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6 The ^{14}C urea breath test

BARRY J. MARSHALL

Introduction

THE FIRST LIQUID based breath test that was used for diagnosis of *Helicobacter pylori* infection was a ^{14}C urea breath test devised by Marshall and Surveyor in the early years when the organism was still named *Campylobacter pylori*[1,2]. They administrated ^{14}C urea in water to fasting patients. At that time the use of ^{14}C for medical diagnosis was well established in gastroenterology for diagnosing other conditions, e.g. detection of fat malabsorption, lactose intolerance, and abnormal liver function[3–5]. The principle of the ^{14}C urea breath test is exactly the same as that for the ^{13}C urea test (see Chapter 5), the only difference being the use of a different carbon isotope. Due to the large amount of the *H. pylori* urease enzyme produced in the gastric mucosa, ingested urea labelled with ^{14}C is broken down to form HCO_3^- and NH_4^+. Since the solubility of CO_2 in acidic gastric juice is low, $^{14}CO_2$ is driven towards the mucosa where it quickly reaches the bloodstream. ^{14}C is a low energy β emitter which can easily be detected by scintillation counting of captured $^{14}CO_2$ from expired breath. This is illustrated in Figure 6.1.

Initially Marshall and Surveyor used 10 μCi (microCuries) of ^{14}C urea and Surveyor has continued to use this dose[2]. This rapid method was validated in a report by Debongnie *et al.*[6]. In that study, it was noted that samples earlier than 15 minutes sometimes gave incorrect (positive) results because the liquid urea solution was exposed to urease enzyme from mouth bacteria.

Graham and Klein[7] reported a ^{13}C urea breath test in which the isotope was measured by mass spectrometry. Because of inherent limitations with the ^{13}C urea method, a liquid meal was used to delay gastric emptying, and ^{13}C was measured in breath collected for 60-90 minutes. Bell *et al.*[8] and Rauws *et al.*[9] used ^{14}C urea in an almost identical fashion and measured cumulative $^{14}CO_2$ excretion. Methods which require patients to ingest a meal have a longer collection time (20–90 minutes) and give lower counts (100–500 disintegrations per minute (DPM)) in breath samples[10–12]. Thus a higher dose of isotope is preferred (3–10 μCi). In the United States, Marshall *et al.* validated a 20 minute 5 μCi test and obtained excellent results in a large series of patients[13].

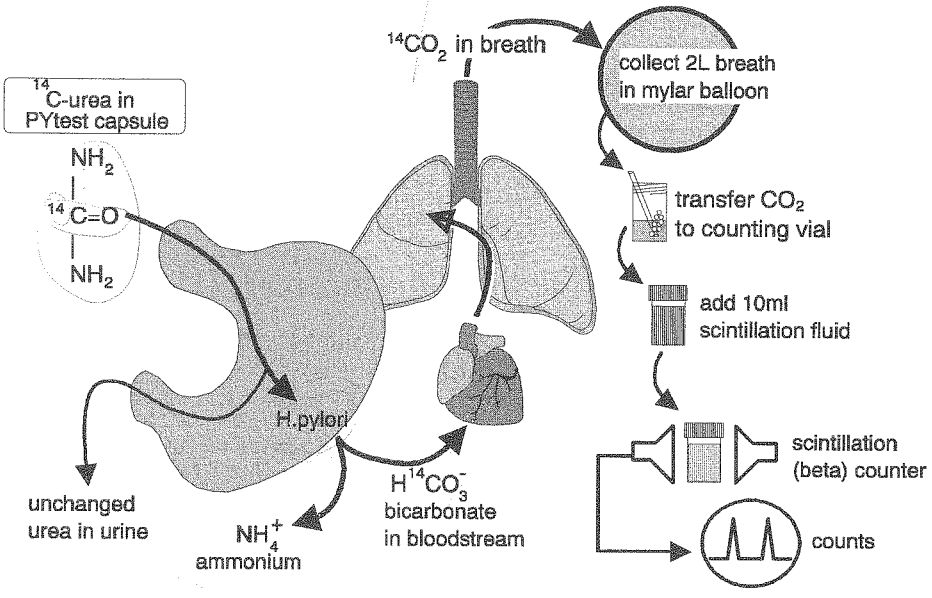

Figure 6.1 *The principle of the ^{14}C urea breath test.*

In order to use the lowest dose possible of ^{14}C urea, contact between oropharyngeal urease and ^{14}C urea must be prevented. If this is not done, then urease in the mouth of normal persons raises the 'negative range' and impairs discrimination between *H. pylori* infected (HP+) and uninfected (HP–) persons. Thus the urea can be supplied to the gastric mucosa in a meal (effective but slows the test), in a drink of water (effective but increases spurious urea hydrolysis from the mouth and causes elevated counts in negative patients) or in a capsule (maintains speed and avoids oral urease). If a rapid test method is used and the urea contacts the oral mucosa, then patients must prepare for the test by thoroughly brushing and rinsing their mouth and diagnostic samples must be delayed 20 minutes to allow $^{14}CO_2$ from mouth urease to dissipate. The highest ^{14}C excretion occurs when isotope is dissolved in 20 mL of water and given to a fasting patient[1,2] but similar high breath counts can be obtained by placing the urea within a quick dissolve capsule which delivers urea immediately onto the gastric mucosa rather than sequestering it within a liquid bolus in the lumen[14]. This method allows the use of very small amounts of ^{14}C urea and is therefore the ^{14}C test of the future.

WHY USE ^{14}C UREA

The ^{14}C urea test is inexpensive. It can be done for about twice the cost of laboratory based serological tests for *H. pylori*. Scintillation counters are commonplace so most hospitals can do the test with no capital outlay. Clearly a breath test is far less costly than endoscopy with biopsy for histology or culture.

The ^{14}C urea breath test is also very convenient. Several methods have been published, but the test can be done in 15–20 minutes and an answer can be obtained 2 minutes later if a scintillation counter is immediately available.

The only drawback of ^{14}C urea is the fact that it is radioactive and therefore is subject to regulations concerning its use. Some of these regulations relate to sensible handling of isotopes but most are designed for much larger doses of other isotopes and are not necessarily appropriate for tiny doses of ^{14}C. Recognising this, the Environmental Protection Agency (EPA) in Australia has recently confirmed that the ^{14}C urea 1 μCi breath test can be used without a nuclear licence because it is so far below the 100 μCi lower limit they have in place for regulation of that isotope (see safety issues below).

The method

1 μCi CAPSULE (PY test®, TRI-MED SPECIALTIES INC, USA)

1 Fast the patient for 6 hours prior to administration of the capsule. The patient should be sitting comfortably at rest. No other preparation is necessary.

2 Instruct the patient to take the capsule with 20 mL of water from a medicine cup.

3 Three minutes after ingestion of the capsule, the patient should drink another 20 mL of water.

NB. Properly dispose of all packages according to your facility's regulations.

4 Collect breath samples at 10, 15 and 20 minutes following ingestion of the capsule. Samples should be collected into an aluminised nylon balloon. Depending on the degree of accuracy required and the cost, one (10 min), two (10–15 min) or even three (10–15–20 min), samples can be analysed.

5 Pump the expired air from the balloon into 1 mmol of hyamine solution in methanol. Add 10 mL of scintillation fluid to the hyamine, close the vial and shake well.

NB. In the United States it is far simpler to mail the balloon(s) to Tri-Med Specialties, Inc. in Virginia for analysis.

Breath CO_2 may also be collected by having the patient blow bubbles directly into the collection fluid. Tri-Med Specialties Inc, sponsor of the ^{14}C urea breath test in the United States, does not endorse the direct method because patients have been known to accidentally drink the caustic collection fluid. If the direct method is used, a safety trap must be placed between the patient and the collection fluid so that there is no possibility of ingestion. An example of a safety trap is shown in Figure 6.2.

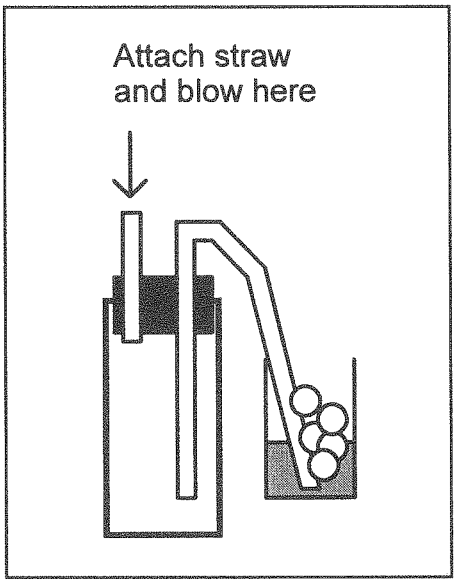

Figure 6.2 *An example of a safety trap that is required when a patient directly exhales into the collection fluid.*

6 Count the emissions in a scintillation counter.

NB. Counting may be performed immediately in which case most patients give a clearly positive or negative result within 2 minutes. If a borderline result is obtained this cannot always be discriminated from chemiluminescence so the sample should be re-counted after an hour or so.

5 μCi LIQUID RAPID TEST

This method is well described in the literature and is quite reliable. However, due to the problem of contact of the urea solution with oral flora resulting in the generation of $^{14}CO_2$ from urease in the mouth, patients need to be prepared carefully for the test, unlike the simple capsule method described above.

1 Instruct the patients to brush their teeth, mouth and tongue with toothpaste and rinse well immediately before the test.
2 Instruct the patient to drink the ^{14}C urea solution.
3 The patient should then rinse his/her mouth several times spitting the water out and making sure that none is ingested.
4 Two to three minutes after taking the isotope, the patient should provide his/her first breath sample.

NB. This sample is a quality control to measure the level of oral urease. If the sample gives a very high reading then a false positive result could occur in a 20 minute sample. If the 2 minute value is normal or low, then the reading at 20 minutes can be believed, even if in the low positive range.

5 Two other samples are taken at 12 and 20 minutes to define the $^{14}CO_2$ excretion curve but in most patients only the 20 minute sample is required to interpret the test. These samples may be collected into balloons or directly into hyamine (as described above).

MEAL BASED TEST

Theoretically, meal based tests are more sensitive than rapid tests because the ^{14}C urea cannot easily be rapidly emptied from the stomach; an event which could give a false negative result. In practice, however, meal based tests are not obviously more sensitive than rapid tests but direct comparison of the two methods has never been performed. In a meal based test, the method used is very similar to that for the ^{13}C urea breath test (Chapter 5).

1 Fast the patients for at least 5 hours or ideally overnight.
2 The patient then eats a high calorie meal.
NB. 'Ensure Pudding' in the United States – equivalent to 150 mL of custard! In Glasgow, McColl's group uses either 200 mL 'Fortisip' or 200 mL milk. In Ipswich, Bell's group uses 350 mL of a liquid meal (unspecified).
3 Take a baseline breath sample.
4 Instruct the patient to brush his teeth and mouth and rinse as above.
5 Instruct the patient to drink the ^{14}C urea solution. Typically this would be 0.1–0.4 Becquerels (MBq) of ^{14}C urea dissolved in 20–30 mL water (2.7–10.8 μCi).
NB. Afterwards, in the case of Bell's method, patients lie down and roll over to make sure that the isotope is evenly distributed over every part of the gastric mucosa.
6 Take breath samples at 20, 40 and 60 minutes.
NB. Usually the breath sample at 20 minutes will determine the patient's status quite accurately[8,11,15].

Results and interpretation

1 μCi CAPSULE TEST

Typical results from a pilot study using the 1 μCi capsule test are shown in Figure 6.3. The subjects were elective patients attending for endoscopy at the University of Virginia. Breath test was performed immediately before upper gastrointestinal endoscopy at which time biopsies were taken for histology, CLOtest and culture.

MEAL BASED TESTS

In Figure 6.4, CO_2 excretion for the liquid meal based test (Bell) is shown as DPM per mmol CO_2 collected. If the smallest dose of isotope and the quickest excretion is desired then omitting the meal seems best. On the other hand, because it coats the mucosa so well, the meal based test probably quantifies the degree of *H. pylori* colonisation better than the rapid test.

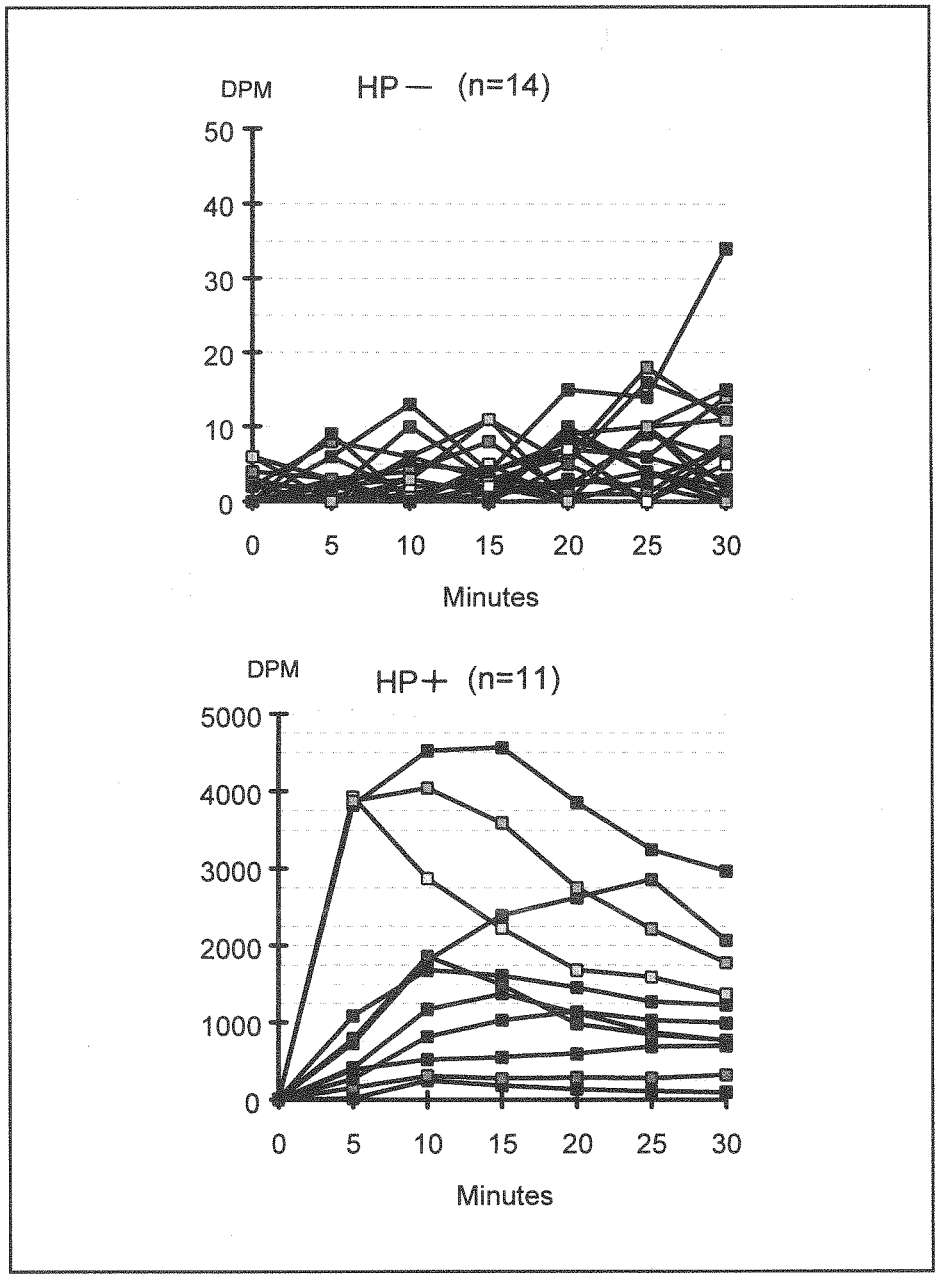

Figure 6.3 *Breath test $^{14}CO_2$ excretion. Typical results from a pilot study using the 1 μCi capsule test. Note: At 10–15 minutes, negative patients give DPM close to background, whereas positive patients average DPM = 1800 and are always >150. The Y axis scale is compressed for HP-positive subjects.*

EXPRESSION OF CO_2 EXCRETION

Initially counts per minute (CPM) should be converted to DPM so that variations

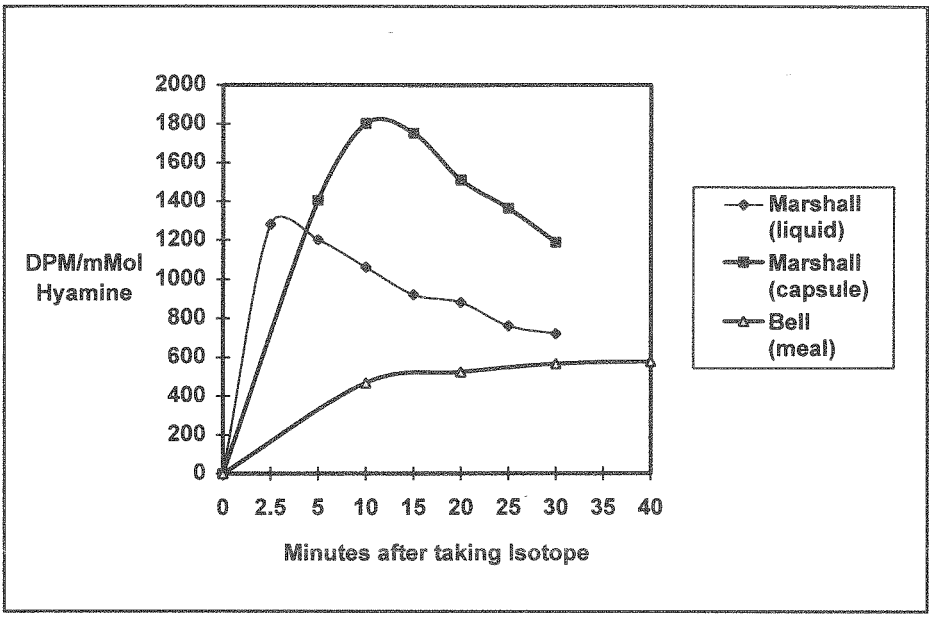

Figure 6.4 *Comparison of CO_2 excretion with various methods (means of HP^+ patients). CO_2 excretion for the liquid meal based test (Bell) shown as DPM per mmol CO_2 collected.*

Table 6.1 Breath sample calculations for δDPM.

Read a blank sample from a normal person who has not taken ^{14}C urea (CPM_B)	55 CPM
Read (CPM_R) a known standard sample (CPM_K)	Reads 90 000 = CPM_R, it is known to be 100 000 = CPM_K
Calculate efficiency (Eff) of the machine = CPM_R/CPM_K	= 90 000/100 000 = 0.9
Obtain CPM_S of breath sample	= 3500
DPMS = $(CPM_S–CPM_B)/Eff$	= (3500–55)/0.9 = 3888

Example values 10 minutes after taking a 1 μCi capsule:	
Negative	49 DPM or less above background (<50 dDPM)
Positive	100 DPM or more above background (≥100 dDPM)
Indeterminate (repeat test)	50–99 DPM above background (50-99 dDPM)

in scintillation counters can be allowed for. Table 6.1 shows how dDPM (disintegrations per minute above background) is calculated.

Many groups convert the δDPM to a value which is independent of the amount of isotope used and the amount of CO_2 collected. This allows easy comparison of results between groups using slightly different methods. An example of such a calculation is given below.

A = DPM given to patient (Becquerels × 60) or (μCi × 2 200 000)

B = mmol of CO_2 collected

C = weight of patient in kg (use 75 in this example)
D = DPM collected (use 1000 in this example)

$$\text{Excretion as \% dose * weight} = \frac{D \times C \times 100}{A \times B}$$

$$\text{thus in the case of Bell's test,} = \frac{1000 \times 75 \times 100}{100\,000 \times 60 \times 2} = 0.625$$

Tips and traps for young players

- Patients undergoing the ^{14}C urea breath test should be prevented from taking any medication which might inhibit *H. pylori*, or prevent contact between the ^{14}C urea and the organism. Therefore, bismuth and antibiotics need to be avoided for 4 weeks prior to the test. Table 6.2 is a list of factors which might interfere with results from the breath test. Further studies are under way to find out if breath tests are sufficiently reliable at earlier times than four weeks. For example, Logan *et al.*[16] found that most patients with relapsing *H. pylori* (failed antibacterial therapy) were positive on breath test a mere 7 days after ceasing therapy (with bismuth).
- If an equivocal test result is obtained after a liquid rapid test, the patient may repeat the test after 2 hours. In the case of a repeated test, a baseline reading is also required. After taking the isotope a second time, breath samples should be taken at 5 minute intervals for 30 minutes to define the breath test curve more accurately.

Table 6.2 Factors which might affect the breath test.

Group	Error	Comment
Recent antibiotic	False negative	Relapse of partially treated *H. pylori* may be slow (2–4 weeks) but most patients are HP+ again after 1 week
Recent bismuth (Pepto-Bismol etc.)	False negative	As for antibiotic above
Sucralfate	False negative	Weak anti HP activity. See antibiotic above
Omeprazole (or other proton pump inhibitors)	False negative	30–50% of patients suppress HP on these agents. HP may be inhibited by alkaline pH caused by local ammonia release. The time required for infection relapse after these drugs is 1–4 weeks but is not known exactly.
Resective gastric surgery	False negative	Isotope may empty rapidly from the stomach
Resective gastric surgery	False positive	Patient may be achlorhydric and have bacterial overgrowth (non-HP urease).
Food in stomach (also bezoar, gastroparesis)	Unknown	Isotope may not come into contact with gastric mucosa. Patient may be achlorhydric and/or have bacterial overgrowth (non-HP urease)

HP: *Helicobacter pylori*

Recommended/current applications

Urease will only be present if the patient is infected with *H. pylori*. Therefore, the test is positive only when bacteria exist on the gastric mucosa. Unlike antibody tests, the breath test gives no reaction when *H. pylori* infection has been cured. Because of this, the test is able to confirm or refute serological (antibody) diagnosis of *H. pylori. For* example, patients with past *H. pylori* infection who still have IgG present in the serum will remain serologically positive for many months[17]. The ^{14}C urea breath test will give a true negative result in such patients.

Since not all *H. pylori* infections can be cured and since cure does not always correlate with clinical response, a proportion of patients will need to be tested soon after therapy. These patients will not be satisfied with a long wait until serologic diagnosis can confirm the patient's *H. pylori* status. In this scenario doctors who do not have a simple breath test available will be tempted to blindly repeat antibiotic therapy or refer patients for endoscopy.

Breath tests do have other advantages, for example they do not require a needlestick. They are completely safe for medical personnel because body fluids are not handled. Breath samples can be sent through the mail with no special preparation.

SAFETY ISSUES OF ^{14}C UREA

Concern with ^{14}C usually arises because of its long half-life, but this is less important for organic compounds such as CO_2 and urea which are rapidly excreted. In the ^{14}C urea breath test, urea is either hydrolysed and expired as $^{14}CO_2$ or excreted unchanged as urea in urine. Because the biological half-life of ^{14}C urea is short, the total cumulative radiation dose received from each breath test is small, far below variations in natural radiation.

Recent data reported by Munster *et al.*[18] indicates that approximately 90% of the ^{14}C from a urea breath test is excreted as CO_2 in expired breath or as urea in urine. This means that after 72 hours, the amount of isotope retained in the body is small. Since the biological half-life of ^{14}C in the body is known, accurate assessment of the cumulative radiation exposure can be done. Stubbs calculated the cumulative lifetime radiation exposure from this test as not more than 0.3 mrem per μCi[19].

To allow various forms of ionising radiation to be compared, all are evaluated in terms of dose equivalent units (DE) expressed in millirems. The average DE from cosmic radiation, terrestrial radiation and endogenous naturally occurring radio-isotopes is a minimum of 88 mrem/year[20]. In the United States, natural radiation is doubled for persons living in Colorado, and may also be increased by aeroplane travel at the rate of 0.3 mrem per hour. Because most people do not concern themselves with the risk of radiation from air travel or living in Colorado, radiation doses of less than 10 mrem per year are regarded as trivial. In addition,

it should be noted that a normal person already carries 0.05 μCi (1.85 kBq) of naturally occurring ^{14}C within body tissues.

A 1 μCi test gives 0.3 mrem, and a 5 μCi test gives 1.5 mrem. Natural radiation in one day gives more exposure than a 1 μCi ^{14}C urea breath test[21] and over 150 such breath tests gives less marrow exposure than a single upper gastrointestinal series[22]. Therefore, it can be strongly argued that the radiation associated with the ^{14}C urea breath test is minimal. With the extremely low dose of ^{14}C and the convenience of the 1 μCi capsule test, this test will be a very useful assay for those who do not have access to a mass spectrophotometer.

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