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Evaluation of the $^{13}\text{CO}_2$ kinetics in humans after oral application of sodium bicarbonate as a model for breath testing

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Abstract. The kinetics of $^{13}\text{CO}_2$ have been investigated following oral administration at five doses between 12.5 and 100 mg of ^{13}C labelled sodium bicarbonate to 10 healthy subjects in a randomized study. Sodium bicarbonate in this study served as a model compound for carbon dioxide/bicarbonate generated in breath tests. Exhalation of $^{13}\text{CO}_2$ into breath was monitored by stable isotope ratio mass spectrometry. The kinetics of $^{13}\text{CO}_2$ were characterized by an apparent terminal elimination half-life of 1 h and a mean recovery of 0.630 of the dose administered. The kinetics were not dose-dependent. These results were in agreement with the findings reported previously after i.v. application of sodium bicarbonate.

Keywords. $^{13}\text{CO}_2$ kinetics, breath tests, exhalation of CO_2 , sodium bicarbonate.

Introduction

Concurrently with the development of stable isotope mass spectrometry, $^{13}\text{CO}_2$ -breath tests have become an important experimental and diagnostic tool in medicine [1]. A special subtype of breath test is the ^{13}C -urea breath test (UBT) for the detection of Helicobacter pylori (HP), which is a major pathogenic factor in ulcer disease [2]. In this test $^{13}\text{CO}_2$ is not produced by the metabolic processes of the patient under investigation, but by the urease activity of the microorganism hosted in the stomach. A characteristic feature of the UBT is the oral administration of the non-radioactive tracer. Since measurable quantities of $^{13}\text{CO}_2$ are exhaled by subjects with HP infections, the test can be used to advantage in the diagnosis and the follow-up treatment of such infections [3]. Several years of experience with the UBT have shown a wide range of test responses in patients with HP infections. One possible explanation for these findings could be the different extent of infection. If this was true, then UBT test results would indirectly reflect the extent of mucosal

lesions, which are thought to be associated with the HP plaques. Little is known, however, about the extent of the intestinal absorption of $^{13}\text{CO}_2$ after orally administered tracer. In particular, a possible dose-dependency of absorption and the absorption kinetics with emphasis on the recovery remained to be investigated. The limited information is partially due to the fact that the amount of urea metabolized by HP cannot be easily determined.

The aim of our study was to evaluate the CO_2 kinetics after oral administration of ^{13}C -labelled sodium bicarbonate as a model to derive basic information for a better understanding of $^{13}\text{CO}_2$ breath tests.

Patients and methods

Subjects

Five female and five male volunteers between 18–45 years of age with a mean body weight of 67 kg (range 51–88 kg) who, on the basis of extensive medical and laboratory screening, were found to be healthy were recruited for this study. The protocol was approved by the local Ethics Committee.

Materials

Gelatine capsules containing 12.5, 25, 50 or 100 mg of ^{13}C labelled crystalline sodium bicarbonate (isotopic purity 99%; Tracer Technologies Inc., Somerville, MA, USA) were supplied by the Department of Pharmaceutical Development of SKD, Göttingen, Germany.

Study design

Each subject was studied prospectively on five occasions when they were given either: A, a single dose of 12.5 mg; B, a single dose of 25 mg; C, a single dose of 25 mg; D, a single dose of 50 mg; E, a single dose of 100 mg sodium bicarbonate in randomly allocated period-balanced sequences. Study days were separated by at

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least 1 week. Each test dose was administered perorally with 100 ml of water after an overnight fast. Additional tap water was allowed *ad libitum* beyond 2 h after dosing. On each study day a breath sample was taken prior to dosing and then at 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210 and 240 min after dosing. Breath samples were collected and handled as described previously [4]. In order to minimize fluctuations in CO₂ production, subjects remained recumbent and fasted during the study period. CO₂ output was measured twice on each study day (Delta Trac, Datex, Helsinki, Finland), before and after profiling, and the mean of both observations was used in further calculations.

Measurement of isotopic ratios

The ¹³CO₂/¹²CO₂ ratios in the breath samples were determined by stable isotope mass spectrometry with a SIRA series II mass spectrometer (VG Instruments, Middlewich, UK). Ion intensities of mass 44 (¹²CO₂) and mass 45 (¹³CO₂) in samples were measured repeatedly in alteration with those in the reference gas. As intensities decreased during measurement in a linear fashion with time, the true intensities at time *t* = 0 were obtained by least squares regression analysis. From these back-extrapolated values isotopic ratios were calculated and corrected for ¹⁷O presence:

$$R = {}^0I_{45}/{}^0I_{44} - (7.5332 \times 10^{-4})$$

Thus for each sample (10 subjects, five regimens, 18 time points) two ratios (sample and reference) were recorded for further processing. The molecular fraction *x_n* of ¹³CO₂ generated from the test dose in each sample was then calculated as follows (see Appendix for details):

$$x_n(t) = R_{s,t} - R_{s,0} * R_{r,t} / R_{r,0}$$

R_{s,t} ratio in sample at time *t*

R_{r,t} ratio in reference at time *t*

R_{s,0} ratio in sample at time 0 (prior to administration)

R_{r,0} ratio in reference at time 0.

The *x_n* were used in the kinetic procedures without conversion to exhalation rates (mmol/min). To avoid unnecessary calculations, results were not transformed to the delta scale, since the focus of the study was on amounts CO₂ excreted. Ratio measurements were sufficiently precise to detect a change of 1*10⁻⁶ in *x_n* (limit of quantitation).

Kinetic methods

The following kinetic parameters were derived: C_{MAX} the maximum observed molecular fraction in the individual profile; T_{MAX} the time point of C_{MAX}; AUC the area under the molecular fraction versus time curves extrapolated to infinity; KE the apparent terminal elimination rate constant.

While C_{MAX} and T_{MAX} were obtained by visual

inspection of the individual profiles, AUC was calculated with the aid of the combined linear/log-linear trapezoidal rule according to [5]. The area beyond the last time point was estimated as x_n(last)/KE. KE in turn was calculated by non-weighted log-linear least squares regression analysis of the last nine data pairs. Recoveries (total fraction exhaled) were calculated from the AUC values and the corresponding CO₂ output and expressed in dose units.

Statistical methods

AUC, C_{MAX} and recoveries were subjected to a two-way analysis of variance (ANOVA). For these calculations our software package GOOSE was used.

Results

All 10 subjects completed the study according to the protocol. The kinetic parameters are compiled in Table 1. CO₂ output ranged from 4.79 to 7.80 mmol min⁻¹ for all subjects on all study days. The day-to-day variation was less than 10% in all subjects. As CO₂ output depends on body weight (Fig. 1) mean values for the different treatments were not calculated, but the average output of each volunteer was normalized by division by the body weight and the mean of the normalized output from all subjects was taken as an estimator of CO₂ output in the population under investigation. This corresponded to an average CO₂ output of 96.9 μmol min⁻¹ kg⁻¹.

AUC values (x_n*h*10⁴) ranged from 1.85–3.05 (mean 2.47) after dose A, from 3.46–6.29 (mean 4.87) after dose B, from 3.60–6.89 (mean 5.03) after dose C, from 7.46–13.20 (mean 9.81) after dose D and from 15.10–25.25 (mean 19.42) after dose E. For the analysis of variance the logarithms of the dose-corrected AUC values were used. While there was a statistically significant difference between the subjects, no such difference was detected between the dose regimens. Furthermore the power to detect a 20% difference between treatments was calculated to be > 0.999.

C_{MAX} values (x_n*10⁴) ranged from 1.62–2.35 (mean 1.95) after dose A, from 2.79–6.04 (mean 4.29) after dose B, from 3.21–5.21 (mean 4.02) after dose C, from 6.97–16.41 (mean 9.43) after dose D and from 12.63–29.74 (mean 18.63) after dose E. The analysis of variance did not report any statistically significant differences (Fig. 2).

In most cases (40/50) the absorption of the test dose was complete within 30 min, in five cases the maximum molecular fraction was reached after more than 50 min. A typical example of an individual molecular fraction vs. time profile is shown in Fig. 3. Generally, absorption and distribution were completed within 50 min. The molecular fractions then declined monoexponentially with time and were well above the limit of quantitation up to 4 h after dosing.

KE values (h⁻¹) ranged from 0.336–0.917 (mean 0.633) after dose A, from 0.512–0.728 (mean 0.632)

Table 1. Kinetic parameters per treatment and subject treatment subject CO₂* C_{MAX}† T_{MAX}‡ AUC§ KE¶ recovery**

A	1	7.36	2.35	15	1.89	0.624	0.567
	2	7.74	1.96	20	1.94	0.531	0.613
	3	6.62	1.73	40	1.85	0.917	0.500
	4	7.50	1.76	25	2.24	0.515	0.685
	5	7.53	2.08	15	3.05	0.335	0.937
	6	5.73	1.62	25	2.53	0.689	0.591
	7	6.15	1.75	60	2.98	0.630	0.748
	8	5.71	1.81	30	2.40	0.581	0.561
	9	5.81	2.23	60	2.86	0.749	0.678
	10	5.17	2.21	30	2.97	0.766	0.626
B	1	7.07	4.51	20	4.36	0.536	0.629
	2	7.77	6.04	20	3.46	0.728	0.548
	3	6.51	4.29	15	4.33	0.614	0.575
	4	7.37	2.96	15	3.85	0.617	0.579
	5	7.66	4.83	15	3.84	0.735	0.600
	6	5.73	5.41	15	5.30	0.598	0.620
	7	6.14	3.37	20	6.16	0.613	0.772
	8	6.38	4.38	25	5.61	0.512	0.730
	9	5.88	2.79	75	5.53	0.722	0.663
	10	5.17	4.34	30	6.29	0.647	0.663
C	1	6.62	4.19	20	4.33	0.590	0.585
	2	7.66	3.39	30	4.01	0.539	0.627
	3	6.63	4.49	60	3.62	1.020	0.490
	4	7.57	3.29	30	4.22	0.578	0.652
	5	7.80	3.21	20	3.60	0.580	0.573
	6	5.10	5.21	20	6.48	0.557	0.674
	7	6.25	3.58	25	5.24	0.616	0.668
	8	5.92	4.85	30	5.98	0.550	0.722
	9	5.84	4.52	50	5.98	0.740	0.712
	10	5.10	3.52	120	6.89	0.659	0.716
D	1	7.16	8.16	15	7.96	0.602	0.581
	2	6.82	8.97	25	8.07	0.628	0.561
	3	6.28	7.96	20	8.27	0.704	0.530
	4	7.63	7.50	20	7.46	0.655	0.581
	5	7.09	7.51	20	8.13	0.674	0.588
	6	4.86	10.35	20	12.89	0.641	0.639
	7	5.81	6.97	40	10.60	0.690	0.628
	8	5.28	8.79	25	9.98	0.643	0.537
	9	6.23	10.82	20	11.50	0.656	0.730
	10	5.41	16.41	25	13.20	0.566	0.728
E	1	6.78	14.44	20	16.92	0.605	0.585
	2	7.42	18.18	20	15.58	0.595	0.590
	3	6.79	20.57	15	17.34	0.611	0.600
	4	7.46	21.73	20	15.10	0.645	0.574
	5	7.11	14.11	25	16.38	0.520	0.594
	6	4.79	21.90	25	24.69	0.603	0.603
	7	6.88	15.20	30	20.74	0.642	0.728
	8	6.03	12.63	50	17.97	0.699	0.552
	9	5.11	29.74	20	25.25	0.550	0.658
	10	4.96	17.77	40	24.27	0.663	0.614

*CO₂-output mmol min⁻¹; †maximum molecular fraction $x_n \cdot 10^4$; ‡time of c_{MAX} min; §area under the molecular fraction time curve $x_n \cdot h \cdot 10^4$; ¶terminal elimination rate constant h⁻¹; **recovery dose units.

after dose B, from 0.539–1.019 (mean 0.642) after dose C, from 0.566–0.704 (mean 0.646) after dose D and from 0.520–0.699 (mean 0.613) after dose E. The terminal half-life of CO₂ was estimated from the KE values to be approximately 1 h. The terminal elimination was independent of dose.

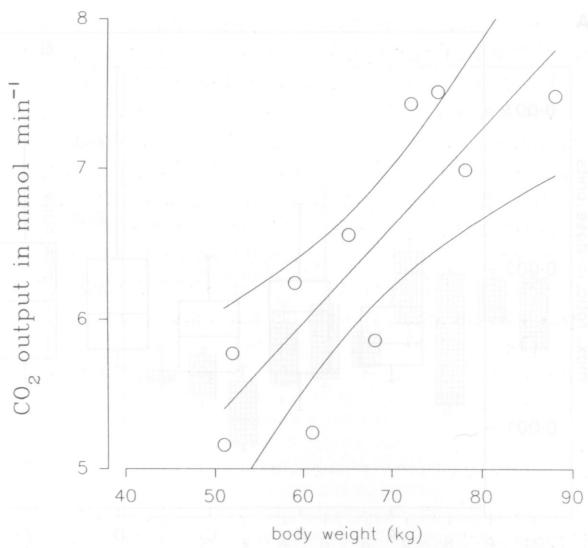


Figure 1. CO₂ output as a function of body weight. Each symbol represents the mean output of a subject (10 observations). straight line: linear regression line $r = 0.8337$ ($P = 0.0038$), curved lines: 95% confidence intervals.

The fraction of ¹³CO₂ exhaled ranged from 0.50–0.94 (mean 0.65) after treatment A, from 0.55–0.77 (mean 0.64) after treatment B, from 0.49–0.72 (mean 0.64) after treatment C, from 0.53–0.73 (mean 0.61) after treatment D and from 0.55–0.73 (mean 0.61) after treatment E. The analysis of variance did not reveal statistically significant differences in the mean recoveries between subjects or dose regimens (Fig. 4). While a slight decrease of recoveries with increasing body weight was observed (Fig. 5), the recoveries did not appear to depend on CO₂ output (Fig. 6).

Discussion

The presently observed CO₂ output (body weight adjusted) of on average 96.9 μmol min⁻¹ kg⁻¹ agrees well with the results after i.v. administration as reported by Irving (101 μmol min⁻¹ kg⁻¹ [6]), Winchell (118–202 μmol min⁻¹ kg⁻¹ [7]), Barstow (139 μmol min⁻¹ kg⁻¹ [8]) and Hoerr (158 μmol min⁻¹ kg⁻¹ i.v., 157 μmol min⁻¹ kg⁻¹ ig [9]). CO₂ output was correlated with body weight thus reflecting the mass of metabolizing tissue. The mean apparent terminal elimination rate constant of 0.63 h⁻¹ is also in excellent agreement with the value 0.60 h⁻¹ [6] and 0.72 1 h⁻¹ [8] found in the literature. The overall mean fraction of ¹³CO₂ exhaled after oral administration of solid sodium bicarbonate (breath recovery) is not different from the results obtained after i.v. infusion or intragastric infusion (Table 2). Obviously after oral administration bicarbonate is rapidly and completely absorbed from the GI tract and mixed with the general CO₂/bicarbonate pool. The size of this pool can be estimated from the terminal elimination rate constant,

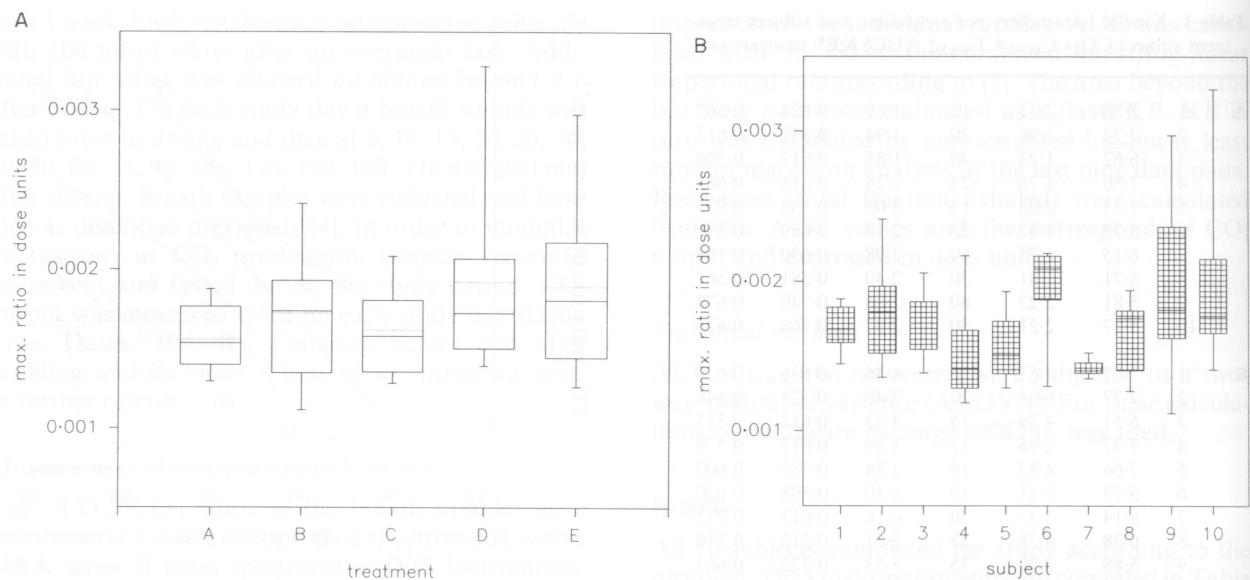


Figure 2. A: Dose-dependency of maximal isotope ratios (C_{MAX}). Each box represents the 10 subjects studied. Lower whisker: 10–25% percentiles, upper whisker: 75–90% percentiles. treatment code see text. B: Subject dependency of maximal isotope ratios. Each box represents the treatments A–E (code see text). Boundaries as described for A. Differences in C_{MAX} are statistically not significant.

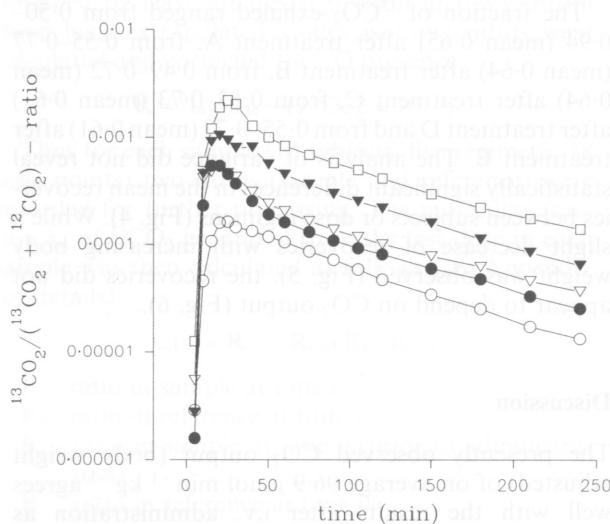


Figure 3. Representative molecular fraction vs. time plot: subject 6. ○: 12.5 mg; ●: 25 mg; ▽: 25 mg; △: 100 mg.

the fraction exhaled, and the CO_2 output to be 16 mmol kg^{-1} . This approach implicitly uses an open one-compartment kinetic model, nevertheless the pool size calculated agrees well with the total pool size of 14.9 mmol kg^{-1} derived from a three-compartment body model [6].

In contrast to the findings reported by Hoerr [9] there was no correlation ($r=0.2229$) between recovery and CO_2 output in our resting subjects (Fig. 6). In our opinion it is likely that the correlation shown by these authors does not indicate a cause-effect relation but a consequence of metabolic changes in their subjects which have influenced both parameters CO_2 output

and recovery simultaneously. Such changes can be induced by feeding [9] as well as by exercise [8].

All studies investigating bicarbonate kinetics have had to face the problem of unaccounted loss of tracer. While in healthy subjects direct excretion of bicarbonate into the urine is negligible [10] irreversible loss due to incorporation into urea could only account for 4% of the amount exhaled. Fecal excretion takes place but appears to be unimportant in this context [10]. As there are no considerable first pass losses to be taken into consideration [9], the missing tracer supposedly vanishes into some 'sink'. Whether bone can fulfil this role or metabolic processes consume CO_2 remains unclear.

On the basis of these data we conclude that the kinetics of bicarbonate after oral administration are essentially identical with those after i.v. injection. Moreover, no dependency of the recovery on the dose administered was observed in the dose range studied.

Since the origin of the CO_2 /bicarbonate is of no consequence for the absorption and elimination processes, CO_2 generated by the cleavage of urea by bacterial urease in the stomach can be concluded to become quantitatively absorbed. Therefore the amount of $^{13}\text{CO}_2$ exhaled after a test dose of labelled urea is a direct and untransformed measure of bacterial urease activity in the stomach. In this context the result of no dose-dependency of the bicarbonate absorption and elimination kinetics is equally important. If a test dose of 75 mg (1.23 mmol) urea is used [4], even complete conversion of the reagent will generate CO_2 amounts, which are completely absorbed as shown in this work. When the recoveries were analysed on a per subject basis, body weight did seem to

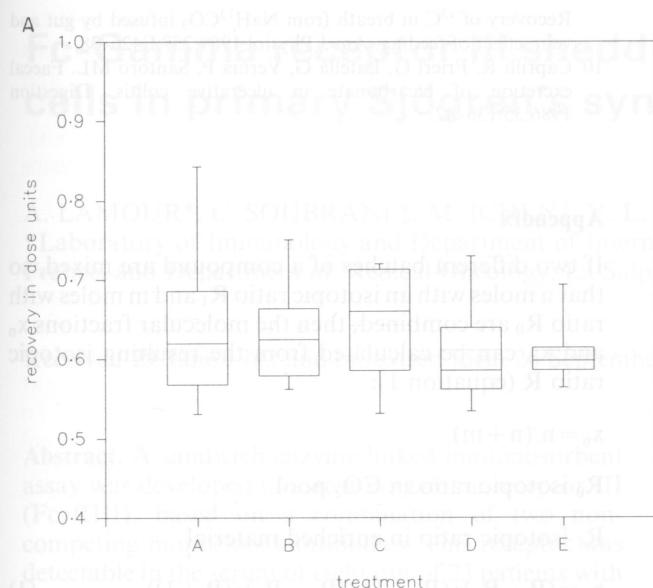


Figure 4. A: Dose-dependency of ¹³CO₂ recovery. Definitions as in Fig. 2. B: Subject dependency of recovery. Definitions as in Fig. 2. Differences in ¹³CO₂ recoveries are statistically not significant.

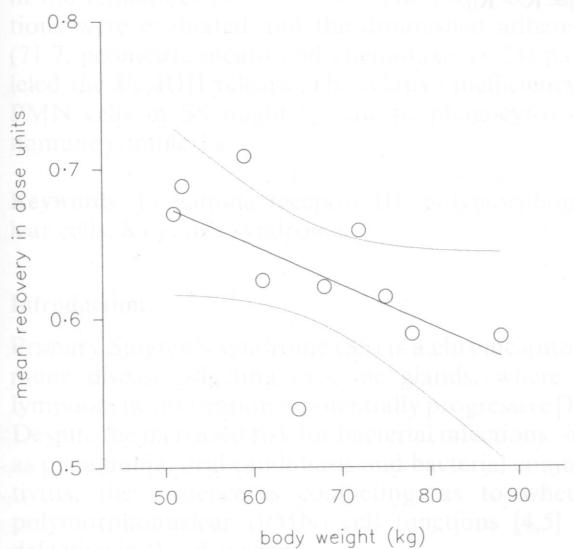


Figure 5. Mean ¹³CO₂ recoveries as a function of body weight. Definitions as in Fig. 1. ($r=0.5974$, $P=0.0682$).

influence recovery. A plot of recoveries vs. body weight (Fig. 5) showed indeed a slight trend towards decreased recoveries with increasing body weight. It remains unclear at the present time, whether such a trend has a physiological basis or is a mere random finding. Literature data [6] when analysed retrospectively did not corroborate these findings.

The elimination kinetics of bicarbonate, i.e. the fact that more than 30% of a given dose are not eliminated, may have implications as far as ¹⁴C-breath tests are concerned. No scientific objective can, in our opinion, justify the use of radiolabelled compounds in humans if such a percentage of the fraction reacted is incorpo-

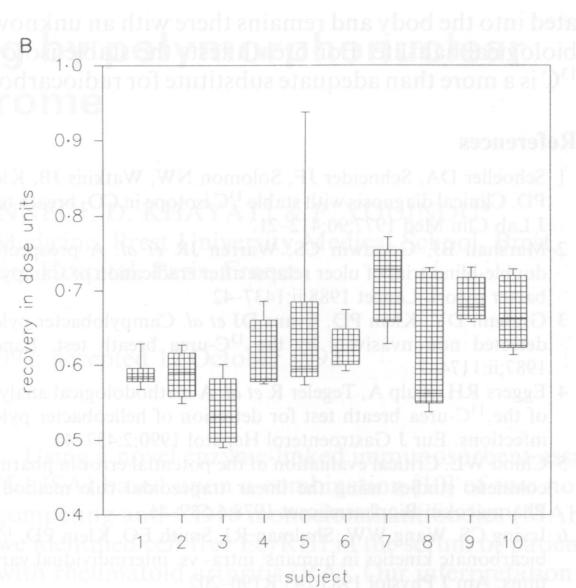


Figure 4. A: Dose-dependency of ¹³CO₂ recovery. Definitions as in Fig. 2. B: Subject dependency of recovery. Definitions as in Fig. 2. Differences in ¹³CO₂ recoveries are statistically not significant.

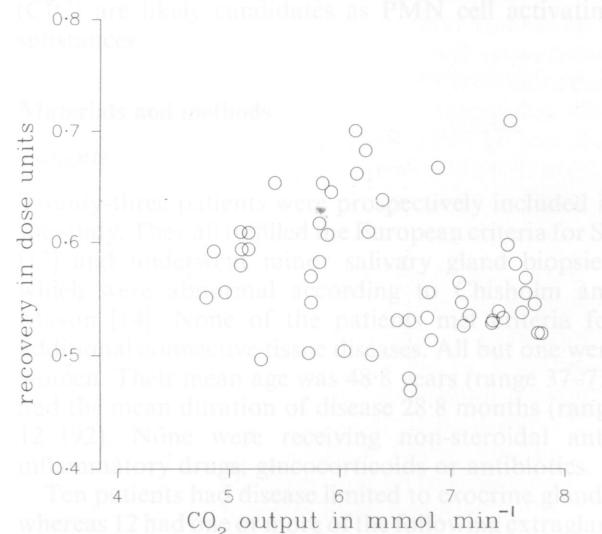


Figure 6. ¹³CO₂ recoveries vs. CO₂ output. Each symbol represents one observation in a subject. ($r=0.2229$, $P=0.1197$).

Controls

With regard to PMN cell function, controls were 45 medical students, 10 healthy volunteers and 10 patients (range 22–57 years) with normal renal function (Table 2).

Table 2. Recovery of ¹³CO₂ in breath

Route	Recovery	Reference	and variation
i.v.	0.51	6	and sex
i.v.	0.67	8	match
i.v.	0.70	9	and race
ig	0.74	9	and age
oral	0.63	present study	(age 20–57)

ated into the body and remains there with an unknown biological half-life. For breath tests the stable isotope ^{13}C is a more than adequate substitute for radiocarbon.

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Appendix

If two different batches of a compound are mixed, so that n moles with an isotopic ratio R_1 and m moles with ratio R_0 are combined, then the molecular fractions x_n and x_m can be calculated from the resulting isotopic ratio R (equation 1):

$$x_n = n/(n+m)$$

R_0 isotopic ratio in CO_2 pool

R_1 isotopic ratio in enriched material

$$x_n = (R - R_0) * (R_1 + 1) / ((R_1 - R_0) * (R + 1)) \quad (1)$$

With the constraints $R_1 \gg 1 \gg R_0$ and $R \ll 1$ (1) can be simplified:

$$x_n = R - R_0.$$

Such changes can be induced by feeding [9] as well as by exercise [8].

All studies investigating bicarbonate kinetics have assumed the absence of unaccounted loss of tracer which is breathable carbon dioxide excretion of bicarbonate and the urine is negligible [10] however the loss due to incorporation in urea equilibrates about 5% of the amount excreted. Renal excretion takes place but appears to be unimportant in the context [11]. As there are two considerable first pass losses to be taken into account, i.e. 5% to the urine and approximately 10% to the lungs [12]. Whether bone can fulfil this role in metabolising plasma consumed CO_2 remains unclear.

In the light of these data we conclude that the kinetics of bicarbonate after oral administration are essentially identical with those after i.v. injection. A dose-response dependency of the recovery in the dose range studied (from 100 to 1000 μM) was observed in the dose range studied (from 100 to 1000 μM). A dose effect will be eliminated once accounted for the absorption and elimination processes. This is generated by the clearance of urea by the liver which is proportional to both bicarbonate and urea. The net effect is that the ^{13}C enrichment in the breath is proportional to the dose of bicarbonate administered. The absorption of bicarbonate is proportional to the bicarbonate concentration in the blood. The net effect is that the ^{13}C enrichment in the breath is proportional to the dose of bicarbonate administered.