

Semiempirical model for diagnostication *Helicobacter pylori* infection by use of ^{14}C labelled urea

Aleksandra Jung,
Marta Wasilewska-Radwanska,
Zbigniew Kopanski

Abstract The main aim of this study was to create a semiempirical model, helpful in estimating severity of the *Helicobacter pylori* (*H. pylori*) infection by using the urea breath test (UBT), when urea labelled ^{14}C has been used for diagnostics. The model consists of four compartments representing stomach (1), blood vascular system (2), lungs (3) and urinary system (4). Mathematical model is based on the balance of radioactive ^{14}C in compartments from 1 to 4. The histological investigations were used as reference methods. Comparison of the results obtained from simulation, which yields dependence of ^{14}C activity on time, to experimental results of UBT, made it possible to determine the ranges of coefficient h_{B} value, which characterized each degrees of severity of *H. pylori* infection: degree 0 (lack of infection) – h_{B} below 0.025; degree 1 (not large) – h_{B} in range 0.025–0.115; degree 2 (moderate) – h_{B} in range 0.115–0.300; degree 3 (significant) – h_{B} above 0.300. It was possible to estimate severity of *H. pylori* infection in clinical practice on the basis of comparing the ^{14}C activity value of experimental points as obtained from the breath test, to the results of simulation with suitable value of the fitted parameter h_{B} indicating degree of severity of infection.

Key words computing modelling • *Helicobacter pylori* infection • kinetics of ^{14}C labelled urea

Introduction

Helicobacter pylori (*H. pylori*) infection of the gastric mucosa is present in the majority of duodenal ulcer patients. The eradication of infection confirmed by an appropriate diagnosis after the treatment appreciably reduces the ulcer relapse rate. Also the very common histological investigations enable to determine the degree of severity of *H. pylori* infection. However, this method is invasive – it requires the mucosal biopsy, in contrast to non-invasive methods detecting *H. pylori* infection, such as urea breath test [8].

Application of urea labelled ^{14}C in diagnostics of *H. pylori* infection in the alimentary canal has already obtained over a ten year history [10, 11]. Diagnosis of *H. pylori* infection with urea labelled ^{14}C is due to the fact that this bacterium produces enzyme urease, which has ability of catalysing the hydrolysis of urea into ammonia and carbon dioxide in accordance with reaction [1]:



Carbon dioxide labelled ^{14}C is removed from the organism with exhaled air and unchanged urea labelled ^{14}C is removed with urine.

Urea labelled ^{14}C was introduced by oral administration as a water solution of total activity 92.5 kBq. Exhaled air was collected in polyethylene bags before introduction of the isotope, then after 2, 5, 10, 15, 20, 25 and 30 minutes after

A. Jung✉, M. Wasilewska-Radwanska
Department of Medical Physics,
Faculty of Physics and Nuclear Techniques,
University of Mining and Metallurgy,
30 Mickiewicza Av., 30-059 Krakow, Poland,
Tel.: +48 12/ 617 29 58, Fax: +48 12/ 634 00 10,
e-mail: jung@novell.ftj.agh.edu.pl

Zbigniew Kopanski
5th Military Clinical Hospital,
1-3 Wrocławska Str., 30-901 Kraków, Poland

Received: 9 October 2001, Accepted: 21 February 2002

the introduction. ^{14}C activity in the collected air was measured with the application of the liquid scintillation method (UBT – urea breath test) [5, 15, 16]. A 24 hour urine collection was also taken. The activity of ^{14}C in each of the excreted samples was estimated separately in a liquid scintillating spectrometer (UUT – urea urine test) [6, 7]. However, results of these tests do not allow to get information on severity of infection of *H. pylori* directly. Therefore, we need to analyse in more detail physical mechanisms involved in the transfer of ^{14}C trace to elaborate a semiempirical model that enables not only to confirm the existing of *H. pylori* infection, but also to determine the degree of severity of this infection in the alimentary canal. The aim of this work is to present such a model based on a compartmental representation of organs involved in the kinetics of ^{14}C .

Methods

The proposed semiempirical model of the kinetics of removing ^{14}C from the organism is schematically presented in Figure 1. The experimental results of measurements of ^{14}C activity in exhaled air and excreted urine were used in this model. It was assumed that compartment 1 represents the stomach, where chemical decomposition of urea under the influence of urease can occur, if *H. pylori* infection took place. Compartment 2 represents the blood vascular system. Both ^{14}C -labelled bicarbonates and ^{14}C -labelled urea are absorbed through a thin layer of mucus of alimentary canal into blood capillary vessels' section and reach blood, then they are transported into lungs and kidneys. Compartment 3 represents the lungs, as organ exhaling $^{14}\text{CO}_2$. Compartment 4 represents the urinary system responsible for removing of urea labelled ^{14}C with excreted urine [14].

The ^{14}C transportation between compartments i and j was described in terms of transfer coefficients k_{ij} . Absorption of

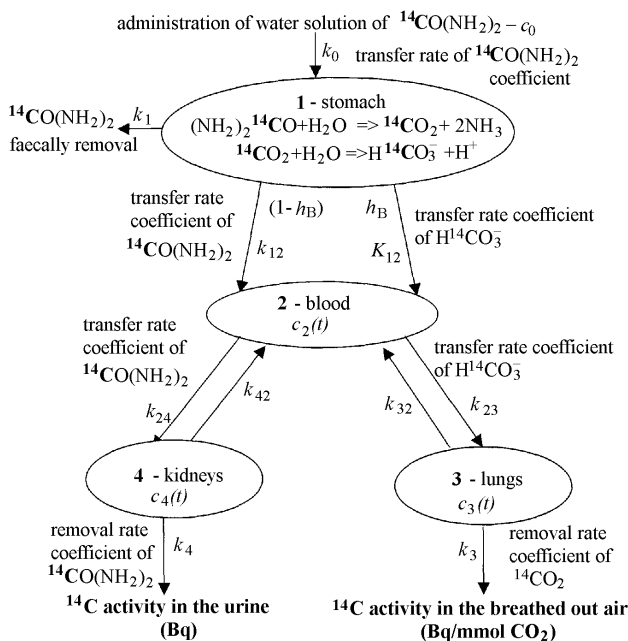


Fig. 1. Scheme of semiempirical model of the kinetics of removing ^{14}C from organism.

^{14}C labelled urea (activity 92.5 kBq) into compartment 1 is controlled by the coefficient k_0 . The partial enzymatic decomposition of urea labelled ^{14}C into bicarbonate ion labelled ^{14}C and ammonium ion takes place in this compartment. The quantity of decomposed urea depends on the degree of severity of *H. pylori* and it was determined by the parameter h_B . This parameter is proportional to the average enzymatic activity of urease of the whole bacterial colony, and thereby to the degree of severity of *H. pylori* infection [4]. This means that the higher value of parameter h_B , the higher amount of ^{14}C labelled urea is decomposed and the higher quantity of radiotracer is removed as $^{14}\text{CO}_2$. Removing of the substance labelled ^{14}C from compartment 1 is described by the coefficients k_{12} for urea labelled ^{14}C and K_{12} for bicarbonate ions labelled ^{14}C . Coefficient k_1 describes the direct removing urea labelled ^{14}C from the alimentary canal. The transportation between compartments 2 and 3 as well as 2 and 4 takes place in both directions, but it is much smaller in the back direction, and it was described by the coefficients: k_{23} and k_{32} as well as k_{24} and k_{42} , respectively. Removing of the final products of metabolism of urea from compartment 3 (carbon dioxide labelled ^{14}C) is described by coefficient k_3 , and removing of urea labelled ^{14}C from compartment 4 is described by coefficient k_4 . The values of transfer and removal rate coefficients between compartments were directly connected to biological half-life time of decay [3]:

$$(2) \quad k_{ij} = \frac{0.693}{T_{b_{ij}}}$$

where: k_{ij} – rate coefficient of radiotracer between compartments i and j [1/h]; $T_{b_{ij}}$ – biological half-life time of decay i.e. the time necessary to half of the quantity of the matter passes from compartment i to compartment j [h].

Then, the described semiempirical model can be presented by a set of the following linear heterogeneous differential equations:

$$(3) \quad \frac{dc_0}{dt} = -k_0 \cdot c_0(t)$$

$$(4) \quad \frac{dc_1}{dt} = k_0 \cdot c_0(t) - k_1 \cdot c_1(t) - k_{12} \cdot (1 - h_B) \cdot c_1(t) - K_{12} \cdot h_B \cdot c_1(t)$$

$$(5) \quad \frac{dc_2}{dt} = K_{12} \cdot h_B \cdot c_1(t) + k_{12} \cdot (1 - h_B) \cdot c_1(t) - k_{23} \cdot h_B \cdot c_2(t) + k_{32} \cdot c_3(t) - k_{24} \cdot (1 - h_B) \cdot c_2(t) + k_{42} \cdot c_4(t)$$

$$(6) \quad \frac{dc_3}{dt} = k_{23} \cdot h_B \cdot c_2(t) - k_{32} \cdot c_3(t) - k_3 \cdot c_3(t)$$

$$(7) \quad \frac{dc_4}{dt} = k_{24} \cdot (1 - h_B) \cdot c_2(t) - k_{42} \cdot c_4(t) - k_4 \cdot c_4(t)$$

where: c_0 [Bq] – activity of administered water solution of urea labelled ^{14}C ; $c_i(t)$ [Bq] – ^{14}C activity in the compartment i , $i=1, 2, 3, 4$; h_B – parameter of severity of infection *H. pylori*; k_0 [1/h] – transfer rate coefficient of the ^{14}C labelled urea administered into compartment 1; k_{ij} [1/h] – transfer rate coefficients between compartments i and j ; K_{12} [1/h] – transfer rate coefficient of decomposed urea from compartment 1 to compartment 2; k_i [1/h] – removal rate coefficients of the ^{14}C labelled urea from compartments 1, 3, 4.

Final balance equation of ^{14}C radioactivity for the present system was formulated as

$$(8) \quad c_0 = \int \frac{dc_1}{dt} dt + \int \frac{dc_2}{dt} dt + \int \frac{dc_3}{dt} dt + \int \frac{dc_4}{dt} dt + A_a(t) + A_u + A_f(t)$$

where: c_1, c_2, c_3, c_4 [Bq] – ^{14}C activity in each compartment 1, 2, 3 and 4; A_a [Bq] – ^{14}C total activity in the breathed out air; A_u [Bq] – ^{14}C total activity in the excreted urine; A_f [Bq] – ^{14}C total activity of removed stool.

The solution of Equation (3) is

$$(9) \quad c_0(t) = c_0(0) \cdot e^{-k_0 t},$$

where: $c_0(t)$ – administered activity of ^{14}C into compartment 1, with $c_0(0)=92.5$ kBq is initial value of ^{14}C activity in administered urea solution.

A change of ^{14}C activity (c_1) in the stomach was described by heterogeneous differential Equation (4). It can be written as

$$(10) \quad \frac{dc_1}{dt} + [k_1 + K_{12} \cdot h_B + k_{12} \cdot (1 - h_B)] \cdot c_1(t) = k_0 \cdot c_0(t)$$

where: $c_0(t)$ is given by Eq. (9).

The solution of Equation (10) is

$$(11) \quad c_1(t) = c_{10} \cdot e^{-k_0 t} + \text{const} \cdot e^{-[k_1 + K_{12} \cdot h_B + k_{12} \cdot (1 - h_B)] t},$$

where

$$(12) \quad c_{10} = \frac{k_0 \cdot c_0(0)}{k_1 + K_{12} \cdot h_B + k_{12} \cdot (1 - h_B) - k_0}$$

with $k_0, k_1, k_{12}, K_{12}, h_B, c_0$ – as it was given in Eqs. (3)–(7).

For the initial condition $c_1(0)=0$ the value of constant $\text{const}=-c_{10}$ was selected and the final solution of Eq. (10) is as follows:

$$(13) \quad c_1(t) = c_{10} \left(e^{-k_0 t} - e^{-[k_1 + K_{12} \cdot h_B + k_{12} \cdot (1 - h_B)] t} \right)$$

The Equation (13) can be directly used to determine the ^{14}C activity in removed stool as $A_f(t)$:

$$(14) \quad A_f(t) = k_1 \int_0^t c_1(\tau) d\tau$$

This activity is neglected in comparison with ^{14}C activity in exhaled air and/or in excreted urine.

The system of Eqs. (5)–(7) consists of three coupled heterogeneous differential equations on the function of ^{14}C activity $c_i(t)$, $i=2, 3, 4$ in individual compartments. A set of three functions $c_i(t)$ is the solution of this equation system. Each function $c_i(t)$ is composed of five exponential functions

$$(15) \quad e^{-\lambda_j t}, \quad j = 0, 1, 2, 3, 4$$

where: λ_0, λ_1 corresponds to the exponents (without time) in Eqs. (9) and (13), respectively; λ_2, λ_3 and λ_4 are power exponents, which were determined on the basis of known partial functions $c_2(t), c_3(t)$ and $c_4(t)$.

Thus, the following form of solution of system of Eqs. (5)–(7) for compartments 2, 3, 4 may be postulated as

$$(16) \quad c_i = c_i(t) = \sum_{j=0}^4 \alpha_{ij} \cdot e^{-\lambda_j t}, \quad i = 2, 3, 4.$$

Coefficients α_{ij} may be found, so that functions $c_i(t)$ fulfil the initial conditions:

$$(17) \quad c_i(0) = 0, \quad i = 2, 3, 4,$$

$$\text{and so } \sum_{j=0}^4 \alpha_{ij} = 0.$$

Input parameters of this model were: c_0 – initially administered activity of ^{14}C ; h_B – parameter determining the severity of the *H. pylori* infection; k_{ij} – transfer rate coefficients between compartments i and j ; K_{12} – transfer rate coefficient of decomposed urea from compartment 1 to compartment 2; k_i – removal rate coefficients of the ^{14}C labelled urea from compartments 1, 3, 4; t – time step, corresponding to experimental time of sampling; and t_{max} – total time duration of the urea breath test (30 min). For all these model parameters and on the basis of the given formula (16), the output functions were determined:

– ^{14}C total activity in the breathed out air:

$$(18) \quad A_a(t) = k_3 \cdot \frac{dc_3}{dt}$$

– ^{14}C total activity in the urine urea:

$$(19) \quad A_u(t) = k_4 \frac{dc_4}{dt}$$

The program was written in ANSI-C. A computer PC was used for calculations. The typical time needed for calculations of the output data for specific input parameters did not exceed 5 seconds on the Celeron 466 PC machine.

Results

The best results of fitting (with the correlation coefficient $r=0.87$) of the theoretical results were obtained for the following values of transfer coefficients: $k_0=166$, $k_1=0.07$, $k_{12}=20$, $K_{12}=9$, $k_{23}=2.26$, $k_{24}=2.16$, $k_{32}=0.13$, $k_{42}=0.09$, $k_3=20$, $k_4=0.12$ (in 1/h), for h_B in the range 0–0.3. The transfer rate coefficients k_{ij} had the same best fitted values for all patients, as it was assumed that kinetics of urea is similar for patients who had no respiratory system, kidneys or metabolic diseases. However, the value of parameter h_B was varying in the range 0–0.3, as the measure of degree of infection severity was different for different patients. The value of parameter h_B was connected to the scale [13] used in the reference endoscopic investigations, describing the severity of *H. pylori* infection. The proposed values of parameter h_B , qualifying each degree of severity of infection of *H. pylori* in alimentary canal carried out properly:

- degree 0 (lack of infection) – up to 0.025,
- degree 1 (not large) – between 0.025–0.115,
- degree 2 (moderate) – between 0.115–0.300,
- degree 3 (significant) – above 0.300.

The difference between these degrees was significant by Kruskal-Wallis test ($p<0.001$).

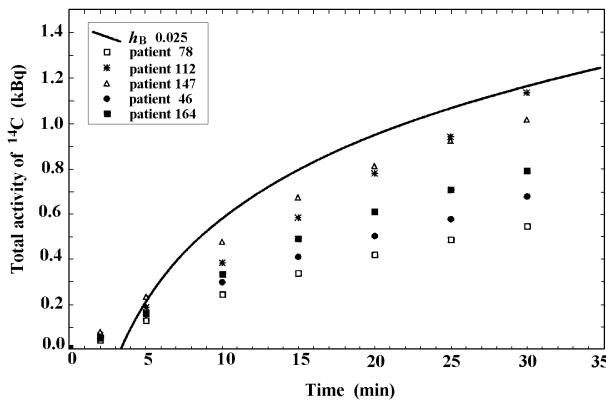


Fig. 2. Total ^{14}C activity in exhaled air vs. time. The points correspond to the experimental UBT results representing lack of *H. pylori* infection in the stomach. The fitted simulation line determines the degree 0.

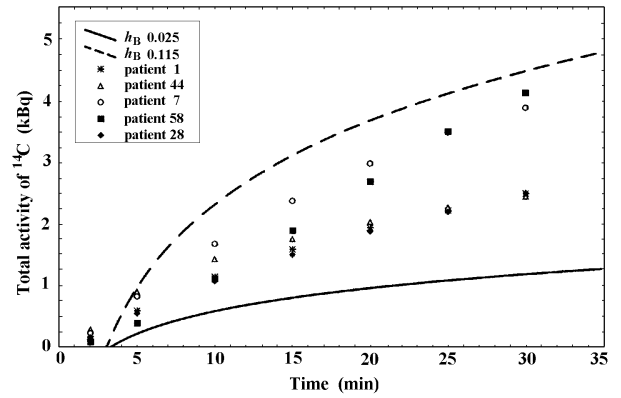


Fig. 3. Total ^{14}C activity in exhaled air vs. time. The points correspond to the experimental UBT results representing not a large degree of *H. pylori* infection in the stomach. The fitted simulation lines determine the degree 1.

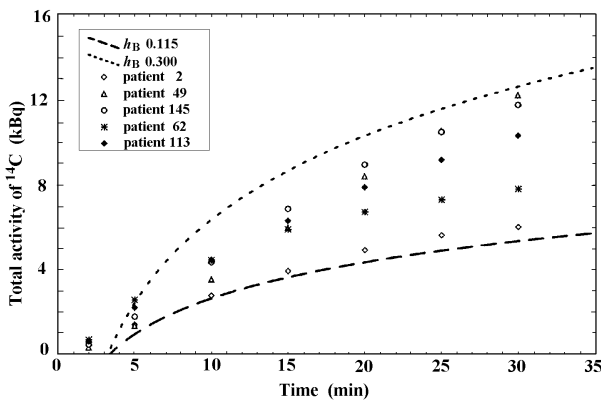


Fig. 4. Total ^{14}C activity in exhaled air vs. time. The points correspond to the experimental UBT results representing a moderate degree of *H. pylori* infection in the stomach. The fitted simulation lines determine the degree 2.

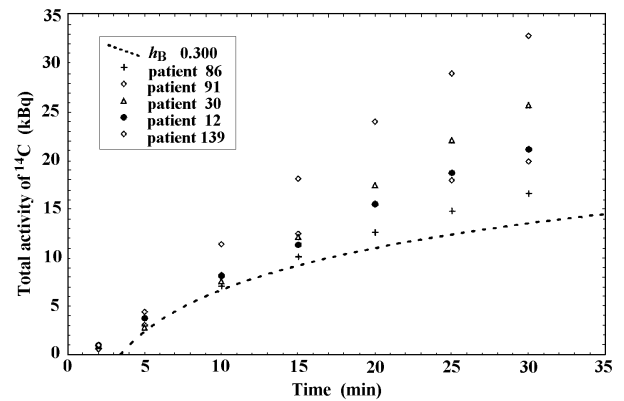


Fig. 5. Total ^{14}C activity in exhaled air vs. time. The points correspond to the experimental UBT results representing a significant degree of *H. pylori* infection in the stomach. The fitted simulation line determines the degree 3.

The statistical error of experimental measurements amounted to 1%; therefore, it was not possible to mark them in the Figures. The error of ^{14}C activity standard, which allowed recalculating cpm into Bq, amounted to 10%.

Representative results of the experimental urea breath test for five non-infected patients and simulation line appropriate to $h_B=0$ (lack of infection) are shown in Fig. 2. Similarly, typical results of the experimental UBT for infected patients with degree 1, 2 and 3 of infection, as well

as simulation lines which determine these degrees of severity of *H. pylori* infection, are shown in Figs. 3–5.

The verification of the presented model was carried out on the basis of literature data on urea breath test results from the publications of the following authors: J. C. Debonnie *et al.* [2], B. J. Marshall *et al.* [10], B. J. Marshall *et al.* [11] and D. A. Peura *et al.* [12]. All results were recalculated to the same value of the initially applied activity of ^{14}C in terms of the total activity in kBq, and then compared with

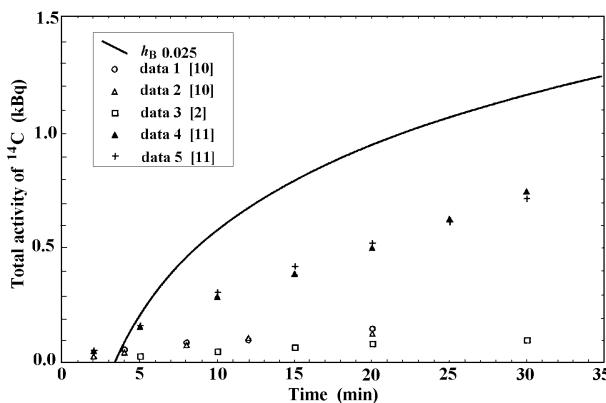


Fig. 6. Total ^{14}C activity in exhaled air vs. time. The points correspond to the experimental UBT results (taken from the literature) representing lack of *H. pylori* infection in the stomach. The fitted simulation line determines the degree 0.

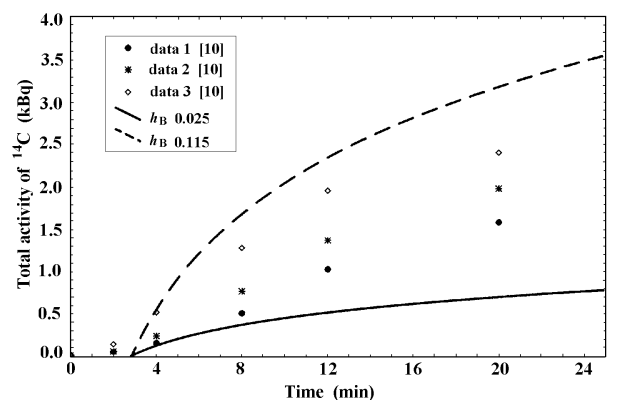


Fig. 7. Total ^{14}C activity in exhaled air vs. time. The points correspond to the experimental UBT results (taken from the literature) representing not a large degree of *H. pylori* infection in the stomach. The fitted simulation lines determine the degree 1.

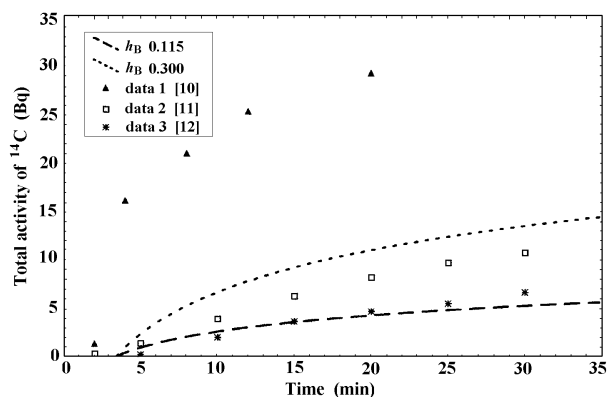


Fig. 8. Total ^{14}C activity in exhaled air vs. time. The points correspond to the experimental UBT results (taken from the literature) and representing moderate and significant degrees of *H. pylori* infection in the stomach. The fitted simulation lines determines the degree 2 and 3.

data obtained by simulation. The results, after the named normalization, are presented in Figs. 6–8.

Discussion

The proposed semiempirical model of kinetics of removing ^{14}C from the organism using the parameter h_B allowed to connect the urea breath test (UBT) results with severity of *H. pylori* infection. Histological investigations were used as a reference method, because they marked by a high diagnostic value and can be treated as the so-called “gold standard” [9]. Results of classification of urea breath tests obtained with the use of the parameter h_B , compared to histological investigation, gave a wrong result in 6 out of 130 cases, which means a strong correlation ($r=0.89$, $p<0.001$). The relative error of theoretical calculations was estimated according to equation:

$$(20) \quad \delta_i = \frac{c_0 - \left(\sum_{i=1}^4 c_i(t) + A_a(t) + A_u(t) + A_f(t) \right)}{c_0} \cdot 100\%$$

where: c_0 [Bq] – activity of administered water solution of urea labelled ^{14}C ; $c_i(t)$ [Bq] – ^{14}C activity in the compartment i , $i=1, 2, 3, 4$; A_a [Bq] – ^{14}C total activity in the breathed out air; A_u [Bq] – ^{14}C total activity in the excreted urine; A_f [Bq] – ^{14}C total activity of removed stool.

This error did not exceed 1% for a typical simulation of urea breath test result ($t_{\max}=30$ min). The error of fitting lines did not exceed 4% for all values of h_B parameter.

The ^{14}C activity of administered urea and total ^{14}C activity in breathed out air during 30 min of the test are needed for determining the severity of *H. pylori* infection in clinical practice. Then, the comparison of points of ^{14}C total activity to simulation line for the adequate value of h_B , gives an information on the degree of severity of infection in an easy way. If the dose of ^{14}C activity initially given to the patient is different from the amount of ^{14}C used in these investigations (92.5 kBq), it is necessary to recalculate the results of simulation for this new value.

In conclusion, the elaborated model of the kinetics of removing ^{14}C from the organism could be useful in clinical diagnosis with ^{14}C labelled urea of severity of *Helicobacter pylori* infection.

Acknowledgments We are grateful to Prof. Andrzej Maksymowicz for his help in computer calculations.

References

1. Bielanski W, Konturek SJ, Dobrzanska MJ *et al.* (1996) Validation of low activity ^{14}C -urea breath test in diagnosis of *Helicobacter pylori* in humans. *Nukleonika* 41;2:57–66
2. Debonnie JC, Pauwels S, Raat Y *et al.* (1991) Quantification of *Helicobacter pylori* infection in gastritis and ulcer disease using a simple and rapid carbon-14-urea breath test. *J Nucl Med* 32:1192–1198
3. Gibaldi M, Perrier D (1982) *Pharmacokinetics*, 2nd ed. Marcel Dekker Inc., New York
4. Jung A, Wasilewska-Radwanska M, Kopanski Z (2000) Determination of severity of *Helicobacter pylori* infection by use of a compartmental model. In: IX Mediterranean Conference on Medical and Biological Engineering and Computing: Medicon 2001. Pula 2:955–956
5. Kopanski Z, Nizioł J, Micherdzinski J *et al.* (1997) Significance of the ^{14}C -urea breath test in the determination of the location infected by *Helicobacter pylori* in the alimentary canal. *Eur J Med Res* 2:136–139
6. Kopanski Z, Wasilewska-Radwanska M, Jung A *et al.* (1999) Diagnostic value of the urine test with ^{14}C -urea in the detection of the *Helicobacter pylori* infection. *Int Med J* 6:109–112
7. Kopanski Z, Wasilewska-Radwanska M, Jung A, Kuc T, Schlegel-Zawadzka M (1998) Selected methodological aspects of the urine test with ^{14}C urea used in the diagnosis of infection by *Helicobacter pylori*. *J Med Phys* 23:193–194
8. Loffeld RJ, Stobberingh E, Arends JW (1993) A review of diagnostic techniques for *Helicobacter pylori* infection. *Dig Dis* 11:173–180
9. Malfertheiner P (1997) *Helicobacter pylori* – base and treatment. Sanmedica, Warsaw (in Polish)
10. Marshall BJ, Plankey MW, Hoffman SR *et al.* (1991) A 20-minute breath test for *Helicobacter pylori*. *Am J Gastroenterol* 86:438–445
11. Marshall BJ, Surveyor I (1988) Carbon-14 urea breath test for the diagnosis of *Campylobacter pylori* associated gastritis. *J Nucl Med* 29:11–16
12. Peura DA, Pambianco DJ, Dye KR *et al.* (1996) Microdose ^{14}C -urea breath test offers diagnosis of *Helicobacter pylori* in 10 minutes. *Am J Gastroenterol* 91:233–238
13. Stolte M (1992) *Helicobacter pylori* Spektrum: von der Gastritis hin zum Malignom. *Leber Magen Darm* 22:91–94
14. Wasilewska-Radwanska M, Jung A, Kopański Z (2000) Determination of severity and localization *Helicobacter pylori* infection by use of ^{14}C labelled urea. *Med Phys* 27:1383
15. Wasilewska-Radwanska M, Jung A, Kuc T, Kopański Z, Cienciała A, Witkowska B (1997) Diagnostics of *Helicobacter pylori* infection by breath test method using urea labelled ^{14}C . In: X National Scientific Conference on Biocybernetics and Biomedical Engineering, Warszawa, pp 162–164 (in Polish)
16. Wasilewska-Radwanska M, Pysklak S, Gilewicz-Wolter J *et al.* (1996) Activity calibration in breath test for diagnosis of *Helicobacter pylori*. *Polish J Med Phys Eng* 2:131–138