Title

CARBON-14-LABELED CYANIDE: RADIOACTIVITY EXCRETION IN MICE AND ESTIMATION OF RADIATION DOSE TO HUMANS

Authors

Tolbert, Bert M.
Hughes, Ann M.

Publication Date

1957-08-15

UCRL-3850

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BERKELEY, CALIFORNIA

UCRL-3850

UNIVERSITY OF CALIFORNIA

Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

CARBON-14-LABELED CYANIDE: RADIOACTIVITY EXCRETION IN MICE AND ESTIMATION OF RADIATION DOSE TO HUMANS

Bert M. Tolbert and Ann M. Hughes

August 15, 1957

Printed for the U.S. Atomic Energy Commission

CARBON-14-LABELED CYANIDE: RADIOACTIVITY EXCRETION IN MICE AND ESTIMATION OF RADIATION DOSE TO HUMANS

Bert M. Tolbert and Ann M. Hughes

Radiation Laboratory
University of California
Berkeley, California

August 15, 1957

ABSTRACT

The excretion pattern of carbon-14 for mice given ip cyanide-C^{14} was determined over a 30-day period. Peak breath excretion of C^{14} occurred in 10 minutes. At the end of 30 days the injected radioactivity was 92.2% excreted in the urine and feces, 25.1% excreted in the breath, and 2.7% remained in the carcass. These data are used to calculate an estimated whole body and kidney radiation dose for the ingestion of cyanide-C^{14} in man.

CARBON-14-LABELED CYANIDE: RADIOACTIVITY EXCRETION IN MICE AND ESTIMATION OF RADIATION DOSE TO HUMANS*

Bert M. Tolbert and Ann M. Hughes

Radiation Laboratory
University of California
Berkeley, California

August 15, 1957

The excretion pattern of radioactivity from C^{14}-labeled compounds has been studied in animals and man for a number of compounds that occur naturally in biological systems. However, no data are available for radioactive cyanide metabolism. Since this is a nonbiological, rather reactive ion, it is possible that an appreciable fraction of the radioactivity from labeled cyanide could be incorporated in the body in a very long-lived form. The purpose of this study is to measure the excretion of carbon-14 from labeled cyanide in mice and to use these data to estimate radiation dose to humans from the ingestion of cyanide-C^{14}. Some metabolic-pathway interpretations are also possible.

EXPERIMENTAL PROCEDURE

Ten mice were each injected intraperitoneally with 0.05 mg of sodium cyanide-C^{14} (about 4 μC) in 0.15 ml of pH 8 solution. These injections were all carried out within a few minutes. The labeled cyanide was prepared by Bernice Fingerman and Richard M. Lemmon, using the sealed-tube reaction of barium carbonate-C^{14}, a potassium mirror, and ammonium chloride.[1] Many attempts at precise direct assay of the carbon-14 in the cyanide solution all met with failure. Scintillation counting, direct plating and Geiger-Mueller counting, Pregl combustion, sealed-tube combustion, and ion-chamber assay gave values ranging from 3 to 6 μC of C^{14} in the injected aliquot. The problem seemed to arise from the volatility of micro amounts of cyanide in small volumes of solution. Such solutions appear to lose appreciable amounts of activity merely on standing for a few hours.

It was decided to use the cumulative urine-feces and breath carbon-14 excretion, together with a carbon-14 assay of the residual carcass activity, as a measure of the total activity actually injected into the mice. The radioactivity in the breath was measured for the first 6 hours for four animals in individual metabolism cages, using continuous ionization-chamber assay of the exhaled carbon-14.[2] Labeled hydrogen cyanide, carbon monoxide, and other such gases are detected by the apparatus, as well as carbon dioxide. The data quoted in this paper are the average values for these four animals. At the end of the 1-, 3-, and 11-day intervals, breath radioactivity was measured by putting four animals in one cage and doing an assay using a sensitive 1-liter ionization chamber.[3,4]

*The work described in this paper was sponsored by the United States Atomic Energy Commission.

The urine-feces excretion data were obtained by placing four mice in a metabolism cage and collecting the combined product. The excreta and washings were homogenized in a Waring blender and diluted to a convenient volume (50 to 150 ml), and a 25- to 100-λ aliquot was transferred to a platinum dish. This aliquot was evaporated to dryness under a heat lamp and the sample counted in a windowless flow G-M counter with an efficiency of about 50%. At the end of the 30-day experiment, sample activities were about double background. Appropriate corrections were made for backscattering, self-absorption, and coincidence, where necessary.

Residual total-body radioactivity was determined as follows: The animal was cut into approximately 1/4-cm^3 pieces, added to 100 ml N KOH, and digested at 50-60°C for 24 hours. Fifty ml of 95% ethanol was added to the digestion mixture, which was then centrifuged. The supernatant was decanted, the volume measured, the mixture shaken to emulsify the fat, and a 50- or 100-λ aliquot pipetted into a platinum counting dish for assay as previously described.

The precipitate, presumably mostly protein, was redissolved in 100 ml of N KOH, and 100λ of the supernatant was assayed for carbon-14. Reprecipitation of this material and assay of the radioactivity showed two-thirds of the activity to remain in this protein fraction. In this procedure the bones are not dissolved, but samples of the bones ground up and counted on direct plates showed that, within limits of the counting error, there was no detectable radioactivity. Samples of fat, counted separately before emulsification, likewise contained negligible radioactivity.

The data on the excretion rate of carbon-14 from mice given cyanide-C^{14} are presented in Table I and Fig. 1. The peak breath-excretion rate occurs 6 to 10 minutes after the injection, whereas the maximum urine and feces C^{14}-excretion rate occurs during the 6 to 24-hour period. At all times urinary and fecal excretion of the carbon-14 is more important than breath excretion, although after the first 10 days these modes are quite comparable in magnitude.

In Table II are presented the cumulative excretion data for carbon-14 from mice given cyanide-C^{14}. It is interesting to note that about 25% of the cyanide carbon is oxidized to CO_2 and appears in the breath. (There may be some labeled hydrogen cyanide in the breath, but the quantity is probably quite small.)

Table III summarizes the radioactive-material balance for the series of animals. The total injected activity present in each mouse was calculated as 4.11 μC. Furthermore, graphic extrapolation of the cumulative excretion of carbon-14 data gives a value approaching 4.11 μC. This value has therefore been used as the injected dose in the radiation-dose calculations presented further on in this paper.

Table I

Daily excretion rate of carbon-14 from mice given cyanide-C^{14}

Interval	Urine and feces (μC/day)	Breath (μC/day)
0 - 6 hr	0.36	1.64[a]
6 - 24 hr	1.72	0.282
1 - 2 d	0.76	0.163
2 - 3 d	0.29	0.054
3 - 4 d	0.19	0.034
4 - 5 d	0.10	0.024
5 - 6 d	0.085	0.0186
6 - 7 d	0.021	0.0151
7 - 8 d	0.023	0.0121
8 - 9 d	0.019	0.0100
9 - 10 d	0.015	0.0085
10 - 11 d	0.013	0.0074
11 - 12 d	0.0070	0.0065
12 - 13 d	0.0087	0.0058**
13 - 14 d	0.0072	0.0055**
14 - 15 d	0.0057	0.0045**
15 - 18 d	0.0042*	0.0035**
18 - 23 d	0.0033*	0.0023**
23 - 26 d	0.0032*	0.0017**
26 - 30 d	0.0028*	0.0011**

[a]See Fig. 1 for breath-excretion-rate curve during the first 6 hours.

*The values are averaged and interpolated from measurements made on the fourteenth, seventeenth, twenty-second, twenty-fifth, and twenty-ninth day after injection.

**These values are obtained by extrapolation.

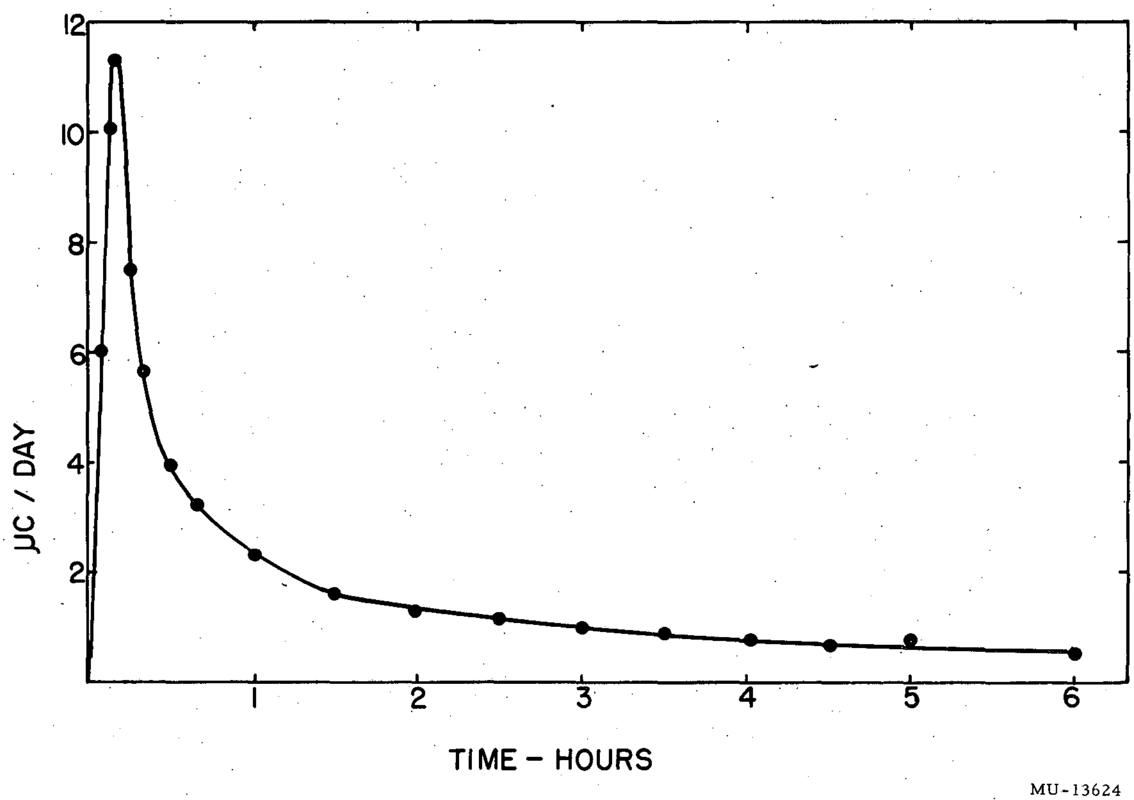

Fig. 1. Rate of breath excretion of carbon-14 after ip injection of cyanide-C^{14} in mice. The injected dose was 4.11 µC and contained about 0.05 mg cyanide.

Table II

Cumulative excretion of carbon-14 from mice given cyanide-C^{14}

Time (days)	Urine and feces (μC)	Breath (μC)	Breath, urine and feces (μC)
0.25	0.090	0.41	0.50
1.00	1.38	0.62	2.00
2.00	2.14	0.78	2.92
3.00	2.43	0.84	3.27
4.00	2.72	0.90	3.62
6.00	2.81	0.91	3.72
7.00	2.83	0.93	3.76
8.00	2.85	0.94	3.79
9.00	2.87	0.95	3.82
10.00	2.88	0.96	3.84
11.00	2.89	0.97	3.86
12.00	2.90	0.97	3.87
13.00	2.91	0.98	3.89
14.00	2.92	0.99	3.91
15.00	2.93	0.99	3.92
3.00	2.95	1.02	3.97
26.00	2.95	1.02	3.97
30.00	2.97	1.03	4.00

Table III

Carbon-14 material balance for mice given cyanide-C^{14}

Time (days)	Carcass Analysis*		Breath, urine and feces (μC)	Summation of C^{14} excreted plus C^{14} in situ (μC)
	Soluble fraction (μC)	Alcohol precipitate fraction (μC)		
8	0.130	0.010	3.79	3.93
15	0.110	0.073	3.92	4.10
22	0.110	0.015	3.97	4.11
30	0.092	0.020	4.00	4.11

*Each value is the average of the analysis of two animals.

DISCUSSION

An important mode of excretion of cyanide is as thiocyanate. In 1932 Lang discovered an enzyme, "rhodanese," which catalyzes the conversion of cyanide to thiocyanate in the presence of thiosulfate.[5] Saunders and Himwich suggest that the function of the enzyme is to form a loose combination with $S_2O_3^{=}$, which then yields sulfur in a form acceptable to CN^-.[6] Rhodanese converts cyanide to thiocyanate almost quantitatively and with fair rapidity.[7]

Thiocyanate is largely distributed in the extracellular fluid and does not penetrate tissue cells to any significant extent,[8] and is bound by serum albumin. In dogs, renal SCN^- clearance bears a straight-line relationship to total CN^- excretion.

Our data show that 10 days after the injection of cyanide 6.5% of the injected dose is still in the animal (70% has been excreted in the urine and feces and 23.5% has been excreted in the breath). Since it is unlikely that any cyanide exists as such at this time, and since thiocyanate is rather rapidly excreted, it would seem probable that this fraction of the cyanide has been incorporated (perhaps via thiocyanate) into some semipermanent body structure.

Since the urine and feces excretion of radioactivity is a little larger than the breath excretion at 30 days, and since this is the converse of the case for glycine-2-C^{14},[9] we belive that at least part of the bound "cyanide" is held as a nitrile or slightly modified CN^- group. Thus, when the given holding molecules are changed by metabolic forces, the cyanide or nitrile is released and appears in the urine rather than in the breath. Alternate fates for this bound "cyanide" would include nucleic acids or substances that are metabolized in high yield to urea. An appreciable fraction of this bound radioactivity appears to be attached to protein molecules. (See Table III.)

The data presented in this paper are based on a single gravimetric dose of 0.5 mg of NaCN per animal. This was sufficient cyanide to cause acute, but short (5 to 10 min), distress-reaction symptoms typical of cyanide poisoning. Preliminary experiments had shown that 0.1 mg NaCN per animal was about the maximum that could be used without danger of killing the animal. We wonder how different the excretion pattern will be if much larger or a smaller quantity of cyanide is used.

ESTIMATION OF RADIATION DOSE

If one assumes uniform distribution of a C^{14}-labeled compound in an animal, it is possible to calculate a radiation-dose rate:

$$\text{millirad/day} = \frac{(N)\ (\bar{E}_{\beta^-})\ (\text{ergs/ev})\ (1000)}{(\text{erg/ g /rad})\ (\text{wt;gm})}, \qquad (1)$$

where N = number of betas per day,

$\bar{E}_{\beta^-}$ = average energy of C^{14} beta (50,000 ev),

ergs/ev = 1.60×10^{-12},

ergs/g/rad = 100

For 100 µC of C^{14} in a 70-kg man, N = 3.20×10^{11} and wt = 70,000. Substituting these data in Eq. (1), we have millirad/day = 3.66.

If one assumes that cyanide excretion in man and the mouse are the same, we can use the mouse excretion data to calculate a series of dose rates over a period of time following administration of a given dose of labeled cyanide. This has been done for 100 µC cyanide-C^{14}, and the data are presented in Table IV and Fig. 2. Integration of the area under this dose-rate-vs-time curve gives the cumulative radiation dose.

Within the limits of the assumptions given above, we see that in 7 days after ingesting 100 µC cyanide-C^{14}, the C^{14}-radiation level in the body will have fallen to natural and cosmic-ray levels. The integrated dose at this time is about 8 mrad. The present weekly-tolerance dose for continuous exposure to whole-body radiation has been set at 100 mrep/week.

An important error in the above assumption is assumption of uniform distribution of radioactivity. This error may be particularly important during the first 2 days, when 50% of the radioactivity is being excreted in the urine and feces. Since most of this activity is in the urine, it is worth while to calculate the dose to the kidneys. In a normal 70-kg man the kidneys weigh about 290 g.[10] Urine volume is 1000 to 1500 ml/day. If we assume that one-third to one-half of the kidney volume contains blood, urine, and other fluids that have the same radioactivity concentration as the urine, then one-tenth of the urinary excretion will be in the kidneys at any one time.

From the data in this paper we calculate that the maximum observed urinary excretion rate is 41% of the injected dose per day (assuming no fecal excretion). This much activity would then produce a maximum radiation rate of 36 millirad per day to the kidneys for a 70-kg man given 100 µC of cyanide-C^{14}.

The integration of the kidney dose rate over the first 5 days gives the following cumulative radiation dosages: 6 hr, 1.93 mrad; 24 hr, 29.6 mrad; 2 days, 35.9 mrad; 3 days, 42.1 mrad; 4 days, 46.2 mrad; 5 days, 48.3 mrad.

Table IV

Radiation dosage from 100 μC cyanide-C^{14} taken into a 70-kg man. Calculated by using excretion data from mice and assuming uniform distribution of radioactivity.

Time (days)	C^{14} in body (μC)	Dose rate (millirad/day)	Cumulative dose (millirad*)
0	100.00	3.66	0.00
1	51.3	1.88	2.46
2	28.9	1.06	4.22
3	20.5	0.75	5.41
5	12.0	0.44	6.76
7	8.6	0.31	7.80
9	7.1	0.26	8.20
12	5.7	0.21	9.00
15	4.7	0.17	9.70
30	2.7	0.10	12.00

*The rad, which is equal to 100 ergs of radiation energy absorbed per gram of tissue, is approximately equal to a rep (83 to 93 ergs/g tissue).

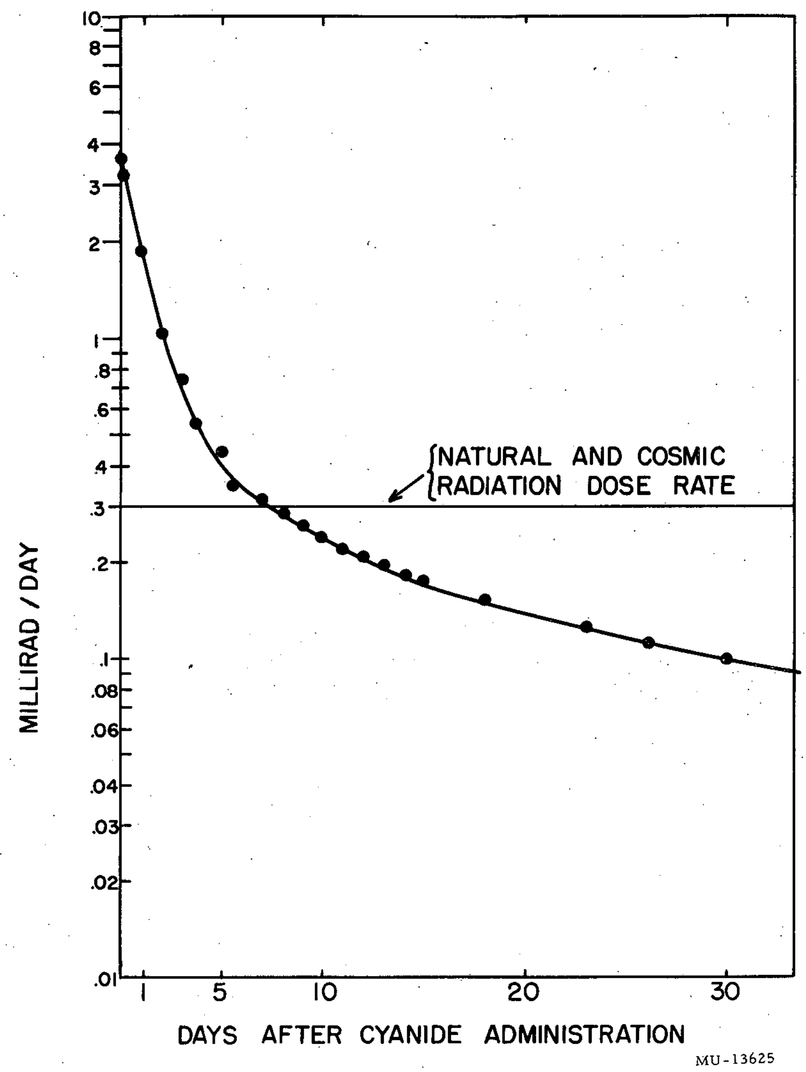

Fig. 2. Daily radiation dose from 100μC carbon-14 administered as CN^- for a 70-kg man (values estimated from mouse data).

REFERENCES

1. Sixma, Hendriks, Helle, Hollstein, and van Ling, Rec. Trav. Chem. 73, 161 (1954).

2. Tolbert, Hughes, Kirk, and Calvin, Arch. Biochem. and Biophys. 60, 301 (1956).

3. Tolbert, Kirk, and Baker, Am. J. Physiol. 186, 269 (1956).

4. B.M. Tolbert, Ionization Chamber Assay of Radioactive Gases, UCRL-3499, March 1956.

5. K. Lang, Biochem. Z. 259, 243 (1933).

6. J.P. Saunders and W.A. Himwich, Am. J. Physiol. 163, 404 (1950).

7. F. Goldstein and F. Rieders, Am. J. Physiol. 173, 287 (1953).

8. L.S. Goodman and A. Gilman, The Pharmacological Basis of Therapeutics (MacMillan, New York, 1955), p. 744.

9. Berlin, Tolbert, and Lee, J. Clin. Invest. 32, 1-4 (1953).

10. H. Gray, Gray's Anatomy, C.M. Goss, Ed., 25th ed. (Lea and Febiger, Philadelphia, 1948), p. 1264.