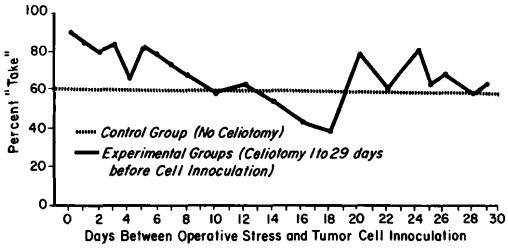

FIG. 1. Relationship of take to time interval between stress (celiotomy) and tumor cell inoculations. Note that when cells were inoculated immediately after stress the takes were 90%, indicating a very low resistance. However, the resistance increased gradually (with a lowering of % takes) for 6 or 7 days; but the resistance continued to increase gradually beyond that of the controls until the 18th day when the takes were only 41% compared to a control level of 60%. Between the 18th and 20th days the resistance decreased, but returned to normal a few days later (by the 28th day).

experimental groups (44 and 41% respectively), as compared with the control group (60%) is statistically significant ($p = .02$). The percent take then rose to a level of 79% in the animals inoculated 20 days after celiotomy. Following this the percent take decreased gradually, returning to normal in the group inoculated with cells 28 days after celiotomy.

Discussion. We have no explanation for this increase in resistance to cancer cells observed 16 to 18 days after celiotomy, other than the supposition that it is related to the celiotomy. Moreover there is no definite explanation of the reduced resistance to cancer cells found so consistently for 2 to 5 days after celiotomy. At first it was thought that it was related to the adrenal response observed in the Selye stress phenomenon. However, the work of Slawikowski(9) suggests strongly that neither the adrenal nor hypothesis exerts a significant influence on this reaction; at least their ablation did not prevent the production of decreased resistance to cancer cells after celiotomy. It is very possible that the liver is a major factor in the phenomenon.

Summary. Approximately one thousand Sprague-Dawley white rats were subjected to the stress of a celiotomy at various intervals (0 to 30 days) before inoculation with Walker 256 tumor cells. The low resistance noted when cells were injected at the time of stress (as measured by a take of 90%) increased gradually from day to day reaching the control value (60%) by the eighth day, but continued to show an increased resistance for several more days, with a take of only 41% on the 18th day. The resistance decreased rather sharply to a take of 79% on the 20th day, coming back to the control level of 60% within the next few days.

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Respiratory Catabolism in Man of the Degradative Intermediates of L-Ascorbic-1-C^{14} Acid. (28371)

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Earlier studies have shown that there is little or no oxidation of the carboxyl carbon of L-ascorbic acid to CO_2 in man(1,2). Recent experiments showed that rapid decomposition of L-ascorbic-1-C^{14} acid occurred when a dilute solution of it remained at room temperature in the dark for 24 to 72 hours. When this material was given to either ani-

mals or humans, $C^{14}O_2$ was increased in the respiratory CO_2; this indicated an increased decarboxylation. Because of these results, a series of studies were performed to determine what the degradative intermediates of L-ascorbic acid are and to show clearly that these degradative intermediates are responsible for the increased decarboxylation in humans of aged ascorbic acid solutions.

Experimental. The L-ascorbic-1-C^{14} acid, which had a specific activity of 1.65 mc/mM, was purchased from the California Corporation for Biochemical Research, Los Angeles. The L-ascorbic-1-C^{14} acid was kept in a solid state and stored in a freezer until it was dissolved in water. Immediately upon dissolving the ascorbic acid in triple distilled water, the homogeneity and purity of the ascorbic acid was checked by determining the specific rotation and by paper chromatography as well as radioautography. The activity of the L-ascorbic-1-C^{14} acid was checked by radioassay.

Two 50 μc vials, both of the same lot number, of the crystalline L-ascorbic-1-C^{14} acid were dissolved by addition of 5.0 ml of demineralized, triple glass distilled water to each of the vials. Both vials were shaken well, and the solution from each vial decanted into an acid-washed screw-cap vial. This solution was then kept in the dark at a temperature of 25° $\pm$ 1°C for the 72-hour experimental period. The vial was kept capped at all times except when it was necessary to open it and determine the optical rotation and prepare the chromatograms. Fifty and 100 lambda aliquots of the solution were immediately spotted on sheets of Whatman #1 chromatographic paper (9 $\times$ 22 inches) and air-dried at low temperature.

The chromatograms were developed in a n-butyl alcohol : acetic acid : water, 250:60:250, solvent system as described by Tegethoff(3). After a 22-hour development period, the chromatograms were air-dried and sprayed with 2,6-dichlorophenolindophenol (4). This procedure was repeated at several 12-hour intervals over a 72-hour period. Dissolving of the crystalline L-ascorbic-1-C^{14} acid in water was taken as time zero in our experiment. Optical rotation of the ascorbate solution was determined at zero time and at 12-hour intervals over the 72-hour period.

Radioautograms were prepared from each chromatographic sheet. The areas of dichlorophenolindophenol decolorization on the original chromatograms were traced onto drawing paper. The chromatograms were then cut into 1½-inch strips and scanned by a 4 π scanner integrator to determine the exact locations and percentages of the C^{14} activity contained on the chromatograms.

Human experiments. Three healthy, normal male volunteers each received, orally, approximately 20 μc of the L-ascorbic-1-C^{14} acid at the following times: Subject No. 1 swallowed 20.13 μc of the L-ascorbic-1-C^{14} acid which was dissolved in distilled water at time zero; Subject No. 2 received 20.13 μc of the L-ascorbic acid solution at the 36th hour; and Subject No. 3 swallowed 16.10 μc of the ascorbic acid solution at the 72nd hour.

Immediately after receiving the tracer quantity of L-ascorbic acid, the men were made to expire directly through a $CaCl_2$ drying train into a 5-liter Cary-Tolbert ionization chamber connected to a vibrating reed electrometer. The $C^{14}O_2$ activity, total CO_2 and volume of flow were recorded automatically on a 6-channel recorder.

Results. Fig. 1 is a diagrammatic presentation of both the chromatograms and the results of the scanning of the chromatographic strips cut from the chromatograms, with the scanner integrator to determine location and percentage of radioactive areas contained on the strips. At time zero the chromatogram shows only one spot at the location with the ratio of fronts of solvent and precipitate (Rf) 0.375 reacting with the 2,6-dichlorophenolindophenol; further, the scanner detected only a trace of activity at the line of application and 97.76% of the activity at Rf of 0.375, thus indicating that the freshly dissolved crystalline L-ascorbic-1-C^{14} acid was essentially a pure and homogenous compound. The 24-hour chromatogram shows decolorization spots occurring at Rf values of 0.12, 0.16 as well as 0.375. The scanner detected activity at Rf 0.12 (peak 1), Rf 0.16 (peak 2) and Rf 0.375 indicating re-

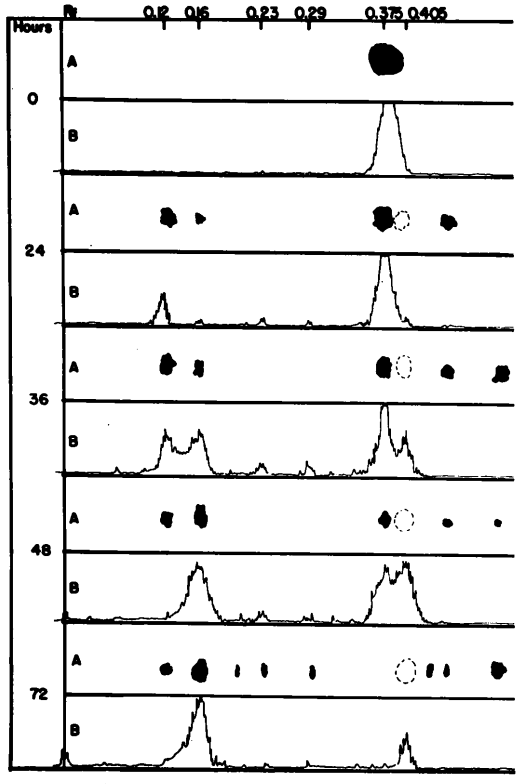

FIG. 1. Chromatographic and radiographic analysis of the degradation of L-ascorbic acid in solution as a function of time. (A) Represents the chromatogram which was sprayed with 2,6-dichlorophenolindophenol. (B) C^{14} activity on chromatogram scanned by 4π scanner integrator.

duced ascorbic acid. Further, a peak was detected at Rf 0.405; this peak corresponds in Rf and lack of color reaction to dehydroascorbic acid.

There was also activity detected at Rf values of 0.23 and 0.29, peaks 3 and 4, respectively. However, these spots were disregarded since they never accounted for over 2% of the total activity detected by the scanner. At the 36th hour, decolorization spots were found at Rf 0.12, 0.16 and 0.375. The scanner showed that peak one accounted for 21.8% of the activity, peak 2, 2.08%, and reduced and dehydroascorbic acid accounted for 43.7 and 30.5% of the activity, respectively. By the 48th hour, peak one had practically disappeared and peak 2 accounted for 41.8% of the activity, while reduced and dehydroascorbic acid accounted for 26.5 and 27.1% of the activity. At the 72nd hour, peak one and reduced ascorbic acid had completely disappeared and remaining was only peak 2 with 79.4% of the activity and dehydroascorbic acid (Rf 0.405), which accounted for 13.3% of the activity. Spots occurred on the chromatograms at Rf values greater than 0.405, commencing with the 24-hour run and continuing through the 72-hour run. However, even though these spots react with the dichlorophenolindophenol, the scanner did not detect any radioactivity in them. These compounds must therefore represent decarboxylation products.

Fig. 2 is a plot of the per cent of total radioactivity as seen by the scanner and shows rate of appearance and disappearance of the various peaks formed by the degradation of the L-ascorbic-1-C^{14} acid solution.

Table I shows the values for the specific rotation obtained on the ascorbate solution at 12-hour intervals from zero time to the 72nd hour. The specific rotation showed a steadily increasing value through the 60th hour, then began to decrease by the 72nd hour. Because of the extreme dilution of the ascorbic acid, the specific rotations as listed

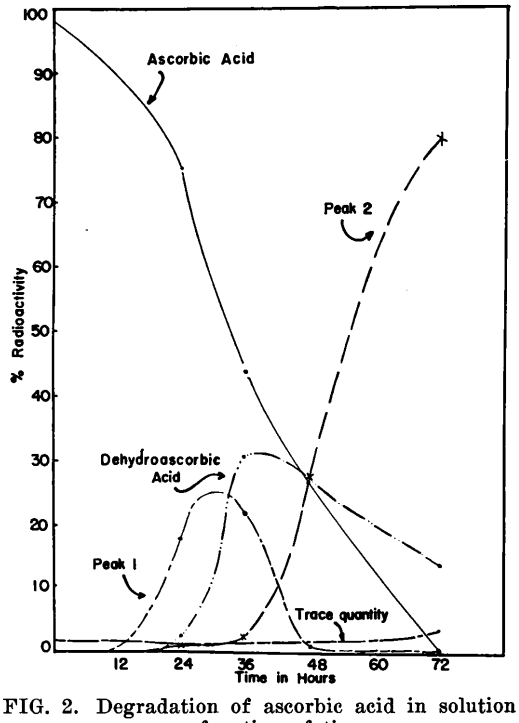

FIG. 2. Degradation of ascorbic acid in solution as a function of time.

TABLE I. Specific Rotation, $[\alpha]^{D}_{25}$, of L-Ascorbic Acid Solution as a Function of Time.

Time (hr)	Specific rotation
Zero	+19.5°
12	+30.0°
24	+35.0°
36	+43.0°
48	—
60	+58.1°
72	+50.0°

TABLE II. $C^{14}O_2$ Excretion *via* the Lungs in the 3 Subjects Who Ingested Fresh and Aged L-Ascorbic-1-C^{14} Acid Solution.

Subject	Time C^{14} ascorbate had been in solution when subject was labeled (hr)	Total μc administered	% of total given, excreted as $C^{14}O_2$
1	Zero	20.13	.089
2	36	20.13	30.6
3	72	16.10	30.6

are subject to an error of ± 2.0°. Reproducibility was checked on each determination and was well within this range.

Table II shows results of the cumulative $C^{14}O_2$ expiration by the 3 men who ingested the L-ascorbic-1-C^{14} acid at times 0, 36 and 72 hours, respectively. After these subjects received the label orally, C^{14} activity of the respiratory CO_2 was measured for a period of 24 hours with the electrometer-ionization chamber analyzer. 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.

Discussion. The L-ascorbic-1-C^{14} acid in solution undergoes the most extensive degradation between the 36-72-hour period after the crystalline material has been dissolved in water. The concentration of the solution used here was 0.053 g per 100 ml. In previous work with non-radioactive L-ascorbic acid solutions of 1 g per 100 ml, these same changes were noted to occur between the 72nd to the 96th hour after the material was placed in solution. The compounds appearing as peaks one and 2 as well as dehydroascorbic acid seem to be the major intermediates in the degradation of ascorbic acid. Further work is being done to identify the compounds deposited on the chromatogram where the major peaks of activity occur.

The work of Hellman indicates that there is practically no oxidation of the carboxyl carbon of L-ascorbic acid to CO_2 in man (<5.0% of dose during a 10-day period) (1). In previous work at this laboratory, it was noted that less than 1% of the total dose of L-ascorbic acid is decarboxylated by man(2).

Our data permit the following conclusion: After drinking a solution of freshly dissolved crystalline L-ascorbic-1-C^{14} acid (proven to be homogeneous by chromatographic and radioautographic tests), a man excretes less than 0.09% of the ingested C^{14} as $C^{14}O_2$. However, when the L-ascorbic-1-C^{14} acid solution has been allowed to stand for a 36-72-hour period and degradative changes have taken place, 2 human subjects then excreted 30.6% of the radioactivity of the total administered dose as $C^{14}O_2$ *via* the respiratory system. A recent observation by Abt *et al.* (5) that man excretes approximately 25% of the total label of L-ascorbic-1-C^{14} acid *via* the lungs could be easily explained if such or similar changes had occurred in the labeled ascorbic acid used in their studies. The true nature of the compounds undergoing decarboxylation in man in these studies cannot be defined from the work presented here except that they are not reduced ascorbic acid. The exact nature and the catabolism of the compounds precipitated at those major spots on our chromatogram not discussed here are being investigated.

Summary. 1. Chromatographic and radioautographic evidence is presented showing that progressive degradative changes occur in L-ascorbic acid dissolved in water and kept at 25°C for a 72-hour period. 2. When a human subject received 20 μc of freshly dissolved L-ascorbic-1-C^{14} acid solution, little or no C^{14} appears in his respiratory CO_2. 3. Men who were given similar samples of L-ascorbic-1-C^{14} acid aged for 36 and 72 hours, respectively, excreted 30.6% of the ingested C^{14} as respiratory CO_2.

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Acceleration of Longitudinal Bone Growth by Intra-Osseous Injection of Papain Protease.* (28372)

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Intravenous injection of papain in immature animals causes profound degenerative changes in all the epiphyseal cartilage plates and arrest of growth(1,2,3). This effect can be produced selectively in the cartilaginous growth plates of a single bone of a limb by injecting the enzyme into the osseous medullary canal and limiting its diffusion by a tourniquet placed proximal to the injection site(4).

During an investigation of the chondrolytic effect of intramedullary injections of papain protease on the epiphyseal cartilage plates of the tibia of young rabbits, it was found that minimal doses of the enzyme would sometimes cause a relative lengthening of the injected bone. The following experiments were performed to study this paradoxical effect of papain.

Materials and methods. Actively growing New Zealand white rabbits, about one kg in weight, were used. They were weighed daily and animals that failed to thrive were excluded from the study.

Papain protease was obtained commercially (Worthington Biochem. Co. and Nutritional Biochem. Co.) in vials containing .03 M cysteine. Activation was also aided by addition of .001 M versene. Phosphate buffer, 0.1 N, pH 7.0, was added to obtain a final enzyme concentration of 1 mg/ml. Assay of each enzyme shipment was performed using iodine^{131}-labelled albumin as a substrate by a modification of the technic of Potter *et al.* (5). Intramedullary injection of the left tibia was performed under intravenous nembutal anesthesia by puncture of the proximal metaphyseal region of the bone, using a No. 22 needle. Injections were given at intervals of 3 or 4 days. A rubber tourniquet on the lower thigh was maintained for 30 minutes following the injection.

A total of 113 animals was used, of which 89 were experimental and 24 control. The experimental animals were subdivided into 5 groups:

1. Seventeen animals received a single intramedullary injection of 0.2 mg of papain protease. Three or more animals were sacrificed serially at 4, 7, 10 and 14 days.

2. Nine rabbits received two 0.2 mg injections of papain protease. Three animals were sacrificed after 10, 14 and 21 days.

3. Forty-one rabbits were administered 3 injections of 0.2 mg papain protease. Thirty-four were sacrificed at 1 month, 7 at 4 months.

4. Fourteen animals received 6 injections of 0.2 mg of papain protease at 3 or 4 day intervals and were sacrificed after 2 months.

5. Eight rabbits were given 3 injections of 0.3 mg of papain protease and were sacrificed at 2 months.

The 24 control animals received a series of 3 intratibial injections, 0.2 ml in volume, containing only buffer solution or 0.2 mg of papain which had been previously inactivated by boiling. Four control animals were sacrificed at 11 days and the remaining 20 at one month.

After sacrifice, the lower limbs were removed by disarticulation at the hip and fixed

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