## Carbon-14 and Tritium Measurement by Means of Bremsstrahlung Emissions

Abstract. *The feasibility of measuring carbon-14 and tritium in vivo has been demonstrated in the rat; thin scintillation detectors were used for the measurement of bremsstrahlung produced by these soft beta emitters. Measurements of tritium in vivo are limited to the study of surface phenomena, whereas bremsstrahlung produced by carbon-14 may be detected from depths of several centimeters.*

Tritium and carbon-14 have found wide application in biological tracer studies. Because these nuclides are pure beta emitters of low energy (maximum beta energy of $^{14}C$ is 155 kev; maximum beta energy of $^{3}H$ is 18 kev), it is difficult to measure their emissions without killing experimental animals, dissecting the organs, and making elaborate preparations of samples (*1*). We are studying the bremsstrahlung produced by these low-energy beta emitters for measurements in vivo and in vitro (*2*). Counting efficiencies and half-value layers in water were first determined for $^{3}H$ and $^{14}C$. The feasibility of utilizing this technique for measurement of $^{14}C$ in small animals was then demonstrated.

Two scintillation detectors were used: a NaI(Tl) crystal (2.54 cm in diameter and 0.1 cm in thickness) with a 5-mil beryllium window, and a CsI(Tl) crystal (11.4 cm in diameter by 0.025 cm) with a 5-mil aluminum window. In both cases the window thickness exceeded the range of the beta radiation of $^{14}C$ and $^{3}H$. The crystals were mounted on photomultiplier tubes, and the output signal was amplified and analyzed by a multichannel analyzer. The performances of the two types of crystals were thus obtained and compared.

Table 1. Average energy ($\bar{E}$) of bremsstrahlung spectra as a function of added absorber thickness. The energies marked with superscripts coincide with the mean energies of the spectra shown in Fig. 1.

| Carbon-14 | | Tritium | |
|---|---|---|---|
| Depth (cm-water) | $\bar{E}$ (kev) | Depth (mm-water) | $\bar{E}$ (kev) |
| 0.0 | 18.7[1] | 0.0 | 6.3[1] |
| .084 | 19.3 | .05 | 6.9[2] |
| .16 | 21.4[2] | .44 | 7.4[3] |
| .32 | 23.5 | .83 | 8.0[4] |
| .55 | 25.7[3] | 1.22 | 8.1 |
| 1.02 | 27.6 | 1.61 | 8.2 |
| 1.80 | 29.7 | | |
| 3.00 | 31.6[4] | | |

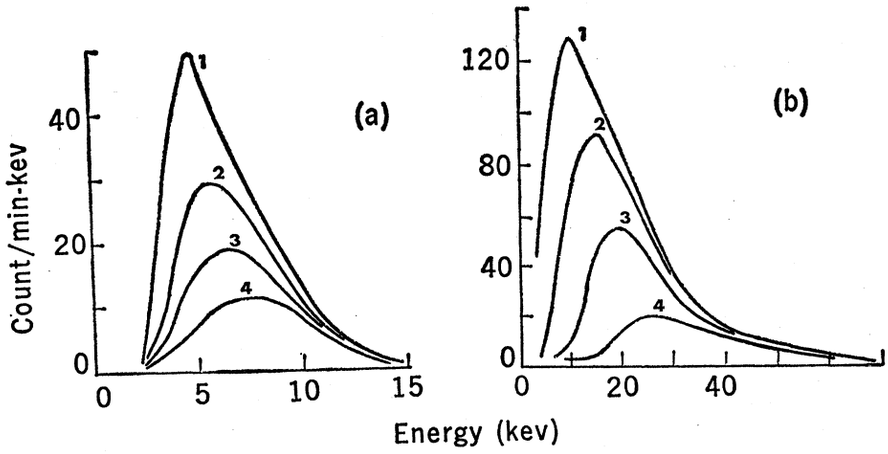


Fig. 1. Bremsstrahlung spectra as a function of added absorber thickness. (a) $^{3}H$; (b) $^{14}C$. The numbers on the spectra refer to values of $\bar{E}$ marked with superscripts in Table 1.

Two sources were used for determinations of the half-value layer. The first contained 500 $\mu$c of tritiated water in a 2-ml volume at a depth of 1.4 mm. The second contained 250 $\mu$c of sodium acetate-$^{14}C$ in 1.5 ml of water at a depth of 2.0 mm. Both solutions were contained in lucite planchets covered by 1-mil mylar. For determinations of half-value layers, the sources were mounted on the bottoms of beakers and incremental quantities of water were added.

The feasibility of counting $^{14}C$ bremsstrahlung in vivo was studied with three rats (320 g). One rat was used for background determinations, and the second was injected intraperitoneally with 100 $\mu$c of sodium acetate-$^{14}C$, and after 1 hour, a period sufficient for the isotope to become uniformly distributed, the animal was killed. The third animal was used to establish counting efficiencies for a relatively concentrated source by killing the animal, exposing the transverse colon, ligating the hepatic and splenic flexures, and injecting the colon with 100 $\mu$c of sodium acetate-$^{14}C$. The colon was then rapidly frozen to prevent diffusion of the source. The peritoneum, musculature, and skin were sutured; and the whole animal was frozen. The bremsstrahlung from the two rats was then measured by placing the detectors 2 cm above either the dorsal or ventral surfaces of the animal.

Bremsstrahlung spectra as a function of absorber thickness are shown in Fig. 1, a and b. In the uppermost curves in those figures there was no added absorber thickness. The bremsstrahlung from the $^{14}C$ source had an average energy of 18.5 kev and an overall counting efficiency of $3.3 \times 10^{-3}$ percent (*3*). The bremsstrahlung from the tritium source had an average energy of 6.3 kev and an overall counting efficiency of $3.6 \times 10^{-5}$ percent.

Figure 2, a and b, shows the reduction in count rate as a function of absorber thickness. Half-value layers were determined from the linear portion of the curve as plotted on a semilogarithmic scale. The half-value layer of the bremsstrahlung was 2.4 cm of water for $^{14}C$ and 0.07 cm of water for $^{3}H$. Table 1 lists experimental data showing the change in average energy with absorber thickness. The average energy is a logarithmic function of the absorber thickness.

Bremsstrahlung spectra obtained

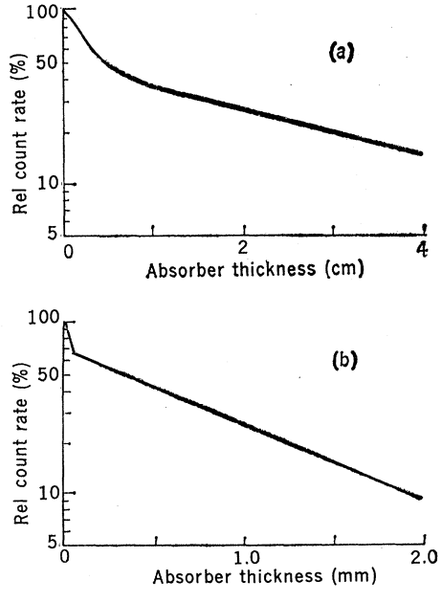


Fig. 2. Reduction in count rate as a function of added absorber thickness; (a) $^{14}C$ bremsstrahlung; (b) $^{3}H$ bremsstrahlung.

Table 2. Counting efficiencies for bremsstrahlung produced in rats. Measurements were made at 2 cm. Each source consisted of 100 $\mu$c of carbon-14.

| Crystal | Surface | Efficiency |
|---|---|---|
| | *Diffuse* | |
| NaI(Tl) | Dorsal | $0.67 \times 10^{-4}$ |
| NaI(Tl) | Ventral | $1.2 \times 10^{-4}$ |
| CsI(Tl) | Dorsal | $0.82 \times 10^{-3}$ |
| CsI(Tl) | Ventral | $1.34 \times 10^{-3}$ |
| | *Colon* | |
| NaI(Tl) | Dorsal | $1.33 \times 10^{-4}$ |
| NaI(Tl) | Ventral | $8.0 \times 10^{-4}$ |
| CsI(Tl) | Dorsal | $2.2 \times 10^{-4}$ |
| CsI(Tl) | Ventral | $6.1 \times 10^{-4}$ |

from the rats are shown in Fig. 3. The control rat was used to measure the background, which was 4.3 count/min for the NaI(Tl) crystal and 104 count/min for the CsI(Tl) crystal. At a 97.5 percent confidence level, the lower limits of detectability for a 30-minute counting period are 1.06 and 5.2 count/min for the NaI(Tl) and CsI(Tl) crystals, respectively (*4*). Dividing these limits by the counting efficiencies, one may determine the amount of isotope that can be detected. The range of activity which may be detected is 60 to 720 nc with the NaI(Tl) crystal and 0.39 to 2.9 nc for the CsI(Tl) crystal.

The average energy of the bremsstrahlung as a percentage of the maximum beta energy differs greatly between the $^{3}H$ and $^{14}C$ sources. This is due to the greater absorption of the low-energy end of the spectrum in the tritium source. The difference in hardening of the spectra can be further seen in the absorption curves. Both sources exhibit a rapid decrease in count rate followed by a more gradual decline as the absorber thickness is increased owing to removal of the low-energy components of the spectra with increasing absorber thickness. The carbon source count rate shows a further reduction of 64 percent before becoming exponential in form, while the tritium source rate is reduced only 33 percent and then becomes linear on a semilogarithmic scale.

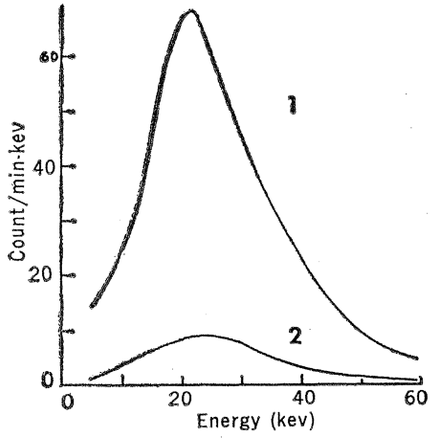


Fig. 3. Selected $^{14}C$ bremsstrahlung spectra from rats. Curve 1, 100-$\mu$c source in colon with the detector over the ventral surface. Curve 2, 100-$\mu$c source diffused throughout body; detector over ventral surface.

Based on the low value of the half-value layer for tritium, studies with tritium appear to be limited to the study of surface phenomena or to the first few millimeters of tissue. Absorption of tritiated substances applied to skin could be studied in this way.

With the greater penetration of carbon-14–produced bremsstrahlung, measurements in vivo become feasible in small experimental animals. With suitable collimation, single organs can be counted. Hence, the detectability of carbon-14–produced bremsstrahlung may help to eliminate the need for serial sacrifice of large groups of animals in some biological experiments, and in other cases eliminate lengthy sample preparations.


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### References and Notes


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3. The average energy is determined from multichannel analyzer data. The crystal window is neglected, and, for low energy photons, it is assumed that 100 percent of the photon energy is absorbed. Average energy $\bar{E}$ is then obtained from the equation:

$$\bar{E} = \frac{\Sigma \text{ channel No.} \times \text{count}}{\Sigma \text{ counts}} \times \frac{\text{kev}}{\text{channel}}$$

4. These minimum counting rates were obtained as follows:

$$\text{minimum count rate} = 1.96(2 \times B)/t,$$

where $t$ is the total counting time and $B$ is the total background count for this counting time.



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## Genetic Mapping of Phenylalanyl-sRNA Synthetase in Escherichia coli


Abstract. *Genetic mapping of the structural gene for a phenylalanyl-sRNA synthetase in* Escherichia coli *was accomplished by the use of mutants with temperature-sensitive or* p-*fluorophenylalanine-resistant enzymes. The structural gene is located at minute 33 of the Taylor-Thoman map of the* E. coli *chromosome, closely linked to a structural gene* (aroD) *for one of the enzymes involved in the biosynthesis of the aromatic amino acids, and distant from the known locations of other aminoacyl-sRNA synthetase genes.*


During the search for mutants of *Escherichia coli* with conditionally expressed lesions in indispensable enzymes, a number of mutants were obtained with alterations in phenylalanyl-sRNA synthetase. One class of mutants was isolated by selection for the ability to grow in the presence of the phenyl-

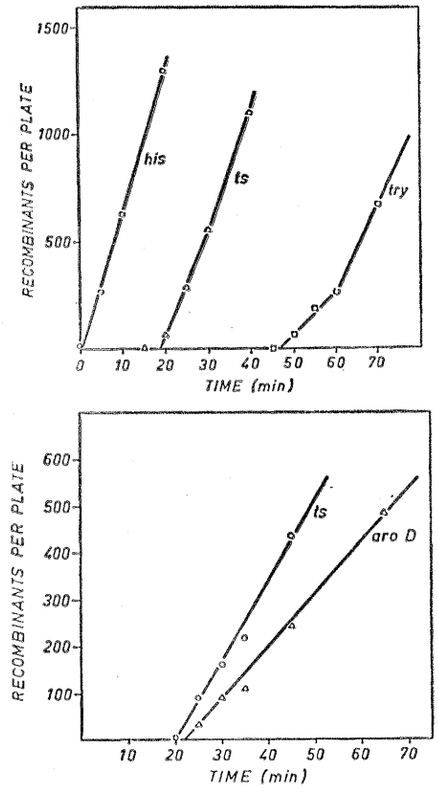


Fig. 1. Appearance of several kinds of recombinants in an interrupted mating experiment between Hfr AT2572a and either (top) NP37021, or (bottom) NP37022. Donor cells were counter-selected by streptomycin. Recombinants for nutritional markers were selected on appropriate mediums at 30°C. Recombinants for temperature-resistant growth were selected on plates incubated at 30°C for 6 hours before shifting them to 40°C.