Systemic Distribution of ^{14}C-formaldehyde from Formocresol-treated Pulpotomy Sites

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J Dent Res 59(3):603-608, March 1980

Introduction.

Formocresol is currently the most popular agent for performing pulpotomies on primary teeth. Histological evaluation of formocresol-treated pulp tissue reveals inflammation and necrosis.[4,5,6,8,11-15] Formocresol has been demonstrated to be toxic to connective tissue.[16-18] *In vitro*, formaldehyde will diffuse through the apical foramen within minutes after placing formocresol in the root canal.[19] In spite of these findings, formocresol remains popular because of its high incidence of clinical success.[1-9]

In a study using Rhesus monkeys, ^{14}C-formaldehyde was found to be absorbed into the systemic circulation within minutes after performing a formocresol pulpotomy.[20] The volume of distribution of the ^{14}C-formaldehyde was larger than the volume of the monkeys, suggesting that either tissue binding or metabolic breakdown of the ^{14}C-formaldehyde occurred.[20]

The purpose of this study was to determine the fate of the ^{14}C-formaldehyde which is absorbed following its application to pulpotomy sites.

Materials and methods.

Two dogs were anesthetized with pentobarbital sodium, 30 mg/kg. Polyethylene catheters were placed in the femoral artery and vein for collecting blood samples and for the infusion of 5% mannitol and creatinine to facilitate urine collections. They were also used to measure the glomerular filtration rate. A Foley catheter was placed in the bladder for collection of timed urine samples. A cuffed endotracheal tube was placed and connected via an expiratory valve to a heavy walled 120 liter bag for the collection of all expired air. Sixteen maxillary and mandibular anterior teeth were isolated with a rubber dam and pulpotomies were performed. After obtaining hemostasis and collecting control samples of blood, urine and expired air, cotton pellets containing 10 μl each of Buckley's formocresol were placed in the pulpotomy sites for five minutes and then removed. The cotton pellets placed in the first dog contained 17 μCi, and in the second dog, 45 μCi each of ^{14}C-formocresol.*

Whole blood samples were collected at 15, 30, 45 and 60 minutes following completion of the pulpotomies. Urine collections were made from 0-20, 20-40 and 40-60 minutes. All expired air was collected continuously from 0-60 minutes. At 60 minutes, the dogs were sacrificed. Tissue samples were removed from the lung, liver, spleen, skeletal muscle, heart and kidney. Cerebrospinal fluid and bile samples were also collected. All tissues and body fluid values were divided by the plasma ^{14}C-formaldehyde activity to obtain a T/P ratio. Ratios exceeding 1.0 indicate there was more ^{14}C-activity in 1 ml of tissue water than there was in 1 ml of plasma water.

Plasma and tissue samples were dissolved in solubilizer† at 40°C with gentle agitation overnight. Bile and spleen samples were decolorized with hydrogen peroxide. All tissues were then dissolved in liquid scintillation cocktail.‡ After allowing chemiluminescence to decay, all samples were counted to at least 10,000 counts in a

Received for publication February 20, 1979.
Accepted for publication April 11, 1979.
Supported by Grant No. DE 03780 from National Institutes of Health, National Institute of Dental Research, Bethesda, MD.

*New England Nuclear
†NCS, Amersham/Searle
‡Aquasol II, New England Nuclear

Packard liquid scintillation spectrometer, followed by repeat counting after the addition of internal standards to correct for variable quenching.

The expired air was bubbled at 100 ml/min through two gas scrubbing traps each containing 50 ml of ethanolamine to trap carbon dioxide. Aliquots of these solutions were counted in the liquid scintillation counter and corrected for quenching; then the total amounts of ^{14}C-activity in both traps were determined.

Plasma and urine samples were analyzed for creatinine,[21] and glomerular filtration rates were calculated from exogenous creatinine clearances. The clearances of ^{14}C-formaldehyde and creatinine were compared to estimate the renal re-absorption and/or excretion of ^{14}C-activity.

Results.

Figure 1 shows the ^{14}C-activity time courses in plasma and urine after placing ^{14}C-labeled formocresol in pulpotomy sites. The open circles connected by the solid line indicate the plasma levels obtained in the first experiment where 16 pulpotomies were performed on the anterior teeth of a 23 kg dog. The plasma activity plateaued after 30 minutes. In the second experiment, using 2.6 times as much isotope in the same number of sites but in a 15 kg dog, plasma ^{14}C-activities (solid circles connected by the solid line) were more than twice as high. Urine ^{14}C-activity (circles connected by dashed lines) generally parallels plasma activity, indicating that ^{14}C-formaldehyde is filtered at the glomerulus. The ratio of formaldehyde clearance to creatinine clearance ranged from 0.20 to 0.26, indicating that 20-26% of the ^{14}C-formaldehyde activity filtered at the glomeruli was excreted in the urine.

Figure 2 compares the ^{14}C-activity in several body fluids at the termination of Experiment 2. The ^{14}C-activity in cerebrospinal fluid (CSF) was approximately one-half the level in plasma. In this experiment, the urine flow rate was relatively low (≈3 ml/min) compared to Experiment 1 and, hence, the urine ^{14}C-activity was considerably higher than that of plasma. Bile had the highest ^{14}C-activity, about twelve times that of plasma.

The ratios of the ^{14}C-activities in various tissues compared to the 60-minute plasma values are listed in Table 1. All activities have been corrected for variable quenching and are expressed as dpm per ml of tissue water. A ratio of 1.0 indicates that the ^{14}C-formaldehyde activity in plasma and

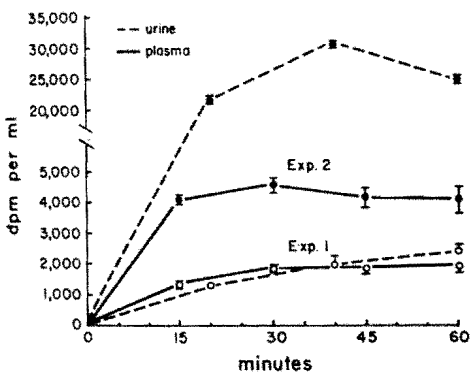

Fig. 1 – Time courses of ^{14}C-activity in plasma and urine after placing ^{14}C-labeled formocresol on pulpotomy sites. Brackets indicate ± one SEM for duplicate samples of plasma and urine at each time period.

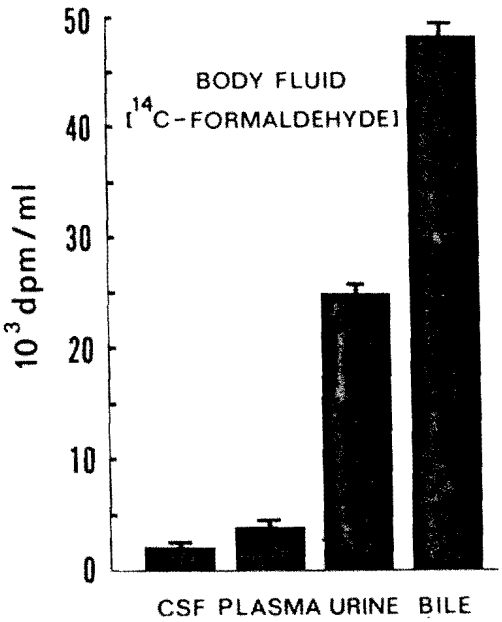

Fig. 2 – ^{14}C-activity in various body fluids. Sixty minutes after performing the pulpotomies in the second experiment, the dog was sacrificed and the indicated body fluids sampled. Brackets indicate one standard error of the mean for triplicate aliquots.

TABLE 1
TISSUE-TO-PLASMA ^{14}C-FORMALDEHYDE ACTIVITY RATIOS OR PLASMA

	T/P[a]
Liver	3.98 ± 0.33 (6)[b]
Lung	2.91 ± 0.09 (4)
Muscle	0.82 ± 0.04 (4)
Heart	1.77 ± 0.11 (6)
Spleen	1.52 ± 0.10 (6)
Kidney	2.26 ± 0.10 (3)
Outer Cortex	2.12 ± 0.10 (3)
Inner Cortex	2.16 ± 0.04 (3)
Outer Medulla	2.68 ± 0.03 (3)
Inner Medulla	3.27 ± 0.28 (3)
Papilla	3.27 ± 0.05 (2)

[a]Tissue-to-plasma ratios expressed as the activity of ^{14}C-formaldehyde per gram of tissue or plasma water.
[b]Numbers in parentheses indicate the number of individual samples analyzed. The indicated values represent the mean ± one standard error of the mean.

interstitial fluid water has equilibrated with that tissue's cell water and that there is no tissue binding. Ratios less than 1.0 indicate that the ^{14}C-activity was restricted from equilibrating completely with all of the tissue water. Ratios greater than 1.0 indicate a concentrating mechanism or tissue binding since there is more ^{14}C-activity in the tissue than can be accounted for by equilibration between plasma and tissue water.

It is evident that liver, lung, heart, spleen, and kidney bind ^{14}C-formaldehyde ($T/P > 1$), while skeletal muscle does not completely equilibrate with plasma ^{14}C-activity. Within the kidney, there is a progressive increase in ^{14}C-activity from cortex to papilla.

The percent of the ^{14}C-formaldehyde dose placed in the pulpotomy sites that was systemically absorbed can be estimated by multiplying the terminal (60-min) plasma activity by the volume of distribution of ^{14}C-formaldehyde.[20] The latter value was determined, in separate experiments, to be 129% of body weight in dogs.

In Experiment 1, the dog weighed 23 kg and, thus, had a volume of distribution of 29,670 ml (Table 2). The terminal plasma ^{14}C-activity was 1913 dpm/ml. The product of these values estimates the total ^{14}C-formaldehyde absorbed systemically. This value, divided by the total ^{14}C dose placed in all of the teeth yields the percentage of the dose absorbed. Table 2 indicates that between 5-10% of the ^{14}C-formaldehyde placed in the pulpotomy sites was actually absorbed systemically. Much more remained in the pulp chamber, but was not absorbed into the systemic circulation.

In the second experiment, we obtained an independent estimate of systemic absorption by multiplying the ^{14}C concentration in each tissue sampled by the total water volume of that tissue or fluid (as listed in standard handbooks). These ^{14}C-activities were then added together along with biliary, urinary, and pulmonary excretions to obtain a total ^{14}C-activity. This total figure did not include the central nervous system, skin, bone or the GI tract and, hence, the value underestimated total absorption.

Table 3 gives these data which total 80% of the systemic absorption as determined by

TABLE 2
QUANTITATION OF ^{14}C-FORMALDEHYDE ABSORPTION

Experiment	Terminal Plasma (^{14}C)[a]	VD[b]	^{14}C dpm Absorbed[c]	^{14}C Dose[d] dpm	%Dose Absorbed[e]
1	1913	29,670	5.68×10^7	5.86×10^8	9.67
2	4075	19,400	7.91×10^7	1.60×10^9	4.93

[a] – ^{14}C dpm per ml plasma.
[b] – Volume of distribution (ml) for ^{14}C-formaldehyde based on previously determined value of 129% of body weight.
[c] – Calculated by multiplying the terminal plasma (^{14}C) by the volume of distribution.
[d] – ^{14}C dose calculated by multiplying the dpm on each pellet by 16 pellets placed in the teeth.
[e] – The percent of dose absorbed calculated by dividing the ^{14}C dpm absorbed by the dose applied X 100.

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TABLE 3
RELATIVE DISTRIBUTION OF ^{14}C-FORMALDEHYDE ACTIVITY THAT WAS ABSORBED

		%Dose*
Biliary	2.42×10^6 dpm	3.06
Urinary excretion	6.24×10^6 dpm	7.89
Pulmonary excretion	2.09×10^6 dpm	2.64
Tissue distribution		
Liver	1.03×10^7 dpm	13.02
Muscle	3.75×10^7 dpm	47.40
Plasma	2.46×10^6 dpm	3.11
Heart	0.78×10^6 dpm	0.99
Lung	0.17×10^6 dpm	0.21
Spleen	0.25×10^6 dpm	0.32
Kidney	0.97×10^6 dpm	1.23
Total	6.318×10^7 dpm	80.50%

*Percent of absorbed dose as determined by the volume of distribution of ^{14}C-formaldehyde multiplied by terminal plasma value (7.91×10^7 dpm absorbed). See Table 2, Experiment 2.

the volume of distribution method. The summing method, however, allows one to see the relative distribution of the ^{14}C-activity in the body. That the two methods agree rather well lends support to their use for quantitating systemic absorption of substances from teeth. While it is interesting that some of the ^{14}C-formaldehyde was metabolized to $^{14}CO_2$ (pulmonary excretion), it accounted for only 2.6% of the total systemic absorption.

Discussion.

The shape of the curve describing the rate of appearance of ^{14}C-formaldehyde in plasma after pulpotomies suggests rapid absorption. The fact that the ^{14}C blood level stabilized after 15-30 minutes could be interpreted several ways. The plateau could be due to the fact that ^{14}C-formaldehyde absorption continues at a rate just equal to the rate at which ^{14}C-formaldehyde is bound to tissue, excreted in the urine, or is metabolized to $^{14}CO_2$ and exhaled by the lungs. Alternatively, the data could mean that there is a rapid initial absorption of ^{14}C-formaldehyde which then disperses into its volume of distribution, including tissue binding, and that this all occurs within the first 15-30 minutes. This interpretation suggests that renal and pulmonary excretion rates are relatively low and that they can not remove ^{14}C-activity from the body fast enough to begin to lower plasma levels in a 60-minute experiment. Work previously reported from our laboratory supports the latter interpretation.[20] In that report, the plasma levels of ^{14}C-formaldehyde were similar regardless of whether the isotope-soaked cotton pellet was left in the tooth for five minutes or for 120 minutes, suggesting that the ^{14}C-formaldehyde absorption had ceased within five minutes. Further evidence in support of that concept came from comparing rates of radioactive iodide absorption from pulpotomy sites before and after treatment of such sites with formocresol. Formocresol compromised the micro-circulation such that absorption of iodide was greatly reduced after only five-minute exposures.

In the present report, the relatively small contribution of renal (7.89%) and pulmonary (2.65%) excretions to the total amount of ^{14}C-formaldehyde absorbed (Table 3) lends further support to the concept that ^{14}C-activity is rapidly absorbed and rapidly equilibrates with its volume of distribution. However, it is only slowly excreted, thus maintaining a relatively high blood level. In this regard, it should be noted that ^{14}C-formaldehyde is filtered at the glomerulus and appears in the urine. The renal clearance of ^{14}C-formaldehyde relative to that of a substance that is filtered and excreted but is neither re-absorbed nor secreted, yields important information. The "filtration marker" in this report was exogenous creatinine (plasma level, 10 mg%). The ratio of ^{14}C-formaldehyde to creatinine clearance ranged from 0.20 to 0.26, which indicates that only 20 to 26% of the ^{14}C-formaldehyde which is filtered is excreted in the urine. The remaining 74-80% is either re-absorbed from the urine and returned to the blood or is bound by the kidney tissue. Probably both phenomena occur, since the tissue to plasma (T/P) ratio of ^{14}C-formaldehyde (Table 1) exceeds unity (indicating binding) but the plasma levels remain relatively constant over 60 minutes. The large renal T/P ratio may be due, in part, to contamination of tissue with urine which was about six times more concentrated than plasma with respect to ^{14}C-formaldehyde.

It is of interest that the bile concentration of ^{14}C-formaldehyde was about twelve times that of plasma (Fig. 2). This may, in part, account for the fact that the liver showed the highest T/P value (3.98) of all of the tissues examined (Table 1). The use of T/P data to establish tissue binding is more easily interpretable in lung, spleen, heart, or skeletal muscle (Table 1), since these tissues are not glandular. In these tissues, T/P values greater than 1.0 (lung, heart, spleen) suggest bindings. The fact that the T/P value in lung tissue (2.91) was the highest observed in non-glandular tissue suggests that the tissue binding which follows systemic absorption of ^{14}C-formaldehyde is quite modest. Although skeletal muscle showed the lowest T/P value, the large mass of skeletal muscle (40% of body weight) accounts for 47.4% of the total absorbed dose (Table 3).

The observation that 2.65% of the absorbed dose was excreted via the lungs (as $^{14}CO_2$) demonstrates that ^{14}C-formaldehyde can be oxidized (Table 3), although the rate at which this occurs is relatively slow. Most standard textbooks of biochemistry list numerous reactions involving tetrahydrofolic acid which serves as a co-enzyme in the transfer of one-carbon fragments during the oxidation of formaldehyde.

The appearance of ^{14}C-formaldehyde in cerebrospinal fluid (CSF) was unexpected. The concentration in CSF was nearly half that of plasma, which suggests that formaldehyde crosses the blood-brain barrier. Future experiments should include a study of the rate at which ^{14}C-formaldehyde appears in CSF, as well as the T/P levels achieved in different parts of the brain.

The use of the volume of formaldehyde distribution, multiplied by the terminal plasma value to calculate total formaldehyde absorption, has been validated in the present report by comparing this value to the sum of values directly measured in individual organs (Table 3). The data in Table 3 account for 80% of that estimated indirectly using the volume of distribution method. Had bone, skin, brain, and gastrointestinal tissues been included, an even closer agreement would have been possible.

Conclusions.

This report confirms our previous finding that ^{14}C-formaldehyde containing formocresol is absorbed from pulpotomy sites and appears in body fluids. The evidence indicates that some ^{14}C-formaldehyde is metabolized to $^{14}CO_2$, although this represents a very small fraction of the total dose absorbed systemically. Tissue binding accounts for most of the systemic absorption. Tissue binding is highest in the liver and lowest in skeletal muscle. The high amount of ^{14}C-activity in bile correlates with the high liver tissue/plasma values and demonstrates formaldehyde concentration by the biliary system. The relatively high tissue/plasma values in the kidney also correlates with the ^{14}C-renal clearance data which indicates re-absorption of filtered formaldehyde.

It is important to emphasize that the quantities of ^{14}C-formaldehyde absorbed are small. These results, in themselves, do not contra-indicate the use of formocresol. They do demonstrate, however, that formocresol is absorbed and distributed rapidly and widely throughout the body within minutes of being placed on a pulpotomy site.

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