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The Metabolism of Rhodium(II) Acetate in Tumor-bearing Mice[1]

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SUMMARY

Rhodium(II) acetate has been shown to have carcinostatic activity in Swiss mice bearing Ehrlich ascites tumors. For metabolic studies, single therapeutic doses of rhodium(II) [1-^{14}C]acetate (24 mg/kg) were injected i.p. into Swiss mice that had been given i.p. implantations 3 days previously of 50-fold 10^6 Ehrlich ascites tumor cells. The tissue distribution and excretion of the rhodium (measured by atomic absorption spectrometry) and the acetate (measured by ^{14}C label) were followed at designated time intervals up to 24 hr after injection.

Rhodium(II) acetate, a neutral cage complex, breaks down to rhodium and acetate ionic species within 2 hr after i.p. injection, as measured by the rapid exhalation of $^{14}CO_2$. Both the rhodium and ^{14}C label disappear rapidly from the ascites fluid, with a small but variable amount of each species being incorporated into the tumor cells. Both species were detected mainly in the blood plasma, and the primary organ of deposition was the liver. No measurable quantity of rhodium was found in the brain tissue. During the first 24 hr following drug administration, only 5% rhodium was eliminated in the urine.

INTRODUCTION

The current history of cancer chemotherapy reveals a continuing interest in the treatment of cancers with compounds formed from the transition metals (4). This interest was recently renewed with the discovery by Rosenberg *et al.* (9, 10) of the broad spectrum antitumor properties of certain platinum compounds, of which, the *cis*-dichlorodiammine-platinum(II) is currently undergoing clinical trial (2). Like some of the platinum complexes, rhodium(II) carboxylates and, in particular, rhodium(II) acetate or propionate have demonstrated carcinostatic activity in Swiss mice bearing Ehrlich ascites tumor cells (1, 3). These complexes (Chart 1) are symmetrical planar molecules with partial aromatic character, capable of forming adducts with a number of biological molecules with electron-donating ligands, such as histidine, adenosine, and its 5′-phosphate derivative (1, 3). As a result of the positive antitumor activity displayed by rhodium(II) acetate, further survival and toxicity studies were undertaken to determine the most efficacious use of the drug for a metabolic study using Swiss mice implanted i.p. with Ehrlich ascites tumor cells. The data in Table 1 show that an increase in survival time and number of 50-day survivors is obtained by increasing the dosage and decreasing the number of injections of rhodium(II) acetate to a single injection of 24 mg/kg. This dose is the same as the LD_{50} value obtained from toxicity studies with 1 i.p. injection of rhodium(II) acetate. In order to investigate the metabolic disposition and excretion of rhodium(II) acetate in the mouse, a single i.p. injection of 24 mg/kg was chosen, because it showed significant antitumor activity at a dosage sufficient for convenient analysis. This type of investigation usually requires a doubly labeled compound, the most ideal of which would be the [^{14}C]carboxyl and the ^{103}Rh derivative. The radioactive rhodium was not available at the time of these studies; therefore, the ^{14}C-carboxyl derivative was synthesized for radioactivity measurements, and the rhodium was followed by atomic absorption spectroscopy.

MATERIALS AND METHODS

Animals, Cells, Drugs, and Instrumental Methods of Analysis. Ehrlich ascites tumor cells were carried in female Swiss albino mice (ARS-Sprague-Dawley; Madison, Wis.) implanted i.p. with 4×10^6 cells. The cells were serially transferred on the 7th day after implantation.

Rhodium(II) acetate was purchased from Matthey Bishop, Inc., Malvern, Pa., and was recrystallized once from hot acetone. The radiolabeled compound was synthesized by ligand exchange with [1-^{14}C]acetic acid (ICN, Irvine, Calif.) by the method of Johnson *et al.* (7). The compound (specific activity, 2×10^7 cpm/mg) was shown to be free of contaminating [1-^{14}C]acetic acid by thin-layer chromatography in Solvent System A (see below). It was dissolved in 0.9% sodium chloride just prior to administration. Sodium [1-^{14}C]acetate was prepared by neutralizing [1-^{14}C]acetic acid with sodium hydroxide (specific activity, 3×10^7 cpm/mg).

All radioactivity measurements were made with the use of a Packard Model 3380 liquid scintillation spectrometer. Aquasol (New England Nuclear, Boston, Mass.) was used for counting liquid samples, and toluene cocktail (6 g of PPO and 0.75 g of POPOP per liter toluene) was used for counting dried samples and strips from chromatographs. Rhodium content of samples was determined with a Perkin-Elmer Model 303 atomic absorption spectrphotometer with a rhodium cathode lamp at the 3434-Å resonance line.

Chromatography. Thin-layer chromatography was performed on silica gel sheets (6061 silica gel without fluorescent indicator; Eastman Kodak, Rochester, N. Y.) using Solvent A, ethyl acetate:acetone:water, 49:36:15; R_F rho-

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dium(II) acetate, 0.7; R_F acetic acid, 0.0. Paper chromatography was performed on Whatman No. 3 paper descending method with solvent system A, R_F rhodium(II) acetate, 0.94; R_F sodium acetate, 0.23; R_F sodium bicarbonate, 0.27. The salts were visualized by spraying the dried chromatograms with bromocresol purple-bromothymol blue (40 mg in 100 ml H_2O). Rhodium(II) acetate travels in this solvent as a single blue spot. Radioactivity on chromatograms was measured by cutting 1-cm strips, which were counted in toluene cocktail.

Survival Studies. Female Swiss albino mice were each implanted i.p. with 4×10^6 Ehrlich ascites tumor cells. Drug treatment was started 24 hr after implantation, and injections were given i.p. once daily for 6 or 3 days. For single-dose survival studies, the mice were implanted i.p. with 8×10^6 Ehrlich ascites tumor cells, and a single i.p. injection of drug was administered 24 hr later. The drug was dissolved in 0.9% NaCl solution and was given in a volume of 0.2 ml. Control mice received 0.9% NaCl solution only. The mice were followed for 50 days; those still alive on Day 50 were counted as living 50 days only.

Toxicity Studies. In order to ascertain the toxic effect of the drug on healthy mice, single i.p. injections of rhodium(II) acetate were administered in doses ranging from 12 to 60 mg/kg. The method of Skipper and Schmidt (11) was used to determine the doses that were lethal to 50 and 10% of Swiss mice. The mice were followed for 100 days; those still alive on Day 100 were considered living 100 days only.

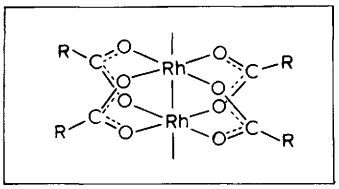

Chart 1. The 6-coordinated cage complex of rhodium(II) carboxylates $[Rh_2(OOCR)_4]$. The axial positions may be occupied by electron-donating ligands. Rhodium(II) acetate, R = CH_3.

Analysis of Tissues for ^{14}C Content. Female Swiss albino mice, 30 to 35 g were implanted i.p. with 50×10^6 Ehrlich ascites tumor cells and used on Day 3 after implantation. At various time intervals after a single i.p. injection of rhodium(II) [1-^{14}C]acetate (24 mg/kg), the mice were sacrificed and the following tissues were removed: tumor, liver, kidneys, spleen, lungs, brain, and a 20-cm segment of small intestine. Tumor cells were centrifuged at $1470 \times g$ for 2 min, and an aliquot of ascites fluid supernatant was mixed with Aquasol and measured for ^{14}C content. The remaining supernatant was discarded and tumor cells were washed either 3 times with 0.9% NaCl solution or twice with ice-cold 5% trichloroacetic acid, followed by 2 washes with ice-cold 95% ethanol. Washed cells were drained and solubilized in Protosol (New England Nuclear), and an aliquot was counted in a toluene cocktail.

The other excised tissues were rinsed with 0.9% NaCl solution and weighed. Tissues were homogenized in 6 ml of 50% methanol in a glass tissue homogenizer with a power-driven Teflon pestle. Homogenate volumes were brought to 10 ml with solvent and incubated at 55° for 5 min in a constant-temperature water bath. Homogenates were then centrifuged for 10 min at $1470 \times g$ in a clinical centrifuge, and supernatants were slowly filtered through Millipore filters (0.45 μM pore size). A 1-ml aliquot was counted in Aquasol. Tissue pellets were washed twice more with 5 ml of 50% methanol, dried in a vacuum, and ground to a fine powder. Ten-mg samples were counted in toluene cocktail.

Analysis of Tissues for Rhodium Content. Tumor, liver, kidneys, spleen, intestines, lungs, and brain were collected from mice, treated as described under "Analysis of Tissues for ^{14}C Content." Tumor cells were separated from ascites fluid by centrifugation and diluted 1:5 with ice-cold distilled water, after the final wash. The ascites fluid was diluted as needed to meet instrument requirements. The other tissues were homogenized in 10 ml of distilled water and oxidized completely with HNO_3 and H_2SO_4, by the method described by Gorsuch (5). Rhodium contents of

Table 1

Effect of dose and schedule of rhodium(II) acetate on Swiss mice bearing Ehrlich ascites tumor

These results are the average of 3 independent experiments.

Experiment	Drug	Dose and schedule	Av. survival time (days)	No. of mice surviving at Day 50/no. tested
1	0.9% NaCl solution	0.9% NaCl solution, 1 dose/day for 6 days	16.8 ± 2.0[a]	0/10
	Rhodium(II) acetate	8 mg/kg, 1 dose/day for 6 days	23.2 ± 5.6	0/10
2	0.9% NaCl solution	0.9% NaCl solution, 1 dose/day for 6 days	15.3 ± 2.0	0/10
	Rhodium(II) acetate	16 mg/kg, 1 dose/day for 6 days	30.1 ± 9.8	1/10
3	0.9% NaCl solution	0.9% NaCl solution, 1 dose/day for 3 days	16.6 ± 3.4	0/10
	Rhodium(II) acetate	16 mg/kg, 1 dose/day for 3 days	38.4 ± 13.1	5/10
4	0.9% NaCl solution	0.9% NaCl solution, once	17.5 ± 1.4	0/10
	Rhodium(II) acetate	24 mg/kg, once	45.2 ± 2.0	6/10

[a] Mean ± S.D.

test samples were determined by atomic absorption spectrometry as the average of 3 measurements alternated with 3 measurements of standard solutions containing ±1 ppm rhodium of the test sample. The detection limit was 0.3 ppm.

Analysis of Plasma and Whole Blood. Mice received 50 units of heparin (Sigma Chemical Co., St. Louis, Mo.) 1 hr prior to the designated sacrifice time. Blood was collected from the chest cavity, after a small transverse incision had been made through the sternum next to the heart, at various time intervals after drug administration. Plasma was obtained by centrifuging whole blood at moderate speed on an IEC clinical centrifuge for 5 min. Plasma and whole blood samples for atomic absorption spectroscopy were processed by the method of Willis (13). For liquid scintillation spectrometry, an aliquot of whole blood was bleached for several hr with 30% hydrogen peroxide. Catalase activity was quenched with a small quantity of methanol; the contents were neutralized and 15 ml of Aquasol were added. An aliquot of plasma was directly mixed with 10 ml Aquasol. Blood cells separated from plasma were washed once with 0.9% NaCl solution then were diluted 1:5 with distilled water for rhodium measurements by atomic absorption spectrometry or dessicated, ground to a fine powder, and 10-mg samples were measured for radioactivity in toluene cocktail.

Measurements of Expired $^{14}CO_2$. Three tumor-bearing mice were given injections of either rhodium(II) [1-^{14}C]-acetate (24 mg/kg) or sodium [1-^{14}C]acetate (17.8 mg/kg) and were immediately placed in a liter Erlenmeyer flask containing potato slices. The flask was immediately closed with a 2-hole rubber stopper containing a short inlet tube and an outlet tube, 4 to 5 inches above the heads of the mice. The inlet tube was connected to an air tank, which was adjusted to have a flow rate of 3 cu cm/5 sec. The apparatus was therefore completely flushed with air every 30 min. The outlet tube was connected to a series of 3 traps made from scintillation vials, each containing 4 ml Protosol. The $^{14}CO_2$ exhaled from the mice was conveniently absorbed by the Protosol and was subsequently counted by the addition of 15 ml toluene cocktail. To ensure no loss of $^{14}CO_2$, a final trap containing 100 ml 50% sodium hydroxide was connected to the apparatus. On completion of the experiment, an aliquot of this solution was neutralized to pH 9, counted by the addition of 10 ml Aquasol, and shown to contain only a small percentage of the total counts. The inlet and outlet tubes of the Erlenmeyer flasks were briefly clamped off at designated time intervals so that the Protosol traps could be changed.

Analysis of Urine. Ten tumor-bearing mice given injections of rhodium(II) [1-^{14}C]acetate (24 mg/kg) were housed in a metabolic cage designed to separate urine from fecal material. With the exception of the stainless steel screen on which the mice sat, the cage was made entirely of glass. The urine from these mice was collected in an ice-cooled test tube for the 1st 24 hr after drug administration, and the volume was measured. Aliquots of this urine were subsequently analyzed for ^{14}C and rhodium content by the method described for the plasma samples. An aliquot of the urine was treated with $Ba(OH)_2$, and the precipitate of $BaCO_3$ was filtered, washed, air dried, and finally counted in 10 ml toluene cocktail. Another aliquot of the urine was treated with Jack bean urease (Sigma Chemical Co., St. Louis, Mo.), 25 units/ml 0.02 M sodium phosphate buffer, pH 7.0, containing 0.5 mg EDTA per ml. After evolution of CO_2 was complete, the urine was counted in 10 ml Aquasol. The difference in counts obtained from equal aliquots of untreated and urease-treated urine indicated the amount of ^{14}C label present in the urine as urea. Urine samples were also obtained from tumor-bearing mice given i.p. injections of rhodium(II) [1-^{14}C]acetate (24 mg/kg) at various time intervals after drug administration. The mice were sacrificed by cervical dislocation and small samples of urine were obtained by pressing their bladders against the edge of a sterile Petri dish. Measured aliquots of these urine samples were spotted on Whatman No. 3 paper, together with standard samples of sodium bicarbonate, sodium acetate, and rhodium(II) acetate. The chromatograms were developed in Solvent System A for 6 hr and were strip-counted for radioactivity.

RESULTS AND DISCUSSION

The results of the survival studies (Table 1) indicated that an increase in average survival time and the number of 50-day survivors is obtained by increasing the dosage of rhodium(II) acetate and decreasing the number of injections. The lethal doses (10 and 50%) of rhodium(II) acetate for single-dose injections were found to be 14 and 24 mg/kg, respectively.

The data (Chart 2) show that both rhodium and ^{14}C rapidly disappear from the ascites fluid during the 1st hr after drug administration and continue to decline for an additional 6 hr. The difference in the contour of the profiles obtained from the 2 methods of analyses 1 hr after drug injection indicated that compound decomposition was occurring. The analysis of 0.9% NaCl solution-washed tumor cells (Chart 3) is similar to that of the ascites fluid, in that levels of rhodium(II) acetate as measured by rhodium content are

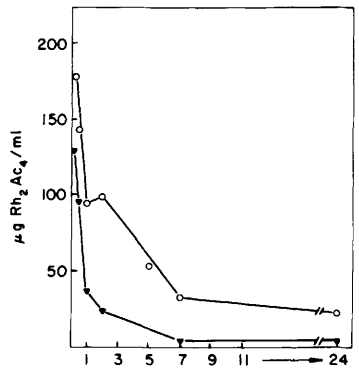

Chart 2. The ascites fluid from Swiss mice previously given i.p. injections of Ehrlich ascites tumor cells and of rhodium(II) [1-^{14}C]acetate (24 mg/kg), as described in "Materials and Methods," was analyzed at various time intervals for rhodium (○) and ^{14}C (▲) content. The quantity of each species present at the designated time intervals was computed as μg rhodium(II) acetate per ml, for comparison purposes only. Each *data point* is the average of 5 individual experiments.

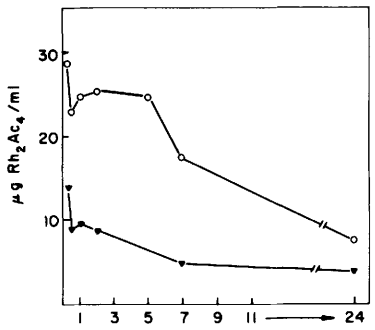

Chart 3. Tumor cells (washed 3 times with 0.9% NaCl solution) from Swiss mice previously given injections of Ehrlich ascites tumor cells and i.p. injections of rhodium(II) [1-^{14}C]acetate (24 mg/kg), as described in "Materials and Methods," were analyzed for rhodium (○) and ^{14}C (▲) content. The quantity of each species present at the designated time intervals was computed at μg rhodium(II) acetate per ml, for comparison purposes only. Each *data point* is the average of 5 individual experiments. The average packed cell volumes at the designated time intervals were: 15 min, 1.6 ml; 30 min, 1.1 ml; 1 hr, 1.0 ml; 2 hr, 1.1 ml; 7 hr, 0.95 ml; 24 hr, 0.05 ml.

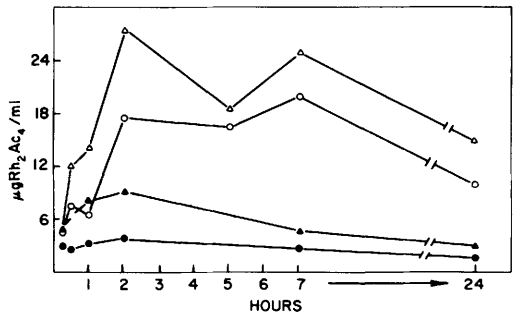

Chart 4. Analysis of blood (at various time intervals) from Swiss mice previously given i.p. injections of Ehrlich ascites tumor cells and of rhodium(II) [1-^{14}C]acetate (24 mg/kg), according to the procedure described in "Materials and Methods"; △, rhodium in plasma; ○, rhodium in whole blood; ▲, ^{14}C in plasma; ●, ^{14}C in whole blood. The quantity of each species was computed as μg rhodium(II) acetate per ml sample for comparison purposes only. Each *data point* is the average of 4 individual experiments.

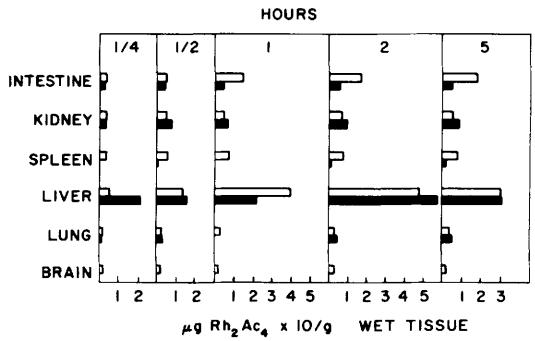

Chart 5. The tissues from Swiss albino mice previously given i.p. injections of Ehrlich ascites tumor cells and of rhodium(II) [1-^{14}C]acetate (24 mg/kg), as described in "Materials and Methods" were analyzed at designated time intervals for rhodium (□) and ^{14}C (■) content. The quantity of each species present in the tissues was computed as μg rhodium(II) acetate × 10/g, wet tissue weight, for comparison purposes only. Each ^{14}C *data point* is the average from 5 separate mice. The rhodium data are the average of 2 experiments, with the use of pooled tissues from 4 or 5 mice for each determination. The 1-hr lung samples were accidentally lost prior to rhodium analysis. A 20-cm segment of the small intestines was analyzed for ^{14}C content. The entire small and large intestines plus content were analyzed for rhodium.

much higher than levels obtained from ^{14}C content. Again, this indicates that the compound may be decomposing so that the ^{14}C label is more rapidly removed than rhodium. A comparison of Charts 2 and 3 reveals that less than 10% of the injected dose is absorbed by the tumor cells. A similar observation was found with an antitumor platinum compound (12), and this is not unusual for antineoplastic agents in general. Analysis of tumor cells washed in ice-cold 5% trichloroacetic acid and then twice with 95% ethanol yielded essentially the same amount of ^{14}C present as cells washed with 0.9% NaCl solution only, which indicates a firm binding of the ^{14}C label to the tumor cell fraction.

The approximately equal quantities of rhodium and ^{14}C detected in the blood plasma at the 15-min time interval after drug injection (Chart 4) indicates that some intact rhodium(II) acetate may be entering the blood stream. However, the subsequent data points show that significant decomposition of the drug probably occurs during the 1st hr after drug administration. Comparison of the amount of rhodium and ^{14}C in the plasma with that of whole blood also indicates that the compound and/or its metabolites are contained almost exclusively in the plasma portion until about 5 hr after drug injection, because plasma constitutes about one-half the volume of whole blood, yet it contained twice the quantity of ^{14}C or rhodium. This conclusion was confirmed by the absence of ^{14}C label and the very low levels of rhodium present in washed blood cells until 5 hr after drug administration.

The results of the analysis of host organs are shown in Chart 5. Considering the heavy metal nature of this compound, it was not surprising to find high levels and possibly a primary deposit occurring in the liver. For the 1st 2 hr after drug administration, the quantities of rhodium detected in the kidneys and intestines increased, indicating that either or both organs may function in the excretory pathway of the drug or its metabolites. The complete absence of rhodium in brain tissue suggests that the compound is probably not absorbed by this tissue. Again, the overall picture obtained from organ analyses indicates that some degree of compound decomposition may occur either prior to or after absorption by the tissues.

Mammalian decomposition of rhodium(II) acetate may generate acetate ions, and the measurement of exhaled $^{14}CO_2$ seemed prudent. The results of this measurement (Chart 6) indicate that large quantities of $^{14}CO_2$ are exhaled and constitute a major metabolite of rhodium(II) acetate. In order to estimate a possible half-life for rhodium(II) acetate in the mouse system, the evolution of $^{14}CO_2$ from rhodium(II) [1-^{14}C]acetate was compared with that obtained from [1-^{14}C]sodium acetate of equivalent acetate concentration under identical conditions (Chart 6). The lag of the rhodium(II) acetate curve behind the sodium acetate curve was assumed to be a measure of the time required for rhodium(II) acetate to generate acetate [Ac] ions. A plot of these data as log percentage [Ac] not expired as $^{14}CO_2$ against time (Chart 7) yields 2 straight lines from either curve. The straight lines obtained during the 1st 2 hr after drug administration provide a basis for assuming that the decomposition of both compounds to $^{14}CO_2$ is governed by 1st-order reaction kinetics. The subsequent change in slope

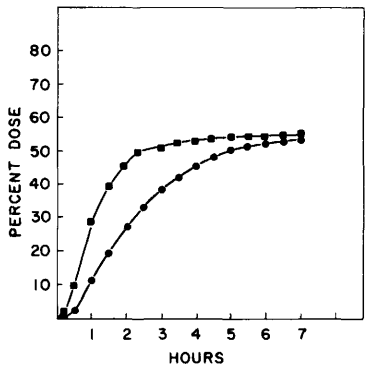

Chart 6. The amount of $^{14}CO_2$ (expressed as percentage injected dose) expired from Swiss mice previously given i.p. injections of Ehrlich ascites tumor cells, as described in "Materials and Methods" and given i.p. injections of either rhodium(II) [1-^{14}C]acetate (24 mg/kg) (●) or sodium [1-^{14}C]-acetate (17.8 mg/kg) (■). Three mice were used per experiment; each experiment was duplicated.

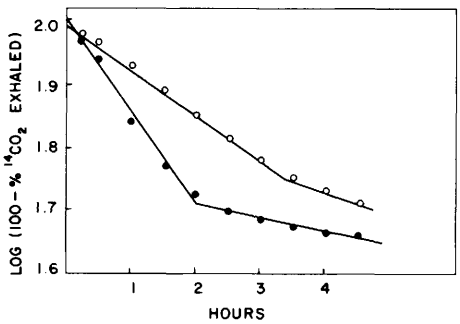

Chart 7. Kinetic analysis of data presented in Chart 6; ○, rhodium(II) [1-^{14}C]acetate; ●, sodium [1-^{14}C]acetate.

of both curves is probably due to a dilution of the expired $^{14}CO_2$ with unlabeled CO_2 as a result of normal metabolism. The rate constant for sodium acetate decomposition was found to be 0.352 h^{-1}. If rhodium(II) acetate (Rh_2Ac_4) decomposition is the result of 2 1st-order reactions:

$$[Rh_2Ac_4] \xrightarrow{k_1} [Ac] \xrightarrow{k_2} [CO_2]$$

then k_1 in this reaction can be obtained by an iterative analysis of the equation:

$$[CO_2]_t = a_0\left(1 - \frac{k_2}{k_2 - k_1} e^{-k_1 t} + \frac{k_1}{k_2 - k_1} e^{-k_2 t}\right)$$

where a_0 represents the initial concentration of rhodium(II) acetate and $k_2 = 0.352$ hr^{-1}. A rate constant of 0.70 hr^{-1} was obtained by this method for the decomposition of rhodium(II) acetate to acetate, and this rate constant yielded a maximum half-life of about 60 min. The effective half-life of rhodium(II) acetate in the mouse may be considerably shorter, since only the half-life of the rate-limiting step has been calculated. It would also be possible that the compound has a much longer half-life than the one obtained from these data if a simple exchange of cellular acetate for radiolabeled acetate on the compound were to occur. However, this alternative does not seem likely due to the kinetic stability of the bridging acetates (1).

From the analysis of the kidneys, it was expected that an appreciable quantity of rhodium(II) acetate might be eliminated from the host in the urine. Also, it was known that *cis*-dichlorodiammineplatinum(II) was excreted primarily in the urine in animals (8). However, urine collected for the initial 24 hr after drug injection contained only 4% of the injected radioactivity and about 5% of the rhodium. Chromatograms of urine samples collected at various time intervals showed that a very small quantity of the ^{14}C label cochromatographed with the rhodium(II) acetate standard. Since all the radioactivity could represent the incorporation of ^{14}C into normal urine metabolites such as urea, urine samples collected during the 1st 24 hr after drug administration were incubated with urease, and this treatment resulted in a 20 to 29% decrease of radioactivity of the urine sample after urease treatment. A similar sample yielded 30% of the radioactivity as $Ba^{14}CO_3$ when treated with $Ba(OH)_2$, indicating the presence of ^{14}C bicarbonate salts in the urine. Therefore, only about 1% of the drug could be excreted intact, and it is apparent that rhodium is excreted in forms other than the original compound.

In conclusion, the effort to elucidate the metabolic fate of this compound is aided by tabulating the quantities of the rhodium and ^{14}C present in all the analyzed tissues at each time interval. Such an analysis showed that 80 to 90% of each species could be accounted for at the 15-min interval, and that about 80% of the ^{14}C label could be found at all other time intervals, whereas only 20 to 25% of the rhodium could be detected. The inability of the combined analysis to account for 75 to 80% of the injected rhodium would indicate that, after a small fraction of the i.p.-injected drug has been absorbed by the tumor cells, most of the drug remaining in the ascites fluid undergoes decomposition to yield a nonabsorbable rhodium metabolite and readily absorbable acetate ions. Rhodium acetate has been shown to react *in vitro* with molecules like cysteine-containing free sulfhydryl groups to form an unstable complex which rapidly breaks down, yielding acetate ions, H^+ ions, and an insoluble precipitate containing rhodium(III) and cysteine (6). In addition, several enzymes known to contain sulfhydryl groups in their active site are inactivated by a small molar excess of rhodium(II) carboxylates (6). It is therefore possible that the injected rhodium(II) acetate may react with sulfhydryl-containing molecules in the ascites fluid, the rhodium being precipitated as an insoluble complex and acetate ions released for further absorption and metabolism. Similarly, the binding of rhodium to membrane components in the lining of the i.p. cavity, particularly proteins containing accessible sulfhydryl groups, could also account for the failure of these analyses to detect all the injected rhodium. The rhodium(II) acetate or rhodium metabolites that are absorbed appear to either remain firmly bound to cellular constituents or are eliminated through the bowel or, to a lesser extent, through the kidneys.

This investigation has led to the following conclusions with respect to the antitumor activity displayed by the compound.

1. Short exposure of tumor cells to an initial high concentration of compound is sufficient to inhibit tumor growth or kill the tumor cells. It is of significance that the packed cell volume of the tumor cells aspirated from the i.p. cavities of the mice at various time intervals up to 7 hr after

a single injection of rhodium(II) acetate (24 mg/kg) remained approximately constant at 1.1 ml. However, a drastic decrease in packed tumor cells to 0.05 ml occurred sometime during the 7- to 24-hr time interval. In addition, a single i.p. injection of rhodium(II) acetate (24 mg/kg) resulted in an average survival time of 45.2 ± 2 days, with 6 out of the 10 mice in the test group alive on Day 50. The average survival time for the 0.9% NaCl solution control was 17.5 ± 1.4 days, with none of the group alive on Day 50 (Table 1). Both these observations suggest that a high percentage of the tumor cells were killed, rather than inhibited, by the compound.

2. The analysis of the tumor cells indicated that the compound was decomposing, with the rhodium remaining firmly bound to the tumor cells. As suggested earlier, the rhodium(II) acetate may react with accessible sulfhydryl groups present on the tumor membrane, or may enter the cell and inhibit enzymes with sulfhydryl groups in their active site. Although there is no definite evidence that the carcinostatic activity of the drug is due to this process, the data presented support such a hypothesis.

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