Biochemical Pharmacology, 1962, Vol. 11, pp. 135–145. Pergamon Press Ltd., Printed in Great Britain.

THE METABOLISM OF 1-AMINO*CYCLO*PENTANE-1-CARBOXYLIC ACID IN NORMAL AND NEOPLASTIC TISSUES*

WILLIAM R. STERLING,† J. FRANK HENDERSON, H. GEORGE MANDEL and PAUL K. SMITH‡

Department of Pharmacology,
The George Washington University School of Medicine, Washington, D.C.

(*Received* 5 *September* 1961; *accepted* 14 *October* 1961)

Abstract—The distribution, excretion and metabolism of the carcinostatic amino acid derivative, 1-amino*cyclo*pentane-1-carboxylic acid, was investigated in normal and tumor-bearing mice. After intraperitoneal injection of ^{14}C-carboxyl-labeled drug, the amount of radioactivity in the blood remained constant for 4 days, while the urinary excretion of this drug also remained at a constant low level during this period of time. An insignificant quantity of $^{14}CO_2$ was liberated. All the tissues and tumors analysed contained significant amounts of radioactivity, almost all of which was in the cold acid-soluble fraction. There was very little incorporation of the drug into protein. Chemical fractionation and chromatography of tissues, tumor cells and urine have indicated that all radioactivity present was in the form of the original compound. The significance of these results is discussed.

1-AMINO*cyclo*PENTANE-1-CARBOXYLIC acid (ACC) is one of the few amino acid analogs known to possess carcinostatic activity and is one of the first new types of antitumor agents discovered through the screening program of the Cancer Chemotherapy Service Center of the National Cancer Institute.[1] It has been reported that the agent inhibits the growth of adenocarcinoma 755, 6C3HED ascites lymphosarcoma and ascites leukemia L4946 in mice, but has no effect on sarcoma 180 and Ehrlich ascites carcinoma. At the same time as this work was done in the United States, the effect of a series of alicyclic α-amino acids on the growth of the Walker carcinoma 256 in the rat was studied in England by Connors *et al.*[2] Only ACC and its glycine ester and peptide had inhibitory activity. These authors have suggested that α-aminoadipic acid might be formed by ring cleavage of ACC *in vivo* and that this compound might then interfere with the metabolism of lysine. Toxicity and preliminary pharmacological data on ACC have been reviewed by Ross *et al.*[1]

Clinical trials of ACC have given variable results. In one study the drug was administered to patients bearing a wide variety of neoplasms, with little or no response. Toxicity in the form of anorexia, nausea and vomiting were noted in some patients receiving 60 mg or more per kg, a circumstance which forced cessation of

* This research was aided by grants of the American Cancer Society (P-144 and T-14) and the National Cancer Institute (CY-308), U.S. Public Health Service, Bethesda, Maryland.

† This work was carried out during the tenure of a predoctoral fellowship (8771) from the Division of General Medical Sciences, U.S. Public Health Service.

‡ Deceased, 6 October 1960.

treatment.[1] However, a recent preliminary report has indicated that ACC caused subjective and objective improvement in three patients with multiple myeloma.[3]

No investigations of the fate of ACC in mammalian systems *in vivo* have been reported. This paper presents experiments conducted to establish the distribution, metabolism and excretion of therapeutic doses of ACC in normal and neoplastic mouse tissues. The results of this investigation indicate that ACC has widespread distribution throughout the animal, and that it is only very slowly excreted. Evidence is presented to show that ACC probably acts in an unmetabolized form. The relationship between the distribution and metabolism of this drug and its pharmacology are discussed.

MATERIALS AND METHODS

An authentic sample of ACC was kindly provided by the Cancer Chemotherapy National Service Center, National Cancer Institute.

ACC was first prepared by Zelinsky and Stadikoff in 1911.[4] Later, Bucherer and Lieb[5] suggested an alternative general method of amino acid synthesis through the hydantoin, starting with a ketone, potassium cyanide and ammonium carbonate. The hydantoin was subsequently hydrolysed to the amino acid with sulfuric acid. By use of this method, Tillson has prepared ACC on a large scale.[6] This procedure was not entirely satisfactory for the synthesis of radioactive ACC on a small scale, however, and the following modification of the method of Bucherer and Lieb was used to prepare ^{14}C-carboxyl-labeled ACC.

To a 60-ml flask were added 5 m-moles of *cyclo*pentanone in 7 ml of 95% ethanol and 5 m-moles of ^{14}C-potassium cyanide (1 mc, New England Nuclear Corporation) in 7 ml of distilled water. The mixture was allowed to stand in an ice-bath for 15 min and was then slowly heated. When the temperature reached approximately 30 °C 20 m-moles of finely ground ammonium carbonate were added and the mixture was refluxed at 58–60 °C for 4 hr to form the principal intermediate, spiro-(*cyclo*pentane-1:5-hydantoin).* The solution was then evaporated to about two-thirds of its original volume, at which point the hydantoin began to precipitate. Anhydrous barium hydroxide, 10 m-moles, was added and the mixture refluxed at 120 °C for 4 days. At the end of this period, more than 90 per cent of the hydantoin had been hydrolysed to ACC. The rate of hydrolysis to the amino acid was followed by paper chromatography of 5-μl aliquots removed daily. After refluxing, the pH was adjusted to approximately 4 with sulfuric acid to precipitate barium sulfate, which was removed by centrifugation. The precipitate was washed once with cold water and the combined supernatant material and washings were evaporated to dryness. The unhydrolysed hydantoin was extracted with a small volume of dioxane, and ^{14}C-carboxyl-labeled ACC was recovered following recrystallization from water.

The identity and purity of the product were established by ascending paper chromatography on Whatman No. 1 paper in *n*-butanol:acetic acid:water (25:6:25). The R_f values of the hydantoin and ACC were 0·94 and 0·55, respectively. The location of the amino acid on the chromatogram could be determined by its radioactivity and by its color after reaction with ninhydrin (the hydantoin does not give a ninhydrin reaction). The position of the ninhydrin-reactive spot, as well as the radioactivity, coincided in R_f

* 1 : 3-Diazaspiro (4 · 4) nonane-2 : 4-dione.

value with that of the ninhydrin-reactive area of the authentic compound. These results were confirmed by paper chromatography in *n*-propanol:water (80:20) and in methanol:pyridine:water (16:4:1). On the basis of these criteria, the ACC synthesized had a radiopurity of more than 98 per cent, a yield of 241 mg (37 per cent) and a specific activity of $1{\cdot}2 \times 10^6$ counts/min per mg.

Male C3H mice,* 20–30 g, and male BDF1 mice,* 18–22 g, were allowed water and commercial laboratory chow *ad libitum*. The C3H mice were implanted intraperitoneally with approximately 2×10^5 6C3HED ascites lymphosarcoma cells, and the BDF1 mice with approximately 7×10^5 adenocarcinoma 755 ascites cells.†

Mice injected with ^{14}C-ACC were placed in a glass metabolism cage in which CO_2, urine and feces could be collected separately.[7] Air entering the cages was freed of CO_2, and the respiratory CO_2 was collected in two towers in series, each of which contained 200 ml of 2 N NaOH. At selected intervals, the contents of the towers were removed and an aliquot was taken for further analysis. Barium carbonate was prepared by standard methods, transferred quantitatively to planchets, dried, weighed, and its radioactivity measured.

Urine and feces were removed from the metabolism cage at intervals and were frozen until analyzed. Radioactivity in the urine was determined after plating and drying an aliquot. The collected feces were weighed, thoroughly mixed and a portion was removed and weighed, dried *in vacuo* and pulverized; the powder was transferred to planchets and its radioactivity measured.

Small quantities of blood were obtained by puncturing the orbital sinus with heparinized capillary tubes of 0·1 ml volume. The contents of the tube were transferred to planchets and the tube was rinsed with distilled water onto the planchet. The planchets were dried at room temperature and their radioactivity was measured. Larger samples of blood were obtained from the thorax in a manner previously described.[8]

At intervals, mice treated with ^{14}C-ACC were killed by cervical dislocation and the tissues removed, rinsed with 0·154 M sodium chloride and weighed. A sample of muscle was obtained from the hind leg of each mouse. The tissues and carcass were frozen and subsequently fractionated into the cold acid-soluble fraction, the hot acid-soluble fraction, and the protein fraction. The tissues were homogenized in cold 0·4 M perchloric acid and extracted two more times with cold 0·2 M perchloric acid. The extracts were combined, neutralized with potassium hydroxide and the potassium perchlorate was removed; an aliquot was taken for radioactivity measurements and the remainder evaporated to dryness *in vacuo* for further analysis. The tissue residue was then extracted with 0·4 M perchloric acid for 30 min at 100 °C, the residue washed once with 0·4 M perchloric acid and the combined extracts were neutralized with potassium hydroxide and the potassium perchlorate was removed; an aliquot was taken for radioactivity measurements. The protein was thoroughly washed with acetone and dried *in vacuo* (no radioactivity was detected in the acetone washes). The dried protein was pulverized, suspended in 25% ethanol and transferred to planchets, dried, and its radioactivity measured.

Ascites tumor cells were removed from the peritoneal cavity by capillary pipette after laparotomy, the ascitic fluid was separated by centrifugation and an aliquot taken

* Obtained from Simonson Laboratories, Gilroy, Cal.

† The tumors were obtained from Dr. G. A. LePage, Stanford Research Institute, Menlo Park, Cal.

for radioactivity measurements. The tumor cells were then fractionated as above. Contamination by blood was absent or minimal in these tumors.

During the course of these studies it was found that not all of the radioactivity present in the hot and cold perchloric acid extracts could be recovered after neutralization with potassium hydroxide. The amount of radioactivity lost during the precipitation of potassium perchlorate was established by experiments in which known quantities of ^{14}C-ACC were carried through the fractionation procedure. It was found that over a fifty-fold range of ACC concentration, a constant fraction equal to 60 per cent of the radioactivity co-precipitated with the potassium perchlorate both in the presence and absence of tissue homogenate. All measurements reported in this paper have been corrected for this phenomenon.

Radioactivity measurements were performed in a windowless gas-flow proportional counter. Samples were corrected for self-absorption and, except where indicated, all measurements included an error of less than 10 per cent.

RESULTS

Excretion

Male C3H mice, 18–22 g ,were injected intraperitoneally with 30 mg/kg of ^{14}C ACC ($7{\cdot}1 \times 10^5$ counts/min). Six of the treated mice were placed in a metabolism cage, and respiratory CO_2, urine and feces were collected at intervals for a period of

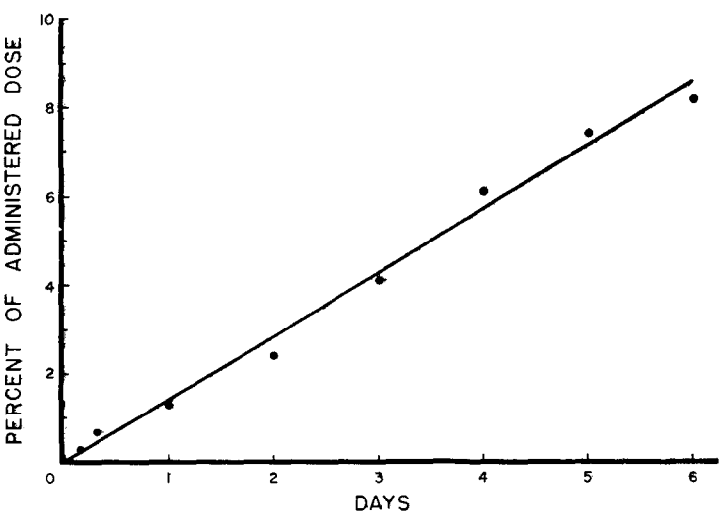

FIG. 1. Cumulative urinary excretion of ^{14}C-ACC. The C3H mice received intraperitoneal injections of 30 mg of ^{14}C-ACC per kg.

6 days. One and four hours and 1, 4, and 6 days after drug administration, two mice from another group which had been similarly treated were taken for blood and tissue analyses as described above.

To examine the possibility that ACC was decarboxylated *in vivo*, expired CO_2 was collected from the six treated mice in the metabolism cage at 4 and 8 hr, and then every day after drug administration for 6 days, and its radioactivity was measured; at no time

did the barium carbonate have a specific activity of more than 0·3 counts/min per mg. The total amount of radioactivity excreted by this route during the 6 days was less than 0·01 per cent of that administered.

The cumulative radioactivity found in the urine over this time period is shown in Fig. 1. At the dose employed, there is a constant, low level of radioactivity excreted daily with a total excretion equal to 8·2 per cent of the administered dose during the 6 days of the experiment.

Feces were collected at the end of the 6-day experiment and assayed for radioactivity as described. A significant amount of radioactivity was recovered in the fecal material, which had a specific activity of 30 counts/min per mg, and accounted for 38 per cent of the administered dose. This finding is of particular interest, since the drug was administered by intraperitoneal injection and not by the oral route.

Distribution

The amount of radioactivity in 1 ml of blood of mice which received a single injection of ^{14}C-ACC is shown in Fig. 2. It may be noted that a near-maximum blood

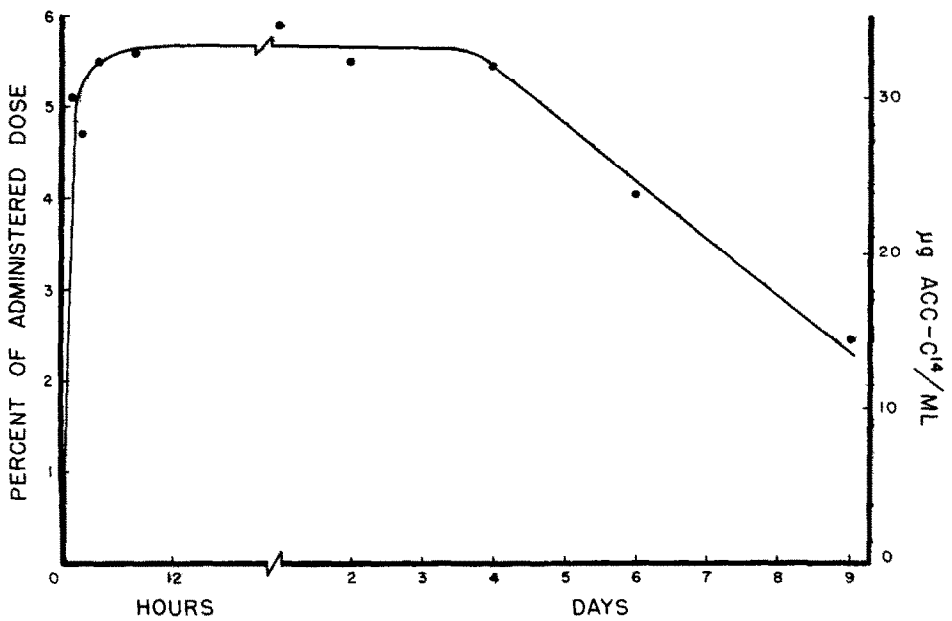

FIG. 2. Blood levels of ^{14}C-ACC. The C3H mice received intraperitoneal injections of 30 mg of ^{14}C-ACC per kg.

level is attained within 1 hr, and that this same level was maintained for 4 days before decreasing slowly to about one-half the initial level at the end of 9 days. At 2 days, approximately 83 per cent of the total blood radioactivity was in the serum.

The distribution of radioactivity in the acid-soluble fraction of the various tissues at several time periods following the administration of ^{14}C-ACC is shown in Table 1;

data from analyses of two mice were averaged. At each time period, the radioactivity in the acid-soluble fraction accounted for at least 98 per cent of the total radioactivity recovered in each tissue. Levels of radioactivity that were close to maximum were attained within 1 hr in all tissues except lung and brain and by 4 hr in these two tissues also. All tissues investigated retained a relatively constant level of radioactivity for a least 6 days. At the end of 24 hr, the distribution of radioactivity, per gram of wet weight, was relatively similar in all tissues except liver.

The data presented in Table 2 show the low levels of radioactivity present in the hot perchloric acid extract and in the protein fraction of the tissues 24 hr after drug administration. These values were representative of the distribution of radioactivity in these tissue fractions at the other time intervals as well. At no time did the amount of radioactivity in these fractions exceed 1·0 per cent of the total radioactivity in the tissue.

TABLE 1. DISTRIBUTION OF ^{14}C-ACC IN THE COLD ACID-SOLUBLE FRACTION OF MOUSE TISSUES*

(Time after the injection of ^{14}C-ACC.)

Tissue	1 hr ACC		4 hr ACC		1 day ACC		4 days ACC		6 days ACC	
	(μg)	(μg/g)	(μg)	(μg/g)	(μg)	(μg/g)	(μg)	(μg/g)	(μg)	(μg/g)
Liver	26·7	16·5	50·5	42·1	25·0	14·4	27·9	24·8	19·8	12·3
Large intestine	22·2	19·2	14·7	16·2	22·6	17·7	16·6	15·8	16·5	19·5
Small intestine	29·8	19·7	31·0	20·0	30·8	16·2	26·7	17·3	35·4	26·7
Spleen	1·4	16·3	2·7	21·2	2·1	16·9	2·0	26·7	1·4	16·7
Kidney	4·2	11·6	4·6	15·0	5·4	14·4	4·3	9·6	4·4	11·8
Lung	1·4	4·7	1·7	12·8	2·0	10·6	2·4	20·0	1·1	6·0
Heart	2·6	17·0	3·2	21·6	2·5	19·0	2·6	20·9	2·1	29·2
Muscle	3·6	9·2	6·8	11·8	5·7	14·2	5·8	15·8	3·5	11·0
Brain	2·3	4·8	7·3	15·4	5·9	13·7	5·8	15·8	6·5	14·8

* C3H mice each received an intraperitoneal injection of 30 mg of ^{14}C-ACC per kg.

Of the radioactivity administered to the animals, after 4 days about 90 per cent could be accounted for either in the animal or the excreta, as is exemplified in Table 3. Of this total, approximately one-third had been eliminated from the animals, mainly in the feces.

Experiments were performed to establish the rate of uptake of ACC into ascites tumor cells *in vivo*. The two ascites tumors used in these studies were the 6C3HED lymphosarcoma, which is sensitive to 30 mg of ACC per kg, and adenocarcinoma 755, which is resistant to this dose.* Thirty milligrams of ^{14}C-ACC per kg were injected

* W. R. Sterling. Unpublished data.

TABLE 2. DISTRIBUTION OF ^{14}C-ACC IN THE HOT ACID-SOLUBLE AND PROTEIN FRACTIONS OF MOUSE TISSUES 24 HR AFTER INJECTION*

Tissue	Hot acid-soluble fraction ACC		Protein fraction ACC
	(μg)	(μg/g)	(μg/mg)
Liver	0·35	0·20	0·005
Large intestine	0·30	0·23	0·006
Small intestine	0·46	0·24	0·006
Spleen	0·06	0·47	0·003
Kidney	0·08	0·21	0·004
Lung	0·05	0·28	0·001
Heart	0·06	0·44	0·003
Muscle	0·07	0·18	0·008
Brain	0·19	0·43	0·002

* C3H mice each received an intraperitoneal injection of 30 mg of ^{14}C-ACC per kg.

TABLE 3. BALANCE OF ^{14}C-ACC DISTRIBUTION*

Source	ACC (μg)	% of administered dose
Organs†	97·2	16·4
Carcass‡	220·8	37·3
Blood (1 ml)	32·2	5·5
Urine	35·0	5·9
Feces§	150·0	25·4
CO_2	<0·1	
Total recovery	535·2	90·5

* Four days after injection of 30 mg of ^{14}C-ACC per kg.
† Cold acid-soluble and hot acid-soluble fractions of organs listed in Tables 1 and 2.
‡ Cold acid-soluble fraction only
§ Equivalents in μg, calculated on the basis of two-thirds of the radioactivity excreted at the end of the sixth day.

intraperitoneally into mice bearing 7-day tumors, groups of two animals were sacrificed ½, 1, 2 and 4 hr later, and the tumor cells were fractionated as described above. The radioactivity present in the cold acid-soluble fraction of the cells is shown in Fig. 3. The initial uptake into the ascites cells is probably attributable to the intraperitoneal route of injection. Thereafter, the radioactivity in the cells decreased and

an equilibrium value was reached in 2 hr in both sensitive and resistant tumors, The concentration of radioactivity in the tumor cells was still higher at 4 hr, however, than that of any of the tissues at this time. The hot perchloric acid extracts and the protein fractions contained less than 2 per cent of the total radioactivity.

Because it had been found that ACC was slowly excreted and remained at a relatively constant level in the blood and tissues for a period of 4 days or longer, it was of interest to determine whether the introduction of new tissue into the mice would effect a redistribution of the drug such that the newly introduced cells would take up the radioactive compound. C3H mice, given a single intraperitoneal injection of ^{14}C-ACC (30 mg/kg) 7 days previously, received an intraperitoneal inoculation of 200 mg (wet weight) of 6C3HED ascites lymphosarcoma cells. These cells were removed from the peritoneal cavities of groups of two mice 24 and 48 hr afterwards, and were fractionated and assayed for radioactivity. As the results in Table 4 indicate, there is uptake of radioactivity into the tumor cells and a concomitant rise in the radioactivity in the blood to the previous peak levels, a finding which suggests that a redistribution of the radioactive compound occurred in the animals.

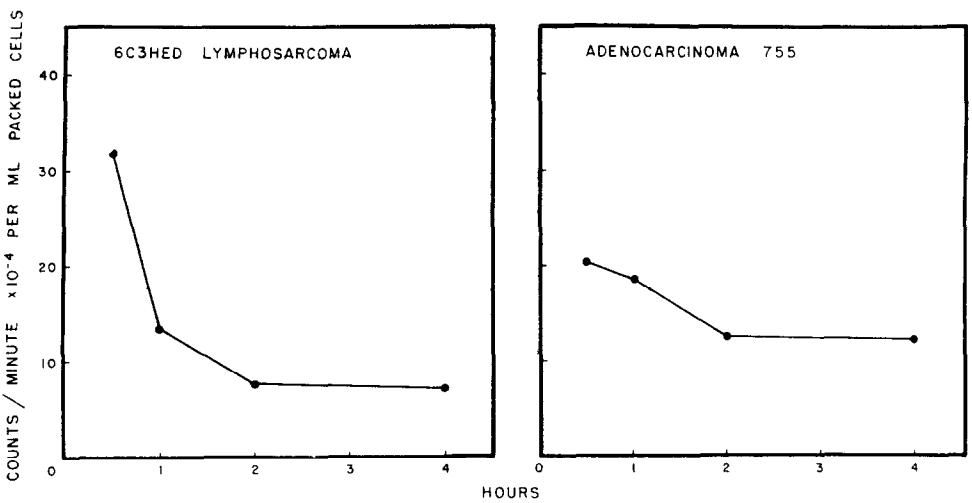

FIG. 3. Distribution of acid-soluble ^{14}C-ACC in ascites tumor cells. Mice bearing 6-day ascites tumors received intraperitoneal injections of 30 mg of ^{14}C-ACC.

Metabolism

The results presented above have indicated a pattern of excretion and tissue distribution of radioactivity in mice following the administration of therapeutic doses of ^{14}C-ACC. The radioactivity found in these various fractions was then subjected to paper chromatographic analysis in order to establish the identity of the compound or compounds which contained this radioactivity. An aliquot of each fraction was analyzed by descending chromatography on Whatman no. 1 paper in one or more solvent systems. The distribution of radioactivity on the chromatogram was then analysed in a Baird–Atomic chromatogram scanner (2π geometry). The sensitivity of this instrument was such that the presence of 0·5 μg of ^{14}C-ACC in a single peak

TABLE 4. DISTRIBUTION OF ^{14}C-ACC IN MICE IMPLANTED WITH TUMOR SEVEN DAYS AFTER DRUG ADMINISTRATION*

Days after drug administration	Days after tumor implantation	Blood	Tumor		
			Cold acid-soluble fraction	Hot acid-soluble fraction	Protein fraction
		ACC (μg/ml)	(μg/g)	ACC (μg/g)	(μg/mg)
8	1	24·1	101·0	1·5	0·006
9	2	32·5	52·7	0·9	0·003
9	Control	14·4			

* C3H mice received 30 mg of ^{14}C-ACC per kg and 7 days later, 200 mg (wet weight) of 6C3HED ascites lymphosarcoma cells.

TABLE 5. RADIOCHROMATOGRAPHIC ANALYSIS OF TISSUES AND EXCRETA OF MICE FOLLOWING THE ADMINISTRATION OF ^{14}C-ACC*

Material analysed	Time after injection of ^{14}C-ACC (days)	Solvent Systems	
		1† R_f(max)	2‡ R_f(max)
^{14}C-ACC	—	0·75	0·50
Urines			
Urine—normal	4 hr	0·76	
Urine—normal	1	0·75	
Urine—tumor§	1	0·72	
Urine—tumor co-chromatographed with ^{14}C-ACC	1	0·72	
Tissues (cold acid-soluble fractions)			
Liver	1	0·75	0·48
Liver—co-chromatographed with ^{14}C-ACC	1	0·74	0·48
Liver	4	0·74	0·51
Large intestine	1	0·75	0·48
Large intestine	4	0·72	
Carcass	1	0·75	
Carcass	4	0·77	
Spleen	4	0·72	
Lung	4	0·75	
Heart	4	0·76	
Muscle	4	0·75	
Brain	4	0·70	
Serum	6	0·72	
Tumor cells§	1		0·49
Tumor cells—co-chromatographed with ^{14}C-ACC	1		0·49
Tumor cells**	9	0·74	

* Male C3H mice received an intraperitoneal injection of 30 mg of ^{14}C-ACC per kg.
† Methanol–water–pyridine (16 : 4 : 1).
‡ *n*-Butanol–acetic acid–water (4 : 1 : 4).
§ C3H mice bearing 6C3HED ascites lymphosarcoma transplanted 7 days previously.
** C3H mice bearing 6C3HED ascites lymphosarcoma transplanted 2 days previously.

could be detected. When the presence of interferring substances prevented the formation of a distinct peak, the area containing all the radioactivity was cut out, eluted with water, and rechromatographed.

Representative results from these experiments, shown in Table 5, indicate that all the radioactivity in the tissue and excreta analysed have R_f values similar to that of authentic ACC. If any other metabolite is formed in the tissues or excreted in the urine, it is present in quantities less than those detectable by these methods.

The radioactive compounds in the feces could not be chromatographed satisfactorily. Hot water, 1 N acetic acid and 1 N ammonium hydroxide all extracted the same amount of radioactivity from the feces. No radioactivity was extracted with ethyl acetate. These findings are consistent with the solubility characteristics of the unchanged drug.

DISCUSSION

This investigation of the distribution, excretion and metabolism of ACC has shown that it is neither metabolized *in vivo* nor incorporated into protein to any significant extent. Although the structure of ACC suggests that it is an amino acid antimetabolite, as it may well be, it clearly does not undergo any of the usual metabolic fates of amino acids. Mickelson[9] has shown that ACC does not interfere with the utilization of natural amino acids by bacteria nor is it utilized in place of the natural amino acids. This compound is taken up into all tissues and in each of these it reaches very similar concentrations, on a wet weight basis. This concentration is maintained in the tissues or remains in equilibrium with the blood concentration for at least 4 days.

The almost constant blood levels during this period and the constant and low urinary excretion are correlated with the maintenance of these constant tissue levels. There is no evidence for concentrative uptake of this amino acid into tissues, as the tissue: plasma concentration ratio is always less than 1. Protein binding experiments were conducted by equilibrium dialysis in 0·05 M phosphate buffer, pH 7·3, against bovine serum albumin over a thirty-two-fold concentration range of ^{14}C-ACC. These experiments indicated that ACC was not bound to plasma proteins to a significant degree.* In addition, the prompt availability of ACC to tumor cells implanted 7 days after drug administration, suggests that ACC is probably in simple equilibrium with the body water and not bound. The relatively large excretion in the feces is not completely understood.

It is of some interest to compare ACC with another unmetabolized amino acid, α-amino*iso*butyric acid (AIB), which has been extensively studied by Christensen and his associates.[10, 11] Although neither compound is either metabolized or incorporated into protein, the only apparent structural similarity is the lack of an α-hydrogen, a characteristic shared by proline. AIB has been tested against the Walker carcinoma 256 and was found to be ineffective.[2] In contrast to ACC, AIB is concentrated in the tissues and an active transport mechanism is involved in this process. The main route of excretion is through the kidneys, and the rate of excretion is more than twelve times that observed with ACC;[12] little AIB is excreted in the feces.[11]

The present findings are pertinent to the somewhat peculiar pharmacological properties of ACC. The toxicity of ACC does not depend on the route of administration,

* W. R. Sterling, unpublished data.

as it is well-absorbed after oral or intraperitoneal administration, with effects that can be compared to those seen following intravenous injection.[1] Investigators have reported an "all-or-none" toxicity after the repeated administration of ACC to animals.[1] In view of its slow excretion and lack of metabolism, it is easy to understand how accumulation of the drug to a toxic concentration could be achieved by chronic treatment. These findings suggest that a single large dose of ACC, or large doses given at weekly or longer intervals, may be equally or more effective in their tumor-inhibitory effects, and much less toxic, than daily injections.

These results have additional consequences with respect to the design and evaluation of carcinostatic antimetabolites of amino acids. It would appear to be important to ascertain the structural requirements for metabolic inertness, and to learn, for instance, whether the absence of an α-hydrogen is truly significant in this regard. It would also appear that amino acid analogs which are not metabolized and which, therefore, may have properties similar to ACC or AIB, should be evaluated for carcinostatic activity in a different manner than that commonly done with other compounds. Administration at much greater intervals than that of 24 hr, usually employed in such tests, might decrease toxicity without substantially affecting carcinostatic potency.

Further studies on the mechanism of action of ACC are in progress.

REFERENCES

1. R. B. Ross, C. I. Noll, W. C. J. Ross, M. V. Nadkarni, B. H. Morrison, Jr. and H. W. Bond, *J. Med. Pharm. Chem.* **3**, 1 (1961).
2. T. A. Connors, L. A. Elson, A. Haddow and W. C. J. Ross, *Biochem. Pharmacol.* **5**, 108 (1960).
3. W. W. Benefiel, J. T. Helsper and G. S. Sharp, *Cancer Chemotherapy Reports* **9**, 21 (1960).
4. N. Zelinsky and G. Stadnikoff, *Z. Physiol. Chem.* **75**, 350 (1911).
5. H. T. Bucherer and V. A. Lieb, *J. Prakt. Chem.* **141**, 5 (1934).
6. H. C. Tillson, M. S. Thesis, The Pennsylvania State University (1948).
7. L. J. Roth, E. Leifer, J. R. Hogness and W. H. Langham, *J. Biol. Chem.* **176**, 249 (1948).
8. J. F. Henderson and G. A. LePage, *Cancer Res.* **19**, 749 (1959).
9. M. N. Mickelson, *Biochem. Pharmacol.* **5**, 165 (1960).
10. H. N. Christensen, A. J. Aspen and E. G. Rice, *J. Biol. Chem.* **220**, 287 (1956).
11. M. W. Noall, T. R. Riggs, L. M. Walker and H. N. Christensen, *Science* **126**, 1002 (1957).
12. T. R. Riggs and L. M. Walker, *J. Biol. Chem.* **233**, 132 (1958).