

Radiation Sterilization of Bacon for Military Feeding

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ABSTRACT

ANELLIS, A. (U.S. Army Natick Laboratories, Natick, Mass.), N. GRECZ, D. A. HUBER, D. BERKOWITZ, M. D. SCHNEIDER, AND M. SIMON. Radiation sterilization of bacon for military feeding. *Appl. Microbiol.* **13**:37-42. 1965.—Sliced, cured bacon, packed in cans and seeded with 6×10^8 spores per can of *Clostridium botulinum* strains 33A or 41B, or with 3×10^6 spores per can of strains 36A, 12885A, 9B, or 53B, was irradiated to various dose levels with γ radiation. Evidence provided by swelling, toxicity, and recoverable *C. botulinum* with 2,200 inoculated, irradiated cans demonstrated that: (i) 4.5 Mrad were more than adequate as a sterilization dose; (ii) the experimental minimal sterilizing dose was 2.0 Mrad, and the theoretical 12-log reduction dose was 2.65 or 2.87 Mrad depending on the method of calculation; (iii) some spoilage occurred at dose levels below 2.0 Mrad; (iv) all visible spoilage of irradiated bacon was due to strains 33A and 12885A only, whose *D* values were, respectively, 0.141 and 0.177 Mrad based on spoilage data, and 0.221 and 0.188 Mrad, respectively, when based on recovery data; (v) toxic cans did not always result in swelling, nor did swollen cans always produce toxic spoilage; and (vi) viable *C. botulinum* can exist for at least 8 months in storage at 30 C without producing visible or toxic spoilage at doses below 2.0 Mrad.

Sterilization of foods with ionizing radiation is a prime objective of the U.S. Army food research and development program. The process preserves foods for long periods of time without refrigeration. Hence, this agency is engaged in establishing prototype processes and guidelines for future commercial radioprocessing of military rations or components. Raw sliced bacon appeared to hold promise for initiating a commercial-type radioprocess, because it: (i) has a relatively short shelf life even under refrigeration, (ii) cannot be thermally processed without destruction of organoleptic properties, (iii) withstands relatively high dose levels without significant loss of consumer acceptance, and (iv) is a relatively poor substrate for the growth and toxin production of *Clostridium botulinum*.

Nearly a decade of developmental effort in our laboratories indicated that prototype uninoculated irradiated foods were found to be free from *C. botulinum*. Among the large number of different foods examined through 1963 were 239 cans of bacon. These cans, of various sizes up to

no. 10, had received dose levels ranging from 1.4 to 4.5 Mrad. They were tested for both botulinum toxin and the presence of viable *C. botulinum* prior to evaluation by consumer taste panels. The findings were negative in every instance (Table 1).

Knudsen (1960) reported that electron-irradiated sliced bacon (dose not indicated), packed in polycel bags, could be kept at 20 C for a somewhat longer period of time than unirradiated controls held under refrigeration. The gain in shelf-life time was not indicated, and the retention of organoleptic quality during this period was considered still acceptable. Heiligman (*unpublished data*) demonstrated that sliced bacon, packed in cans and irradiated to 4.5 Mrad with γ rays, maintained high consumer-preference ratings for a period of 2 years at storage temperatures of 21 and 38 C. Ambient temperature storage yielded somewhat higher acceptance scores than the elevated temperature (6.8 and 6.2 hedonic scale points, respectively; a drop of only 0.4 and 0.8 points from the unstored controls).

There are no reports in the technical literature incriminating bacon as a cause of botulism. Nevertheless, it must be assumed that bacon can harbor *C. botulinum*, and that a radioprocess, to be considered microbiologically safe, must

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TABLE 1. *Microbiological safety of uninoculated irradiated bacon*

Year examined	Irradiation dose (Mrad)	No. of cans	
		Irradiated	Free from <i>Clostridium botulinum</i> and toxin
Before 1957	1.4	24	24
	2.3	24	24
1957	Unknown	22	22
	4.5	1	1
1958	2.8	2	2
	4.2	20	20
1959	4.5	38	38
	4.7	4	4
1960	4.5	14	14
	4.2	26	26
1961	4.2	1	1
	4.5	8	8
1962	4.5	28	28
	4.5	25	25
1963	2.5	1	1
	4.5	1	1

destroy this organism. This paper describes studies designed to examine: (i) the effectiveness of 4.5 Mrad to sterilize sliced bacon packed in cans and deliberately infected with large numbers of *C. botulinum* spores, (ii) the dose safety margin provided by 4.5 Mrad, (iii) the possibility of reducing the 4.5 Mrad dose, and (iv) the *D* values of the *C. botulinum* strains used.

MATERIALS AND METHODS

Test organisms. *C. botulinum* strains 33A, 36A, 12885A, 9B, 41B, and 53B were used. Of 102 strains screened in phosphate buffer, 33A, 36A, 41B, and 53B represented the group of highest radioresistance to γ rays, and 12885A and 9B were of intermediate resistance (Anellis and Koch, 1962). Strain 12885A was reported by Wheaton, Pratt, and Jackson (1961) to be the most radioresistant of five *C. botulinum* organisms tested in five different foods. Spores were prepared by the method of Anellis and Koch (1962), except that distilled water was used, instead of phosphate buffer, for washing and suspending the individual spore crops.

Food preparation. The bacon used complied with the military specification MIL-B-35030A, 7 August 1959, under the following description: slab, frozen, smoked, derinded, Type A, Style 1, with a curing salt content as indicated in Table 2. The bacon was cut into strips with a sanitized slicing machine. Batches of 20 slices were placed aseptically between sheets of sterile parchment paper, the slices overlapping along the longitudinal axis, with the leaner portions face up. The packages were boxed and kept at -28.9 C until used.

TABLE 2. *Chemical analysis* of curing compounds in bacon*

Compound	Range†	Median
Water	18.2-34.4%	27.8
NaCl	4.2-6.3%	5.3
NaNO ₂	1.3-9.5 ppm	3.2
NaNO ₃	134-215 ppm	163

* Association of Official Agricultural Chemists, 1960.

† Duplicate determinations were made on six individual samples of bacon. Each sample consisted of the entire pooled contents of five replicate 300 × 200 randomly selected sealed cans of bacon.

Inoculation. Prior to inoculation, the required number of packages were thawed overnight at 5 C. A 2-ml amount of a heat-activated (80 C for 10 min) suspension of *C. botulinum* spores, appropriately diluted, was added onto the surface of each set of 20 bacon strips with a sterile 2-ml syringe. The inoculum was then spread uniformly over the surface and between each strip with a sterile hockey stick-shaped glass rod. The excess water on the bacon was permitted to evaporate into the air. The loss of added moisture occurred within 30 min of air exposure, as determined by experiment.

Canning. Each batch of 20 strips was rolled up aseptically in the parchment, inserted into a 300 × 200 C-enamel can, and cut with a sterile knife to can-size lengths. One rolled package of bacon yielded five full cans of approximately 100 g of meat per can.

The bacon-filled cans were evacuated to 50.8 cm of Hg below atmospheric pressure, and filled with nitrogen, which was filtered through sterile cotton, to atmospheric pressure before sealing. Pairs of sealed bacon cans were then sealed in 307 × 409 metal cans and packed in wet ice.

Two inoculated bacon packs were prepared. Pack 1 consisted of 260 cans inoculated with strain 33A and 260 cans with 41B. Each can contained approximately 6×10^6 spores. This pack was intended to test both the adequacy of 4.5 Mrad to sterilize bacon, and the effect of doses below 4.5 Mrad. Pack 2 comprised 1,640 cans in four 410-can lots, inoculated with the strains 36A, 12885A, 9B, or 53B, respectively, at a level of about 3×10^6 spores per can. The latter pack was designed to establish the minimal radiation sterilization dose. A total of 2,300 cans of bacon, including various irradiated and unirradiated controls, were used in this study.

Irradiation. Irradiation was carried out with γ rays from spent fuel rods at the Argonne High Level Gamma Irradiation Facility (Argonne National Laboratory). Each large can was centered in urn position no. 3, the position of greatest dose uniformity (Swope, 1960), with identical size cans containing ice (-17.8 C) inserted into positions

no. 1T, 2, 4, and 5B to maintain the contents of the sample cans in the range of 1.7 to 10 C during irradiation. All available rack positions were utilized. If vertical doses in the large cans varied by more than $\pm 2\%$ from top to bottom, the cans were removed at the half-way dose, inverted 180°, and reimmersed for the remainder of the exposure. Ferrous sulfate dosimetry (Swope, 1958) was conducted by Argonne personnel, with identical sample cans for determining dose rate.

Bacon Pack 1 was irradiated in the range 1.0 to 4.5 Mrad in 0.5-Mrad increments, with lots of 20 cans per dose per strain up to 4.0 Mrad, and 100 cans per strain at 4.5 Mrad. Pack 2 was subjected to doses of 1.5 to 3.0 Mrad in 0.5-Mrad levels, in lots of 100 cans per dose per strain.

After irradiation, the cans of bacon were removed from the larger cans and stored at 30 C. Pack 1 was incubated for 4 months and Pack 2 for 8 months. Periodic examinations were made for product spoilage.

Enumeration of C. botulinum and indigenous anaerobes. Most probable number counts were made on unirradiated, unincubated control cans of bacon Pack 1 by four methods: (i) washing the bacon with water in a 1-quart (0.946 liter) Mason jar, (ii) washing with fat emulsifying diluent (Capps, Wollam, and Hobbs, 1949), (iii) homogenizing the bacon in a blender jar with water or (iv) with the emulsifier. The entire can contents were used with sufficient diluent to make a 1:5 dilution. Additional serial dilutions were prepared with water or emulsifier. A 1-ml amount of the diluted samples was inoculated into each of five replicate screw-cap tubes (20 × 150 mm) containing 20 ml of Wynne's broth (Wynne, Schmieding, and Daye, 1955). The tubes were incubated at 30 C for 6 weeks, examined for turbidity, and the most probable number estimated by use of the Fisher and Yates (1953) statistical tables. All positive tubes were tested for botulinum toxin by the mouse neutralization assay described below.

Assay of bacon cans for C. botulinum spoilage. Swollen cans were examined after an additional 2 weeks of incubation after visible spoilage. Flat cans of Pack 1 were tested after a 4-month incubation period, and Pack 2 after 8 months of incubation. Cans were opened aseptically, and the entire contents were transferred to sterile 1-quart Mason jars. Sterile distilled water was added to yield 1:5 dilutions. The jars were shaken vigorously 50 times by hand to separate the slices of bacon, then for an additional 3 min on a shaking machine. Samples of the wash water were centrifuged at 2,000 rev/min for 30 min, and one mouse (15 to 20 g) per sample was injected intraperitoneally with 1.0 ml of supernatant fluid. Every sample producing symptoms of mouse intoxication within 4 days of injection was retested on two unprotected mice; two mice were protected with 0.5 ml of antitoxin A, another two mice with 0.5 ml of antitoxin B, and two unprotected mice received 1.0 ml of supernatant fluid which had been boiled for 15 min.

To detect for the presence of viable *C. botu-*

linum, 1.0-ml samples of wash water were inoculated into each of five tubes of Wynne's broth, the tubes were heated at 80 C for 10 min to activate any viable spores present, and incubated at 30 C. An identical set of inoculated tubes were incubated without heat activation. Anaerobic growth within 6 weeks was confirmed for *C. botulinum* with a mouse toxicity test.

Calculation of radioresistance. The equation of Schmidt and Nank (1960) was used to compute the radiation *D* value (dose needed to reduce the spore population by 90% or one log cycle) of the test organisms. Partial spoilage data as well as recovery of *C. botulinum* from nonspoiled cans were employed in these calculations.

Assuming that a microbiologically safe radio-process should conform with a 12-log cycle spore reduction of *C. botulinum*, the sterilizing dose was estimated in two ways:

$$D \times 12, \quad (1)$$

and

$$S + (D \times n) \quad (2)$$

where *S* is the experimentally established minimal sterilizing dose based on flat, nontoxic, sterile cans (2.0 Mrad) for the actual spore density used, and *n* is the number of log cycles needed to adjust the initial spore density used to a hypothetical 10^{11} spores.

RESULTS

Recovery of C. botulinum spores. Blending of bacon Pack 1 controls with water proved to be unsatisfactory for recovering viable organisms. A heavy top layer of fat separated out in which

TABLE 3. Comparative recoveries of *Clostridium botulinum* spores from unirradiated bacon

Method	Strain 33A ^a		Strain 41B ^a	
	No. of samples	Avg MPN per g	No. of samples	Avg MPN per g
Emulsifier wash ^b	3	3.5×10^3	3	7.4×10^3
Emulsifier blend ^c	3	4.0×10^3	3	2.8×10^3
Water wash ^d	3	9.5×10^3	3	8.7×10^3
Water blend ^e	1	— ^f	1	— ^f
Average MPN		5.7×10^3		6.3×10^3

^a Initial inoculum was approximately 3,000 spores per gram.

^b Washed bacon surfaces with fat emulsifying diluent.

^c Comminuted bacon in blender jar with fat emulsifying diluent.

^d Washed bacon surfaces with water.

^e Comminuted bacon in blender jar with water.

^f Very heavy separation of fat layer prevented representative sampling.

TABLE 4. Effect of γ irradiation on spoilage of cans of bacon inoculated with spores of *Clostridium botulinum** (bacon Pack 1)

Strain no.	No. of 300 \times 200 cans used	Radiation dose (Mrad)	No. of samples		
			Swollen	With botulinum toxin	With viable <i>C. botulinum</i>
None 33A	40	0.0	19	0	0
	20	0.0	12	8	17
	20	1.0	1	5	8
	20	1.5	0	1	2
	100†	2.0-4.0‡	0	0	0
	100	4.5	0	0	0
41B	20	0.0	4	0	18
	20	1.0	0	0	7
	20	1.5	0	0	0
	100†	2.0-4.0‡	0	0	0
	100	4.5	0	0	0

* Inoculation level was 600,000 spores per can.

† There were 20 replicate cans per dose.

‡ Increases in 0.5 Mrad increments.

many of the bacteria could be trapped, making representative sampling of the diluent impracticable. Washing the clostridia from the bacon surfaces with water yielded most probable number estimates approximately equal to those obtained by washing or blending with fat emulsifier (Table 3). Hence, all mean counts, acquired by the three latter techniques, were averaged to yield 6×10^3 spores per gram of bacon for both strain 33A and 41B, or about 6×10^5 spores per can.

Employing the surface-water wash method on bacon Pack 2 controls gave mean most probable numbers of 3×10^4 spores per gram, or 3×10^6 spores per can of bacon, for each of the strains 36A, 12885A, 9B, and 53B.

Sterilizing dose range for bacon. Of 200 cans of bacon seeded with 6×10^5 spores of *C. botulinum* (33A, 41B) per can and irradiated to 4.5 Mrad, not one can evidenced visible swelling and toxicity after 4 months of storage at 30 C (Table 4). Neither viable spores nor vegetative cells could be recovered after this prolonged incubation period. A radiation level of 4.5 Mrad appeared to be more than adequate as a sterilizing dose for cans of bacon containing a *C. botulinum* load of 6×10^5 spores per can.

An additional 200 inoculated cans of bacon, exposed to 2.0, 2.5, 3.0, 3.5, and 4.0 Mrad in lots of 20 cans per strain per dose, were found to be nonswollen, sterile, and free from toxin (Table 4). At 1.5 Mrad, none of the cans out

of 40 swelled; yet, one can contained toxin and two cans contained viable *C. botulinum*. However, at 1.0 Mrad, one can out of 40 was swollen and toxic, and harbored viable *C. botulinum*. Another four flat cans had toxin and viable botulinum organisms, and an additional ten flat cans had no toxin but contained viable botulinum organisms. Only 12 of 20 cans of unirradiated bacon inoculated with strain 33A were swollen; of these spoiled cans, only eight had botulinum toxin. Viable botulinum organisms were found in a total of 17 cans. Strain 41B produced just four swollen cans out of 20; though toxin appeared to be absent from all 20 cans, viable *C. botulinum* was recovered from 18 samples.

All the spoilage which occurred at 1.0 and 1.5 Mrad exposures was caused by strain 33A only. Strain 41B was apparently killed at 1.5 Mrad, and remained viable but dormant at 1.0 Mrad in 35% of the cans.

No *C. botulinum* organisms or their toxins could be recovered from 40 uninoculated, unirradiated cans of bacon, although 47.5% of the samples were visibly spoiled after the 4-month incubation period. This spoilage was caused by the

TABLE 5. Effect of γ irradiation on spoilage of cans of bacon inoculated with spores of *Clostridium botulinum** (bacon Pack 2)

Strain no.	No. of 300 \times 200 cans used	Radiation dose (Mrad)	No. of samples		
			Swollen	With botulinum toxin	With viable <i>C. botulinum</i>
36A	10	0.0	1	10	7
	100	1.5	0	0	29
	100	2.0	0	0	0
	100	2.5	0	0	0
	100	3.0	0	0	0
12885A	10	0.0	2	9	9
	100	1.5	1	0	3
	100	2.0	0	0	0
	100	2.5	0	0	0
	100	3.0	0	0	0
9B	10	0.0	2	10	9
	100	1.5	0	0	15
	100	2.0	0	0	0
	100	2.5	0	0	0
	100	3.0	0	0	0
53B	10	0.0	0	10	6
	100	1.5	0	0	9
	100	2.0	0	0	0
	100	2.5	0	0	0
	100	3.0	0	0	0

* Inoculation = 3 million spores per can.

TABLE 6. Radiation sterilization dose for bacon inoculated with spores of *Clostridium botulinum*

Strain no.	Spores per can	No. of cans	Total spore inoculum	D value (Mrad) based on		Sterilization dose (Mrad)				
				Spoilage data ^a	Recovery data ^b	Exptl ^c	Actual no. of spores used ^d		Adjusted to 10 ¹¹ spores and reduced to 10 ⁻¹ spore ^e	
							Spoilage data	Recovery data	Spoilage data	Recovery data
33A	6 × 10 ⁵	20	1.2 × 10 ⁷	0.141	0.221	2.0	1.69	2.65	2.55	2.87
36A	3 × 10 ⁶	100	3 × 10 ⁸		0.214	2.0		2.57		2.54
12885A	3 × 10 ⁶	100	3 × 10 ⁸	0.177	0.188	2.0	2.12	2.26	2.45	2.47
9B	3 × 10 ⁶	100	3 × 10 ⁸		0.205	2.0		2.46		2.52
41B	6 × 10 ⁵	20	1.2 × 10 ⁷		0.160	2.0		1.92		2.63
53B	3 × 10 ⁶	100	3 × 10 ⁸		0.199	2.0		2.39		2.50

^a Number of swollen cans.

^b Number of flat cans containing recoverable *C. botulinum*.

^c Flat, nontoxic, sterile cans.

^d Decimation through 12 log cycles with equation $D \times 12$.

^e Decimation through 12 log cycles with equation $S + (D \times n)$.

indigenous nonputrefactive bacon microflora, as demonstrated by taxonomic examinations.

Minimal sterilization dose. A total of 1,200 cans of bacon, containing 3×10^6 spores per can and irradiated to 2.0, 2.5, and 3.0 Mrad in lots of 100 cans per strain per dose, were found to be nonswollen, nontoxic, and sterile (Table 5). At 1.5 Mrad, 399 cans out of 400 were neither swollen nor toxic. Only one of the 400 cans, harboring strain 12885A, was visibly spoiled but did not contain toxin. However, at this dose, 29% of the samples containing strain 36A, 3% of the cans seeded with 12885A, 15% infected with 9B, and 9% inoculated with 53B contained viable *C. botulinum*. Unirradiated controls had practically 100% toxic spoilage, but only 12.5% of these cans were visibly swollen.

Radioresistance of *C. botulinum*. Information in Tables 4 and 5 was employed to calculate *D* values. Strains 33A and 12885A were the only two of the six strains used which produced visible spoilage below the 2.0 Mrad level. The *D* values of these two strains, based on the commonly used visible swelling data were, respectively, 0.141 and 0.177 Mrad (Table 6). However, on the basis of the more severe recovery tests, strain 33A appeared to be somewhat more resistant to radiation than 12885A ($D = 0.221$ and 0.188 Mrad, respectively).

Strains 36A, 9B, 41B, and 53B did not cause visible spoilage of bacon, thus, *D* values were computed on the basis of recovery data only. The *D* values of these strains were, respectively, 0.214, 0.205, 0.160, and 0.199 Mrad (Table 6).

The theoretical 12*D* dose computed for each strain used was in nearly every instance higher

than the experimental sterilizing dose observed. In general, the highest theoretical sterilizing doses were obtained by the mathematical adjustment of the initial spore inoculum to 10¹¹ spores, and decimating them through 12 log cycles with the equation $S + (D \times n)$ rather than with $D \times 12$.

DISCUSSION

Bacon Pack 1 has yielded evidence that, under closely controlled experimental conditions, a dose of 4.5 Mrad was more than adequate as a safe sterilization process under conditions of unrealistically high levels of contamination with *C. botulinum* (6×10^5 spores per can). Furthermore, the data strongly indicated that the sterilizing dose could safely be lowered to somewhere in the range between 2.0 and 4.5 Mrad. Occasional botulinum spoilage can be expected to occur at 1.0 and 1.5 Mrad under these highly artificial conditions.

Bacon Pack 2 defined more accurately the minimal radiation sterilization dose. It verified initial results (Table 4) that bacon could be sterilized with doses of 3.0, 2.5, and 2.0 Mrad, even when the meat contained 3×10^6 spores per can (Table 5). These results were based on nonswollen, nontoxic, sterile cans rather than on spoilage data.

If a microbiologically safe radioprocess must conform to a 12 log cycle spore reduction (12*D*) of *C. botulinum*, the computed dose should be based on either recovery data or on spoilage results in which the unirradiated total inoculum is mathematically adjusted to 10¹¹ spores or, $S + (D \times n)$. Calculation of a 12*D* dose by the

equation $D \times 12$ may not always provide the maximal safety. For example, strain 33A, with an apparent spoilage D value of 0.141 Mrad, gave a computed $12D$ ($D \times 12$) of 1.69 Mrad, whereas the experimental sterilizing dose was 2.0 Mrad (Table 6). In this instance, the margin of safety against botulism is seriously diminished. However, in the case of strain 12885A, the spoilage $12D$ dose of 2.12 Mrad is adequate from a safety standpoint. Under the conditions of the experiment, the latter strain appeared to be more active physiologically than 33A. Thus, although 12885A yielded a higher D value based on spoilage data, in reality strain 33A was of higher radioresistance, as demonstrated by recovery of the test organism from bacon.

Using recovery data and the most radioresistant strain tested, a theoretically safe radioprocess for bacon packed in cans should be 2.65 or 2.87 Mrad, depending on the method of calculation (Table 6). This is 0.65 or 0.87 Mrad higher than demonstrated by actual experiment.

Bacon, generally, is a relatively poor medium for the germination and growth of *C. botulinum* even under grossly infected conditions (Table 4 and 5). The organism can remain viable for at least 8 months of storage at 30 C without producing visible or toxic spoilage at doses below 2.0 Mrad, although there appears to be a potential danger of botulinum spoilage in unirradiated cans. It may also be noted that toxic cans did not always result in swelling, nor did swollen cans always produce toxic spoilage. Greenberg, Silliker, and Fatta (1959) reported similar results with thermally processed cans of cured hams. They attributed the phenomenon to the inhibitory property of the salts present in the cured meat.

It should be emphasized that the margin of public health safety built into a calculated 2.65 to 2.87 Mrad radioprocess for bacon packed in cans is assured by three factors:

(i) The sterilizing dose of 2.65 or 2.87 Mrad is 0.65 to 0.87 Mrad higher than the actual dose observed to be necessary for sterilization of bacon inoculated with 6×10^5 to 3×10^6 *C. botulinum* spores per can.

(ii) The sterilization dose of 2.65 or 2.87 Mrad is computed to accomplish a theoretical destruction level of 12 log cycles for the most radioresistant strain used (i.e., 10^7 spores to 10^{-5} , or 10^{11} spores to 10^{-1}). Available data suggest that the incidence of total anaerobic spores in raw meats is very low (Steinkraus and Ayres, 1964), and, of this population, the proportion of spores of *C. botulinum* is considerably lower. This organism has never been reported to occur in bacon.

(iii) At the end of the incubation periods, all unspoiled cans of both inoculated bacon packs were opened and examined for the presence of toxin and for viable *C. botulinum*. This subculture technique is a more severe test for sterility than the partial spoilage method commonly used for calculating analogous processes. Hence, this method increases significantly the confidence of the safety margin built into a 2.65 or 2.87 Mrad sterilizing dose.

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