take slightly but based on a 24 g increase in bw, the food intake/100 g bw was reduced 4.02%. The combination of 2 or 3 hormones in all cases depressed food consumption/100 g bw.

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Verification of L-Ascorbic-1-C^{14} Acid Catabolism to Carbon^{14} Dioxide in the Human by Liquid Scintillation Counting. (29747)

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We have previously noted a new excretory pathway for ascorbic acid catabolism in man, the respiratory tract(1). Our data were obtained from 4 human volunteers. We were the first to establish that in man and the monkey(2), the major portion of ascorbic acid is excreted within the first few hours following ingestion, both through the expired air and the urine in a manner similar to that reported for the guinea pig(3,4).

Baker *et al*(5) reported on the catabolism of D-glucuronolactone-6-C^{14} and L-ascorbic-1-C^{14} acid. They noted that L-ascorbic-1-C^{14} acid was synthesized from D-glucuronolactone-6-C^{14} in the human. This is not in agreement with Grollman and Lehninger(6). Similarly their statement(5), that no L-ascorbic-1-C^{14} acid was catabolized and expired as $C^{14}O_2$ in man, is in contradiction to our reported findings(1).

Later Baker *et al*(7) studied the catabolism of the degradative intermediates of L-ascorbic-1-C^{14} acid in man and noted that, in contrast to intact ascorbic acid, when the degraded material was ingested by human subjects, carbon^{14} dioxide was found in the expired air. They stated that in 2 human subjects given the degraded substances "C^{14} activity of the respiratory CO_2 returned to background activity in 6-13 hours. However, C^{14} activity of the respiratory CO_2 of the subjects was measured at 24-hour intervals during a 5-day period following the first day of experiment."

Baker concluded that Abt *et al*(1) used similarly degraded L-ascorbic-1-C^{14} acid and that the observed carbon^{14} dioxide was therefore not a true metabolite.

The instability of vitamin C in solution is a well established fact(8). We have been cognizant that "Ascorbic Acid is one of many compounds that may undergo degradation during aging in solution"(9).

Due to the importance of accurately establishing the catabolism of ascorbic acid and its relation to human nutrition, we have obtained new data from a fifth volunteer, which we report here. We are presenting a paper chromatogram and a radiochromatogram prepared from the labeled L-ascorbic-1-C^{14} acid used in the human study here presented. This has been a routine procedure in all our

experiments, to show the purity of the radioactive ascorbic acid at time of ingestion. The radioactivity of the exhaled $C^{14}O_2$ was measured with a liquid scintillation counter* according to a method devised in our laboratory(10). This additional information is presented to confirm our original report(1).

Experimental materials and methods. A healthy male volunteer, on an adequate vit C dietary intake, orally ingested 192 μc of freshly prepared L-ascorbic-1-C^{14} acid *immediately* following its solution in water. Aliquots of this freshly prepared solution of L-ascorbic-1-C^{14} acid were used for chromatographic studies for proof of radiochemical purity and for preparation of the standards.

The single ingested amount of radioactive ascorbic acid was calculated on the basis of 1 μc per lb body weight. A total of 192 μc of carbon^{14}, incorporated in the C-1-position of 35 mg of ascorbic acid, were given.† Specific activity was 5.5 μc per mg of ascorbic acid.

The expired aid was collected at set intervals in Douglas Bags of 30 liter capacity, during 3-minute collecting periods. The initial sample of expired air was collected from the volunteer one-half hour following ingestion and thereafter at hourly intervals through the seventh hour. Following this, samples were collected at the 10th, 12th, 24th, 26th, 30th and 36th hours. Samples thereafter were collected twice daily during the 24-hour through the 72-hour period; then samples were collected at intervals through the fifteenth day, a total of 25 samples following initial ingestion of the radioactive ascorbic acid. Cumulative data showing the fraction of L-ascorbic-1-C^{14} acid catabolized as $C^{14}O_2$ are shown in Table I.

TABLE I

Time following ingestion of L-ascorbic-1-C^{14} acid (days)	Fraction of ingested L-ascorbic-1-C^{14} acid catabolized to $C^{14}O_2$	
	Per hr (%)	Cumulative (%)
.125 (3 hr)	2.55	4.7
.5	.29	10
1	.30	13.6
2	.035	14.6
3	.037	15.4
7	.012	18.8
15	.009	21.7

Carbon dioxide contained in the expired air in the Douglas Bag is absorbed directly in the counting vial, which contains one ml of one molar hyamine hydroxide solution and 2 ml of methanol. The alkaline absorber is completely neutralized by one millimole of carbon dioxide; therefore the amount of radioactivity in each vial represents the fraction of the ingested amount of radioactive ascorbic acid metabolized to carbon^{14} dioxide per millimole of carbon dioxide.

The expired air from the Douglas Bag is recirculated with the help of a rotary pump through the vial containing the alkaline absorber. Ten minutes are sufficient for complete neutralization of the alkaline absorber.

The counting vial, charged with one ml of hyamine hydroxide and 2 ml of methanol, is placed inside a glass tube of 15 cm length with an outer diameter of 5 cm. Two rubber stoppers serve to make the system gas tight. The stopper on the bottom has an indentation wide enough to secure the vial and the upper stopper has 2 holes holding 2 glass tubes, one used as the inlet and the other as the outlet. The inlet tube, whose end is funnel-shaped, extends 1 cm above the absorbing solution. A drying tube, filled with calcium chloride, is placed between the Douglas Bag and the inlet tube to absorb the moisture in the exhaled air. Tygon tubing and polyethylene coupling are used throughout the system for making connections. Two samples are prepared from each bag and after adding 14 ml of Liquifluor in toluene to the vial, the vial is placed in the liquid scintillation counter and the radioactivity is determined.

Preparation of standards. The sample of L-ascorbic-1-C^{14} acid prior to counting in the liquid scintillation counter was prepared by 2 different methods. In one method an aliquot of the freshly dissolved radioactive ascorbic acid was placed on a piece of filter paper, dried and oxidized in the modified Schöniger combustion flask to carbon dioxide and water(11). The carbon dioxide was absorbed in 25 ml of hyamine hydroxide and

* Packard Tri-Carb Liquid Scintillation Spectrometer, Model 314E.

† Synthesis was carried out in authors' laboratory(1).

the radioactivity of 1 ml aliquots of the hyamine hydroxide solution was determined in the liquid scintillation counter.

The second method consisted of dissolving an aliquot of the same solution of radioactive ascorbic acid in methanol. One ml of the methanol solution was placed in a counting vial along with 1 ml of methanol and 1 ml of hyamine hydroxide. The solution was saturated with carbon dioxide in the absorption apparatus by passing inactive exhaled air from a Douglas Bag over it for 10 minutes. Fourteen ml of Liquifluor in toluene were added and the vial was counted in the liquid scintillation counter.

Both methods gave the same results, 2.34 $\times$ 10^8 cpm for 35 mg L-ascorbic-1-C^{14} acid. This corresponds to 192 μc C^{14}, since an internal C^{14} standard prepared in an identical manner showed a counting efficiency of 55%.

Determination of radiopurity. The melting point of the radioactive ascorbic acid was 186°, unchanged by admixture of authentic ascorbic acid.

An aliquot of the radioactive ascorbic acid was placed on a filter paper strip, dried and developed in an ascending chromatogram in a butanol, acetic acid water (4:1:5) system for 18 hours. A sample of inactive ascorbic acid was run on a second strip of filter paper. The location of the radioactive ascorbic acid was determined by viewing it under an ultraviolet light, and was found to be at the same distance from the origin as the inactive ascorbic acid. A radiochromatogram was prepared and scanned in a windowless chromatogram scanner. The peak of the radiochromatogram curve corresponded to the ascorbic acid spot on the paper chromatogram (Fig. 1).

Calculation. For calculation the exact normality of the hyamine hydroxide and the amount of carbon dioxide exhaled per hour by the human subject must be known.

The hyamine hydroxide was titrated with 0.1 normal hydrochloric acid and was found to be 0.985 normal.

The hourly amount of CO_2 exhaled by the subject was taken from our own determinations, as well as from standard text books (12). Hourly excretion was calculated on

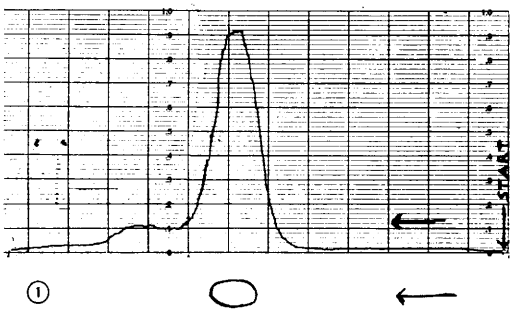

FIG. 1. Radiochromatograms and paper chromatograms of L-ascorbic-1-C^{14} acid. Specific activity: 5.5 μc per mg. Developing solvent: butanol, acetic acid, water (4:1:5). Upper curve shows peak of carbon-14 activity and paper chromatogram shows location of ascorbic acid.

the basis of 1 mole of carbon dioxide per hour.

The total amount of C^{14} radioactivity ingested was 2.34 $\times$ 10^8 cpm. One ml of hyamine hydroxide 0.985 molar neutralizes 0.985 millimole of CO_2. In each sample we determined the fraction of the total ingested radioactivity converted to $C^{14}O_2$ in 0.985 millimole of CO_2. The data obtained at intervals were then calculated as fraction of the ingested material per mole of CO_2, which corresponds to an average amount of carbon dioxide exhaled per hour. From these data cumulative excretion was computed.

Results. With the improved method here described, we have been able to extend the period for measuring excretion of exhaled $C^{14}O_2$ to 15 days following ingestion of radioactive ascorbic acid (Fig. 2). The shape of the curve constructed from these data corresponds in every respect to the curves obtained from experiments on the 4 subjects

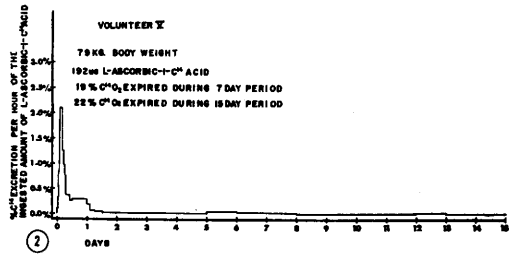

FIG. 2. Newly presented data for human subject V showing carbon^{14} excretion rates of expired air followed for 15 days. Carbon^{14} excretion for 7 days was 10.5%, for 15 days 21.6% of the total ingested activity.

previously reported(1). Following ingestion of the radioactive ascorbic acid an appreciable amount of $C^{14}O_2$ is present in the expired air within the first half hour and reaches its peak by the third hour. Thereafter there is a precipitous decline in excretion of $C^{14}O_2$ which levels off by the 10th and 12th hours. The specific activity of $C^{14}O_2$ per mole of CO_2 is above 1% per hour of the ingested radioactivity during the 2- and 3-hour periods. Specific activity is below 1% per hour except for the period from 2 to 4 hours following ingestion (Fig. 2). After 24 hours specific activity falls to 0.1% of the ingested amount of radioactive ascorbic acid.

Conclusion. As previously pointed out in the literature, a certain portion of ascorbic acid administered to the human could not be accounted for before the availability of radioactive compounds(13,14). We have shown that this unknown balance can now be accounted for as carbon dioxide excreted in the exhaled air. The catabolism of ascorbic acid is relatively slow in the human. An appreciable amount of radioactive ascorbic acid must therefore be ingested in order that the catabolized carbon dioxide can be detected in man. It is therefore necessary to ingest quantities in the range of 1 μc of L-ascorbic-1-C^{14} acid per lb of body weight to measure catabolized $C^{14}O_2$ in the expired air with an ionization chamber or a liquid scintillation spectrometer. Important contributions to human nutrition and the nutritional requirements of vit C may be derived from the demonstration of this new human excretory pathway in the catabolism of ascorbic acid.

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Effect of Alterations in Serum Electrolytes on Production of Endogenous Pyrogen.* (29748)

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It is generally believed that fever is mediated by a humoral substance released from leukocytes in response to some form of tissue injury. The pyrogenic material then enters the circulation to exert its influence on the thermoregulatory centers of the brain, thereby initiating the constellation of physiological events which culminate in fever. This fever-promoting substance, termed endogenous pyrogen (EP), has been demonstrated

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