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Microwave Acid Digestion of Human Body Fluid for Trace Elements Measurements

Chi-Ching Chan*, Chem-J Yen, Chao-Chin Hu, Wen-Kang Chen

Trace metal measurements in body fluid by atomic absorption spectrophotometer require preliminary digestion of the sample by strong acid. Microwave radiation has been used to heat the acid, which dramastically shortens the time required for digestion and facilitates the preparation for large numbers of samples to analysis.

We have applied this technique to the digestion of small amount biological samples by modifying a commercially available microwave digestion bomb. Digestion proceeds rapidly (≦22 mins) in the double close-vessel that eliminates contamination or loss from volatilization.

Standard Reference Material from National Institute of Standards and Technology bovine serum (1598), bovine liver (1577a), and "Second-Generation" Biological Reference Material human serum are analyzed to verity the accuracy of this technique. We assessed the applicability of this technique to analysis for trace elements in biological specimens.

Key words: body fluid, trace element, microwave digestion

Introduction:

Trace metal measurements in biological specimens by atomic spectroscopic techniques reguire preliminary digestion of the sample[1]. This digestion process is the limiting factor as to how much time the entire procedure takes and also as to the efficiency of recovering the actual amount of metal present in the smaple[2]. It became clear that the sample and target preparations are critical steps in the analysis of such sample type. Metals can be released from the tissue by combusting the organic tissue at high temperature (the dry ashing method of digestion), or by combusting the organic tissue using heat and concentrated acid (the wet ashing method). Both techniques have certain limitation including excessive time, risk of contamination of possible loss of metals by voltilization, excessive manipulation of sam-

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ple and incomplete digestion[2,3].

Using microwave energy as the heat source in acid digestion was first demonstrated eighteen years ago[4]. Recently comercial microwave digestion system have become available for laboratory use, and several studies have been published[1-10]. When it is used with sealed microwave-transparent vessels, temperatures above the boiling point of the acid are produced. This drastically shortens the time needed for digestion[6,8-9] and facilitates the preparation of large numbers of specimens for anlysis[1]. Microwave heating is rapid, safe, and programnable. It can offer advantages over traditimal wet or dry washing techniques[2]. This techniques depend on the direct coupling of electromagnetic radiation with the mineral acid solvents used in decomposition. Because high temperatures and pressure are obtained in sample preparation time, control of the temperature and pressure during these closed vessel digestions in critical both to the efficiency and reproducibility of the digestion as well as to the safety of the operation[4].

We have applied this technique to the digestion of small amounts of biological sample such as serum, urine by double close-vessel digestion constitution including commercially available microwave digestion vessel and place two 7 ml Teflon vials in it[11]. Standard Reference Material from National Institute of Standards and Technology bovine serum 1598, bovin liver 1577a, and "Second-Generation" Biological Reference Material human serum are analyzed to verity the accuracy of this technique. The detection limits obtainable when using the modifying microwave digestion method is also determined.

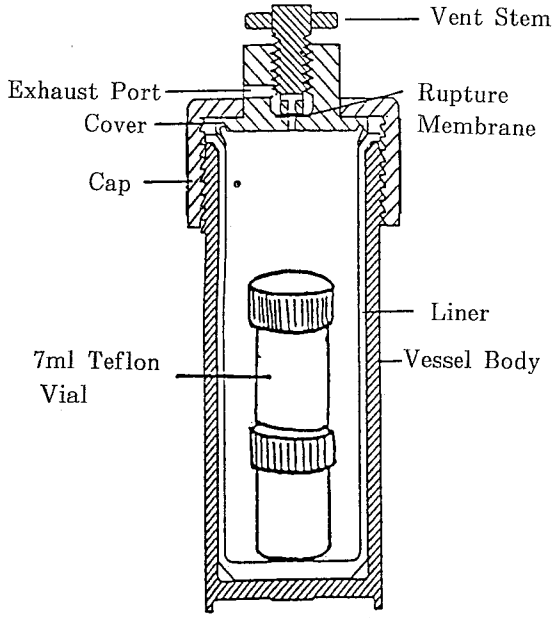
Materilas and Methods:

Apparatus:

A laboratory microwave sample preparation system, Model MDS-2000, puchased from CEM corp, Matthews, NC, is used. The oven has a variable power range to 100% full power (630W±50W) in 1% increments, a variable timing range from 1 second to 59 minutes 59 seconds, and pressure control system to monitor and control pressure conditions inside sample vessels. A maximum of five sequential stages of varying power and time intervals can be programed, and 20 multi-stage programs can store in the computer memory.

The double close-vessel digestion constitution including a lined digestion vessel consists of an outer pressure vessel and chemically resistant 100 ml inner liner which is placed two 7 ml Teflon vessels in it (Fig 1). Because the maximum number of digestion vessels used in a microwave digestion oven are twelve, we can digest twenty four samples in one time.

Fig.1. Double Close-Vessel Digestion Bomb.



A clean laboratory and laminar-flow hood capable of producing class 100 air (100 particles per cubic meter) are required for all specimens handling to avoid to possibility of contamination from environment. All laboratory wares were thoroughly cleaned by soaking in nitric acid solution (50%, V/V) for at least 24 hrs, and rinsed with deionized water followed by another rinsed with double distilled deionized water.

Atomic absorption spectrometric analysis are performed with a model 5100 PC instrument utilizing on electrothermal graphite atomizer, equipped with Zeeman background correction (5100 PC with 5100 ZL Zeeman Furace Module, PERKIN-ELMER Corp. NorWalk). Table 1 list the instrumental settings for analysis in this work. All elemental quantifications are determined by comparing spectrometric data with the appropriate aqueous calibration curves.

Table 1. Instrumental Conditions of Graphite Furnance AAS.

	Cu	Fe	Zn	Pb
Method	GFAAS	GFAAS	GFAAS	GFAAS
Lamp Energy/wave length (nm)	70/324.8	61/248.6	63/213.9	69/283.3
Matrix modifer	None	None	None	None
Dry 1				
Temperature (℃)	110	110	110	110
Ramp/Hold (sec)	1/30	1/20	1/30	1/30
Gas Flow (ml/min)	250	250	250	250
Dry 2				
Temperature (℃)	130	130	130	130
Ramp/Hold (sec)	10/30	10/30	10/30	10/30
Gas Flow (ml/min)	250	250	250	250
Pyroysis				
Temperature (℃)	500	900	200	300
Ramp/Hold (sec)	10/20	10/20	10/20	10/20
Gas Flow (ml/min)	250	250	250	250
Atomize				
Temperature (℃)	2000	2100	1400	1400
Ramp/Hold (sec)	0/5	0/5	0/5	0/5
Gas Flow (ml/min)	0	0	0	0
Clean Out				
Temperature (℃)	2400	2400	2400	2400
Ramp/Hold (sec)	1/5	1/2	1/2	1/5
Gas Flow (ml/min)	250	250	250	250

Condition Reference: Modified by Running the THGA (Transersely Heated Graphite Atomized) Graphite Furnace: Techniques and Recommended Condition (Perkin-Elmer corp.)

Reagents:

High-purity water used for both preparative and cleaning purpose is produced by double distillation of deionized water by bi-distillation apparatus (DESTAMAT, Heraeus, Quarzglas, Kleinostheim). Nitric acid and perchloric acid used for microwave acid digestion are of suprapur grade (E. Merck). Nitric acid for clean glassware are of GR grade (E. Merck or BDH). Working standard of atomic absorption reference solutions are prepared by diluting a required amount of the stock solutions (E. Merck) with double distilled deionized water. The validation of the methods developed in this study are carried out by using NIST Standard Reference Materials bovine serum 1598, bovine liver 1577a., and "Second-Generation" Biological Reference Material human serum.

Specimens:

Samples of human serum and urine taken from students of 19-25 years in Chung-Shan Medical and Dental College are collected in low metal contamination injector or containers. All blood and urine from donors are delivered to the laboratory within two hours and followed by centrifugation at 3000 rpm for 10 mins. The supernatnat are put into acid-washed plastic tubes and stored at -70℃.

Procedure:

Microwave acid digestion by double-close vessel

(1) Transfer 1 ml Human serum or urine sample directly into the 7 ml Teflon vials then add 500 ul concentrated nitric acid and 200 ul concentrated perchloric acid as described by Chi-Ren Lan[11] with slight modification. Prepare blanks that contain only water and acid. After capping the liners, seal them in the digestion vessels. Place the assembled units, twelve vessels at a time, in the trunable of the microwave oven and irradiate at variable power and time controlled by the program stored in computer memory (Table 2). Remove the digestion vessels from the microwave oven when they are sufficiently cooled and pressure in the vessels are decreased. Dilute sample and blanks with double distilled deionized water after digestion to the total volume of 10 ml, then transfer to acid-washed plastic tubes and stored at 4℃ until measurement the concentration of Zn, Cu, Fe, Pb.

(2) SRM Bovine serum 1598, bovine liver 1577a, and Biological Reference Materials human serum are digested by different programs as described above. The metal concentration is analyzed to verity the accurary of this techniques.

Preparation of Standards:

Standards of Cu, Fe, Pb and Zn for calibration are prepared by serial dilution of the stock solution with double distilled deionized water to produce concentrations within each metal's calibration range. Calibration curve for the determination of each elements are shown in fig 2. Spiking standards of Cu (1500 ug/L), Fe (2000 ug/L), Zn (500 ug/L) and Pb (10 ug/L) are adding in mixed serum specimen before microwave acid digestion. The recovery of each metal is listing in Table 3.

Quantification of metals concentration:

Determination of metals followed digestion procedures described is performed with Graphite Furnace AAS (PERKIN-ELMER 5100PC). Samples solution analyzed for Pb in human serum run without further dilution. Other metal in different samples are diluted with water in order to ensure that sample

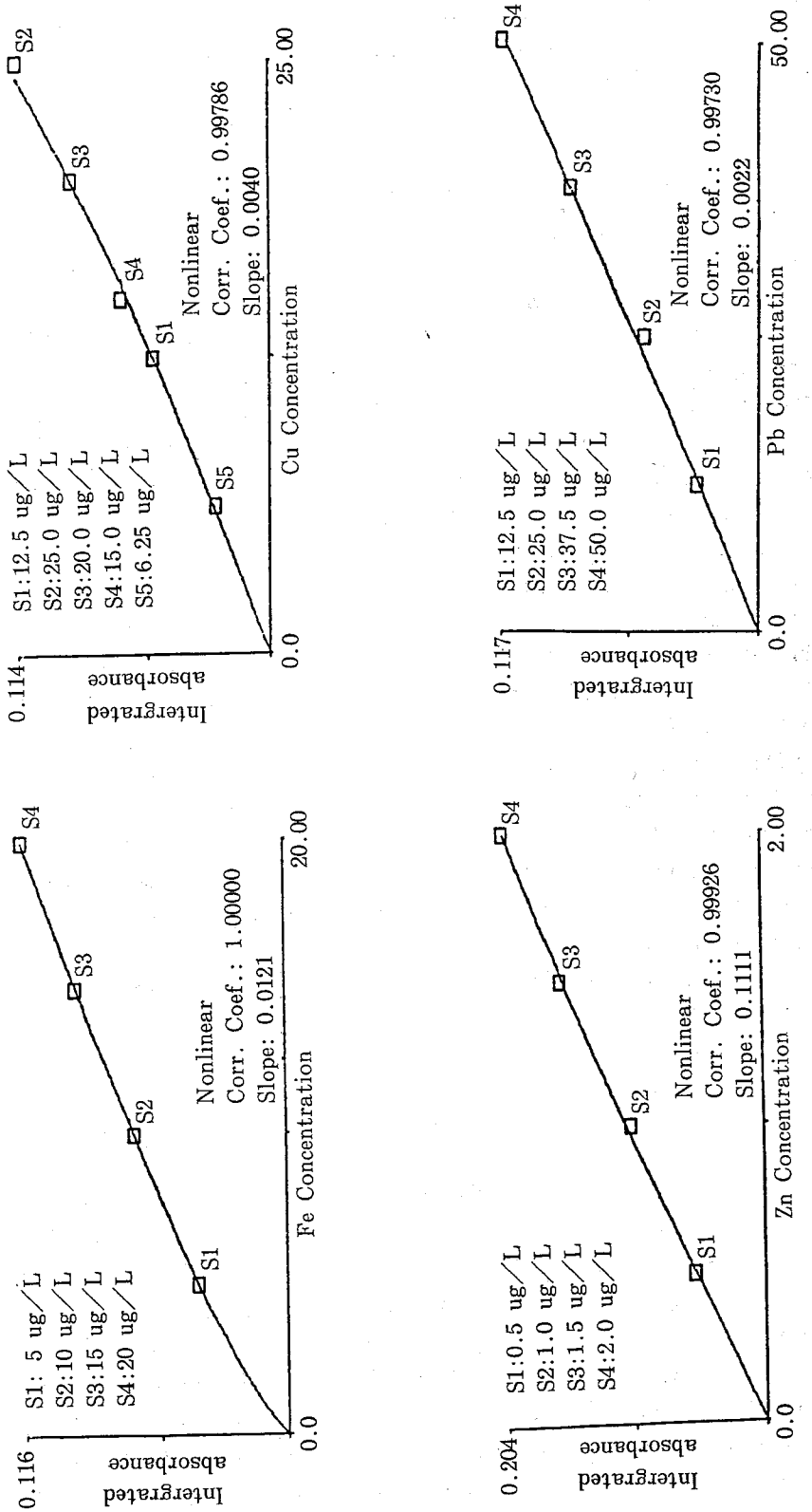


Fig. 2. Calibration Curve for the Determination of each Element in this Work.

Table 2 The Program of Microwave Oven for Digestion of Human Serum and Urine.

File name	Program / Stage	Power (%)	Pressure (psi)	Run Time (min)	TAP Time (min)	FAN Speed (%)
Serum2	1	40	60	03 : 00	02 : 00	100
	2	60	85	05 : 00	03 : 00	100
	3	75	130	08 : 00	03 : 00	100
	4	90	150	03 : 00	03 : 00	100
	5	100	180	03 : 00	03 : 00	100
Urine1	1	30	60	03 : 00	02 : 00	100
	2	50	85	05 : 00	03 : 00	100
	3	70	130	08 : 00	03 : 00	100
	4	90	150	03 : 00	02 : 00	100
	5	100	180	03 : 00	01 : 00	100

a. Number of Vessels: 12
b. Acid: HNO_3 plus $HClO_4$

Table 3. Recovery Values (%) for Spike Samples of Human Serum Analysized by Microwave Digestion.

	Cu	Fe	Zn	Pb
Sample Concentration (ug/L) [a]	1148 ±15.9	3975 ±73.4	783.8±155.3	4.6±0.6
Adding Spike Concentration (ug/L)	1500	2000	500	10
Measure Spike Concentration (ug/L) [b]	1649.7±39.8	2063.4±57.1	490.9±106.2	9.3±1.1
Average Recovery (%) [c]	110	103	98	93

a, b Sample concentration are obtain from five mixed serum specimens.

c Average recovery (%) = $\frac{\text{Mean of measure of spike concentration}}{\text{Adding spike concentration}}$

concentration within each metal's calibration range.

The optinization of the atomization temperature program is one of the most important considerations in Graphite Furnance AAS analysis. The instrumental parameters are given in Table 1.

Result:

We used the acid-digestion microwave techniques to analyze biological specimens for Zn, Cu, Fe and Pb. Quality assurance is monitored through the determination of sample spiked standard solutions and NIST Standard Reference Materials. Average spiked sample recovery values for each metals range from 93-110% (Table 3). The good recovery values of this techniques indicate

a low loss or contamination of metals during the microwave acid-digestion process.

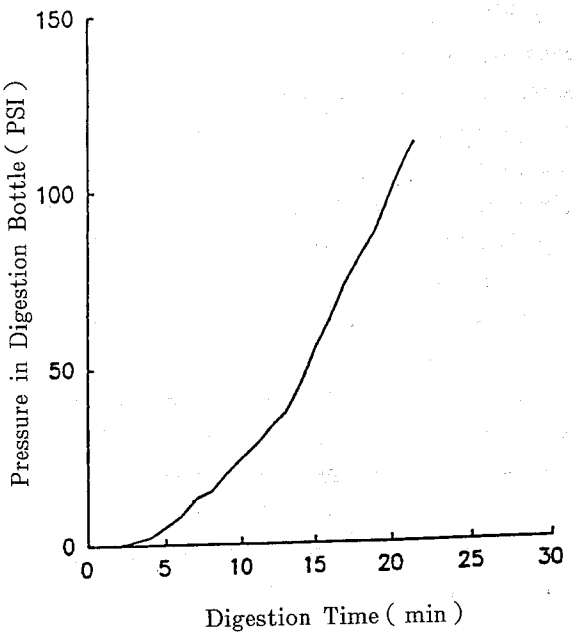
Table 4 summarizes the results obtained for NIST-certified bovine liver (SRM 1577a), bovine serum (SRM 1598) and Biological Reference Material human serum analyzed by the microwave acid-digestion procedure. The detection limit of the preposed method, defined as three times the standard deviation of the analytical results[14] for SRM, are also listed in Table 4. After digestion by microwave heating, the metals in human serum and urine are determinated by atomic absorption spectroscopy. The mean and standard deviation (SD) values of the concentration of the metals in biological samples are listed in Table 5. Except lead in human serum and copper in urine, most of the metals concentration in body fluid are resemblance to reference data from previous studies[15-20].

Discussion:

Microwave acid digestion offers certain advantages over conventional wet ashing. Microwave digestion is faster and requires less of the analyst's time than dose wet ashing; the microwave unit allows programming of time and power cycle so that once a method has been setup, sample digestion can be automated[2]. In this study, we determine that a carousel of 24 samples can be digested within 22 mins by the double close-vessel digestion system. On the other hand, wet ashing and dry ashing digestion takes about 4 hours or more of almost constant attention[2].

Microwave heating of acids occurs much more rapidly than conduction heating used to raise to the temperature of acids using a hot plate[10]. If heating is done in a sealed pressure vessel, the maximum temperature will rise dramatically[10,12,13]. However, the pressure in a sealed container will increase quickly during microwave heating [10]. In addition to partial pressure of the acids, reaction products (such as CO_2, NO) will rapidly increase pressure in sealed vessels[4,10]. Because Teflon is transparent to microwave radiation, it does not absorb heat energy directly from the microwave but does adsorb heat from the sample though conduction. Pressure rather than temperature is more often limited parameter for nitric acid and organic sample digestions. A microwave oven with pressure control system is used for digested biological specimens in this work. Fig 3 shows the pressure profile of the samples with acids in a double-closed vessel exposed to microwave radiation. For safety reason, we apply lower radiation power in the beginning of digestion to slowly ramp the press and temperature[4].

Fig.3. The Pressure Profile of the Sample with Acids in a Double-Closed Vessel Exposed to Microwave Radiation.



Recovery values for serum sample, spiked with varying amounts of Cu, Fe, Zn and Pb are analyzed in this work (Table 3). Average recovery values for microwave acid-digestion ranged from 93-110%. This results proved that the double closed-vessel digestion system can avoid loss or contamination of metals.

We use this technique to analyze human serum for Pb, Fe, Cu, Zn, human urine for Cu, Fe. The NIST SRM bovine serum 1598, bovine liver 1577a, and BRM human serum are also analyzed to verity the accuracy of this technique. Table 4 summarizes the results obtained for standard reference materials analyzed by the method developed in this work. The experiment results agree with the certified (recommended) values. The microwave oven, mixed acid digestion system is a suitable dissolution technique for a wide range of biological specimen types.

Metals concentration in biological specimens are showed in Table 5. Expect Pb in human serum and Cu in urine, most of them are resemblance to previous studies[15-20].

The survey of the two articles literature[21,22] shows that widely divergent reference values have been reported for trace elements in blood plasma or serum of healthy persons. Certainly, some of the disparities may be interpreted as biologic variations due to age, sex, pregnancy, physiologic conditions, dietary habits, environmental or occupational exposure, geographic circumstances, or similar influences[22]

The different concentration of lead between this work and previous studies may be caused by different sample collection. The values for Pb refer to human whole blood[16], and we are obtained from human serum. Tsuchiya[23] reports the lead in erythrocytes is about 16 times than in plasma, the concentration of lead in serum obtained by this work are at normal range.

The reasons of urine copper concentration obtained by us are lower than reference data including: 1. Only a random urine sample is collected for analyze in this work. On the other hand, 24 hours urine sample are collected for analyze in other studies [16,17]. 2. Copper is mainly excreted via the bile, as shown in animals and in human beings[17]. The biliary excretion is about 100-fold higher than excreted into urine.

In conclusion, this reasearch study proves that the microwave digestion system with double-closed vessel reported is an efficient technique to prepare biological body fluid samples for determination of elements.

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Table 4. Quality Control Using Standard Reference Materials in the Work.

Element	Centified value (Mean±SD)	This Work (Mean±SD)	Program of[a] Microwave Acid Digetion	Detection[b] Limit (3×SD)
SRM 1577a				
Pb (ug/g)	0.135± 0.015	0.143±0.037	Serum 2	0.111
Cu (ug/g)	158 ± 7	163.25 ±9.40	(Urine 1)	28.2
Zn (ug/g)	123 ± 8	117.35 ±6.97		20.91
Fe (ug/g)	194 ±20	195.53 ±8.41		25.23
SRM 1598				
Cu (ug/g)	0.72 ± 0.04	0.80 ±0.19	Serum 2	0.57
Zn (ug/g)	0.89 ± 0.06	0.93 ±0.17		0.51
Fe (ug/g)	2.55 ± 0.10	2.89 ±0.27		0.81
BRM Human Serum				
Cu (ug/g)	11.1 (10.7−11.5)[c]	11.81 ±0.09	Serum 2	0.27
Zn (ug/g)	9.6 (9.4− 9.8)	10.87 ±1.41	(Urine 1)	4.23
Fe (ug/g)	25.9 (24.4−27.4)	25.91 ±0.32		0.96

a: Program of microwave acid digestion are listed in Table 2.
b: Detection limit are defined as three times the standard deviation (SD) of the analytical results.
c: Centified value with, in parentheses, 95% centified limits.

Table 5. Metals Concerntration in Biological Specimens.

Sample Element	Sample Number	Mean±SD	Reference Data[a]
Human Serum			
Pb (ug/L)	22	19.65± 17.78	<200−350[b]
Fe (ug/L)	53	1366.49±591.61	800−1200
Cu (ug/L)	53	959.30±183.51	815−1370
Zn (ug/L)	53	766.75±311.69	800−1100
Human Urine			
Cu (ug/L)	25	17.05± 7.85	30−60[c]
Fe (ug/L)	25	116.25± 65.71	100−150

a: Reference data are obtained from previous studies (15−20)
b: Reference data of Pb concentration are obtained from human whole blood. Concentration of Fe, Cu, Zn in this work are obtained from serum.
c: Reference data of Cu, Fe concentration are obtained from 24 hours urine sample, concentration of Cu, Fe in this work are obtained from random urine sample.

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偵測人體體液中微量元素之微波酸消化前處理技術之研究

詹騏璟* 嚴正傑 胡超群 陳文貴

以原子吸收光譜儀偵測生物樣品之微量元素時，許多樣品需熱強酸分解，以降低基質之干擾效應。近年來，密閉式微波酸消化法開始被應用在樣品前處理步驟，不僅可以節省消化樣品的時間及同時處理多個樣品，並且能避免樣品在消化過程中之污染或漏失。

為了配合小量體積之體液樣品的微波消化處理，本實驗以內容積7毫升之鐵氟龍小瓶盛裝檢體及酸液，放入內容積100毫升之微波消化瓶中，組合成雙層密閉式微波消化瓶，供體液樣品之消化分解用。利用此技術處理之全部消化過程少於22分鐘，即能取得消化完全之樣品溶液。

SRM 1598牛血清標準品，SRM 1557a牛肝標準品以及人類血清標準品以此微波酸消化前處理技術分解，分析其微量元素含量，驗證方法之準確度，同時將此前處理流程應用於血清尿液樣品，偵測其微量元素之含量。

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