Model experiments and remarks on the radiocarbon dating of the Shroud of Turin

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Abstract

Model experiments were carried out using ^{14}C labelling technique on linen samples to study the probability of ^{14}C contamination of the Shroud of Turin in exchange reactions, which could influence its radiocarbon dating. The stable labelling of the linen samples was proven through the reaction of $^{14}CO_2$ and carbonates originating from tap water absorbed on the linen. Investigations were carried out to study the stable bond of ^{14}C using the cleaning procedures of the three radiocarbon laboratories. It was shown that these procedures could not remove all „fresh" ^{14}C contamination of environmental origin bonded on the Shroud during the past centuries and isotope fractionation caused by bacteria and fungi present on the Shroud could also result in the ^{14}C enrichment in the cellulose of the Shroud. All these effects should have been taken into account during the methodologically correct dating process to avoid the determined younger age of the Shroud in contradiction to other dating methods.

I. Introduction

Since 1951 several scientific investigations have been carried out studying the age of the Shroud of Turin. The results of more than 50 scientists representing about 40 fields of science supported more and more the originality of the Shroud confirming its authenticity, thus proving that assumption, which was mainly based on traditional respect until the end of the XIXth century.

Therefore, the results of the radiocarbon (^{14}C) dating[1] caused a serious shock. This is an absolute method, which was selected and carried out in 1988. As it is widely known, the concordant results of the investigations carried out in the three laboratories (Zurich, Oxford, Tucson) concluded that the Shroud originated of the Middle Ages and dated back to the period of 1260 – 1390 (Damon et al[1]).

It is well known, that many symposia have been organized to investigate the reason of this incredible contradiction between the results of these various branches of science and the radiocarbon dating. These meetings were not able to resolve the apparent paradox. Several outstanding scientists have criticized the dating procedure, but absolute evidence to prove its incorrect execution has not been provided.

It was e.g. suggested that the material of the Shroud have been exposed to such environmental effects (like dust, contaminated air, pollen, fungi bacteria, wax incense, scents etc), which could have affected the result of the dating procedure. Events such as the fire in Chambery in 1532 could have increased the concentration of ^{14}C, thus influencing the result of the dating[2]. It was pointed out already 10 years before the radiocarbon tests that the material of the Shroud contained various microorganisms, spores, bacteria, fungi and other particles, which had been deposited on it during the past centuries. The presence of these microorganisms on the Shroud could have affected the results of the dating procedure, thus its age could not be measured even roughly.

In spite of all these doubts no further experiments using material of the Shroud have been carried out to our knowlegde so far, although it has become even clearer that only experimental evidence can dispel the view whether the Shroud is only a mediveal piece of linen, one of the many beautiful, historical and archeological objects. This would, howewer, require another sample of the Shroud, which, taking into account all necessary canonical permission, seems unlikely.

Therefore, we decided to carry out experiments using linen samples of the present age to study those physical, chemical and microbiological effects, which could have affected the Shroud during proven historical events. These investigations have been performed by using the ^{14}C tracer method.

Methods

The experiments were carried out using raw linen and according to the practice of the laboratories taking part in the dating process, 50 mg samples were cut and investigated. The carbon content of the linen samples was 44.4 % and the water absorption capacity of the linen samples was double of the weight of the samples.

$Ba^{14}CO_3$ (3.288 GBq/mM, 54.8 mCi/mM) and glucose(U) - ^{14}C (both of Amersham, analitycal grade) were used as labelled compounds. Lactic acid (Merck, puriss) was used to liberate $^{14}CO_2$, while $Ba(OH)_2$ (0.05 M, Riedel – Haan, a.g.) was used to measure the microbiologically produced $^{14}CO_2$. In the course of the cleaning procedures the effects of HCl (1 M), NaOH (1 M), NaOCl (2.5 %) (all from Merck, puriss) were studied. Tartaric acid, citric acid, L-ascorbic acid, glucose and oleic acid (all from Merck, puriss) were used in the exchange reaction studies. The ^{14}C adsorption studies as well as the exchange reactions on the linen samples were carried out in a special apparatus. The Clinisosol^{TM} liquid scintillation coctail (Institute of Isotopes Co. Ltd) was used in the radioactivity measurements performed with a liquid scintillation spectrometer (Berthold BF 5000).

The electron irradiation studies were performed with the 4 MeV energy LPR-4 type linear electron accelerator of the Institute of Isotopes.

In the radioactivity studies five measurements were taken of each sample and three samples were measured at each individual experiment. The uncertainty of the results was estimated by calculating the standard deviation of the measured data (1s).

Measurement procedures and results

1. Investigation of the bond of ^{14}C on the linen samples

The ^{14}C adsorption of the linen samples was studied in a set of measurements. Wet and dry linen samples (50 mg each), calcium carbonate and calcium carbonate - magnesium carbonate mixture, isolated from drinking water were placed into the 2 L volume chamber of a special apparatus. Carrier-free $Ba^{14}CO_3$ (0,081 mg) was used to produce $^{14}CO_2$, which was liberated by feeding lactic acid to the radioactive carbonate and the radioactive gas was filled through a mercury valve into the reaction chamber. After 12 hours exposition the apparatus was evacuated removing the non–adsorbed $^{14}CO_2$ from the chamber through an alkaline absorber. The linen samples were dried at room temperature and their radioactivity was measured in liquid scintillation spectrometer using 2.0 ml distilled water and 15.0 ml Clinisosol^{TM} cocktail. 10.0 mg samples of calcium carbonate and calcium carbonate – magnesium carbonate were weighed followed by the measurement of their radioactivity. The experimental data are shown in Table 1.

The measurement data show that ^{14}C bonded stable on the dry and wet linen samples. The samples did not loose their radioactivity after several months of storage in a closed vessel. The data also show that more radioactivity was adsorbed by calcium carbonate – magnesium carbonate mixture isolated from drinking water.

Similar results were observed, when the adsorption of $^{14}CO_2$ was investigated on linen samples by using distilled water containing $^{14}CO_2$. The radioactivity of the samples was measured after they were dried (Table 2.). It is seen that the linen samples were ^{14}C enriched under these experimental conditions too.

2. Investigation of exchange reaction with ^{14}C in different organic substances

Since the linen sample consists of organic compounds the exchange reaction with $^{14}CO_2$ was studied in the above mentioned apparatus using organic model substances such as glucose, oleic acid, L-ascorbic acid, tartaric acid, citric acid. Small but definite amount of radioactivity has bounded into the organic compounds as seen in Table 3. The biggest effect was found in the case of glucose, which is the fundamental unit of cellulose.

3. Investigation of exchange reaction with $^{14}CO_2$ on linen by electron irradiation

Similar investigations concerning the exchange reaction were carried out on linen samples during electron irradiation. The textile samples were placed into the Suprasil reaction cuvette, which is equipped with greaseless stopcock for e.g. gas saturation. This cell was deareated before irradiation and then filled with $^{14}CO_2$. The cuvette was then placed 30 cm below the scanner of the electron accelerator window for irradiation. In order to achieve more homogenous irradiation a 1 mm thick Al scatter plate was placed below the accelerator window to scatter the vertical electron beam on the sample cell. The dose rate was measured by using the ethanol-monochloro-benzene dosimeter solution filled into a similar cuvette as used for the textile samples. After determining the electron dose rate (kGy/min), the textile samples were irradiated to 15 kGy dose under $^{14}CO_2$ atmosphere. The data in Table 6. show that much more stable bond was observed on the linen after electron irradiation, which was not removable either with acidic or with alkaline treatment.

4. Stability of the labeled linen samples after chemical cleaning

To study the stable bond of ^{14}C by the exchange reaction and electron irradiation we used the cleaning procedures of the three laboratories. The results of these experiments are summarised in Table 4., 5. and 6.

In the case of using acidic and alkaline treatments the radioactivity measured on the linen samples decreased to 0.85 %. Applying 2.5 % NaOCl cleaning solution also after the acidic and alkaline treatments the radioactivity measured on the linen samples decreased to 0.3 %.

5. Microbiological investigation with glucose(U)-^{14}C.

The existence of various microbes on the Shroud of Turin has alredy been shown. In order to model the effect of microorganisms, investigations were carried out with microbes isolated from the linen samples used in our experiments to study their degradation effect on glucose(U)-^{14}C. Therefore the microbes isolated from the linen samples were incubated in 0.5 % peptone solution. Thereafter 10 ml sterile solution of

glucose(U)-^{14}C (5 µCi/2,8 µg glucose) and15 mg non-radioactive glucose was mixed and incubated with microbes isolated from the linen. The degradation of glucose(U)-^{14}C to $^{14}CO_2$ was investigated at 25 ^0C in a suitable apparatus. The $^{14}CO_2$ evolved, was absorbed in 0.05 M $Ba(OH)_2$ solution and the molar radioactivity of isolated and dried precipitate of $Ba^{14}CO_3$ was measured in LSC in Clinisosol^{TM} cocktail. Our results proved that the microbes isolated from the linen samples degrade the glucose(U)-^{14}C very rapidly, utilizing it as carbon source in their metabolism. The results also show that the microbes can convert the glucose(U)-^{14}C to $^{14}CO_2$ rapidly and the molar radioactivity of the bonded $^{14}CO_2$ in the form of $Ba^{14}CO_3$ was lower than that of the glucose(U)-^{14}C and inactive glucose mixture resulting in a calculated biological isotope effect between 14-19 %, which caused enrichment in^{14}C of the residual glucose.

Discussion

We believe that these model experiments imply that in the course of the well-documented fire in Chambery an excess of ^{14}C could have bonded on the linen from the water used during extinguishing the fire. This water contained ^{14}C in the form of dissolved carbonates and its radioactivity was in equilibrium with the atmosphere in 1532. This excess ^{14}C could get on the linen not only as dried salt, but could also exchange with the Ca-Mg-carbonates present on the linen from the previous centuries. These processes could „rejuvenate" the Shroud. It is known that ^{14}C-^{12}C exchange reaction can be very important depending on the circumstances. It is essential to mention that ^{14}C atoms originating from the reaction of ^{14}N atoms with the neutron component of cosmic radiation are oxidized quickly to $^{14}CO_2$ in the atmosphere and than exchange with the dissolved carbonates in the waters. In aqueous solution this exchange reaction between ^{14}C and ^{12}C in mild alkaline medium (e.g. $NaHCO_3$) is particularly very fast. It was found that, even the relatively small concentration of CO_2 in the air can lead to serious exchange losses when $NaH^{14}CO_3$ solution evaporates[3]. Similarly it is known that under humid condition in the air the radioactivity of $Ba^{14}CO_3$ samples can decrease through exchange with the CO_2 content of air. This exchange

was found to be 37.4 % during 65 hours[4]. Our measurements indicated similar exchange in Ca-Mg mixture (Table 1).

All of these exchange reactions might have influenced the results of radiocarbon dating. In 1966 Sellstedt et al[5] already published that the exchange reactions can lead to false results in the course of the radiocarbon dating of carbonates originating from animal fossils in wet soil. According to their investigations the radiocarbon dating was correct only in those cases, when the collagen-bonded ^{14}C was measured.

We tried to carry out the cleaning procedures similarly to those three laboratories with ^{14}C-labelled linen to study whether these procedures could remove all adsorbed or bonded „^{14}C impurities" got on the Shroud during the centuries, or whether stable bonding of ^{14}C exists in the exchange reactions. It is an important issue, since the stabilized ^{14}C, bonded to the cellulose in chemical process, cannot be removed either by acidic, by alkaline or by oxidative procedures. The three radiocarbon dating laboratories considered to examine samples of the Shroud of Turin microscopically to identify and remove any „foreign" material by different physical and chemical procedures before the radiocarbon dating. It was not known to what degree dirt or other contaminants (i.e. pollens, carbon, wax, etc) might affect the linen samples influencing the ^{14}C content of the Shroud and thus the result of the dating process. Hence they performed different physical and chemical procedures both on the control samples and on the samples of the Shroud. The laboratories stated that the cleaning procedures revealed no evidence of contamination because there were no significant differences between the results obtained.

Our cleaning procedures were performed on our ^{14}C labeled linen samples after 7 months of storage (Table 5.). This treatment, which involved both strong acidic and alkaline treatment removed most of the ^{14}C content, because the main part of the radioactivity was present in the form of carbonates. The stable labeling, however, which occured during 7 months could not have been removed quantitatively either by acidic or by alkaline treatment (0.85 %). It was reduced to 0.3 % by the strong oxidative treatment.

Our measurements indicate that in the course of the labeling experiments with ^{14}C stable exchange reaction occured, which could not be removed completely either by acidic or by alkaline and oxidative treatments. All these observations confirm the

possibility that, different chemical changes could have been taken place in the cellulose of the Shroud of Turin during the centuries. Elementary carbon could have deposited on it and in the fire of Chambery in 1532 exchange reactions could have occured with the $^{14}CO_2$ content of the atmosphere as well as from the Ca-Mg carbonates and natural $^{14}CO_2$ containing water used for fire extinguishing..The ^{14}C content of this stable labelling was in equilibrium with the ^{14}C content of the atmosphere in 1532 and with the actual ^{14}C content of the environment resulting in exchange reactions even until the storage of the Shroud under controlled conditions. It has also been shown that there is a high probability of a very significant enrichment which caused more than 20 % deviation in calendar age of both old and modern linen by ^{14}C during gas phase isotop exchange reaction[2].

High energy irradiation might have caused also changes in the material of the Shroud. Here, it is important to emphasize the results of the exchange reaction with $^{14}CO_2$ during electron irradiation (Table 6). The experimental data demonstrate that more stable bond of ^{14}C was observed after high energy radiation, which was not removable by any cleaning procedure.

It is known that the cellulose molecules can change seriously, particularly after a long time[6]. The following chemical processes are responsible for these changes:

1) Oxidation

   Primary ^{-}OH to aldehyde or carboxyl groups

   Secondary OH groups to aldehyde (cleavage of C – C bond), ketones, or carboxyl groups

   Terminal reducing groups to carboxyl groups

2) Depolimerisation
3) Substitution of hydroxyl with organic or inorganic radicals
4) Hydrolysis
5) Replacement of alcoholic hydrogen
6) Graft addition reactions with free radical sites, generated via various free radical reaction initiators.
7) Base exchange reactions

   Fundamental glucose units liberates from the cellulose macromolecules (depolymerisation).

Substitution's reaction of -OH groups with organic radicals

Substitution of alcoholic hydrogen

Reductive endgroup-reaction

8) Addition reaction with the free radicals (effect of U.V. radiation)

These chemical reactions, especially oxidation, depolymerisation, formation of sugar, aliphatic acid and alcohol can proceed in the presence of bacteria and fungi, which were clearly identified on the Shroud. A thick complex of Lichenotelia, which consists of bacteria and fungi, was verified by Leoncio Garza-Valdes[7] on the Shroud and the cultures using Krumbein K-2 media were done from samples taken from the Shroud. The Lichenotelia caused on the fibre of the Shroud a heavy deposit of about 500 μm thickness. It means 25 – 50 % transformation of the cellulose fibers in the Shroud. In connection with this observation Mattingly has proposed a hypothesis that bioplastic coating on the Shroud produced the error in the radiocarbon analysis of the three laboratories. Howewer, it is sure that microorganisms involved in biopolymer production did not fix atmospheric carbon because they are not photosynthetic organisms and used the cellulose or the degradation product of cellulose carbohydrates as carbon sources in their metabolism. Therefore the biopolymer product should have shown the same carbon age, that was determined by Damon et al[1]. It was also a previous hypothesis that the presence of algae on the Shroud could not be excluded either due to the favourable storage conditions (i. e. humidity, temperature, semi-darkness). The algae could bond the actual ^{14}C through photosynthetic pathway, producing stable labelling on the linen, which cannot be removed by chemical cleaning procedures. The investigation made by STURP in 1978 did not find any pigments that are involved in photosynthesis. Howewer, the degradation products of pigments might be present in the Shroud, but the area when the radiocarbon sample was taken was relatively dark. There is, howewer, a more significant factor. It was verified that the endproduct of the decomposition reaction caused by microorganism (bacteria, fungi) are CO_2 and H_2O. Taking into account the isotopic effect in the splitting of ^{14}C – ^{12}C bond the microorganisms could build ^{12}C from the cellulose or decomposition product of cellulose of the Shroud into their organism to a higher degree, than ^{14}C, which was then metabolized and emitted as $^{12}CO_2$ in a higher amount than $^{14}CO_2$. Still some

methylotrophic bacteria use C_1 compounds which are simple organic molecules that contain no carbon – carbon bonds. This isotopic fractionation can achieve more than ^{13}d – 70 ‰.[8] We could isolate from our model linen such microorganism filamentous bacteria that were able to decompose glucose(U)-^{14}C – inactive glucose mixture. It is also known that, the discrimination against ^{14}C in biological systems can achieve more than 15 %. We found in our experiments 14 – 19 %. This isotopic effect could cause an enrichment in ^{14}C of residual linen material in the Shroud during the centuries, resulting in the change of isotope ratio and thus the „rejuvenation" of the Shroud.

Conclusions

1. Our model experiments which were carried out with ^{14}C labelling technique on linen samples proved the probability of ^{14}C contamination of the Shroud of Turin resulting in ^{14} – ^{12}C exchange reactions, which could influence – even if in small degree - the results of radiocarbon dating of the Shroud. The stable labelling of the linen samples was proven through the reaction of $^{14}CO_2$ and carbonates of tap water adsorbed by the linen. The stable labelling could achieve 0.85 %. Gas phase exchange reaction could not be excluded too, which increased the amount of ^{14}C in the fire of Chambery but the degree of this reaction is difficult to calculate.
2. We have also used ^{14}C labeling to examine stable bond of ^{14}C using the cleaning procedures carried out by the three laboratories during the preparation of the samples of the Shroud for their radiocarbon dating. Our results indicate that the cleaning procedures could not remove all ^{14}C impurities bonded on the Shroud during the previous centuries.
3. Taking into consideration the chemical change of the cellulose by microbiological processes and the environment, the stable bond of new ^{14}C on the Shroud is very likely. A conclusive proof is the existence of the isotope effect, i.e. the discrimination towards ^{14}C by microorganisms in the decomposition reaction of the cellulose caused by bacteria and fungi resulting in the enrichment of ^{14}C in the cellulose of the Shroud. Therefore we have to consider the following reason. The conventional radiocarbon age was calculated by the three laboratories using procedures suggested by Stuiver and Pollach[9] with normalisation to d ^{13}C = - 25 ‰. The mean value of

$^{14}C/^{12}C$ ratio was 0.9180, which results in 706 radiocarbon age (i.e. 1282 calendar age). The real measured ratio before the normalization was 0.8777 (resulting in 1081 radiocarbon age, i.e. 907 calendar age). Of course we could not carry out experiments on the sample of the Shroud for determination of isotopic fractionation by filamentous bacteria or fungi, but an isotopic fractionation higher than 14 % can be imagined. Due to the biological isotope effect caused by the microorganism on the Shroud, however, further correction should have been carried out by 14 – 19 %. Nevertheless, we do not know how many percent of the cellulose of the Shroud was degraded by the microorganism – particularly in the area of the removed sample on the Shroud. When the Shroud was 1988 years old at the time of radiocarbon dating, the measured ratio would have been 0.7510 and the normalised ratio 0.7860. On the basis of the results of our experiments we have to consider the following correction:

S error of the radiocarbon age determination:

1) Microbial isotopic fractionation –d = -14-19 %
2) Stable exchange reaction: 0.85 %
3) Not removed other ^{14}C impurities: 0.3 %

Calculation

The results of the calculation are presented in Table 7.

Hence these corrections resulted in lower $^{14}C/^{12}C$ ratios as were measured in the radiocarbon dating, which means that the material of the Shroud must be much older than the age calculated by the three laboratories. Although the three laboratories carried out precise measurements, in consequence of the combined effects discussed above, they have measured higher isotopic ratios and there was no chance to accomplish the radiocarbon dating of the Shroud of Turin correctly. The little, non representative invalid sample chosen for the radiocarbon dating from the Shroud contributed to all these effects. Consequently the real age of the Shroud cannot be determined exactly when we do not take into account the measurable data mentioned above in the calculation of the real age. In our opinion the elimination of these

modifying factors is the reason why the radiocarbon dating resulted in the younger age attributed to the Shroud of Turin in contradiction to other dating methods (e.g. vanillin method). Therefore it would be necessary to measure microscopically the real thickness of the bacteria and fungi and to collect more microbiological samples from the Shroud particularly in the neighbourhood of the location of the previous sampling for the correct determination of the main factor: the microbiological isotope fractionation using glucose(U)-^{14}C.

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Sample	**Mass** (mg)	**Fixed ^{14}C** (dpm)
Dry linen	51.2	10480 ± 102
Wet linen	50.4	34570 ± 186
$CaCO_3$	4.2	284740 ± 533
Ca-$MgCO_3$	7.4	377310 ± 614

Table 1. Fixed ^{14}C radioactivity in exchange reaction on different linen and carbonate samples (samples were dried before the measurement of the radioactivity).

Sample	**Mass** (mg)	**Radioactivity of labelled water** (dpm)	**Fixed ^{14}C on linen** (dpm)	(%)
Dry linen	50.8	2190 ± 47	230 ± 15	10.4
Wet linen*	50.6	2140 ± 46	300 ± 17	14.0
Wet linen	50.2	2300 ± 48	260 ± 16	10.9

Table 2. Fixed radioactivity on different linen samples from ^{14}C-labelled distilled water. *These samples were dried before measurements of radioactivity.

Sample	Fixed radioactivity (dpm/g)
Tartaric acid	3420 ± 58
Citric acid	1020 ± 30
L ascorbic acid	5160 ± 71
Glucose	11800 ± 108
Oleic acid	3040 ± 55

Table 3. Investigation of exchange reaction with $^{14}CO_2$ in different organic substances

Sample	Radioactivity	
	(dpm)	(%)
^{14}C-labelled linen	53000 ± 230	100
1 M HCl cleaning solution	1529 ± 39	2.8
1 M NaOH cleaning solution	2785 ± 52	5.3
1 M HCl cleaning solution	386 ± 20	0.7
Released $^{14}CO_2$ from the linen	47850 ± 220	90.3
^{14}C-labelled linen after the cleaning	450 ± 20	0.85

Table 4. Effect of the cleaning procedures on the radioactivity of the ^{14}C-labelled linen.

Sample	Radioactivity	
	(dpm)	(%)
^{14}C-labelled linen (21.2 mg)	66889 ± 260	100
1 M HCl cleaning solution	1831 ± 42	2.7
1 M NaOH cleaning solution	1432 ± 38	2.1
2.5 % NaOCl cleaning solution	2304 ± 48	3.4
1 M HCl cleaning solution	233 ± 15	0.35
Released $^{14}CO_2$ from the linen	60889 ± 246	91.1
^{14}C-labelled linen after the cleaning	200 ± 14	0.3

Table 5. Effect of the cleaning procedures on the radioactivity of the ^{14}C-labelled linen

Sample	Radioactivity	
	(dpm)	(%)
^{14}C-labelled linen	47094 ± 210	100
1 M NaOH cleaning solution	2076 ± 45	4.4
1 M HCl cleaning solution	246 ± 15	0.5
Distilled water cleaning solution	10 ± 3	0.02
Released $^{14}CO_2$ from the linen	42557 ± 206	90.3
^{14}C-labelled linen after the cleaning	2205 ± 47	4.7

Table 6. Effect of the cleaning procedures on the radioactivity of the ^{14}C-labelled linen after electron irradiation.

Microbiological degradation of cellulose samples, %	? error, %	Corrected $^{14}C/^{12}C$ ratio	Normalised value of corrected ratio	Radiocarbon age / **Calendar year**
10	2.85	0.8527	0.8929	950 / **1038 +/- 47**
20	4.55	0.8383	0.8768	1092 / **896 +/- 40**
30	6.25	0.8229	0.8607	1240 / **748 +/- 33**
40	7.95	0.8080	0.8451	1390 / **598 +/- 27**
50	9.65	0.7931	0.8295	1550 / **438 +/- 20**
20	4.95[x]	0.8343	0.8726	1127 / **861 +/- 38**
30	6.85[x]	0.8176	0.8552	1290 / **698 +/- 31**
40	8.75[x]	0.8009	0.8377	1470 / **518 +/- 23**
50	10.65[x]	0.7843	0.8203	1640 / **348 +/- 15**

Table 7. Calculated radiocarbon and calendar age from corrected $^{14}C/^{12}C$ ratio. ? error originates from: (1) microbiological isotopic fractionation (mean value :17 %; [x] data: 19 % isotopic fractionation). (2) stable isotopic exchange (0.85 %); (3) non-removed impurities (0.3 %).