City between the 25th and 30th of December 1949. The extensive trial record was published in English in 1950 (2). The servicemen confessed that the specific functions of Units 731 and 100 were to investigate the weapons utility of the pathogens that cause 'plague, cholera, gas gangrene, anthrax, typhoid, and paratyphoid' (3). However, it is clear from their testimony that, of the pathogens investigated by Unit 731, the highest priority was to weaponise *Bacillus anthracis* and *Y. pestis*. Of the three methods whereby humans can contract plague, the Japanese decided to concentrate on two methods for dispersing BW agents, one that used explosive force to disperse a formulation containing *Y. pestis* as an aerosol over targeted populations and a second type that depended on dispersing fleas infected with *Y. pestis* to cause bubonic plague in population centres.

There were two groups of victims of Japanese BW. The first group was those people whom Unit 731 used as subjects in their inhumane laboratory experiments, which involved infecting subjects with different pathogens and recording the results. According to historical records, more than 3,000 Chinese anti-Japanese patriots, civilians, Soviet citizens, Mongolians and Koreans were used as human subjects and infected with various pathogens by different methods, including passive oral infection, injection, bites by infected vectors, and exposure to aerosols created by exploding bombs (4). Most of these subjects died almost immediately, but some survivors were vivisected after they contracted various diseases.

The second group was Chinese civilians and soldiers. Unit 731 manufactured large quantities of *Y. pestis* cells that were used to contaminate blood fed to many thousands of fleas. The fleas were placed in Uji bombs that were carried by aircraft and released on Chinese population centres. As a result, plague among humans and rats became epidemic in Chinese provinces. As for the total number of Chinese deaths due to Japanese BW, one estimate by a Chinese scholar is that: '...during Japan's invasion of China Biological Warfare activities were carried out in more than twenty provinces and cities, causing more than 200,000 casualties among the Chinese people' (5). While the Chinese population suffered, Japan's BW programme and its weapons had no discernible useful military effects on the outcome of its aggression in China and elsewhere.

## United States' biological warfare programme

The US started its BW programme in 1942, following the precedent set by the United Kingdom (UK) and Canada. The reason for doing so was that their intelligence agencies had come to the incorrect conclusion that Germany had an operational BW programme, so they had to defend themselves against it and contemplate developing their own biological weapons. The UK and Canada closed down their offensive BW programme during the 1950s, but retain substantial defensive capabilities to this day. The US continued its offensive BW programme until 25 November 1969, when President Richard Nixon terminated it by executive order (6). Like the UK and Canada, the US continues to maintain a strong, comprehensive defensive BW programme.

After the Second World War ended, the US BW programme conducted a study as to which pathogens should be considered as possible BW agents (7). Eventually, 39 microbial and toxin agents were screened and, from these, *B. anthracis* and *Y. pestis* were given high priority to be weaponised as lethal agents. However, some time during the 1950s, the decision was made to give *Y. pestis* a much lower priority. The reasons for the decision were:

- *Y. pestis* was less virulent than *B. anthracis*
- *Y. pestis* stored in wet solution had a short half-life
- *Y. pestis* experienced a marked drop in virulence after lyophilisation and storage
- open-air testing indicated that *Y. pestis* loses viability rapidly when aerosolised (8).

While *B. anthracis* was the favoured lethal agent of the US BW programme, it had in fact weaponised seven agents for use against humans (see Box 1) before the programme was terminated in 1969. Of these seven, five were zoonotic pathogens – *B. anthracis*, *Brucella* species, *Coxiella burnetii*, *Francisella tularensis* and Venezuelan equine encephalomyelitis virus (VEEV). All biological weapons and the agents that they contained were destroyed during 1970 and 1971 while reporters and foreign representatives observed.

## Iraq's biological warfare programme

After initial explorations in the late 1970s, Iraq's BW programme commenced in earnest in 1985. By the time Operation Desert Storm ended with a ceasefire in February

**Box 1**  
**Agents weaponised by the United States before 1969**

**Bacteria:**

*Bacillus anthracis*

*Brucella species*

*Coxiella burnetii*

*Francisella tularensis*

**Viruses:**

Venezuelan equine encephalomyelitis virus

**Toxins:**

Botulinum neurotoxin

Staphylococcal enterotoxin B

1991, Iraqi scientists had investigated the BW potential of five bacterial strains, one fungal strain, five viruses, and four toxins. In addition, two bacterial species, *Bacillus subtilis* and *B. thuringiensis*, were developed for use as simulants (simulants are non-pathogens used for testing purposes). Two pathogenic bacteria were studied – *B. anthracis* and *Clostridium perfringens* (the cause of gas gangrene). Research on *B. anthracis* was initiated in 1985 at Muthanna State Establishment, the principal Iraqi chemical and biological weapons facility, but was transferred to a research laboratory at Salman Pak in 1987. Beginning in early 1990, scientists at the Foot and Mouth Disease Centre at Al Manal began to investigate five viruses for their potential use as biological weapons agents. Two viruses, Crimean-Congo haemorrhagic fever virus and yellow fever virus, were found to be unsuitable since they required vectors for dispersal. The remaining three, enterovirus 17, human rotavirus and camelpox virus, were researched further, and a large egg incubator was obtained to mass produce these viruses. However, only certain growth characteristics of the viruses were clarified before Iraq's BW programme was terminated in 1990 (9).

Iraq's chemical arsenal included 250-lb (LD-250) and 400-lb (R-400) bombs. Based on successful chemical warfare, some of these bombs were adapted to hold 60 litres and 85 litres of biological solution, respectively. The walls of the chambers containing biological payloads were coated with an inert epoxy paint to protect bacteria from the toxic effects of contact with metal. Testing determined that, of the two bomb types, the R-400 was the more suitable as a biological weapon. In 1990, 200 R-400 biological bombs were produced; of these, 100 were filled with botulinum toxin, 50 with *B. anthracis*, and seven with aflatoxin. These biological bombs were deployed at two sites and ready for immediate use.

Iraq had procured 819 Scud missiles from Soviet Bloc countries before 1991 and by itself had manufactured

about 80, most of which proved to be faulty. The Scud had a range of 300 km and could carry high-explosive payloads of up to one tonne. Some Scuds, renamed Al Huseyns, were re-engineered by Iraqi engineers to double their range, although the trade-off was reduced payload capacity. In 1990, the Muthanna State Establishment received a special shipment of 100 Al Huseyns (10). Of these, 13 were filled with botulinum toxin, ten with aflatoxin, and two with *B. anthracis* (11). The warheads of an unknown number of SAKR-18 122-mm rockets were filled with botulinum toxin, aflatoxin, or the simulant *B. subtilis*, for field testing, but, as far as is known, no biological rockets were actually deployed. From the account above, it can be seen that only one zoonotic pathogen, *B. anthracis*, was actually used as a payload in Iraqi weapons and one virus, camelpox virus, was being investigated for its BW potential. In the end, unlike chemical weapons, the Iraqis never used their biological weapons for any purpose.

## The Union of Soviet Socialist Republics' biological warfare programme

The oldest national BW programme, of the longest duration, and the most comprehensive, was that of the USSR. The most complete history of this programme has been told by M. Leitenberg and R.A. Zilinskas (12). They explained that the programme had two generations, with the first spanning 1928 to 1971 and the second 1972 to 1992. A summary of the Soviet programme is presented below.

### The first-generation programme: 1928–1971

In 1925, the Director of the Soviet Military Chemical Agency, Dr Yakov Fishman, set up a small BW laboratory in Moscow, eventually to be called the Scientific Research Institute of Health, and appointed Nikolay N. Ginsburg to be its head. More importantly, in 1928 Fishman submitted a laboratory progress report to the Commissar for Defence, Kliment Y. Voroshilov, that had four parts: first, a description of Ginsburg's investigations that demonstrated the feasibility of BW; second, an assessment of the potential uses of bacteria for the purposes of warfare and sabotage; third, a plan for the organisation of military biology; and fourth, a plan for organising defences against biological attacks (13). The second part included a description of how a team led by Ginsburg was weaponising *B. anthracis*, a pathogen they had found well suited for BW purposes since its spores are both virulent and hardy. The Ginsburg team also investigated the BW potential of *Vibrio cholerae* and *Y. pestis*.

Fishman's report appears to have stimulated the Revolutionary Military Council to issue a secret decree in 1928, ordering the establishment of an offensive BW programme (14). Thus, the Soviet BW programme's first generation commenced. The decree's implementation led to the Soviet Union acquiring a large BW programme even before it entered the Second World War in 1941. Soviet prisoners of war were extensively debriefed by German intelligence agents, who found that this programme was conducted in three institutes in the Moscow region, including Ginsburg's institute (renamed the Workers' and Peasants' Red Army [RKKKA] Biotechnology Institute); four institutes in the Leningrad region (now St Petersburg); and an open-air test site, code-named Aralsk-7, on Vozrozhdeniye Island in the Aral Sea (15).

There is hardly any information about the Soviet BW programme's activities during the Second World War. However, it is important to note that, when the USSR attacked Japanese forces that had occupied Manchuria in 1945, they captured the ruins of the Japanese BW facilities and some of its workers (see above). The Soviets learned a great deal from the Japanese programme, especially from its investigations and testing of *Y. pestis* strains. At that time, scientists knew little about what made *Y. pestis* infectious and virulent (knowledge that did not become available until a virulence-associated plasmid was first described in 1981). So the Japanese experience probably helped the Soviets to determine which *Y. pestis* strains were the most virulent to humans, information that would have been helpful when selecting strains for their weaponisation efforts (16).

The first-generation Soviet BW programme can be characterised as having assessed known pathogens for weapons potential and employing three classical applied microbiology techniques – mutation, selection and propagation – to weaponise five bacterial pathogens (*B. anthracis*, *Burkholderia mallei*, *Coxiella burnetii*, *F. tularensis* and *Y. pestis*) two viruses (VEEV and *Variola* virus) and botulinum neurotoxin. Of these eight agents, six are zoonotic. Although there are some overlaps between the US and Soviet BW programmes, the major difference is that the Soviets weaponised two contagious agents, *Variola* virus and *Y. pestis*, while the US eschewed contagious pathogens for offensive purposes.

### **The second-generation programme: 1972–1992**

In 1971, the Central Committee of the Communist Party and the USSR Council of Ministers issued a decree, stamped 'of special importance', that laid the foundations for a new system of acquiring modern biological weapons and specified how to pay for it. The decree formally marked the beginning of the 'modern' second-generation Soviet BW programme. Soon after, Decree No. 99 of the USSR's Ministry of Defence (MOD) established the 15th Directorate to direct

the BW programme (17). Further, the Politburo ordered the establishment of an entirely new organisation composed of institutes, production plants and storage facilities dedicated to BW. This organisation was named Biopreparat. Although an ostensibly civilian organisation, its head was Colonel General Yefim I. Smirnov, who also headed the 15th Directorate. Biopreparat's main responsibility was to manage a large programme, code-named 'Ferment' in Russian (which translates to 'Enzyme'), whose objective was '...to develop a second generation of biological weapons using genetically modified strains, which would be of greater military value than existing natural strains. It planned to introduce new properties into disease organisms, such as antibiotic resistance, altered antigen structure, and enhanced stability in the aerosol form, making delivery of the agent easier and more effective' (18). The MOD set up a new classification level, called 'series F' clearance, which was higher than Top Secret, as Ferment became operational. In addition, a new and highly secret Interdepartmental Scientific–Technical Council on Molecular Biology and Genetics, whose cover designation was P.O. Box A-3092 (19), was established to provide scientific direction to Ferment. The highly regarded virologist and academician Victor M. Zhdanov was appointed as its chair.

Ferment initially focused on traditional agents, such as *B. anthracis*, *B. mallei*, *F. tularensis*, *Y. pestis* and VEEV but, within a few years, its scientists were also investigating filoviruses, Junin virus and Machupo virus (20). Alongside its offensive research and development programme (R & D), Biopreparat institutes performed defensively directed R & D under a programme code-named 'Problem 5'. Its lead agency was the N.F. Gamaleya Institute of Epidemiology and Microbiology, but its R & D was mostly performed by six institutes that comprised the anti-plague system. Its major objective was to develop vaccines and treatments for the pathogens weaponised under Ferment and foreign threat agents discovered by Soviet intelligence. Two reports written by researchers at the James Martin Center for Nonproliferation Studies contain the history and organisation of the anti-plague system, including Problem 5 (21, 22).

The Soviet BW programme reached its apex in the late 1980s. At that time it had four components. The first was comprised of the MOD's three biological institutes and an open-air test site. The second was Biopreparat, which had five major institutes and about 35 supporting facilities. The third was the Ministry of Agriculture, with six institutes and an unknown number of supporting facilities. The fourth was Problem 5, as described above. At that time, an estimated 60,000 personnel operated the Soviet BW programme. Since there have been no defectors from any of the MOD's biological institutes, little is known about the BW-related R & D that was conducted within their walls. Conversely, many scientists who once worked for Biopreparat have

either defected or, after the Soviet Union was dissolved in December 1991, succeeded in relocating to countries such as Israel, the UK, the US and elsewhere. Accordingly, there is a considerable amount of information about the R & D conducted at Biopreparat institutes.

By the time Russian President Boris Yeltsin ordered the MOD to close down its offensive BW programme in 1992, its scientists had created several unique bacterial and viral constructs. For example, scientists at the MOD's Scientific Research Institute of Epidemiology and Hygiene in Kirov City and Biopreparat's State Research Centre for Applied Microbiology (SRCAM) developed an especially virulent *Y. pestis* strain that was resistant to the existing EV strain vaccine. In a related project, the same Kirov team developed *Y. pestis* simulants based on strains of *Y. pseudotuberculosis* and *Y. enterocolitica*. Although some strains of these zoonotic pathogens can cause low-order gastrointestinal disease in humans, other strains are non-pathogenic and thus were used as simulants for *Y. pestis* in institutes with low biosafety levels and field tests of candidate weapons systems. At SRCAM, Zaviyalov worked to develop a so-called 'F1 minus' strain of *Y. pestis*. The reason for doing so was that, in Western countries, standard serological tests had been used for many years to detect antibodies to the F1 protein (these tests are still the basis for surveillance and diagnosis of plague in infected humans and animals). By using an F1 minus strain of *Y. pestis* in their biological weapons, the Soviets would have made it more difficult for the attacked population to identify the causative pathogen of the resulting disease outbreak and begin timely treatment. An F1 minus strain of *Y. pestis* was indeed created but it was taken over by the MOD, so its fate as a BW pathogen is unknown. Yet other Soviet scientific teams sought to eliminate epitopes on the surface of classic BW agents, so as to make them unrecognisable to the diagnostic techniques and vaccines possessed by Western countries, and to develop strains of *B. anthracis*, *B. mallei*, *B. pseudomallei* and *Y. pestis* that were resistant to ten antibiotics (S. Popov, personal communication, 2007). Other bacterial strains, as well as viruses, that were weaponised are listed in Box 2.

After the Soviet Union was dissolved in December 1991, the new Russian President, Boris Yeltsin, came to terms with the knowledge that the USSR had operated an offensive BW programme in violation of the Biological and Toxin Weapons Convention. In response to this new knowledge, on 11 April 1992, he issued Edict No. 390, 'On Ensuring the Implementation of International Obligations Regarding Biological Weapons', which ordered that offensive BW programmes be shut down (23). On the same day, Yeltsin promulgated a decree that led to a 50% reduction in the staffing levels of the MOD and Biopreparat institutes and a 30% cut in their funding. In practice, an even more severe downsizing occurred, with individual institutes undergoing personnel decreases ranging from 50% to over 90%.

## Box 2 Agents weaponised by the Union of Soviet Socialist Republics before 1992

### Bacteria:

*Bacillus anthracis*  
*Brucella* species  
*Burkholderia mallei*  
*Burkholderia pseudomallei*  
*Coxiella burnetii*  
*Francisella tularensis*  
*Legionella pneumophila*  
*Yersinia pestis*

### Viruses:

Marburg virus  
 Venezuelan equine encephalomyelitis virus  
 Variola virus

### Toxins:

Botulinum neurotoxin

However, as of the time of writing, no one outside the Russian Federation has any information on the fate of the agents weaponised by the Soviets or the military scientists who did this work.

## Conclusion

The US Department of Health and Human Services and Department of Agriculture have identified pathogens and toxins that have the potential to pose a severe threat to public health and safety, to animal or plant health, or to animal or plant products. These are called 'select agents'. The Centers for Disease Control and Prevention's Division of Select Agents and Toxins (DSAT) administers the Federal Select Agent Program, which regulates the possession, use and transfer of select agents that cause human diseases (24). Accordingly, DSAT regulates 46 agents and toxins, including the small number of bacteria and viruses that in the past were weaponised by the national BW programmes discussed above. Of these 46 agents, 13 are bacterial species and 23 are viruses.

When one examines these regulated agents, it is clear that, out of the 13 bacterial species, 11 are zoonotic pathogens and, out of the 23 viral species, 22 are zoonotic pathogens. Furthermore, of the 13 bacterial species, seven have in the past been weaponised by one or more of the four national BW programmes discussed above, while three viruses were weaponised.

It is impossible to foretell which pathogens are most likely to be acquired and used by either nations or terrorist groups

to arm coming biological weapons. However, if and when it is done, it is most likely that the future biological armourer will select one or more of the pathogens listed in Table I.

**Table I**  
**Select agents that potentially pose a severe threat to public, animal and/or plant health and safety: Tier 1**

Bacteria	Viruses
<i>Bacillus anthracis</i>	Ebola virus
Botulinum neurotoxins	Marburg virus
Botulinum neurotoxin-producing species of <i>Clostridium</i>	Variola major virus (smallpox virus)
<i>Burkholderia mallei</i>	Variola minor virus (alastrim)
<i>Burkholderia pseudomallei</i>	
<i>Francisella tularensis</i>	
<i>Yersinia pestis</i>	

A subset of select agents have been designated as Tier 1 because they ‘...present the greatest risk of deliberate misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence, and pose a severe threat to public health and safety’ (see Table I).

In 1943, the US military decided to institute a BW programme. As it did so, its commanders chose to weaponise

two agents – botulinum neurotoxin and *B. anthracis*. After having conducted applied research for about a year, the military decided to concentrate its weaponisation efforts on the second. The reason for choosing *B. anthracis* was that it ‘...gave greater public prominence as a potential agent for biological warfare than any other micro-organism. Its wide range of pathogenicity for man and domestic animals, its ease of cultivation in the laboratory, and the remarkable resistance of its spores are desirable features that undoubtedly favored its selection as one of the principal agents to be investigated at Camp Detrick’ (25).

It is the author’s belief that the reasons set forth in 1943 for weaponising *B. anthracis* are as valid today as they were then. Therefore, whether it is a proliferant nation or a terrorist organisation that deploys a biological weapon in the future, that weapon’s payload will most likely be *B. anthracis* spores. The second possibility, which is somewhat probable, is that terrorists, not nations, will acquire and use botulinum neurotoxin to contaminate food or beverages (26).

## Acknowledgements

Special thanks are given to Ms Helen Zilinskas for her excellent editing.

## Brève histoire des programmes d’armements biologiques et éclaircissement sur les agents pathogènes d’origine animale utilisés en tant qu’armes biologiques

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### Résumé

Chacun des milliers de microorganismes différents qui affectent la santé et la sécurité des populations humaines, animales et végétales de la planète peut être transformé en arme biologique, c’est-à-dire faire l’objet de procédures de recherche et développement visant à mettre au point des espèces ou souches destinées à charger des systèmes d’épandage, des bombes, des roquettes ou des missiles à puissance létale. Néanmoins, de nombreuses études historiques sur les armements démontrent que le nombre de bactéries et de virus effectivement militarisés est limité. L’auteur explique que la plupart des agents pathogènes militarisés au cours du xx<sup>e</sup> siècle par le Japon, l’Union des Républiques soviétiques socialistes (URSS), les États-Unis et l’Irak étaient de nature zoonotique. Si une nation ou un groupe terroriste devait acquérir des armes biologiques à l’avenir, la charge utilisée serait probablement constituée d’agents pathogènes zoonotiques.

**Mots-clés**

Agent pathogène zoonotique – Arme biologique – Bioterrorisme – États-Unis – Guerre biologique – Irak – Japon – Militarisation – Union des Républiques soviétiques socialistes.



## Breve historia de los programas de armas biológicas y el uso de patógenos animales como agentes de guerra biológica

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**Resumen**

En potencia, cualquiera de los miles de microorganismos distintos que afectan a la salud y seguridad de las poblaciones mundiales de humanos, animales o plantas puede ser convertido en un arma, o dicho de otro modo: servir de objeto de investigación y experimentación con el propósito de crear especies o cepas que puedan constituir la carga mortífera de sistemas de vaporización, bombas, cohetes o misiles. Con todo, numerosos estudios de historia de la guerra dejan patente que solo unas pocas especies de virus y bacterias han sido efectivamente transformadas en armas. Como deja claro el autor, la mayoría de los patógenos que en el siglo xx convirtieron en arma el Japón, la Unión de Repúblicas Socialistas Soviéticas (URSS), los Estados Unidos y el Iraq eran zoonóticos. Si en el futuro una nación o un grupo terrorista hubiera de procurarse armas biológicas, lo más probable es que estas estuvieran cargadas con un patógeno zoonótico.

**Palabras clave**

Arma biológica – Estados Unidos – Guerra biológica – Iraq – Japón – Patógeno zoonótico – Terrorismo biológico – Transformación en arma – Unión de Repúblicas Socialistas Soviéticas.



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