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A STUDY OF GALLIUM-72

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A Study of Gallium[72] [1]

Summary and Conclusions

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During the years 1949–51, the staff of the Medical Division of ORINS spent a major portion of its effort on a study of the therapeutic use of gallium[72]. This report summarizes the problems which were encountered, the results obtained, the studies completed, and the resulting clinical judgments.

Since gallium is still a relatively unknown element and its isotopes are only rarely used in biology, the studies began with problems in physics, proceeded through chemistry, biology, and pharmacology, and ended with an assessment of clinical results.

The physical characteristics of the gallium isotopes were restudied. A method of standardization of activity was established for routine use. Methods were developed for tissue assay, and a number of new devices were set up to measure activity. Protection of personnel was a major problem. A device for remotely controlled injection of the active material was designed and built. A urine storage container was constructed, and a system of shielding certain patients was instituted. During the study, a method of preparing gross autoradiograms was developed. Microscopic autoradiograms were not satisfactory because of the short half-life of the isotope and its multiple radiations. Fifteen possibly acceptable compounds of gallium were investigated, of which the citrate proved the best.

A number of methods of preparation were used, both to purify materials for pile irradiation and for conversion to the citrate complex for animal and patient use. A method for stable gallium tissue analysis was adapted for use in long-term studies.

The toxicity of various compounds was investigated in rats, dogs, swine, and man. These studies included checking the toxicity of other dyes and reagents going into the preparation of the drug as finally used in patients.

The distribution and excretion patterns and some elements of metabolic behavior were studied in approximately 400 animals of five different species. Finally, the isotope was used in test doses in 34 patients with various types of cancer. In 21 patients full therapeutic testing was done. The main groups of patients had osteogenic sarcoma with metastases to the lung, or extensive plasmocytoma. The remainder had soft-tissue tumors, metastatic to bone.

The interpretation of the therapeutic value of gallium[72] based upon these data is as follows:

1. Localization of the Element: When gallium citrate in large doses was injected into the blood stream, it rapidly accumulated in bone and viscera. About 50 per cent was excreted by the kidneys within the first twenty-four hours. Following this period, there was either a redistribution or a differential excretion of the retained material so that at five to ten days after injection there was further accentuation of the differential localization in areas of actively metabolizing bone. There were specific deposits of material in soft-tissue metastases from osteogenic sarcoma. This deposition was not uniform; instead, it was highly variable and appeared to follow not the tumor cells themselves but the sites of early calcification. Concentration of the element occurred also in areas of osteoblastic metastases in plasmocytoma

[1] From the Medical Divison, Oak Ridge Institute of Nuclear Studies, under contract with the United States Atomic Energy Commission. The summary was presented in part at the Thirty-seventh Annual Meeting of the Radiological Society of North America, Chicago, Dec. 2–7, 1951.

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and in metastases to bone from soft-tissue carcinoma. Apparently it was deposited in the immediately adjacent areas of normal tissue reaction to tumor. This localization, when uniform, is theoretically excellent for therapeutic purposes, but the differential concentration in relation to visceral organs is optimal only at a time which is many days post-injection.

2. *Localization of the Radiation:* The pattern of absorption in a tissue of the radiant energy from a radioactive isotope does not necessarily follow the pattern of deposition of the chemical. About two-thirds of the total radiation from gallium^{72} is in the form of high-energy gamma emission and, no matter where the chemical is deposited within the body, there will be ionization produced in distant organs.

It takes about four half-lives for gallium^{72} to segregate significantly into localized areas of deposition within the tumor. During these first four half-lives, the chemical is distributed *via* the blood stream to nearly all tissues, but especially to the viscera. Hence, the pattern of absorption of the radiant energy in tissues will be that of total-body irradiation for an appreciable fraction of the first half-life and primarily visceral irradiation for the subsequent three half-lives.

If the assumptions and conditions of the familiar Marinelli, Quimby, and Hine formula are accepted, it is possible to calculate a first approximation to the radiation localization pattern. If the calculations are based upon uniform distribution of the isotope in 1 gram of tissue in the center of a small and a large cylinder of tissue, the proportions of energy absorbed due to the separated beta and gamma components are as follows:

	Total Radiation	Beta Energy Component	Gamma Energy Component
Human-sized cylindrical mass	100%	26%	74%
12 × 2.5-cm. cylindrical mass	100%	59%	41%

The localization in the body of that portion of the energy absorbed due to the beta component will be similar to the localization of the stable carrier chemical. No matter where the stable form is localized, however, the energy absorbed due to the gamma component is in effect a total-body irradiation. Thus, during the early hours following injection, when most of the isotope decays, the energy released favors uniform total-body irradiation. Four half-lives post-injection, when the stable form begins to show a higher differential localization in tumor than in abdominal organs, the beta component predominates in the localized area of the tumor, but there is still a considerable gamma component which gives total-body irradiation. During this period of most favorable deposition of the stable chemical, however, over 90 per cent of the radioactive atoms have decayed.

It appears from the picture of the deposition of the chemical and the pattern of ionization from the radiation that two main features must be coordinated in a radioactive chemical used for internal therapy: (*a*) the rate of deposition of the carrier chemical into favorable areas, and (*b*) the rate at which the radioisotope decays. With gallium^{72}, the relative concentration of the isotope is favorable for therapy after about three to five half-lives. Iodine, for comparison, is favorably localized in less than one half-life. Since therapeutic effectiveness depends, to a large measure, upon the ratio of rate of chemical deposition to rate of decay, gallium^{72} is therapeutically ineffective.

3. *The Toxicity of the Chemical:* The toxicity of gallium citrate is relatively low in rats, but comparatively high in dogs. Man is probably somewhere in between. The knowledge gained from animal work is not transferable to man. One patient was given stable gallium in a manner which simulated the usual therapeutic trial. The dosage was continued until the toxicity picture was demonstrated but discontinued before it approached damaging levels. This patient, who received as much or more

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stable gallium than most patients who were treated with gallium^{72}, showed mild anorexia, malaise, and a significant lowering of the leukocyte and red cell values. From estimates of this and the clinical data, it is guessed that the LD 10 for man is about 20 mg. Ga^{+++}/kg. body weight.

4. *The Radiation Toxicity:* The clinical picture of gallium^{72} toxicity usually began with the third or fourth dose of gallium^{72} citrate at dose levels of 1.5 to 5.0 mg. Ga^{+++}/kg. given twice a week. There was first anorexia, which progressed through malaise and vomiting to weight loss greater than expected from the disease. Occasionally, especially when lower dosage was continued over longer periods, there developed a severe, dry, maculopapular, desquamating skin rash. The leukocytes began to decrease in number along with the development of malaise and progressed downward with increasing severity following each dose. Hemoglobin and hematocrit values showed a severe depression, but this developed more slowly than the leukocytopenia. After the gallium^{72} doses were discontinued, there was a continued decrease in the blood values for about one to two weeks. As the patient began to feel better, they returned to normal. With the beginning of a second course of gallium^{72} therapy, the same general picture was repeated, but at an accelerated rate and with greater severity.

It is difficult to isolate the radiation toxicity from the stable gallium toxicity, since both appear to have common features. From tissue respiration studies in a Warburg apparatus and from the clinical pictures in animals and in man, it is believed that there was a subacute total-body irradiation sickness which showed up early in the course of treatment. This was accentuated upon the later development of chemical toxicity. In cases where a major portion of the bone marrow was relatively untouched by the disease, the toxicity picture was reversible. In plasmocytoma, seriously affecting the bone marrow, toxicity developed more rapidly and with a greater severity and was less likely to be reversible.

Thus, it appears that there are two factors contributing to the overall toxicity of gallium^{72} used in internal radiation therapy. One is total-body irradiation; the other is metal toxicity. The two together are synergistic. Superimposed in already debilitated tissues, these two factors are sufficient to make supposed therapeutic doses lethal.

The conclusions reached on gallium^{72} do not necessarily hold true for gallium^{67}. Gallium^{67}, when given with a small non-toxic amount of carrier, may have the same favorable end distribution and has a very low energy emission. With a 3.24-day half-life, the effective radiation may be spread over a long enough period to effect a significant tumor irradiation without overwhelming total-body irradiation. There will be no chemical toxicity to augment the total-body irradiation. However, for the same reasons that gallium^{67} may be more effective in tumors, it may be more lethal to bone marrow. Gallium^{67} should be regarded as an entirely new problem, to which little of the knowledge gained with gallium^{72} is applicable.

CONCLUSIONS

The staff of the Medical Division of the ORINS are of the opinion that gallium^{72} is not a therapeutic agent in cancer of bone, for the following reasons:

1. Although the isotope was highly concentrated in many areas of tumor, in every case areas of low concentration occurred.
2. Because of the short half-life of the isotope, the relatively high concentration in tumors develops after most of the radiation energy has been expended.
3. Undesirable total-body irradiation effects become prominent at subtherapeutic dosage levels.
4. Development of chemical gallium toxicity was a limiting hazard in long-continued dosage.

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I. Physics and Radiation Characteristics of Gallium[72]

MARSHALL BRUCER, M.D., and H. D. BRUNER, M.D.

In 1948, Dudley and his co-workers at the Naval Medical Research Institute became interested in the toxicity of gallium and its compounds. During the course of their investigations, it was observed that this element had a predilection for bone. Gross autoradiograms, obtained following a trial use of the radioisotope gallium[72] in rats, showed it to be localized with a high degree of specificity in areas of rapidly growing bone. The suggestion was made by Dudley that gallium might be a vehicle for carrying radiation to bone tumors.

In 1949, the staff of the Medical Division began its investigations of the pharmacological properties of gallium in animals. Much of the previous work of the Navy group was repeated, and a search was made for more suitable compounds. Methods of radioassay and standardization were restudied. By early 1950, there was sufficient evidence for beginning the therapeutic use of radiogallium in man, and in May of that year, the first patient, with terminal osteogenic sarcoma, was given gallium[72]. From May 1950 through the summer of 1951, 21 patients with metastatic tumors of a wide variety of types, mostly involving bone, were treated. An additional 34 patients were given tracer or test doses of gallium[72].

PHYSICAL NATURE OF GALLIUM

Gallium's first claim to attention was the fact that it is one of the three elements whose properties were known before the elements themselves were discovered. In 1871, it was a blank spot in the periodic table, but Mendeleef predicted its properties from its periodic relationships. It was finally discovered in 1875 by Boisbaudran in a French zinc ore. It is now known to be widely distributed in the earth's crust but always in minute quantities. There are no large deposits and present production is as a by-product of aluminum and zinc processing. Gallium ranks fifty-seventh among the elements in abundance in the earth's crust and is present in a concentration of the order of 10^{-11} per cent. This makes it considerably less abundant than gold (10^{-9} per cent) but far more abundant than radium (10^{-12} per cent).

Gallium is a member of Group III B in the periodic table. The two higher Z members of this group have been relatively rare until recently and are not important industrially. Gallium has atomic number 31 and an average atomic weight of 69.72. In pure form it is a bluish white metal with a melting point of 29.78° C., and a boiling point close to 2,000° C. Gallium differs from the usual metals in that, like water, it expands upon solidifying; the density of the liquid is 6.093 and of the solid 5.903.

There are at least ten isotopes of gallium, ranging from $_{31}Ga^{64}$ through $_{31}Ga^{73}$. Only two of these are stable, $_{31}Ga^{69}$, which is 60.2 per cent abundant and $_{31}Ga^{71}$, which is 39.8 per cent abundant. The isotopes with weights 72 and 73 are found in minute amounts in uranium fission products. Of the eight artificially produced radioactive isotopes, four have half-lives measured in terms of minutes, one five hours, and one nine hours; all of these are useless therapeutically. The experience gained from working with 14-hour Ga^{72} indicates that a 14-hour half-life is at the lower limit of practicability for isotopes to be used in patients. Thus, of all the gallium isotopes, only the 14.30-hour Ga^{72} and the 78-hour Ga^{67} are useful. The preferable Ga^{67} isotope can be made with a $(p, 2n)$ reaction that is efficient only with high-energy protons, but when this project began no cyclotron was available to us for the production of protons of this high energy. The Ga^{72} isotope can be made either with fast or thermal neutrons. The fast neutron reaction has an unknown, but probably low, cross section and would demand the use of separated Ga^{73}. Therefore a thermal neutron

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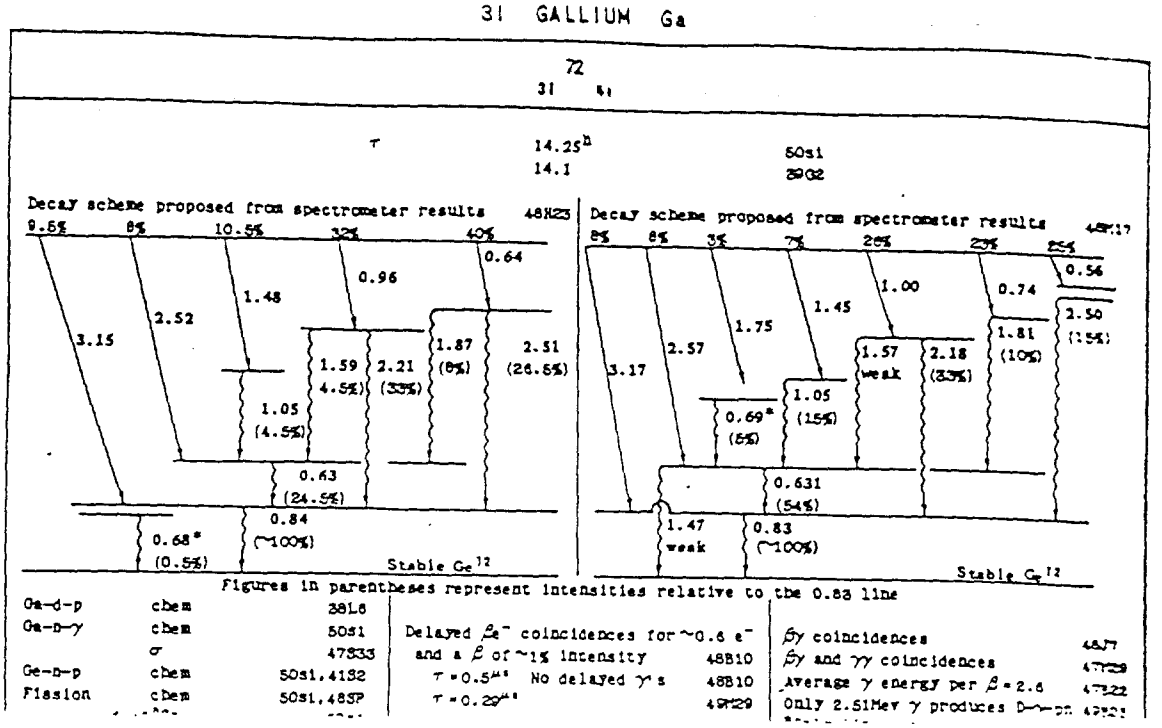

production at the beginning of this project.

The activation equations for the thermal reaction are as follows, where A equals specific activation, N the number of target atoms, σ the cross section (Th-n), f the neutron flux, t the irradiation time, and T the half life.

$$A = N\sigma f\left(1 - e^{\frac{-0.693t}{T}}\right)$$

For ${}_{31}Ga^{69}$ as the target,

$${}_{31}Ga^{69}(N, \gamma)\ {}_{31}Ga^{70^{20.5m}}\ {}_{32}Ge^{70} + \beta^-$$
$$(60.2\%)\ (\sigma = 1.4 \times 10^{-24})$$

For ${}_{31}Ga^{71}$ as the target,

$${}_{31}Ga^{71}\ (N, \gamma)\ {}_{31}Ga^{72^{14.3h}}\ {}_{32}Ge^{72} + \beta^- + \gamma$$
$$(39.8\%)\ (\sigma = 3.4 \times 10^{-24})$$

Thus, there will be roughly 1.7 times more Ga^{72} formed than Ga^{70}, and within three hours after removal from the neutron flux, the twenty-minute Ga^{70} activity will be less than 1 per cent of the total.

Of the seventy thermal neutron-produced isotopes with half-lives falling between twenty-first in rank of efficiency of production. Its low cross section predicts that the specific activity will be low, and under the best present conditions there is obtained 1 mc. of Ga^{72} per 4.7 to 5.2 mg. of stable gallium. Separated Ga^{71} is available, and with its use the specific activity can be increased fourfold, but this is still insufficient for practical purposes. A higher neutron flux would greatly improve the specific activity problem, but such reactors are not generally available, and the low-specific-activity material (1 mc., 5 mg. $Ga^{69,71}$) had to be used in this project.

Two alternative partial decay schemes for Ga^{72} are shown in Figure 1. The predominant elements of decay as stated by Haynes *et al.* (1) are shown in Table I. The probable dosage rate in terms of gamma intensity has been calculated for the major components of the decay scheme as shown in Table II.

Obviously a considerable portion of the

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energy is discharged as high-energy gamma emission. Such an isotope, no matter where located in the body, must yield considerable total-body irradiation. It can be calculated that in terms of the radiant energy absorbed from a uniform dosage throughout the tissues of man, the ratio of beta to gamma components would be about 20β to 58γ. This relationship will be reversed in a small animal such as the rat. Therefore, there can be no direct transference of radiation dosimetry from smaller animals to man.

TABLE I: PREDOMINANT ELEMENTS IN DECAY OF Ga^{72}

Predominant Beta Rays		Predominant Gamma Rays	
Energy (mev)	Frequency (%)	Energy (mev)	Frequency (%)
3.15	9.5–20	2.51	26
2.52	8.0–18.5	2.21	31.5
0.95	32	1.87	7.5
0.64	40	1.59	4.5
		1.25	2
		1.05	4.5
		0.84	100
		0.68	2
		0.63	18.5

The beta-ray dosage was calculated from the formula of Marinelli, Quimby, and Hine: $D\beta = 88\ \bar{E}\beta TC$ where $\bar{E}$ equals the mean energy of the beta rays weighted by their frequency, T the half-life in days, and C the microcuries disintegrating in 1 gram of tissue. By this formula, the beta dosage was calculated at 20.62 rep/mc. of Ga^{72} completely decaying in 1 gram of tissue. Similarly, we used the formula $D\gamma = K\gamma Cg$, where $K\gamma = 1.44\ t \times 10^{-3}$, t is the half-life in hours and g is a calculated geometric factor. Using weighted frequencies and mean energies, the gamma-ray dosage for a standard size man equals 58.41 r/mc. of Ga^{72} (complete disintegration) in 1 gram of tissue in the center of the body. This roentgen dosage is based upon uniform distribution of gallium throughout the body so that the radiation cross-fires the given gram of tissue in the center of the body. The same dosages calculated for the rat are 14.45 r for the gamma component, while the beta dosage remains the same as that for man.

TABLE II: RADIATION INTENSITY OF COMPONENTS OF THE DECAY OF Ga^{72}

Gamma (mev)	$I\gamma$ r/mc.-hr. at 1 cm.	Per Cent Frequency	$I\gamma$ Contribution to Total γ-Radiation $I\gamma \times$ %/100
2.51	11.25	26.0	2.925
2.21	10.25	31.5	3.229
1.87	9.3	7.5	0.697
1.59	8.3	4.5	0.373
1.25	6.9	2.0	0.138
1.05	6.0	4.5	0.270
0.84	4.9	100.0	4.900
0.68	4.1	2.0	0.082
0.63	3.8	18.5	0.703
TOTAL			13.317 r/mc.-hr. at 1 cm.

Since autoradiographic studies have shown that gallium is not uniformly distributed even in small volumes of tissue, and since the metabolic pattern continuously undergoes rapid change during disintegration of gallium, these calculations are of limited usefulness and are to be interpreted as order-of-magnitude values in assessing the radiation dosage to tissues. Three other methods of estimating radiation dosage which were based on measurements external to the body were investigated, but all were inadequate for the same reasons that calculated dosages were inadequate. By default, therefore, the hematological picture appeared to be the most practical method of assessing the radiotoxic effects of therapeutic amounts of Ga^{72}.

STANDARDIZATION OF GALLIUM

A detailed statement of standardization methods for Ga^{72} has been published as ORO-44 by the Technical Information Service, USAEC (2). Briefly, two methods were described, an electroscope calibration using gamma radiation and a comparison of the beta rays against an Ra DEF standard.

A Lauritsen electroscope was calibrated against a known source of radium and converted to millicuriage by use of specific gamma roentgen intensities by methods described by Marinelli, Quimby, and Hine

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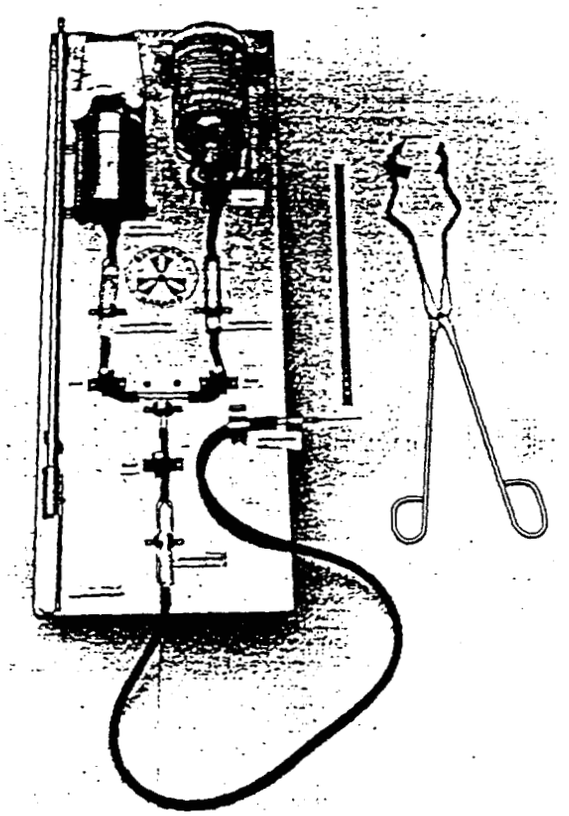

Fig. 2. Special intravenous injection system. For detailed description, see text.

(3). Similar measurements were made on a source of Ga^{72} and the calculation based upon the Iγ value computed above from the incomplete but best available decay scheme described by Haynes.

A beta calibration against a U. S. Bureau of Standards Ra DEF standard was run a number of times as a check on the electroscope standardization. Beta absorption curves were run on samples with constant geometry and all sources of variations kept to a minimum.

HEALTH PHYSICS

The dosages employed in patients were at first 10 to 20 mc. repeated weekly. After a time they were increased to 50 to 100 mc. repeated twice weekly. With the development of severe toxic reactions, the dosages were reduced to the 40- to 50-mc. range repeated twice weekly. During the entire period of this study 12 curies of Ga^{72} were

There were four main sources of radiation hazard which demanded special attention. The first was the area surrounding the chemical preparation of the gallium nitrate as it arrived from the reactor. The second was during the injection of the material into the patient. The third was around the patient for the subsequent twenty-four hours, and the fourth was the collection of excreta saved for analysis.

To reduce radiation dose during chemical preparation of gallium for patient use, special 6-inch lead shields were constructed inside each of two fume hoods which were used alternately. The highest total body dosages received by the chemists averaged about 55 mr/day, but this low level was achieved only after considerable redesign of the handling procedures.

Since it was necessary to inject gallium by the intravenous route, supratolerance dosages were received by personnel until a special intravenous injection system was devised, as shown in Figure 2. This device has since been found practical for use with almost all intracavitary or intravenous injections.

The infusion apparatus was designed to be autoclaved as a unit, set up at the bedside, and operated from a distance. A standard parenteral fluid bottle was fastened to the board at a slightly higher level than the shielded wide-mouth flasks. The two flasks drained by gravity into parallel visual drop tubes; these in turn drained through a T-tube into the common drop tube, which was connected by rubber tubing to an intravenous needle. Tubing clamps were placed so as to control the drainage of either flask into the intravenous needle or to allow the saline to back-up into the shielded flask for rinsing. On each tubing clamp there was fitted a 1/4-inch hexagonal nut which fitted a hex spin-tight wrench fixed on the end of a 3-foot wand.

In operation, the radioactive solution was kept behind shielding while the saline infusion was started. When the infusion was flowing at a slow steady drip, the radio-

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mouth flask. The usual safe distance from this time on was 3 to 4 meters from the apparatus. By means of the wand, the rate of mixture of radioactive solution and saline was controlled from 3 feet, and the body was about 5 feet away. Since the radioactive fluid was tinted by a *p*H indicator, the rate of flow through each drop tube and the color intensity of mixture in the common drop tube could be observed from across the room. The usual infusion took ten to thirty minutes. When all of the radioactive solution had drained from the shielded flask, the common clamp was closed and the saline was washed back into the flask. This rinse and two subsequent rinses were allowed to run into the vein without further dilution by saline. With three rinses, the residual activity in the shielded flask and tubing was negligible and the apparatus could be safely removed from the room. The entire assembly was washed, reassembled, and sterilized in an autoclave as a complete unit ready for the next use.

With all other methods of injecting radioactive materials, the period of injection was found to be the time of maximum exposure of nurses and staff. By use of this apparatus, the injection exposure became a relatively innocuous procedure.

The third area of radiation hazard was around the patient. Measurements of the 6.25 mr/hr. isodose lines around the patient at various times after injection gave patterns of which an example is shown in Figure 3. Immediately after injection of 20 mc. of Ga^{72} citrate, this isodose line is approximately a circle 4 meters from the center of the patient. Because Ga^{72} has a short half-life and is excreted rapidly *via* the urine, the isodose line rapidly regresses toward the patient. By two hours the maximum permissible isodose line is 3 meters from the patient, and by sixteen hours 1 meter. After two days it is close to the surface of the body so that there is little hazard to the nursing personnel carrying on ordinary hospital routines.

Since ordinary plaster walls provide almost no shielding, all personnel were kept

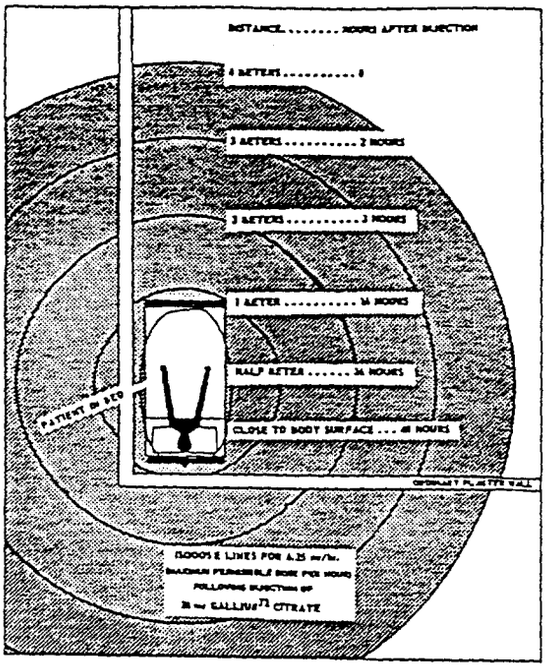

Fig. 3. Isodose lines for 6.25 mr/hr. following injection of 20 mc. gallium[72] citrate.

away from adjacent hallways and ward rooms during the initial period after injection. Other patients having radioactive therapy probably received a negligible fraction of their own dosage from exposure to a gallium patient, but patients not under radioactive therapy and staff members were shielded from active patients. A detailed statement of the health physics precautions taken for gallium and other isotopes in the Medical Division Hospital has been published (4).

During surgical operations, a radioactive patient may provide a considerable spot dose to personnel who acquire doses approaching tolerable limits during their routine work. For example, during a two-and-one-half-hour operation to remove the left lung of a patient who had been given a 20-mc. test dose of Ga^{72}, the surgeon and his first assistant each received a total of 400 mr to their hands as measured on film badges placed within the palms of their rubber gloves. Badges under their collars registered 30 mr. The anesthetist for this operation received a total-body exposure of 40 mr.

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During the chemical preparation of Ga^{72} citrate, the exposure to the hands can be considerable. During the hour required to prepare 4 ampules of Ga^{72} citrate, the chemist received 530 mr to his right hand and 355 mr to his left. During autopsies on patients who had been given radioactive gallium just prior to death, total-body exposure to the pathologist was as high as 628 mr over the two- or three-hour procedure.

One of the major sources of radiation hazard was from the area set aside for storage of urine collected from active gallium patients. During the first twenty-four hours after injection, up to 50 per cent of the injected dose of Ga^{72} was excreted, so that twice-weekly collections on as few as two or three patients gave a continuously supratolerable level within a radius of 4 meters. In order to contain this radiation, a urine storage vault (Fig. 4) was built.

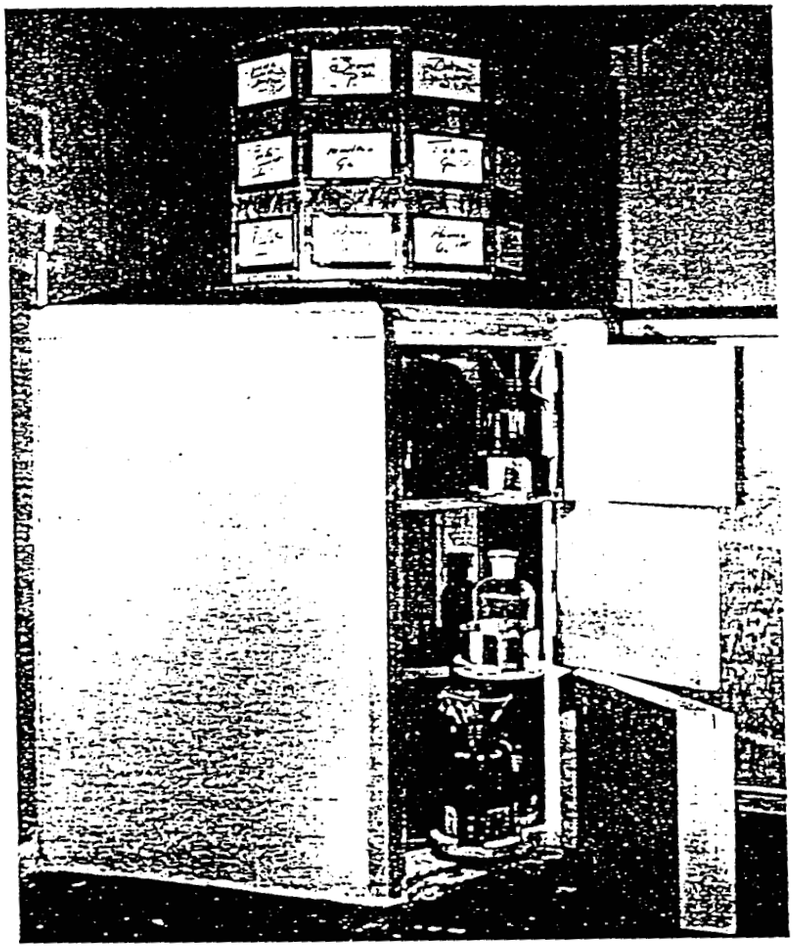

Fig. 4. Urine storage vault.

ASSAY AND SAMPLE COUNTING PROCEDURES

A standard pseudo-wet-ashing procedure was used to estimate the amount of radiogallium in tissue samples. A weighed amount of tissue was placed in a 10-ml. volumetric flask. Concentrated HNO_3 was added and heated gently until the tissue was completely dissolved. Water was added to volume and the contents were then poured directly into the top half of a 50-mm. standard Petri dish. Previous studies had indicated that there was no preferential distribution of Ga^{72} into fat

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globules. A standard survey meter can be used to estimate the amount of activity in the 10-ml. volumetric flask in the event that dilution is necessary in order to obtain counting rates between 300 and 3,000 per minute.

The sample was placed in a carriage which slid with reproducible geometry beneath a Geiger-Müller tube mounted in a horizontal lead shield. The tube was a 30 mg./sq. cm. glass envelope carbon cathode tube sold by Herbach and Rademann, Philadelphia.

The usual standardization procedures were used to equate counts per minute to millicuries. The efficiency of counting was routinely between 1.55 and 1.80 per cent. Efficiency of counting was sacrificed for precision of counting plus speed and ease of preparation of large numbers of samples. Only one transfer of a sample was necessary in this procedure, and only two pieces of glassware were exposed to contamination. The apparatus was easily cleaned with Dreft or Tide, but not with ordinary soap powders.

Certain special instruments were devised for routine counting procedures. The urine of patients contained large quantities of Ga^{72} during the first twenty-four hours, so that extreme degrees of dilution were required for conventional counting methods, and in the process there was appreciable personnel exposure. For this reason the following system was devised:

A D.C. electrometer for amplification of ion chamber currents was connected to an ion chamber especially built for urine measurements by Mr. Floyd M. Glass of the Instruments Division, ORNL, Oak Ridge. The urine was collected in 2-liter machine-made Pyrex bottles; at the end of the collection period, water was added and mixed to the 2,000 c.c.-mark. The bottle was placed in a lucite rack in constant geometrical relationship to the ion chamber. The readings of the electrometer at different range scales were made directly as millicuries of gallium according to direct calibration from samples of known millicuriage. The range of accurate readings was from 10 microcuries to 35 millicuries total in 2,000 c.c. The precision was of the order ± 2 per cent.

The method involved no transfer, no dilution, and a minimum contamination of glassware. The daily samples could be measured in a tenth the time necessary with conventional methods, and with negligible exposure of personnel.

II. Gallium Chemistry of Biological Interest

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The primary problem in the chemistry of gallium was related to finding a compound both biologically acceptable and easily synthesized at high levels of radioactivity.

BASIC CHEMISTRY

Because gallium has only recently become available in a pure state, relatively little is known of the chemistry of this element. Its chemical properties are quite similar to those of the more widely known aluminum. Its usual valence is +3, but stable +2 salts and a +1 oxide are known. The metal is stable in air but an oxide film forms in moist air or oxygen. Gallium is relatively resistant to both alkali and acid but, when heated with a mixture of HCl and HNO_3, it will slowly react to form the chloride. Several salts can be made by the direct action of halides in the presence of heat. The sulfides, sulfates, nitrate, and phosphate have been formed, as well as a few organic salts. A sesquioxide is produced by heating a gelatinous hydroxide of uncertain structure between 900 and 1200°.

Most gallium salts are hydrolyzed in aqueous solution and can be kept in solu-

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tion only when strongly acidic or basic. This is particularly true of the inorganic salts (5).

In choosing a compound suitable for toxicity and distribution studies and for patient use, the work of Dudley and Levine (6) was most helpful, although several compounds were studied in addition to those prepared by these investigators. The criteria used for acceptability of possible compounds were:

(*a*) Complete solubility in water and 0.9 per cent NaCl solution at 20 to 42° C.

(*b*) Stability of the solution.

(*c*) Low rate of release of gallium ions after injection of the compound into animals.

(*d*) Ability of experimental animals and man to metabolize safely the non-gallium moiety.

(*e*) Production of a sterile compound.

(*f*) Speed of synthesis from gallium nitrate.

With these criteria, the complexing abilities of several acids and bases were studied. Hydrochloric, carbonic, phosphoric, ascorbic, maleic, tartaric, acetic, salicylic, oxalic, benzoic, and succinic acids and ethanolamine and diethanolamine were unsatisfactory. The complexes formed with gluconic, mandelic, and lactic acids were suitable in that they remained in solution at a *p*H range of 7.2 to 7.6 but were undesirable otherwise (7).

The compound that was decided upon for animal and human studies was gallium citrate. One of the chief reasons for choosing this compound was the fact that it could be prepared quickly and easily in sterile solution. The final solution could be administered within two to three hours after the bombarded gallium nitrate was removed from the nuclear reactor.

STABLE GALLIUM CITRATE

All of the stable gallium citrate used in the accompanying studies was prepared according to the method of Dudley (8), with a few unimportant modifications. Analyses of several batches of this compound gave a gallium content of 23 to 24 per cent as contrasted with the 25 to 26 per cent value reported by Dudley.

RADIOGALLIUM CITRATE

Purified gallium nitrate was prepared according to Dudley (8) and sealed in quartz ampules, which were scored around a slightly indented neck. When the bombarded material was received, the ampule was placed in a plastic holder and the top was removed by hitting the scored neck a sharp blow. For each 300 mg. of gallium as the nitrate, 4 ml. of 6 *N* HCl was pipetted into the ampule. A heat lamp was placed over the ampule and the solution was stirred by drawing the material into a pipette and allowing it to flow back. When the solution appeared complete, the material was filtered. Filtration was necessary because quite often one or two small particles would not dissolve under these conditions. While the nature of these particles was not determined, it was thought that they were small amounts of the relatively insoluble oxide. The ampule was rinsed once with 1 ml. of 6 *N* HCl and twice with 2 ml. of water. The washings were also filtered. The total filtrate was added to a mixture of 8 ml. of 20 per cent citric acid solution, 3 ml. of a saturated water solution of bromcresol purple and 15 ml. of chloretone solution (5 mg. per ml.). This mixture was agitated by an air stirrer. A solution of 15 *M* NaOH was added dropwise until a definite purple color was produced. A final *p*H of approximately 7 was determined by means of *p*H paper. The average gallium concentration in the final preparation was 9.6 mg. per ml. The solutions and glassware used were carefully cleaned and sterilized by autoclaving, so that the final product was aseptic.

When the solutions of this radiogallium citrate stood for a few days, a definite Tyndall effect was seen, and it became more pronounced with prolonged standing. Several weeks after preparation, there was a definite precipitate. Because of these facts and the nature of the animal distribution of the gallium, the possibility that at least

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part of the gallium citrate was in a colloidal form continued to be a problem throughout of the animal and human studies. No attempts were made to determine whether any of these small colloidal particles were present in the freshly prepared solutions, but all indications were that they did exist.

ION-EXCHANGE GALLIUM CITRATE

The possibility of the formation of colloidal particles and the knowledge of the ease with which gallium is hydrolyzed led to a study of another method of synthesis of radiogallium citrate (9). As has been pointed out, the ion-exchange method of preparation was desirable in all respects except that the mol ratio of citric acid to gallium was 5 to 1 as contrasted with a ratio of 1.2 to 1 for the simple radiogallium citrate preparation. While the distribution and excretion of the ion-exchange complex was somewhat different from that of the simple prepared complex, this difference might have been due to the greater amount of citric acid rather than to a difference in the degree of hydrolysis. Whatever the reason, the complex formed by ion-exchange was impracticable because of the high citric acid concentration of the solution.

CHEMICAL GALLIUM DETERMINATION

The method used for the analysis of gallium in tissues was based on that of Dudley (10). The original method was for 5- to 10-gm. samples of tissue, while the sample size used in obtaining the results in the accompanying reports was 0.5 to 1.5 gm. The reagents were reduced in the same proportion. It should be added that reliable results were obtained only when the chloroform extractions of gallium were made by ten minutes of mechanical shaking.

III. Effect of Internal Irradiation on Respiration of Rat Liver Slices[4]

JESSE D. PERKINSON, JR., PH.D., and ROBERT S. INGOLS, PH.D.

Since there exists no means of determining the radiation dosage derived from an internally administered radioisotope except by use of calculations such as those of Marinelli, Quimby, and Hine (3), it seemed desirable to find some measurable biological effect of radiation which could serve as a basis for comparison of the effectiveness of different isotopes and perhaps of external roentgen irradiation. The results reported here are from a preliminary attempt made to determine whether the inhibition of total tissue respiration, or oxygen consumption, was proportional to the amount of internal irradiation that the tissue received.

Gallium^{72} was used as the source of internal radiation because of the interest in this isotope as an irradiator of bone tumor. Since it had been shown that gallium concentrates significantly in the liver (11), knowledge concerning the effect of the metal and of radiation from the isotope was desired. The decay scheme for Ga^{72} is incomplete, so that a true roentgen equivalent physical (rep) value for radiation dose was impossible (12). The liver of the rat was used because of the relatively extensive data available concerning the effect of physiological variables such as age, weight, and sex on the metabolism of that tissue. A large accumulation of data exists concerning distribution of gallium in the liver (13).

Barron (14), in 1946, was apparently the first to show conclusively that whole-body roentgen-irradiation of rats inhibited the oxygen consumption of liver slices and that some enzymatic systems were more sensitive than others. Until that time the literature was quite confused.

There is a paucity of reports concerning

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the metabolic effect of internally administered isotopes. Barron *et al.* (15) studied the effect of intravenously injected plutonium on the metabolism of several tissues. They found that some phases of liver metabolism were inhibited in two series of rats that had received either 1 or 2 mg. of $PuO_2(NO_3)_2$ per kilogram and were killed two to ten days later. In other series, however, the same inhibitions were not observed. Barron suggested that the apparent lack of relationship between radiation dose and degree of metabolic inhibition might be attributable to an unequal distribution of plutonium and to the relatively shallow penetration of alpha rays.

ACUTE EXPERIMENTS

Experimental: Male Wistar-Carworth strain rats weighing between 200 and 250 gm. received by tail vein either a mixture of stable gallium and gallium^{72} citrate or stable gallium citrate alone, so that all animals received equal amounts of metal. Amounts of Ga^{72} equivalent to 1.7 mc. per kilogram were injected as accurately as the solution volumes could be measured with a tuberculin syringe. The injections were made with the rats under light ether anesthesia and just after 0.2 ml. of 10 per cent calcium gluconate solution was given, as described in Section IV of this series.[5] At two, six, twelve, eighteen, twenty-four, forty-eight, seventy-two, and ninety-six hours after injection, one animal which had received Ga^{72} and its stable gallium control were sacrificed and the livers removed. Portions of the same lobe of each liver were used for Qo_2, glycogen and water content determinations and for histologic studies and activity measurements. It was determined that the anesthesia and injection technic did not affect the Qo_2. Twelve rats were anesthetized with ether and injected by tail vein with calcium gluconate and saline equal in volume to that administered to the rats that received gallium citrate solutions. At six hours after injection the liver slice oxygen consumption was not different from that of the untreated normal animals.

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TABLE III: LIVER Qo_2 VALUES FOR UNTREATED RATS AND THOSE THAT RECEIVED EITHER STABLE GALLIUM PLUS Ga^{72} OR ONLY STABLE GALLIUM.*

(Number of animals used to obtain the average values are in parentheses)

Time (hr.)	P	Stable Qo_2	P	Radioactive Qo_2	P
2	<0.01	4.9(4)	<0.20	5.3(4)	<0.01
6	<0.01	5.6(7)	<0.02	4.2(7)	<0.01
12	<0.02	6.8(4)	<0.04	5.1(4)	<0.01
18	<0.01	7.6(4)	<0.01	5.0(4)	<0.01
24		6.1(12)		5.8(12)	
48		6.3(4)		6.5(4)	
72		6.7(4)		5.7(4)	
96		5.9(4)		6.2(4)	

* Normal Qo_2 = 6.2 av. (22)

All rats used in these studies received a stock diet of Rockland Checkers *ad libitum*.

One interest in these studies was the possible application of the results to the use of Ga^{72} in human beings, so that the rats were not fasted, as they usually are before Qo_2 determinations, since the patients would not be in a fasting state. It has been shown that glycogen acts as a metabolically inert substance in liver Qo_2 determinations (16). In measuring liver Qo_2 and glycogen content of 22 untreated rats, it was found that consistent Qo_2 values could be obtained by making an empirical correction for the glycogen content, so that all values are reported with this correction.

The Qo_2 determinations were made with standard Warburg technics, using Krebs-Ringer phosphate buffer solution in a saturated O_2 atmosphere. No substrate was added. The data from four or five flasks with slices from the same liver were averaged for each Qo_2 value.

Results: 1. Qo_2 Determinations: The average Qo_2 for untreated normal rats of the same age, weight, and sex as the control and experimental animals was found to be 6.2 μl. of O_2 per milligram of dry weight tissue per hour. In Table III, the number of animals killed at each period after injection is given in parentheses together with the probability values for the statistical

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TABLE IV: Qo_2 VALUES FOR RATS RECEIVING STABLE AND RADIOACTIVE GALLIUM

("Mg./kg." refers to the average amount of gallium that was injected)

Hours	Stable		Radioactive	
	Qo_2 (gly)	mg. Ga/kg.	Qo_2 (gly)	mg. Ga/kg.
2	4.9	8	5.3	8
6	5.6	9	4.2	9
12	6.8	9	5.1	9
18	7.6	9	5.0	8
24	6.1	10	5.8	10
48	6.3	12	6.5	10
72	6.7	9	5.7	9
96	5.9	8	6.2	9

animals. It can be seen that the differences between the values for the untreated animals and those receiving either the stable gallium or Ga^{72} plus stable gallium are highly significant through the eighteen-hour period. The differences between the stable gallium and the Ga^{72} plus stable gallium values are significant at six, twelve, and eighteen hours after injection.

The Qo_2 and radioactivity data summarized in Figure 5 show that the stable gallium alone had an inhibitory effect on the oxygen consumption for six to twelve hours

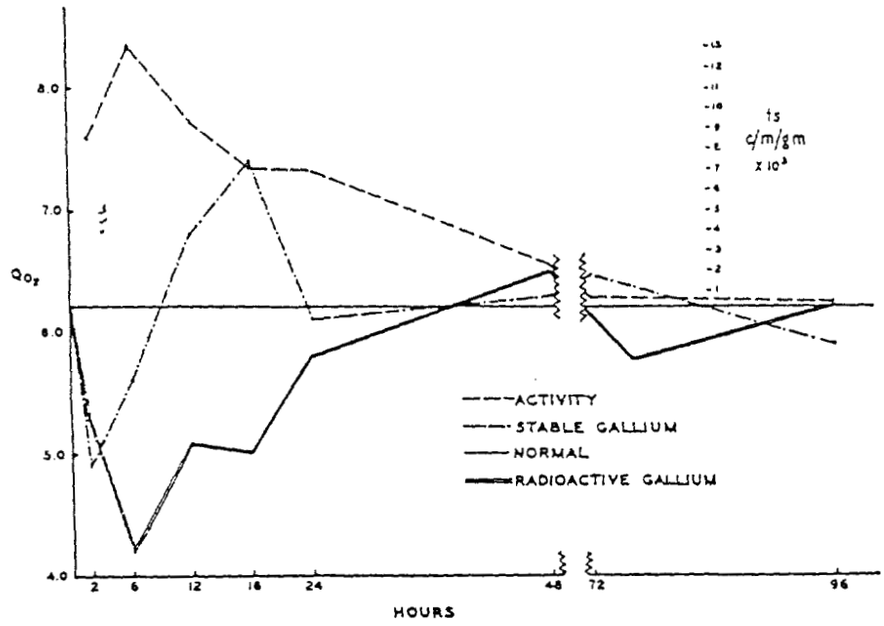

Fig. 5. Change in Qo_2 following administration of stable and radioactive gallium. "ts" refers to the fact that the activities are those that existed in the liver at the time of sacrifice.

validity of the differences found. These probabilities were calculated according to "Student's" formula. The left-hand "P" column refers to the differences between the Qo_2's for the animals that received only stable gallium and the value for the untreated normal animals. The center column refers to the differences between the stable gallium control animals and those receiving radioactive plus stable gallium. The right-hand column refers to the differences between the rats that received radioactive gallium plus stable gallium and those for the untreated normal after injection but that the Ga^{72} plus stable gallium produced a still greater decrease in the Qo_2 and that the inhibition persisted for eighteen to twenty-four hours. The radioactivity of the liver samples is expressed as counts per minute per gram of tissue at the time the animals were sacrificed. The obvious proportionality between the Qo_2 and the activity present at the time of sacrifice is seen better in Figure 6, were Qo_2 is expressed as a function of activities. That the Qo_2 differences were not due to different doses of gallium can be seen in Table IV, where it is shown that the amounts of gal-

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TABLE V: GLYCOGEN CONCENTRATIONS IN LIVER FOLLOWING GALLIUM INJECTION

Hours after Injection	Per Cent Glycogen in Liver	
	Stable Gallium	Radioactive plus Stable Gallium
2	1.7	2.0
6	0.8	0.7
12	1.6	1.9
18	4.1	2.1
24	3.9	2.0
48	2.7	3.1
72	4.8	3.1
96	4.8	4.2

lium were essentially the same for the experimental and control animals.

2. *Histology:* There was no evidence of histologic abnormality in any of the liver sections.

3. *Glycogen and Water Content:* In Table V are shown the average glycogen concentrations in livers from animals sacrificed at the different time intervals. It can be seen that the glycogen content was lower in both the irradiated and non-irradiated livers for the first twelve hours after injection, but that this lower level persisted longer in the rats that received the radiation. While the trauma due to the injection procedure and the gallium itself may have affected the liver glycogen store, the radiation had an additional effect.

There was no measurable effect of either the gallium or the radiation on the water content of the liver.

CHRONIC EXPERIMENTS

Experimental: Since the radiation derived from Ga^{72} inhibited the liver oxygen consumption up to twenty-four hours after injection, it was important to learn whether repeated injections of stable and radioactive gallium would have a more permanent effect. Since such an experiment would give information as to whether untoward reactions in patients who received Ga^{72} were due to the chemical toxicity of gallium or to radiation combined with chemical toxicity, 8 male Wistar-Carworth rats weighing between 200 and 250 gm. were injected by tail vein twice a week for four weeks with an average single radiation dose of 1.7 mc./kg. so that they received an average total of 14.5 mc./kg. The average single gallium dose was 9 mg./kg. or an average total of 75 mg./kg. Eight other rats were injected according to the same schedule with equal amounts of stable gallium. All rats had free access to food and water. In order to obviate the already demonstrated twenty-four-hour period of inhibition of liver oxygen consumption that resulted from Ga^{72} radiation, these rats were sacrificed ninety-six hours after the last injection and the Q_{O_2} of sliced liver sections determined as in the preceding experiments. Stable gallium content of the same liver lobe was determined for all animals.

Results: The average results of these repeated doses are presented in Table VI,

TABLE VI: AVERAGE FINDINGS AFTER REPEATED GALLIUM INJECTIONS

	Stable Gallium	Radioactive plus Stable Gallium
Q_{O_2}	5.1 (4.3–5.8)	5.1 (4.6–6.0)
W.B.C.	15,000 (10,000–18,000)	9,800 (7,000–13,000)
Hb.	13(12–14)	13(11–17)

with the ranges in parentheses. The Q_{O_2} was inhibited to the same significant degree in the animals that received the stable gallium as in those receiving Ga^{72} plus stable gallium. The radiation produced no effect that lasted as long as ninety-six hours after the last injection, while the accumulated gallium did inhibit respiration at that time.

As shown in Table VI, the radiation from Ga^{72} produced a mild leukopenia, while the stable gallium was without effect. The hemoglobin was reduced to the same degree in both groups of rats, so that this amount of radiation did not affect hemoglobin production, while gallium inhibited it.

Liver damage could not be seen histologically.

The irradiated rats lost weight during the four-week experimental period with the exception of one animal that gained a total of only 8 gm. The average weight loss was 26 gm. Five of the stable gallium

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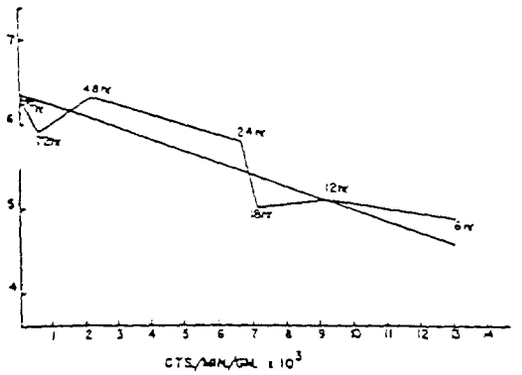

Fig. 6. Relationship between Qo_2 and gallium activity. The activities in counts/min./gm. × 10^3 are those that existed in the livers at the time of sacrifice.

rats lost weight but the average weight loss was only 2 gm. In the irradiated rats diarrhea occurred an average of sixteen days after the first injection, while in the stable gallium rats this symptom did not develop until the twenty-fourth day.

The average gallium content of the stable gallium rat livers was 2 μgm. per gram (range, 2–3) and that of the irradiated rat livers was 4 μgm. per gram (range 1–7).

DISCUSSION

These experiments were made in an attempt to learn whether a biological effect of radiation might be used as a basis for comparing one internally administered radioisotope with another. No attempt has been made to arrive at any physical expression of radiation dose.

Figure 6 is presented as a general statement of the relation that was found to exist between the relative amount of radiation present at the time the rats were sacrificed and the effect of the radiation on total oxygen consumption by the liver. Several factors enter into this statement. It is obvious from Figure 5 that the liver tissue recovers quickly from the effect of this amount of radiation, so at all times there is a recovery process which influences the overall measurement. Since the peak of gallium concentration is reached sometime between two and twelve hours (Fig. 5), the decrease of gallium content, and therefore of radiation dose, after that time will produce less inhibition of the oxygen consumption.

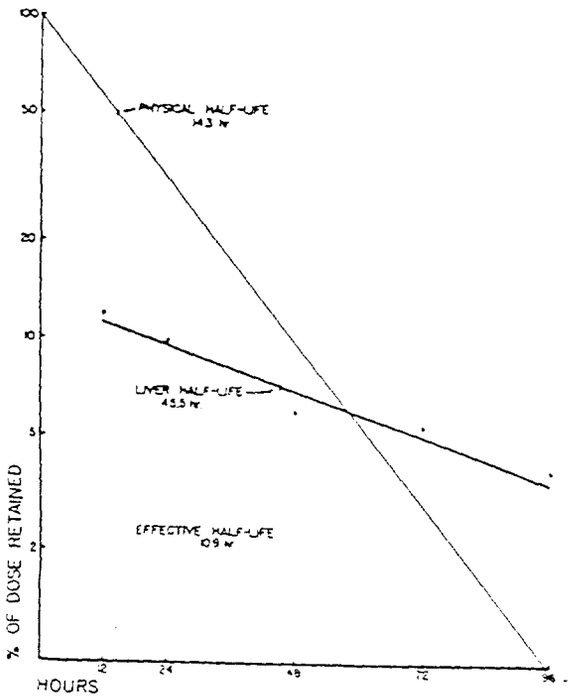

Fig. 7. Biological half-life of gallium[72] in liver. The effective half-life was calculated according to Marinelli *et al.* (3).

In Figure 7 is shown the physical decay of the Ga^{72}. Not only is the gallium concentration in the liver beginning to decrease sometime during the six- to twelve-hour period, but what gallium is there is contributing less radiation per unit weight from the moment it is injected.

An examination of Figure 8 shows that sometime during the eighteen- to twenty-four-hour interval the decrease in liver activity becomes chiefly a function of the physical decay of Ga^{72}. It is interesting to note that the Qo_2 inhibition is evident until this same time.

Considering the fact that the total oxygen consumption is the sum of many different metabolic reactions, it is understandable that a correlation between amount of internal radiation and the Qo_2 would not be completely consistent from measurement to measurement. As Barron has shown, some enzymatically controlled reactions are more sensitive than others (14), so

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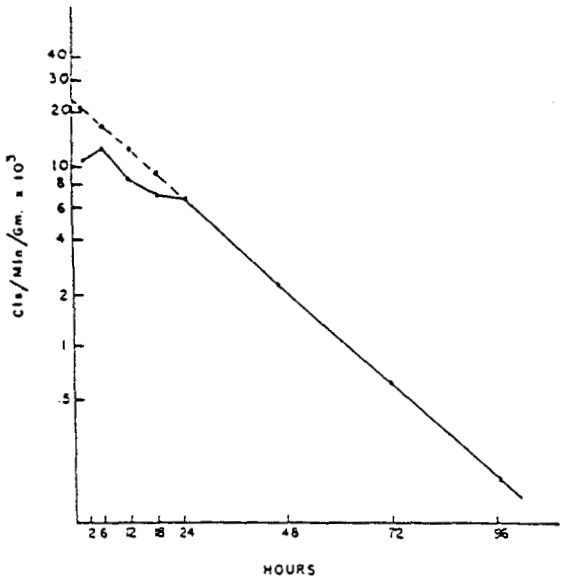

Fig. 8. The stability of gallium concentration in the liver. The solid line is an expression of the determined activity in liver at the indicated times after injection. The broken line was obtained by extrapolating the constant part of the activity curve. Activities calculated by means of the decay constant for Ga^{72} are shown to be in agreement with the extrapolation.

that it is possible that a single enzyme system could be more strictly correlated with the amount of radiation. With this idea in mind, experiments are being conducted to learn at what level of internal P^{32} irradiation liver Qo_2's are minimally and maximally affected. With this background, it is planned to study the effect of the minimal amount of P^{32} radiation which will affect different enzyme systems and to compare these results with the effects of other internally administered isotopes.

It was possible that the radiation from Ga^{72} might not have produced an inhibition of oxygen consumption without the presence of gallium. In order to test this possibility, Ga^{67}, with a specific activity of essentially 100 per cent, is being used in similar studies. The results so far obtained show that the internal radiation is effective even when chemically negligible amounts of gallium are present.

In summary, it has been demonstrated that internal radiation derived from Ga^{72} inhibits the oxygen consumption of rat liver slices and that the degree of inhibition is related to the dose of radiation. The Qo_2 of liver slices from control animals that received stable gallium was inhibited slightly during the two- to twelve-hour period after injection.

Repeated injections of stable gallium produced a liver Qo_2 inhibition which was apparent ninety-six hours after the last injection. Repeated injections of Ga^{72} plus stable gallium produced no additional effect.

The glycogen content of livers from the stable and radioactive gallium rats was less than normal. This decrease persisted longer in the irradiated animals.

At the levels of radiation and gallium dosage used, there was no histologic evidence of liver damage during the period of Qo_2 inhibition.

With repeated injections, leukopenia seemed to be the result of radiation, while the hemoglobin reduction was due to the effect of stable gallium. Gastrointestinal disturbances were produced by both stable and radioactive gallium, but appeared much sooner in the Ga^{72} animals.

IV. Toxicity of Gallium Citrate in Dogs and Rats

H. D. BRUNER, M.D., BETTY M. COOPER, M.D., and DONALD J. REHBOCK, M.D.[6]

Studies on the toxicity of gallium citrate in dogs and rats were begun in October 1949 and continued during the following year. At that time, Dudley and his associates (6, 10, 11, 17) had published the basic data which suggested that radioactive isotopes of gallium might segregate in osseous tissue in amount sufficient to be therapeutically useful against neoplasms of bone. By virtue of these data and per-

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sonal communication with Commander Dudley, it was possible to select the gallium citrate complex as the most biologically acceptable form of the metal which could be rapidly synthesized at the 500-mc. level (see Section II of this series).

Because this radiochemically prepared solution of gallium citrate contained extraneous material not present in solutions of the recrystallized pure complex, both forms were employed for these toxicity studies, but without noticeable difference in results. Only the intravenous route of administration was used, since radioactive gallium can be given to man solely by that route. Dudley and Levine (6) showed that gallium was not absorbed from the gut and only partially from subcutaneous and intramuscular deposits. Such unabsorbed radioactive material would produce severe radiation damage at the point of injection.

Following intravenous injection of the citrate complex, the animals exhibited generalized hyperirritability. If enough of the solution were given, they displayed opisthotonos and died acutely with respiratory paralysis. Presumably an acute hypocalcemia created by the citrate was responsible for death. Experiments on rats demonstrated that calcium given just prior to the gallium citrate prevented these acute deaths, and by trial and error it was established that the minimal effective prophylactic dose was approximately 2 mols of calcium ions per mol of citrate. This ratio suggests that much of the citrate was rapidly mobilized from the complex immediately following injection. Thus, deaths occurring within twenty-four hours after injection were not regarded as gallium-induced. In view of these observations the prophylactic administration of calcium as Calcium Gluconate Injection (U.S. P.) became an integral part of the administration of gallium citrate and was used in this 2:1 mol ratio preceding all injections of the citrate into man and animals.

EXPERIMENTAL METHODS

A total of 137 male rats of the Wistar-Carworth albino strain, weighing 200 to 275 gm., were used. After injection they were caged in pairs and given Checkers and water *ad libitum*. The injection procedure is important, since the apparent toxicity can be artificially increased by poor technic. The injections *via* the tail vein were made under light ether anesthesia in the belief that the rat suffered less trauma from the ether than from the struggle which otherwise takes place; also, such injections can be made with precision. The vein was entered with a 26-gauge needle attached by a short length of gum tubing to a tuberculin syringe filled with saline. While the saline was being injected slowly, the required amount of 10 per cent calcium gluconate was given by puncturing through the gum tubing; after the calcium had been washed in, the desired amount of gallium citrate was injected through the tubing, and it was then washed in with the remaining saline. This procedure was used in all rat experiments. It was especially advantageous when the gallium solutions were highly radioactive, in that the operators thus received a minimal amount of radiation. All injections were made *very slowly* and as aseptically as possible.

The dogs were healthy mongrels of both sexes weighing 5 to 14 kg. and caged individually. Commercial food was supplemented with bones. All injections were *via* the external saphenous or basilic veins and without anesthesia.

Four preparations of the citrate complex were used. The first was a radiochemical preparation described in Section II. This, the least pure form, is not taken through a crystallization stage. The second was a recrystallized sample obtained from Dudley containing 23.05 per cent Ga. The third and fourth were samples prepared from 98.5+ per cent purity metal, differing only in the number of recrystallizations and analyzed 22.5 per cent Ga. The latter three samples were dissolved in distilled water in amounts sufficient to give approximately an 8 per cent solution *after* adjustment to *p*H 7.2–7.4 with NaOH and passage through a No. 42 Whatman filter.

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Filtration removed approximately 30 per cent of the gallium originally present and eliminated the initial opalescence of the solutions. The gallium concentration of the solution was then determined by direct analysis. In all cases, the doses have been calculated and expressed in terms of gallium element, not the gallium citrate complex.

By using normal red blood cells as osmometers, the filtered solutions were adjusted to approximately isosmotic concentrations with distilled water or NaCl solutions. In order to be able to administer the highest doses to rats, 1.5 times isosmotic solutions were prepared.

RESULTS

At a given dose level, at least two and usually three of the samples described above were used, but because there were no apparent differences between the preparations, the data have been combined.

1. *Toxicity of Single Doses in Rats:* No deaths due to gallium itself were observed in rats receiving doses up to 223 mg. Ga/kg. body weight. After higher doses, deaths did occur within twenty-four hours, but it is questionable if they were the result of toxicity of the metal. It proved impossible to administer larger amounts of gallium citrate without laying the data open to challenge on the ground of injecting too large a volume of solution, too hyperosmotic a solution, or unrealistic amounts of Ca and citrate ions. Thus, it is possible to state only that the 10-day LD 50 for rats was greater than 220 mg. Ga/kg. body weight.

For the first four to eight days after injection of the higher doses, the rats ate little, ceased to groom themselves, experienced mild diarrhea, and lost about 15 per cent of their body weight. By the twentieth day, however, they appeared normal, and by the thirtieth day had made up the initial weight loss. Histologic study of such recovered rats showed only mild cloudy swelling of the kidneys and increased hemosiderin in the spleen. Rats receiving lower doses were proportionally less affected At the height of the reaction, the sections showed mild to moderate renal tubular damage and lymphoid degeneration.

2. *Toxicity of Single Doses in Dogs:* Single injections of one of the above four samples of gallium citrate were given to 50 dogs in doses ranging from 10.6 to 47.0 mg./kg. body weight. The per cent surviving fifteen days later, computed by the cumulative method, was linearly related to the log of the dose. The 10, 50, and 90 per cent lethal dose values were 10.5, 18.2, and 41.1 mg./kg., respectively. Many of the animals living beyond fifteen days were severely debilitated and recovered very slowly; 3 were sacrificed when it became obvious that they would not recover.

Most dogs vomited soon after the injection, and all refused food and rapidly lost weight. Nearly every animal suffered from diarrhea; the stools were foul and blackish, and gave positive tests for blood. Those animals which survived exhibited the least anorexia and gastrointestinal upset. Debility and weakness progressed rapidly in those which died; death was preceded by coma. There were no outstanding characteristics in the clinical picture. Two females in early pregnancy aborted about twelve hours before death.

The urine samples always contained many red blood cells, granular and hyaline casts, and + to ++++ albumin according to the sulfosalicylic test, but no sugar. The blood urea nitrogen of animals dying after the fourth day ranged between 190 and 300 mg. per cent, but was not especially elevated in dogs dying twenty-four to seventy-two hours after injection.

In some dogs living more than fifteen days, the hemoglobin values were essentially unchanged, while in others the level was reduced to as much as 40 per cent of the control value. In general, the depression of hemoglobin was proportional to the degree of debility.

Rigor mortis appeared at once after death. At autopsy, the gastrointestinal tract contained only a semifluid chocolate material which gave a positive test for

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blood, but gross bleeding points were not found. The kidneys were enlarged and pale. The lymph nodes all over the body were about three times normal size and firm. Bronchopneumonia was frequent and probably terminal.

Histologically, the renal damage resembled that of mercury poisoning. The tubular destruction ranged from cloudy swelling to severe necrosis with sloughing and blockade. Practically every section of the lymph nodes showed marked nuclear fragmentation and necrosis of the lymphoid elements, with polymorphonuclear infiltration; in some sections the latter was so intense as to suggest a purulent reaction. Figure 9 is a photomicrograph of a typical reaction. This histologic picture seems to have no counterpart so far as human pathology is concerned. The spleen showed a milder degree of this lymphoid reaction. The liver, pancreas, heart, intestine, and bone marrow were negative except for hyperemia; in the lung there was lobular pneumonia of recent origin.

Histologic sections of dogs surviving the acute phase were essentially negative except for mild hyperplasia of the lymph nodes, aplastic changes of the bone marrow, and mild to moderate signs of previous tubular damage. Nevertheless, according to chemical analyses, the liver, spleen, and kidney contained appreciable amounts of gallium (8 to 70 μgm./gm. of wet weight). The bone usually retained more, while the lymph nodes had least. Presumably this metal is held in a biologically inert form.

In animals which died within forty-eight hours after administration of the metal, these changes were only partially developed. Presumably the metal can cause acutely lethal biochemical changes in tissues prior to the more slowly developing uremic syndrome, which also appears to be a result of metal poisoning.

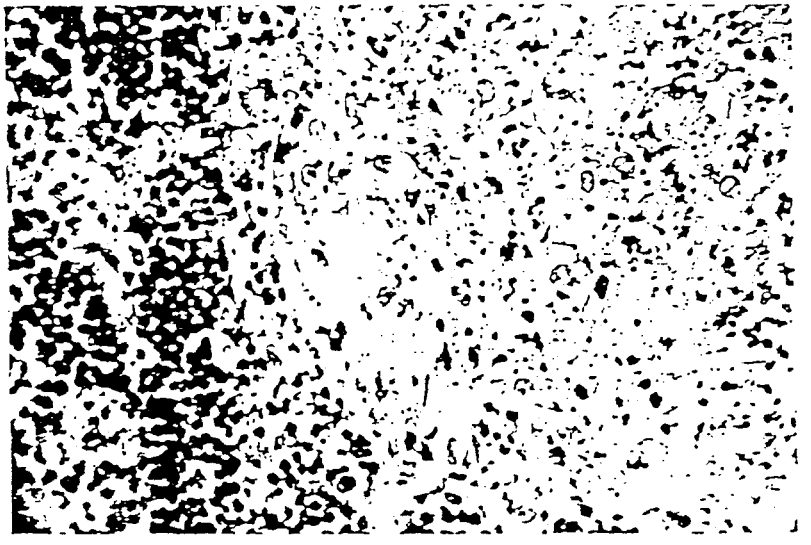

Fig. 9. A part of a follicle and germinal center of the lymph node of a dog dying seventy hours after receiving 35 mg./kg. of gallium citrate intravenously. The nuclear fragmentation and necrosis of the lymphoid elements are quite prominent. The polymorphonuclear infiltration is much greater in other sections, but is obvious here. Such nodes had a lower content of stable gallium metal than other tissues such as liver, which showed no damage. Similar nodes and sections have been forwarded to the Armed Forces Institute of Pathology. ×450.

3. *Toxicity of Repeated Small Doses in Dogs:* Twenty-four dogs, in 3 groups of 8 each, received gallium citrate intravenously in doses of 5.0, 3.5, and 2.5 mg. of gallium per kilogram of original body weight twice a week until death. The immediate reaction by the gastrointestinal system to these smaller doses was compara-

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tively mild, but the toxicity was otherwise accentuated. At the 5 mg./kg. dose level, 6 dogs died after the second dose (10 mg./kg. total) and the remaining 2 after the third. At the 3.6 mg./kg. level, 4 dogs died after the second dose (7.0 mg./kg. total) and 2 each after the third and fourth doses. At 2.5 mg./kg., 2 dogs each died after the third, fifth, seventh, and eighth doses. Dudley (18) has observed this summative effect at 5 mg./kg. on dogs and also on rabbits at the 10 mg./kg. level; it is therefore not a species peculiarity.

Anorexia, debilitation, and weight loss were of about the same degree as with the single large dose, but, being more spread out, were not quite so obvious. The first dose at these levels produced only a moderate illness, but the course became abruptly worse when the second dose was given. Granular casts and erythrocytes were present in large numbers in the urine following the second dose, and albumin was present in ++ to ++++ amounts. The hemoglobin and hematocrit levels decreased moderately in the longest surviving dogs, while the leukocyte count usually increased without particular change in the differential count.

At death, the blood urea nitrogen values ranged from 175 to 250 mg. per cent, but the histologic sections of the kidney suggested a milder degree of damage. The lymph-node reaction was qualitatively the same as in the single dose series, but distinctly more moderate in degree.

DISCUSSION

These data demonstrated such a widely different species sensitivity to intravenously administered gallium citrate that it was impossible to anticipate the probable toxicity in man. The cautious trials reported in Section VI of this series indicate that man is to be grouped with the dog but probably is more resistant. Man withstood the twice weekly doses of 2.5 mg./kg. or more much better than the dog and with total doses as large as 66.7, 70.2, and 111.6 mg./kg. spaced over six to fourteen weeks failed to show lymphadenopathy or urinary changes indicative of renal damage. Nausea, vomiting, anorexia, and debility did occur in man, but in relation to the dose were not nearly as severe as in dogs. Various types of skin reactions, leukopenia, and/or hemorrhages signaled the discontinuance of therapy. The percentage of the dose which was excreted increased as the size of the dose increased, but in absolute terms increased amounts of the metal were laid down in the body when larger total doses were given. This point is discussed further in Section VII of this series (pp. 588–590).

Since a single dose of gallium given to man usually contained between 50 and 75 mc. of Ga^{72}, these toxic reactions cannot be ascribed solely to either the radiation from Ga^{72} or the metal carrier. In fact, it is not unlikely that the two forms of injury complement one another.

Dudley, Henry, and Lindsley (19) compared the toxicity of subcutaneously administered gallium citrate in five species. The ten-day LD 50 values were: mouse, 600 mg. Ga/kg.; rat, 110 mg./kg.; rabbit, 45 mg./kg.; dog and goat, less than 15 mg./kg. In young rats weighing 50 to 100 gm., a value of 230 mg./kg. was found. The LD 50 for intravenous administration in 10 dogs was in the neighborhood of 15 to 20 mg./kg., equaling that reported above. Experiments with gallium lactate (6) demonstrate, however, that the intravenous route approximately doubles the apparent toxicity of the metal.

The toxic syndrome described by Dudley *et al.* (19) in rabbits which had received gallium lactate or citrate subcutaneously agrees in essentials with that observed in dogs in these experiments. In both species the immediate cause of death was uremia secondary to acute damage to the renal tubules. The detailed biochemical studies of Dudley and his associates, on rabbits, supplement the above histologic data on dogs. It should be pointed out, however, that the data presently available do not specify the manner by which gallium intrinsically damages cells. The deaths which occurred twenty-four to forty-eight

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hours after injection were without outstanding histologic lesions, so that the uremic syndrome is perhaps to be regarded as one of the effects of the metal that is particularly prone to kill because of its relation to excretion. Other points for which no explanation can be offered are: (*a*) the lymph-node reaction, seen chiefly in the dog; (*b*) the large species variability in toxicity; (*c*) the excessive effects of repeated small doses of the metal.

The general toxicity experiments can be summarized as follows:

1. The ten-day lethal dose for rats following intravenous administration of gallium citrate was greater than 220 mg. Ga/kg. body weight. Larger amounts could not be given for chemical and physiological reasons.

2. The fifteen-day LD 50 for dogs after intravenous administration of the same preparations was 18.2 mg. Ga/kg. The data plotted as per cent surviving *vs.* log of dose fell very close to a straight line.

3. Dogs given fractional doses intravenously twice a week succumbed after smaller total doses of metal. The toxicity seemed to be accentuated.

4. The clinical syndrome resembled an acute debilitating disease involving particularly the gastrointestinal tract and terminating by uremia. Histologically, the kidneys were chiefly involved as in mercury poisoning. In dogs, a novel feature was an acute lymphadenitis with polymorphonuclear reaction to lymphoid degeneration. These lesions were repaired in animals which survived.

5. The large species differences made useless further work for the purpose of estimating the probable toxicity of the metal for man.

V. Distribution Studies on Gallium[72] in Rats

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The following series of experiments on rats was carried out preliminary to the use of Ga^{72} for the treatment of osteogenic and related neoplasms in man. The reports by Dudley and his associates (6, 10, 11, 17, 20) established that the metal had a definite affinity for bone and actively metabolizing osseous tissues but, because of the tediousness of the chemical method of assay, their data were limited in scope and not adequate as a basis for designing clinical procedures. Subsequently, and concurrently with the experiments reported here, they used the Ga^{72} tracer method to obtain additional data (19, 21–23), which in general agree with those below.

These experiments were planned as prototypes of the clinical studies. Since the metal had never been administered to man, these animal experiments were the sole guide to what might be observed clinically. It was recognized that quantitative data from the rat could not be transferred directly to human beings but that the general biological relations obtained in the rat should hold in man. The objections which might be raised to using the rat for this purpose would also apply to any other species.

The various compounds of gallium used in these experiments were always injected intravenously. This is the only route suitable for clinical administration of the radioactive isotope, since incomplete absorption from subcutaneous or intramuscular deposits would lead to local necrosis; the metal is not absorbed from the gastrointestinal tract (6).

In these experiments the animals were sacrificed at regular intervals to secure a complete sequence of the changing concentrations of metal and isotope in the various tissues. A total of thirty-four tis-

[7] Commander, MC, USN. The opinions or conclusions in this report are those of the authors and are not to be construed as necessarily reflecting the views or endorsement of the Navy Department.

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sues or parts of organs and the excreta were analyzed. Special attention was given to small but vital structures such as the adrenals, pituitary, and eye, where a comparatively low concentration of isotope might result in a disproportionate amount of overall dysfunction. Also, there was always the possibility that the metal might localize selectively in one of these tissues.

Whether Ga^{72} will selectively localize in human osteogenic neoplasms cannot be answered by animal experiments. Even if there were a comparable experimental osteogenic tumor, the final answer would have to come from observations on patients. On the other hand, the experiments below supply information which ought not to come from observations on man or cannot on account of the sporadic and uncertain nature of autopsy material.

EXPERIMENTAL PROCEDURES

The rats were randomized healthy adult males of the Carworth-Wistar strain, weighing 240 ± 30 gm. All injections were given *via* a tail vein while the rat was under light ether anesthesia lasting less than ten minutes. The possible trauma of anesthesia was considered preferable to the struggle or application of force otherwise necessary.

The following procedure was used for intravenous injections: A tuberculin syringe was connected to a 26-gauge needle by a very short piece of intravenous gum rubber tubing and filled with sterile saline. When the needle was in the vein and the saline flowing in very slowly, the solution of isotope in another syringe was added to the inflowing saline by puncturing the connecting tubing. When calcium gluconate was used, it was injected prior to the isotope and washed in with saline. The injection was made very slowly; three to five minutes were required to administer the total volume of 1.5 to 2.0 ml. Because the solution of isotope was not sterile and no attempt was made to sterilize the rats' tails, the procedure should be termed "clean" rather than "sterile"; there was never any evidence of systemic infection in these or other similarly injected animals.

After injection, the rats lived in a special metabolism cage from which the feces and urine could be collected without cross-contamination (24). They were supplied with Checkers and water *ad libitum*. At specified intervals, the rats were slowly viviperfused with 150 to 200 ml. of heparinized saline under light pentobarbital anesthesia. Removal of the blood was necessary because at six and twelve hours after injection the concentration of Ga^{72} was higher in blood than in many tissues, while later it was lower. The tissues to be analyzed, either the whole organ or portions of it, were weighed, transferred to volumetric flasks, dissolved in concentrated HNO_3 with the aid of gentle heat, and made to volume. What remained of the carcass was also dissolved and treated like the samples. The total dose of Ga^{72} was obtained from the sum of the samples and the carcass rather than the supposedly injected dose, which was found to be highly unreliable, particularly when small volumes were given.

The animals were dissected in a standard manner, and in the case of skin, muscle, salivary glands, lymph nodes, etc., the same regions or parts were taken. The right femur was dissected out, freed of skeletal muscle, and the ends of the femur separated from the shaft by oblique tension on the epiphyseal lines. The marrow was not removed from any bone sample. The four incisor teeth were extracted together with their pulpy roots; the latter, according to autoradiographs, were the most active areas of the entire animal. The activity of the skeleton was computed as 7.06 per cent of the body weight times the activity of the femur, which was considered the average for bone (25).

The counting system for Ga^{72} consisted of a 30 mg./sq. cm. carbon cathode glass tube mounted horizontally in a horizontal lead shield. A plastic carriage holding the sample in a 5-cm. machine-made Petri dish moved on a track to a fixed point beneath the tube. The Petri dishes held 10 cm. of the liquid digest and, where possible,

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a 10 ml. volumetric was used for digesting, so that the transfer consisted merely of c ally pouring its contents into the dish. The efficiency of counting, based on millicuriage calculated from electroscope measurements related to a NBS radium needle (Section I), varied between 1.55 and 1.80 per cent with different tubes. The background counting rates ranged from 25 to 34 counts per minute. The samples were counted to give standard deviations of ±2 per cent or less except where the net counting rate was only 1 or 2 times background where ±5 per cent or less was accepted.

Three different representative chemical forms of gallium were examined as follows:

(*a*) $GaCl_3$, which is an ionized soluble salt at *p*H 2.0–2.2 and below; above *p*H 2.2 an insoluble hydroxide forms. The Ga^{+++} ion reacts with nearly every anion in the blood, including proteinate to form insoluble precipitates.

(*b*) Gallium alizarinate, which is an insoluble but highly dispersed chelate or lake at *p*H 7.4. The exact composition was indefinite and it was prepared in small b es as needed.

(*c*) Gallium citrate, which is a stable, soluble complex at *p*H 7.4. It reacts with proteins very slowly, if at all.

Two preparations of the citrate complex were used, the details of which are given in Section II: a very pure complex produced on an ion exchange resin surface having 5 mols of citrate per mol of Ga^{+++} and a less purified but rapidly prepared complex having ~1.2 mols of citrate per mol of Ga^{+++}. This form is identified by the term "radiochemical." It was used routinely for the patients. Apparently the citrate ion was rapidly mobilized after injection because the animals went into hypocalcemic tetany and died unless treated prophylactically with Ca^{++} as Calcium Gluconate Injection (U.S.P). By trial and error, the dose of Ca^{++} was found to be 2 mols per mol of citrate.

Except for one series, only enough radioactivity was injected to give suitable counting rates on the intended day of sacrifice. This resulted in a corresponding variation in dose of carrier, *i.e.*, inert $Ga^{69,71}$. This was not a constant variation, since the specific activity of the gallium was not constant. The maximum specific activity was slightly over 1 mc./5 mg., but usually it was lower due to physical decay during preparation of the solution. To minimize this "batch effect" and to control variations arising from changes in weather, food intake, health of the colony, etc., two rats intended for each sacrifice time over the whole experiment were injected from each freshly prepared batch.

RESULTS

The data from each of the five series of experiments were identical in type and are presented in similar form. In the tables the numbers of rats injected for sacrifice at each time interval and the dose ranges in millicuries per kilogram are shown at the top of each column. Below are the average cumulative percentages of the total dose which were retained or excreted, and the distribution of the excreted fraction between the urine and feces.

The three most useful ways of presenting the distribution data are given in the two lower sections of each table and the companion figure. The *total organ content in per cent of retained dose* indicates the distribution of gallium present in the animal but without reference to the actual amount present. This is useful in judging relative shifts of gallium content on an organ level. Multiplying the retention data by these percentages gives the values used to construct the figures. For brevity, only the organs showing an important uptake of gallium are shown. The skin and skeletal muscle, which comprise about 70 to 75 per cent of the rat's weight, contribute most of the activity lumped under "Remainder."

The *differential absorption ratio*, the DAR of Marinelli (3), is defined as

$$\frac{\text{Activity per gram of tissue}}{\text{Total retained activity/body weight}}$$

By normalizing the gallium content in a tissue relative to dose, excretion, and ani-

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TABLE VII: DISTRIBUTION OF Ga^{72} IN MALE RATS SACRIFICED AT INTERVALS AFTER INTRAVENOUS INJECTION OF Ga^{72}-LABELED $GaCl_3$ AT *pH* 2.0–2.2

(Each value is the average of the number of rats at the top of the column)

Number of rats	5	5	4	5	5
Time sacrificed (hr.)	6	12	24	48	96
Dose					
mg. Ga^{+++}/kg.	0.7–2.6	0.8–6.8	0.8–3.6	1.5–8.0	2.4–8.0
mc./kg.	0.02–0.56	0.02–1.35	0.16–0.80	0.41–2.94	0.47–1.62
Per cent of original dose retained	75.8	61.7	59.8	40.9	32.9
Per cent of original dose excreted	24.2	38.3	40.2	60.1	67.1
Fraction in:					
Urine (%)	99.7	97.1	91.3	93.0	88.9
Feces (%)	0.3	2.9	8.7	7.0	11.1
Total organ content in per cent of retained dose					
Blood plasma*	8.9	3.1	3.1	1.1	0.7
Blood cells	0.7	0.5	0.4	0.1	Negligible
Liver	27.9	19.4	23.2	11.8	19.6
Spleen	0.6	1.0	1.2	0.8	1.4
Kidney	1.4	3.0	2.4	5.9	5.7
Skeleton†	29.4	38.2	30.7	46.2	44.7
Remainder	31.2	34.7	39.0	34.1	27.9
Differential absorption ratio					
Blood plasma	2.6	1.5	0.9	0.3	0.2
Blood cells	0.2	0.2	0.1	Negligible	Negligible
Liver	7.4	5.0	4.9	3.0	3.8
Spleen	2.6	2.8	5.0	2.9	5.1
Kidney	1.6	3.0	2.7	6.1	5.6
Femur	4.2	5.4	5.6	6.4	6.8
Teeth	3.0	4.9	4.6	6.8	12.3
Skeletal muscle	0.2	0.1	0.3	0.2	0.1
Thymus	0.5	0.3	0.4	0.3	0.5
Heart	0.2	0.2	0.2	0.1	0.2
Lung	0.6	0.5	0.6	0.4	0.6
Skin	0.8	0.7	1.1	0.3	0.4

* Plasma and blood cells computed as 7.5 per cent of the body weight times the determined hematocrit.
† Skeleton computed as 7.06 per cent of the body weight times the activity per gram of femur (11).

mal size, the DAR greatly facilitates intercomparison of animals within a group, as well as intergroup and interseries comparisons. Also, because the roentgen is defined in terms of volume, this normalized activity per gram bears a direct relation to the relative dose of radiation received by tissues except in the case of bone.

Each value in the tables is the average of the number of rats at the top of the respective column. In general, these averages truly represent the central tendency, but occasionally a single, or rarely two, high or low values of unexplained origin threw the average out of line. The standard deviations of these means adjusted for small numbers were nearly always within 30 per cent of the mean; the exceptions were within 50 per cent. The mean and median values nearly always coincided. The use of larger numbers of rats per group would have given smaller variances, but the basic information secured by averaging 4 to 7 rats would not have been essentially improved by doubling the number of animals.

1. *The Distribution of $GaCl_3$*

In interpreting these data, it should be kept in mind that gallium existed as Ga^{+++} in this preparation. On entering the vein, it probably reacted with several blood constituents to form precipitates, but there is no information on what these reactions are, or on the subsequent history of the reaction products. Four batches of $GaCl_3$ were prepared in physiological saline at *pH* 2.0 to 2.2; they contained 2 to 4 mg. of Ga^{+++} per milliliter.

The excretion data in Table VII show that most of the excretion took place during the first twenty-four hours after injection and that the urinary system carried nearly

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all of the load. Thereafter, the urinary and fecal excretion were about equal. The appearance of Ga^{72} in the six-hour fecal samples suggests that the metal was excreted by the mucosa of the large intestine. In separate acute experiments,[9] Ga^{72} appeared in hepatic duct bile within three to five minutes after intravenous injection of $GaCl_3$ or gallium citrate. The concentration of Ga^{72} in the bile paralleled the concentration in the plasma, but was some five times greater. The progression of maximal Ga^{72} activity through the gastrointestinal tract, which was analyzed in five sections, indicates that the duodenal region was the source of the most of the activity in the feces. Because the pancreas and the wall of the gut showed low relative concentrations of Ga^{72}, the liver was assumed to be the source of the activity. No part of the genitourinary tract other than the kidneys showed appreciable concentrations of Ga^{72}.

The "biological half-life" (3) was twenty-one hours. The animals which received the highest doses of gallium metal excreted a larger fraction of the dose.

Table VII shows that the liver had the highest relative concentration initially; subsequently it decreased more or less in parallel with the plasma concentration. The differential uptakes of the spleen and kidneys at first were relatively low, but increased with time. The femur (as representative bone) had the highest differential uptake of the twenty-four-hour samples but still only slightly higher than the next three tissues. The incisor teeth showed the highest absorption ratios; most of the activity was in the pulpy roots. The remaining twenty-nine tissues, including skin, muscle, glandular, lymphoid, and nervous tissue, some of which are listed in Table VII, gave no evidence of segregation.

Figure 10 demonstrates that the liver and plasma rapidly lost their initial content, while the spleen, kidney, and skeleton retained essentially the Ga^{72} which they had at twenty-four hours. Therefore, the increased differential absorption ratios of the bone, spleen, and kidney were the result not of a continuing uptake of Ga^{72}, but of the loss of Ga^{72} from the liver and soft tissues such as skin and skeletal muscle.

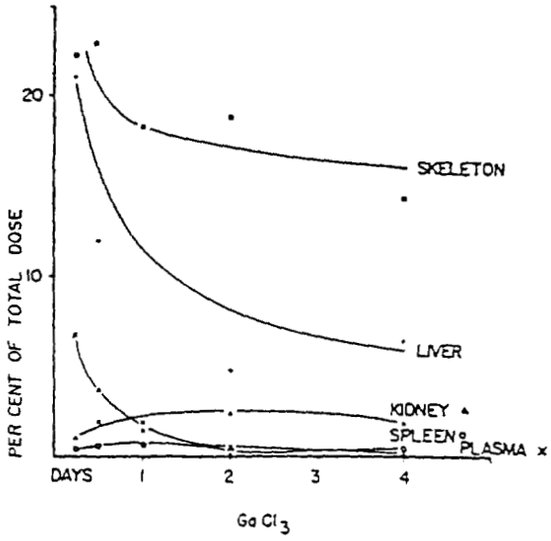

Fig. 10. The percentages of the original total dose of Ga^{72} retained by the organs showing the highest differential absorption ratios. The metal was injected intravenously as $GaCl_3$ at *pH* 2.0 diluted with physiological saline solution.

Comment: Because 68.8 per cent of the initial number of radioactive Ga^{72} atoms decay during the first twenty-four hours following injection, that period is relatively the most important with respect to radiation dose to tissues. Thus, during the first twenty-four hours, the liver received more radiation per gram of tissue than did bone; subsequently, the bone received a higher dose than the liver or other tissues, but the difference after twenty-four hours was comparatively minor and did not compensate for the larger difference during the first twenty-four hours. In any case, the total dose to the bone over the ninety-six hour period (6.7 half-lives) was of the same order as that to the liver and was far short of the five- to ten-fold difference that would be the minimum for therapeutic purposes. In addition, the liver and viscera were subjected to a cross-fire of γ radiation from the Ga^{72} distributed over the trunk; the long bones of the limbs and the bones of the head escape much of this cross-fire (*c.f.* Marinelli, Quimby and Hine, 3).

[9] Performed by Dr. J. W. Archdeacon.

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TABLE VIII: DISTRIBUTION OF Ga^{72} IN MALE RATS SACRIFICED AT INTERVALS AFTER INTRAVENOUS INJECTION OF Ga^{72} ALIZARINATE AT *p*H 7.4

(Each value is the average of the number of rats at the top of the column)

Number of rats	4	6	6	4	4	6
Time sacrified (hr.)	6	12	24	48	72	96
Dose						
mg. Ga^{+++}/kg.	0.2–0.4	0.3–0.4	0.2–0.5	0.3–1.2	0.4–1.7	0.7–1.2
mc./kg.	0.05–0.09	0.06–0.10	0.04–0.11	0.04–0.23	0.07–0.31	0.17–0.28
Per cent of original dose retained	88.2	85.6	58.8	65.9	60.7	58.2
Per cent of original dose excreted	11.8	14.3	41.2	34.1	39.3	41.8
Fraction in:						
Urine (%)	97.8	74.3	76.8	77.3	69.4	
Feces (%)	2.2	25.7	23.2	22.7	30.6	
Total organ content in per cent of retained dose						
Blood plasma*	0.6	0.4	1.3	0.3	0.2	Negligible
Blood cells	0.1	0.2	0.2	0.1	0.2	Negligible
Liver	55.1	51.3	28.8	50.5	46.8	48.3
Spleen	4.0	1.7	4.9	5.5	4.8	7.6
Kidney	1.5	4.4	6.9	2.4	2.0	0.7
Skeleton†	14.7	18.6	51.7	24.1	27.7	20.4
Remainder	24.0	23.4	6.2	17.1	19.4	23.0
Differential absorption ratios						
Blood plasma	1.0	1.1	1.9	0.3	0.2	Negligible
Blood cells	0.3	0.5	0.3	0.1	0.2	Negligible
Liver	14.4	14.4	7.7	13.7	11.3	13.0
Spleen	7.3	4.4	15.3	16.1	15.5	18.6
Kidney	2.0	5.9	9.5	3.5	2.8	1.0
Femur	1.9	2.6	7.2	3.4	3.8	2.9
Shaft	2.2	2.5	7.2	3.7	3.7	3.3
Ends	1.5	2.7	7.2	3.0	2.9	2.4
Heart	0.4	0.3	0.4	0.2	0.2	0.2

* and † See footnotes to Table VII.

In summary, gallium given intravenously as $GaCl_3$ was not distributed differentially to any one tissue rapidly enough or to a sufficient degree to suggest that this form of gallium ought to be administered therapeutically in man. This form of gallium, soluble at *p*H 2.0–2.2, or below, is pharmacologically undesirable for intravenous purposes because it may result in embolism and local irritation.

2. *The Distribution of Gallium Alizarinate*[10]

The high differential absorption ratios of the spleen and liver in the $GaCl_3$ experiments suggested that particulates had formed which the reticulo-endothelial tissue of these organs phagocytized. Thus, these differentials were the result of physicochemical properties which resulted in a non-specific distribution.

To test this possibility, gallium was prepared in a soluble particulate form, a chelate or lake with alizarine. A 10 per cent solution of alizarine red in 4 *N* NH_4OH was added to a solution of $GaCl_3$; on neutralizing this with 1.66 *N* acetic acid, a precipitate formed which was washed three times, then suspended in water and put into solution by use of $NaHCO_3$ and heat. The lake was freshly prepared for each series of rats injected.

The twenty-four-hour data in Table VIII were completely out of line due to anomalous values from 2 of the 6 rats sacrificed at that time. The average of the other 4 rats would have fitted into the sequence but the values on these 2 animals were included since 6 other rats which received the same preparation for sacrifice at later periods did not show anomalous values.

The total excretion of Ga^{72} and its distribution between the urine and feces (Table VIII) were distinctly different than after injection of $GaCl_3$ or gallium citrate. The excretion was comparatively small

[10] These experiments were made by Joe B. Nash.

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during the first twelve hours after injection, and the total excretion was only about two-thirds of that in the other experiments. T[illegible] ticipate the findings in later experiments, this was not the result of low doses of carrier metal. The high percentage of Ga^{72} in the feces was a result of the decreased urinary excretion, because the absolute amount in the feces was similar to that in the other experiments. No value can be given for the biological half-life, but it was more than four days, and by graphical extrapolation ~ 10 days.

Immediately after injection, the liver showed the highest differential absorption (Table VIII). The spleen was about half as active, and bone about one-seventh as active. As the experiment proceeded, the spleen became the most active tissue, passing the liver between twenty-four and forty-eight hours. At no time was bone more than one-fifth as active as the liver or spleen. The liver contained nearly half of the Ga^{72} which was retained, and the skeleton 20 to 25 per cent. In terms of the original dose (Fig. 11), the liver lost about 40 per cent of the Ga^{72} which it had initially, while the spleen and bone retained t[illegible] initial uptake or perhaps added to it. T[illegible] plasma was nearly cleared of Ga^{72} by six hours, in contrast to the forty-eight-hour exponential decrease following administration of the other two gallium compounds.

Comment: The rapid disappearance of the injected material combined with the high differential uptakes of the spleen and liver point to removal of the particulate matter by phagocytic action of the reticuloendothelial cells. On the other hand, the presence of Ga^{72} in tissues such as heart and red cells (Table VIII) indicates that some of the gallium was loosely bound to the alizarine or possibly was liberated by action of the body metabolic processes. In the face of this contradiction, these experiments cannot be regarded as typifying the distribution that would result if an insoluble, inert colloid formed when the gallium was injected intravenously. It is safe to conclude, however, that gallium in particulate form does not show a localization favoring bone. It should be injected in a form which will minimize *in vivo* precipitation.

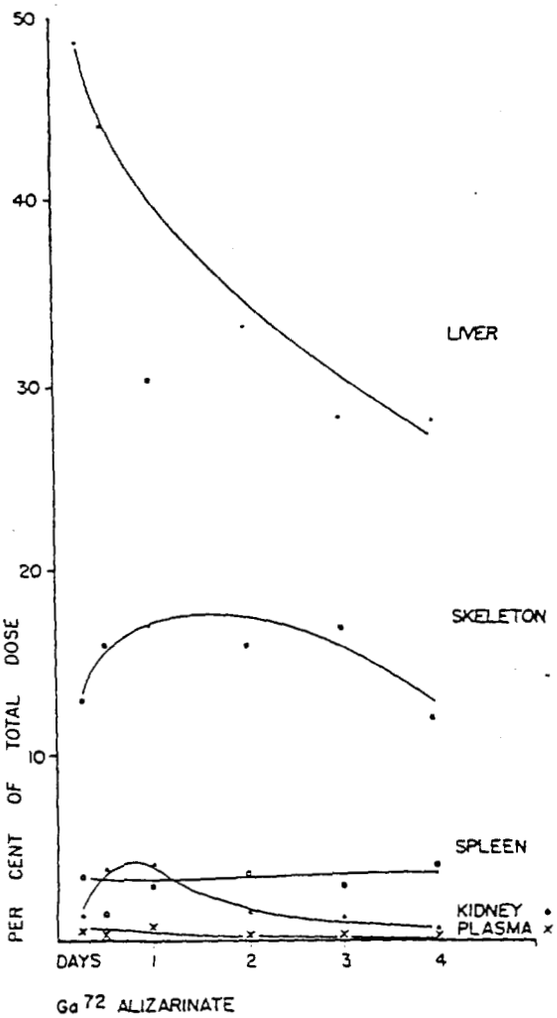

Fig. 11. The percentages of the total dose of Ga^{72} retained by the organs showing the highest differential absorption ratios. The gallium was injected as the alizarinate at *p*H 7.4 diluted with physiological saline solution.

3. *The Distribution of Gallium Citrate: Ion Exchange Preparation*

Work by Dudley (8) indicated that the citrate complex was preferable for intravenous use. A number of other organic and inorganic compounds of gallium were examined chemically (Section II), but none was found more acceptable than the citrate. Manufacture of the pure citrate using Ga^{72} was out of the question because of handling

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TABLE IX: DISTRIBUTION OF Ga^{72} IN MALE RATS SACRIFICED AT INTERVALS AFTER INTRAVENOUS INJECTION OF Ga^{72} CITRATE (pH 7.4) PREPARED ON AN ION EXCHANGE COLUMN

(Each value is the average of the number of rats at the top of the column)

Number of rats	4	4	4	4	4
Time sacrificed (hr.)	12	24	48	72	96
Dose					
mg. Ga^{+++}/kg.	0.2–0.6	0.4–1.4	1.4–2.3	6.0–6.5	6.0–6.9
mc./kg.	0.01–0.02	0.02–0.06	0.06–0.10	0.25–0.27	0.25–0.29
Per cent of original dose retained	74.4	72.8	46.5	27.7	20.5
Per cent of original dose excreted	25.6	27.2	53.5	72.3	79.5
Fraction in:					
Urine (%)	88.6	94.8	91.5	90.8	90.6
Feces (%)	11.4	5.2	8.5	9.2	9.4
Total organ content in per cent of retained dose					
Blood plasma*	8.8	4.2	1.4	1.1	0.3
Blood cells	0.4	0.5	0.4	0.5	Negligible
Liver	10.8	11.5	10.5	4.0	5.8
Spleen	1.1	1.2	1.8	0.5	0.6
Kidney	1.2	1.3	1.4	1.1	1.5
Skeleton†	29.3	27.4	53.6	58.5	67.8
Remainder	48.4	53.9	30.9	34.3	24.0
Differential absorption ratios					
Blood plasma	2.5	1.2	0.4	0.3	0.1
Blood cells	0.1	0.1	0.1	0.2	Negligible
Liver	2.8	3.2	2.7	1.0	1.4
Spleen	4.0	5.2	3.9	2.0	2.6
Kidney	1.5	1.8	1.9	1.6	2.3
Femur	4.3	3.9	8.1	8.7	9.8
Shaft	3.7	3.4	6.1	8.9	9.3
Ends	5.2	4.6	8.9	8.5	10.1
Teeth	3.4	4.3	8.1	9.7	11.6
Calvarium	2.4	2.7	4.4	5.0	7.3
Lung	0.8	0.8	0.7	0.7	0.8
Heart	0.4	0.3	0.3	0.1	0.2

* and † See footnotes to Table VII.

problems and the twenty-four hours necessary for the synthesis. An alternate method of quickly synthesizing a pure citrate complex was developed which made use of the surface properties of an ion exchange resin (9). The material so prepared was never administered to patients, because, as was learned here, the citrate can be rapidly mobilized from the complex and is potentially lethal. The best preparation had a ratio of 5 mols of citrate per mol of gallium. Accordingly Ca^{++} was administered prophylactically in the ratio of 2 mols of Ca^{++} per mol of citrate.

Results: The data have been summarized in Table IX and Figure 12. As with $GaCl_3$, excretion of Ga^{72} proceeded rapidly during the first twenty-four hours after injection and tapered off exponentially. The total excretion was 80 per cent of the injected dose, in contrast to 60 per cent for $GaCl_3$. Most of the excretion took place by way of the kidney, and it continued for seventy-two hours instead of being confined chiefly to the first twenty-four hours after injection. The fecal excretion was greatest between twenty-four and forty-eight hours.

The liver, spleen and bone had about the same differential absorption ratios twelve hours after injection (Table IX); at ninety-six hours, the differential absorption ratio of the femur was seven times that of the liver and four times that of the spleen. This shift in favor of the bone began to take place between twenty-four and forty-eight hours. Figure 12 shows that the differential favoring bone occurred by virtue of the bone losing relatively less of its initial uptake than did the liver, plasma, kidney, and spleen. In this series of experiments the ends of the bone were somewhat more active than the shaft; in the other experiments the shaft was definitely more

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active. The calvarium, a typical flat bone, was less active than the femur, but the changes of differential absorption ratio paralleled those of the femur. The mandible, not shown in Table IX, had a differential absorption ratio about midway between the femur and calvarium.

Comment: In terms of radiation dose, the bone did not receive a differential which would be therapeutically useful, since the differential absorption ratio in favor of bone occurred after 70 per cent of the radiation had been expended. Nevertheless, this was an improvement over the $GaCl_3$ distribution.

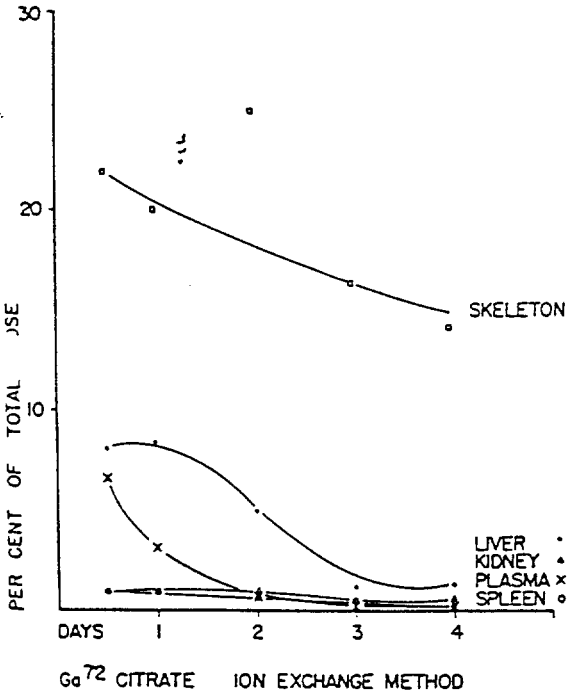

Fig. 12. The percentages of the total dose of Ga^{72} retained by the organs following intravenous injection of the gallium citrate complex at *p*H 7.4 and preceded by calcium gluconate. The citrate was prepared on an ion exchange column (9) and had 5 mols of citrate per mol of gallium.

4. *Distribution of Gallium Citrate; "Radiochemical" Preparation (Variable Dose)*

The "radiochemical" preparation of gallium citrate contained approximately 1.2 mols of citrate per mol of gallium. It was impure in that NO_3^-, Cl^-, and Na^+, introduced during the preparation, were not removed and the citrate was not separated or crystallized. On the other hand, this synthesis plus an assay of radioactivity required less than ninety minutes. Ca^{++} was employed in the usual proportion of 2 mols per mol of citrate.

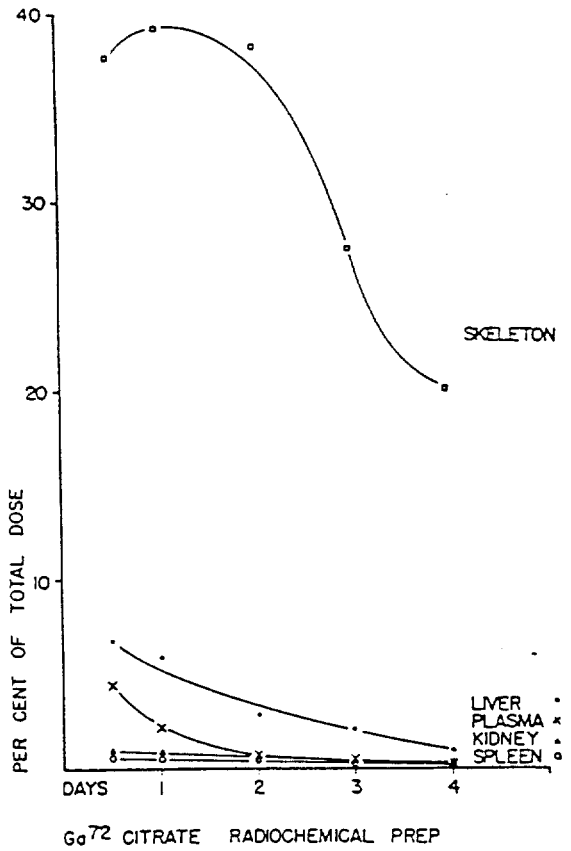

Fig. 13. The percentages of the total dose of Ga^{72} retained by the organs following intravenous injection of the gallium citrate complex prepared by the "radiochemical" method described in Section II. The solution contained 1.2 mols of citrate per mol of gallium at *p*H 7.4. The dose of carrier gallium increased with the length of time before sacrifice.

Results: As with the ion exchange preparation, the most rapid excretion occurred during the first twenty-four hours after injection and continued at a reduced rate over the next two days; 77 per cent of the initial dose was excreted in ninety-six hours (Table X). The urine was the chief route of excretion, during the early observations, but after a longer interval the urinary and fecal loads were about equal. The biological half-life was 20.6 hours.

In these experiments, where there was a

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TABLE X: DISTRIBUTION OF Ga^{72} IN MALE RATS SACRIFICED AT INTERVALS AFTER INTRAVENOUS INJECTION OF Ga^{72} CITRATE (*p*H 7.4) USING VARIABLE DOSES OF THE "RADIOCHEMICAL" PREPARATION

(Each value is the average of the number of rats at the top of the column)

Number of rats	5	5	5	5	5
Time sacrified (hr.)	12	24	48	72	96
Dose					
mg. Ga^{+++}/kg.	0.5	0.5	2.1	6.8	26.0
mc./kg.	0.08	0.11	0.45	1.4	5.3
Per cent of original dose retained	65.0	59.4	47.5	37.5	23.4
Per cent of original dose excreted	35.0	40.6	52.5	62.5	76.6
Fraction in:					
Urine (%)	85.3	94.2	91.7	90.3	89.7
Feces (%)	14.7	5.8	8.3	9.7	10.3
Total organ content in per cent of retained dose					
Blood plasma*	6.8	3.6	1.0	0.6	0.5
Blood cells	3.9	3.1	2.0	1.1	0.1
Liver	10.4	9.8	5.9	5.3	3.9
Spleen	1.0	1.1	0.8	0.4	0.3
Kidney	1.3	1.3	1.3	0.9	0.8
Heart	0.1	0.1	0.1	0.1	Negligible
Skeleton†	58.0	65.9	80.6	73.8	89.2
Remainder	20.5	15.1	8.3	17.8	6.2
Differential absorption ratios					
Blood plasma	1.5	0.8	0.3	0.2	0.1
Blood cells	0.1	0.1	0.1	0.1	Negligible
Liver	2.1	2.1	1.3	1.1	0.9
Spleen	2.1	2.2	1.7	1.2	1.0
Kidney	1.7	1.5	1.4	0.9	0.9
Femur	8.1	9.3	11.4	11.3	14.1
Shaft	10.2	11.7	13.5	13.6	18.2
Ends	6.3	7.4	9.5	8.8	10.5
Teeth	2.6	2.7	5.1	5.6	6.8
Calvarium	3.5	4.4	6.1	5.6	8.7
Heart	0.3	0.2	0.2	0.1	0.1
Lung	0.7	0.5	0.3	0.4	0.2
Lymph nodes	0.6	0.7	0.7	0.3	0.3

* and † See footnotes to Table VII.

wide variation in the amount of carrier gallium injected, it became clear that the percentage of the total dose which was excreted was related to the size of the dose of metal: The higher the dose, the larger the percentage excreted; however, in absolute milligrams of gallium, more of the metal was retained after the larger doses. This effect is further discussed below.

The differential absorption ratio of the femur twelve hours after injection was four times higher than that of the next highest tissues, the spleen and the liver (Table X). This difference increased as the experiment proceeded; at forty-eight, seventy-two, and ninety-six hours the bone was 6.7, 9.5. and 14 times more active than the next highest tissue. As shown in Figure 13, the soft tissues retained only one-fourth or less of their initial content, while the bone retained about half.

The shaft of the femur was not purely cortical bone, since the epiphyseal line, which has a very high uptake according to autoradiograms (17) (Section IX), remained attached to the shaft. The marrow, which was not removed from the shaft, could hardly be the source of the activity, since the trabecular bone of the ends of the femur was still more cellular, but less active. The calvarium, composed of tabular bone and some marrow, was about half as active as the femur.

Comment: This series, in contrast to the preceding, showed differential absorption ratios which would support the use of Ga^{72} to irradiate osteogenic neoplasms in man. The data indicate that the bone received four to five times as much radiation as the liver and possibly fifteen to twenty times as much as the soft tissues during the first twenty-four hours following injection.

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Subsequently, the differential in favor of bone was still larger, but only 30 per cent the activity remained. However, two sources of generalized radiation not immediately apparent from the tables tend to reduce the differential. One is the Ga^{72} circulating in the plasma and red cells; this results in whole-body irradiation, but it appears to be negligible. The other is the activity stored in the urinary and gastrointestinal tracts prior to excretion; this is significant in view of the ~ 50 per cent of the dose which was excreted during the first day.

It is suggested tentatively that gallium citrate manufactured by this particular method has a special affinity for bone and little affinity for the viscera. The relatively low uptake by the spleen and liver probably is associated with the non-particulate nature of the complex, and later with slow liberation of the metallic ions. These observations would seem not to be sporadic experimental artifacts, since four different batches of the radiochemical preparation were used.

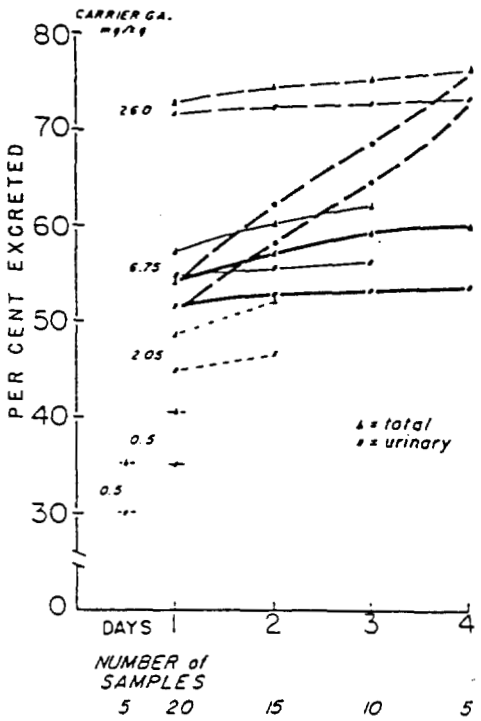

Fig. 14. The urinary and total excretion data of Table X broken down to show the relation of dose of metal to per cent excreted. See text for discussion.

Relation of Size of Dose of Gallium to Amount of Gallium Excreted

The data collected in Table XI were taken from sections *1*, *3*, and *4* above, together with one value from Section X, the last of this series. They demonstrate clearly that an increased dose of gallium was associated with an increased percentage of the dose excreted in the urine during the first twenty-four hours after administration. Despite this increased percentage excretion, the retained milligrams of gallium per gram of tissue increased as the dose increased. Data taken from the clinical studies in Section VI show a similar relationship. There was a definite tendency for a given individual to exhibit a consistently high or low rate of excretion at the different dose levels; age, sex, and severity of illness had no apparent relation to this tendency. The last column of Table XI brings out the fact that relatively more of the dose was excreted by way of the urine as the dose increased. The significance of these two relationships is not evident.

TABLE XI: EXCRETION OF Ga^{72} IN THE URINE OF RATS IN PER CENT OF THE INJECTED DOSE DURING THE FIRST TWENTY-FOUR HOURS

	Dose of Ga^{+++} in mg./kg.	Per cent Excreted	Urine/feces*
$GaCl_3$ (Result A)	0.8–3.6 1.5–8.0 2.4–8.0	36.7 53.8 52.3	10.5 22.0 19.4
Ion exchange gallium citrate (Result C)	0.4–1.4 1.4–2.3 6.0–6.5 6.0–6.9	22.8 44.8 67.4 74.2	4.9 14.4 43.2 32.3
"Radiochemical" gallium citrate (Result D)	0.5 2.1 6.8 26.0	35.0 44.9 54.9 71.7	6.3 11.7 20.2 60.7
Carrier-free gallium citrate (Section X)	Less than 10^{-4}	21.4	2.3

* The ratio of the relative amounts in urine and feces.

Examination of the excretion data of the preceding experiment revealed a peculiarity

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TABLE XII: DISTRIBUTION OF Ga^{72} IN MALE RATS SACRIFICED AT ONE, TWO, AND FOUR DAYS AFTER INTRAVENOUS INJECTION OF GALLIUM CITRATE (*pH* 7.4), THE "RADIOCHEMICAL" PREPARATION

(E alue is the average of the number of rats at the head of the column. Same dose series)

No. of rats	6	7	7
Time sacrificed (hr.)	24	48	96
Dose			
mg. Ga^{+++}/kg.	28.5	28.5	28.5
mc./kg.	4.8–6.3	4.4–6.1	4.4–6.3
Per cent of original dose retained	29.5	26.4	24.3
Per cent of original dose excreted	70.5	73.6	75.7
Fraction in:			
Urine (%)	94.0	90.7	90.7
Feces (%)	6.0	9.3	9.3
Total organ content in per cent of retained dose			
Blood plasma*	1.2	1.2	0.5
Blood cells	0.2	0.1	0.1
Liver	8.2	6.7	5.5
Spleen	0.1	0.3	0.3
Kidney	2.4	1.4	1.0
Heart	0.3	0.3	0.3
Skeleton†	72.4	77.0	79.5
Remainder	15.2	12.5	12.8
Differential absorption ratios			
Blood plasma	0.3	0.3	0.1
Blood cells	Negligible	Negligible	Negligible
Liver	2.4	1.9	1.7
Spleen	0.4	1.4	1.2
Kidney	1.4	1.7	1.1
Femur	10.3	11.3	11.2
Shaft	11.6	13.9	13.1
Ends	8.8	8.7	9.5
[illegible]h	5.3	7.2	7.1
[illegible]ph nodes	0.2	0.3	0.3
Heart	0.1	0.1	0.1
Lung	0.3	0.4	0.4

* and † See footnotes to Table VII.

series were slightly higher, and for the femur and its components were slightly lower than those in the "variable dose" series (Table X). The differential favoring bone over the next highest tissue was 4 at twenty-four hours and 8 at ninety-six hours; the corresponding ratios in the variable dose series were 4 and 14, respectively. The distribution among the organs in per cent of the retained dose was essentially the same as that for the variable dose series except that the ninety-six-hour value for the skeleton was lower and the liver content was consistently slightly higher.

The curves in Figure 15 are flatter and placed lower on the scale than those in Figure 13. This follows from the higher retention figures of Table X which, as pointed out before, are of uncertain interpretation. When allowance is made for the dose-retention effect, Figures 13 and 15 are quite similar.

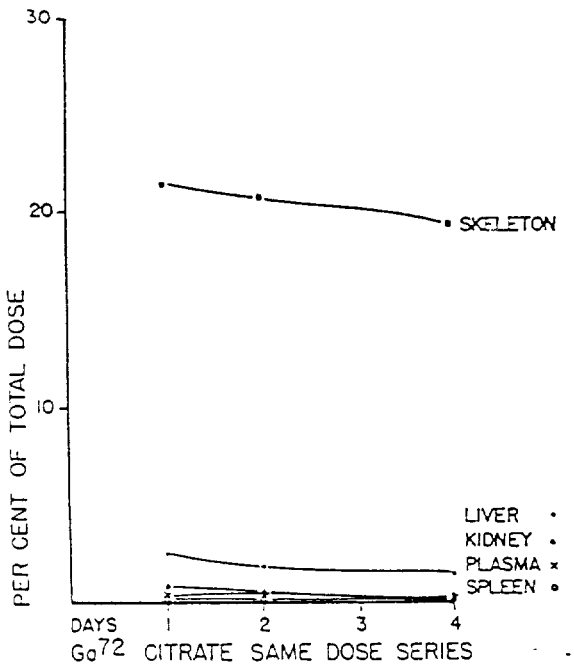

Fig. 15. The same as Fig. 13 except that all animals received 28.5 mg./kg. of carrier gallium.

Comment: The data demonstrate that the size of the dose of metal had negligible effect on its percentage distribution in the rats and its relative concentration in the various tissues. In terms of weight of metal, however, the gallium retained in the body was greater. For example, 50 per cent retention of a 2.5 mg./kg. dose would be 1.25 mg./kg., in contrast to 20 per cent retention of 25.0 mg./kg., which would be 5 mg./kg.

The clinical implications of this experiment are: A larger dose of inert carrier metal would increase the amount of metal and associated radiation which would pass through the kidneys and urinary tract; this is undesirable. On the other hand, small repeated doses ought to lead to more rapid accumulation of metal in the tissues and limit the radiation dose because of metal toxicity. This is a serious problem where the specific activity of the radioisotope is low, as with Ga^{72}.

While this experiment was performed as

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a control on the effect of dose of carrier metal, it also partially controlled the possibility that the concomitant dose of radiation influenced the results. The twenty-four-hour data checked with the twenty-four-hour data in Table X from rats receiving only about 2 per cent as much radiation. The experiments in Section X also agree that the radiation dose was not an important factor.

DISCUSSION

According to the experiments reported here, the physicochemical state of the gallium compound influenced the distribution of the metal following intravenous administration. When gallium was given as an insoluble particulate at *p*H 7.4, the liver and spleen exhibited the highest relative concentrations of the metal. When it was injected as a soluble citrate complex at *p*H 7.4, the bone showed the highest differential uptake, and this increased during the following three days. The differential favoring bone was the result of the bone taking up initially a larger fraction of the dose and holding it more firmly than the next three highest tissues, the liver, spleen, and kidney. From the standpoint of administering radiation by use of a radioactive isotope, this way of achieving a differential uptake is less preferable than if the bone took up increasing amounts as the experiment proceeded, regardless of the liver and other organs.

The gallium citrate complex prepared by addition of predetermined amounts of reagents gave the highest differential absorption ratios for bone. This was fortunate in view of the difficulties of synthesizing a chemically pure compound with some 500 mc. of an isotope whose half-life is 14.3 hours. The differential absorption ratios obtained with this complex were good enough to justify its use in clinical studies. However, unless a dilute solution of citrate complex is infused very slowly, Ca^{++} as Calcium Gluconate Injection (U.S.P.) should be injected prophylactically.

The data clearly show that a smaller fraction of the dose of gallium was retained as the dose increased from 0.5 to 28 mg. of Ga^{+++}/kg.; a similar effect was demonstrated for man. This dose effect, however, apparently was confined to the excretion-retention processes, since neither the differential absorption ratios nor the percentage distribution data were affected.

A secondary result of this primary dose-retention effect is that the excretory and detoxifying mechanisms will be put under stress out of proportion to the increase of absolute amounts of Ga^{72} taken up by the bone. This damage may be automatically limited, however, in that man probably cannot tolerate single doses of gallium, as gallium citrate, in excess of 10 mg./kg. The data here do not indicate what level of carrier gallium might be the best compromise between absolute retention, relative retention, and potential toxicity for man.

Because of the short half-life of Ga^{72}, the differential radiation dose to bone is greatly influenced by the degree of rapidity with which the differential uptake in bone takes place. Obviously, the above data on the rat gave no clues on how rapidly a practical absorption favoring bone will be established in man, if at all. The practical value of these rat data is that they show that such a differential radiation dose can be obtained. Only clinical studies on man, using biopsy or autopsy material, can give the necessary information. Whether osteogenic neoplasms will take up gallium is a still further extension.

SUMMARY

1. Gallium labeled by Ga^{72} was injected intravenously into five different series of rats for the purpose of studying the distribution of the metal in animal tissues. The experiments were designed so as to serve as a prototype for the subsequent clinical studies.

2. Following the injection of $GaCl_3$, an acidic (*p*H 2.0), ionized salt which probably reacted with the blood constituents, there was no distinct differential localization of the element during the first day. Thereafter, the bone had the highest differential

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absorption ratio, but this was due to the fact that the other tissues retained their initial content of gallium less firmly than bone. The excretion was chiefly *via* the kidney and exceeded the 50 per cent level at slightly more than twenty hours after injection. Because Ga^{72} has a 14.3-hour half-life and the development of the differential was slow, the liver and bone received about the same cumulative radiation doses.

3. Gallium alizarinate, a soluble particulate lake or chelate, gave a distribution entirely different from the chloride. The spleen and liver had very high differential absorption ratios; the bone was never more than 20 per cent as high. The ten-day total excretion was 50 per cent, with the urine and feces contributing about equally. This distribution appears to be associated with phagocytosis of the particles by the reticulo-endothelial system, but it is probable that some gallium was loosely bound to the alizarine or some of the lake was broken down by the body metabolic processes. The experiment indicates that a particulate form of gallium or a compound which reacts to give fine particles on injection would not segregate to a sufficiently high degree in bone.

4. A very pure gallium citrate complex prepared on an ion exchange resin had 5 mols of citrate per mol of gallium and required prophylactic use of Ca^{++}. More than 50 per cent of the dose was excreted during the first twenty-four hours after injection, chiefly by way of the urine. The bone at twenty-four hours after injection had about the same differential absorption ratio as the liver and spleen, but due to the fact that all other organs retained the metal less firmly than bone, its differential absorption ratio at ninety-six hours was four and seven times greater than that of the next two tissues. The tendency toward a differential favoring bone was definite, but because it developed so slowly, the differential radiation dose to bone was not sufficient for therapeutic purposes.

5. Another type of gallium citrate complex was prepared by adding predetermined amounts of reagents to the activated Ga^{72}-NO_3; it had 1.2 mols of citrate per mol of gallium, but also required a prophylactic injection of Ca^{++}. By twenty-four hours after injection the differential absorption ratio of the femur was four times that of the liver and spleen, and at ninety-six hours some fourteen times. As in the previous experiment, this increasing differential was the result of the bone losing relatively less of its initial uptake than did the other tissues. The cumulative differential radiation dose to bone was large enough to warrant trial of this preparation of gallium in man.

6. An examination of the excretion data of the preceding experiments demonstrated that the percentage of the dose which was excreted increased as the size of the dose increased. Also, the ratio of urinary to fecal excretion increased with the size of the dose. The ninety-six-hour urinary excretion by 62 patients showed a similar relation to the dose. These changes in excretion suggested the possibility of an effect of size of dose (metal and/or radiation) on the differential absorption ratios and the distribution of the retained fractions.

The dose-excretion relation was responsible for the observation that different average excretion curves could be obtained depending on the choice of method of calculation.

7. All rats received the same large dose (28.5 mg. Ga^{+++}/kg.) of the radiochemically prepared citrate complex regardless of the time of sacrifice. The excretion pattern corresponded to that of animals in the preceding series which received similar high doses. On the other hand, the differential absorption ratios and the percentage distribution of the retained doses were similar to those of animals which had received doses only 2 to 5 per cent as large. Thus, large doses of carrier gallium had no significant effect on the distribution of the metal in the tissues, although the excretory pattern was definitely affected. Concomitant variations in the radiation dose appeared to be unimportant in causing these dose effects.

8. The rat data indicate that the radio-

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chemical preparation of gallium citrate is suitable for use in clinical studies. They do not provide information as to what dose will give the highest specific uptake in bone consonant with the least metal toxicity and total-body irradiation.

VI. Clinical Studies with Gallium[72]

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BACKGROUND OF THE CLINICAL TRIAL OF GALLIUM

Since radiogallium seemed to have selective deposition in bone and primarily in centers of osteogenic activity, it was considered worth while to investigate the therapeutic possibilities in human tumors involving the skeletal system. Since there was very little previous clinical experience with this isotope, a flexible program was started and was continuously modified as experience indicated.

In general, two groups of patients were studied. In one group a definite attempt was made to produce a therapeutic effect with Ga^{72} treatment. The second group was composed of patients in whom no therapeutic attempt was made, but rather in whom localization or uptake of Ga^{72} in the various tumors or sites was studied. All the patients in both groups, with one exception, had ultimately fatal neoplasms not amenable to curative surgery or radiotherapy. Those in the "therapeutic" group were, in general, in a more advanced stage of disease and were completely beyond even palliation from conventional forms of therapy. In many instances, the initial tracer studies helped to determine whether or not a prolonged therapeutic attempt would be undertaken. Bone tumors of all types which were available to us, both primary and metastatic, were studied, and occasionally tumors of other types when opportunity arose. In this latter group were three cases of chronic leukemia, one of Hodgkin's disease, and a few other neoplasms without skeletal involvement.

MODE OF ADMINISTRATION AND DOSAGE

The radioactive gallium was available only on Mondays and Thursdays, because of the schedule of the Oak Ridge National Laboratory Reactor Division. Because of the 14.3-hour half-life, the isotope was administered shortly after it was removed from the reactor. The program of biweekly treatment was believed to be fairly satisfactory and might have been chosen even if the availability of the isotope had not been a determining factor. It would be quite impractical to give a therapeutic amount in a single dose. Such a dose would produce personnel hazards insurmountable at the present time and, because of individual sensitivity to radiation effect, it would be impossible to estimate accurately the proper dose. Further, the toxicity of the stable gallium precluded giving all of the desired dose at a single time. Fractional treatments at three- and four-day intervals appeared to allow for a reasonable protraction of the treatment and adjustment of the individual dose; but it is pointed out that this was a rather arbitrary factor in the experimental trials.

The gallium was always administered intravenously. Very little work has been done on the absorption of gallium from the gastrointestinal tract, and it might be feasible to give this material orally. However, the extremely short half-life makes this route of administration undesirable. Even if the gallium were very rapidly absorbed, the stomach and upper small intestine would receive a high dosage of radiation. It is obvious that the ratio of absorption time to half-life is an important factor in determining the relative amount of radiation delivered to the stomach and small intestine as compared with other tis-

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sues of the body; when this ratio is high, as it must be in the case of a very short half-life isotope, there is a distinct disadvantage in the oral route. The intravenous route also has the advantage of assuring a known dosage.

Normal tissue and whole-body tolerances for amounts of radiogallium necessary to produce a significant effect upon malignant tissues were unknown. Preliminary calculation and small-animal experiments had indicated that the accepted radiation dosimetry as applied to other isotopes would be of little value in calculating radiation dosage to tissues. It was therefore necessary to utilize the hematologic picture to assess the damaging effects of whole-body irradiation, and clinical and roentgenographic experience in evaluating a therapeutic response. In the "therapeutic" group, doses which were believed to be moderate were given and gradually increased to a toxic level. In two patients our estimates of safe dosage limits were in error and the radiogallium is believed to have hastened death.

The doses, which ranged from 10 to 100 mc., were always given by an intravenous drip apparatus described earlier in this study. The material was poured into this apparatus from a bottle held with tongs, and no close handling of the radioactive gallium was necessary. Doses below 10 mc. were generally given with a 10 c.c. syringe contained in the metal syringe protector which has previously been described. The large doses could be measured quite satisfactorily by means of a Lauritsen electroscope. The small doses represented fractional volumes of larger samples assayed by the electroscope, and these small doses often were not very satisfactorily measured in the syringe, because of bubbles, which obscured the exact degree of filling and also because of the residual gallium which remained in the syringe after injection.

PROGRAM OF STUDY

In all patients studied the diagnoses were confirmed by histologic sections, except for a few with malignant plasmocytoma and leukemia, in whom the diagnosis was based upon cytologic studies. The studies in the Medical Division included a routine history and physical examination. Routine urinalyses and extensive x-ray examinations were performed. Blood chemistry examinations included determinations of serum calcium, phosphorus, alkaline and acid phosphatase. Kidney and liver function tests were obtained in the patients who were to receive large amounts of gallium. Additional work was done as seemed desirable in individual cases.

Frequent—in some cases daily—hematologic studies were performed. These included a white count, differential count, chamber eosinophil count, and estimate of platelets. At less frequent intervals, hemoglobin determinations, hematocrit readings, and red cell counts were done. The bone marrow from many of the patients was examined before, during, and after therapy. The blood chemistry determinations, particularly calcium, phosphorus, and phosphatase, and the studies of liver and kidney function were repeated during and after the courses of the therapy in an effort to detect various changes due to radiation or stable metal toxicity. Repeated x-ray examinations were probably the most important method of evaluating the course of the disease. Thirty-five-millimeter colored photographs were also used in some patients to show comparative sizes of external lesions.

METHODS OF STUDY OF LOCALIZATION OF GALLIUM[72]

Methods used to determine the localization of gallium[72] varied with the individual patient, depending upon the type of lesion and suitability for biopsy. In many patients external radioassays were made. Most of these were done with a highly collimated Geiger-Müller tube, which made possible rather exact localization. Patients who received very small doses of gallium could not be studied with this instrument because of its relative insensitivity, and

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for these a tube with less directional shielding was used.

Biopsies were performed upon many patients, usually forty-eight hours after the last dose of gallium. This time interval was rather arbitrarily chosen; it was believed to be long enough to allow differential localization and short enough to give suitable levels of activity for study with a reasonable dose to the patient. In most instances an attempt was made to obtain a portion of the neoplastic lesion with an area of adjoining normal tissue, which for the most part was bone. We also tried to obtain skin, muscle, and any other easily available, relatively normal tissue to serve as a basis of comparison. Blood appeared to be a most suitable normal tissue for this purpose, and blood radioactivity levels were obtained simultaneously with the biopsies in many cases. In each biopsy study, the tissues were carefully studied grossly and divided into portions for specific determinations. Radioassays were made upon acid digests of tissue, and for each type of tissue an adjacent area was saved and labeled for histologic sections. Gross and microscopic autograms were also prepared on many of the biopsy specimens, and some of the tissues from which gross autograms were made were subsequently studied by histologic sections corresponding to the various areas of the gross autogram. Routine histologic sections were made on every biopsy, but during the early part of the study these were not always chosen and labeled in such a way that the exact tissue at the points of activity in the gross autogram could be identified.

Autopsy material was obtainable for 11 of the patients who received large doses. When feasible, an effort was made to administer a dose of radioactive gallium at a suitable time before the expected time of death so that radioassays could be made. In one patient radioactive phosphorus and gallium were given simultaneously, in another sulfur and gallium, in an effort to study comparative distributions. At the autopsies, the usual gross and histologic studies were carried out, and extensive radioassays and autoradiograms were also obtained.

TOXIC MANIFESTATION

One of the chief problems throughout this study was the evaluation of the toxic manifestations which developed. Since there was no previous human experience with stable gallium, the manifestations of toxicity to the metal were unknown. The characteristics of total-body irradiation damage or radiation damage arising primarily from an isotope localized in bone were quite well known; but since there is no way of measuring the effective dosage to the bone marrow of this type of radiation, there is a lack of quantitative data to evaluate chemical *versus* radiation effects. Because of the fact that it was not possible to obtain a high specific activity of Ga^{72}, it was necessary to give amounts of stable gallium which we believed to be somewhat toxic.

Patients usually tolerated the first one or two doses very well and many of them said they could not notice any effects whatever from the treatment. Some felt rather drowsy during the six or eight hours following the intravenous infusion. This drowsiness tended to persist uniformly in individuals who received many injections. Patients who received only three or four injections of gallium did not notice delayed effects. Those who received "therapeutic" amounts began to notice anorexia after the third or fourth dose, and with continued treatments severe nausea and vomiting developed. Some patients maintained good appetites but vomited promptly after each meal. Others refused to eat any significant amount after they were well into the course of treatment. Many could eat breakfast but would not take any other meal during the day. As the course of treatment progressed, vomiting immediately after administration of the dose became prominent. In some individuals whose personalities did not lead us to believe that they would be particularly open to suggestion, a "conditioned reflex" vomiting occurred as soon as they saw the gal-

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lium coming into the room. A frequent symptom was a peculiar metallic taste during the period of treatment. The gastrointestinal symptoms were the most uniform manifestations of toxicity with large doses. Two young patients, however, who received rather large amounts had only minimal anorexia.

Prominent dermatologic changes were seen in many patients, but they were not uniform in type or predictable in degree. They usually developed after the fifth or sixth injection of gallium or within the second, third, or fourth week of treatment. Mild degrees of folliculitis were sometimes seen, while in other instances an extensive maculopapular rash developed, and in a few cases there was a generalized exfoliative dermatitis. Itching was very severe with almost all of these lesions. Some of them were associated with rather prominent edema of the skin and subcutaneous tissue. All of the dermatologic manifestations cleared up within a few days after cessation of therapy.

In two patients very small retinal hemorrhages were noted during the latter part of the gallium therapy.

Hematologic changes were prominent and were usually of primary importance in determining when the treatments should be discontinued. After from one to five "therapeutic" doses of gallium, depending on the dose size, the changes in the blood picture became apparent. The first of these changes was a relative lymphopenia, often associated with a prompt decrease in the total white cell count. Somewhat later the platelets decreased in number. Slight changes in the red cell values began to occur quite promptly, but the more severe effects were delayed and the anemia usually reached its greatest severity some weeks after the end of the course of treatment. Bone marrow studies done before treatment and during the greatest hematologic depression showed that the gallium treatment caused a profound hypoplasia of all the marrow elements. Red cell precursors, plasma cells, reticulo-endothelial elements, and a few other primitive cell types were the chief nucleated forms persisting in these very hypoplastic marrows.

Recovery was seen in the hematologic picture rather soon after cessation of gallium therapy in most instances. The red cell regeneration, however, lagged behind that of the white cells and platelets. Clinical manifestations of the severe bone marrow depression consisted of a susceptibility to infection, particularly pneumonia, and in some instances a prominent bleeding tendency. Of two patients who died during profound bone marrow depression, one had evidences of both bleeding and infection, while in the other infection was most prominent. These two patients died in spite of antibiotics, blood transfusions, and toluidine-blue therapy.

In the other patients, who had milder bone marrow depression but prominent gastrointestinal symptoms, many types of symptomatic therapy were used, including pyridoxine, Dramamine, sedatives, antihistaminics, and intravenous fluids. Several patients with bone marrow depression received blood transfusions during the course of therapy.

It was difficult to determine to what extent the toxic manifestationf could be attributed to radiation alone and to what extent they were caused by stable metal toxicity. There was little doubt that both factors were important, but the relative role played by each was difficult to quantitate. A single patient, No. 110088, was given a series of injections of stable gallium comparable to those in a therapeutic course of radioactive gallium. A small tracer amount of radiogallium was administered with each dose, for measurement purposes. It was believed that this patient received too little radiation to be significant clinically. In general, he tolerated the course of treatment with much less evidence of toxicity than did the patients who received a large amount of radiation. He maintained his weight, although he had some nausea and anorexia. During the course of treatment, he had a viral or fusospirochetal infection, which was believed to be incidental, but which impaired his general

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TABLE XIII: SUMMARIES OF CASES IN WHICH Ga^{72} WAS GIVEN IN LARGE DOSES

(N.D., No data; 0, None; +, Little; ++, Moderate; +++, Pronounced)

Case, Sex, and Age	Diagnosis	Mc. Ga^{72} Mg. Ga, and Mg./Kg.	Hematologic Depression	Anorexia Nausea Vomiting	Skin Rash	Evidence of Concentration in Tumor: External Counting	Evidence of Concentration in Tumor: Operation or Biopsy	Evidence of Concentration in Tumor: Autopsy	Clinical Impression
P.D.G. 010003 ♀14	Osteogenic sarcoma, with pulmonary metastases. Primary amputated	50.3 mc. 251.4 mg. 8.5 mg./kg.	0	0 (due to gallium)	0	++	N.D.	+++	Patient received a relatively small amount of gallium when in nearly terminal condition. No evidence of effect on course of the disease
C.R.M. 010007 ♂40	Plasma-cell myeloma	162.5 mc. 813.0 mg. 13.7 mg./kg.	+	+	+++	0	N.D.	N.D.	Within 10 days after start of treatment, mass (? plasmocytic lesion) on alveolar ridge decreased in size. Hypoproteinemia and generalized edema developed during gallium treatment. No distinct effect of gallium on course of illness
W.C.A. 010008 ♂43	Osteochondrosarcoma. Primary present	511.6 mc. 2511.0 mg. 33.0 mg./kg.	++	+++	++	+	++	N.D.	Tumor became softer and larger during course of therapy. No definite effect of gallium on course of disease Death at home. No autopsy
T.F.P. 010009 ♂26	Osteogenic sarcoma	383.4 mc. 1889.7 mg. 28.6 mg./kg.	++	++	+	++	+	N.D.	No discernible effect of gallium on clinical course Death elsewhere seven and a half months after first tracer Ga^{72}. Autopsy performed elsewhere
H.D. 010012 ♂19	Osteogenic sarcoma. (?) Pulmonary metastases	606.6 mc. 2390.5 mg. 30.1 mg./kg.	+++	+++	+	0	0	N.D.	Pneumonectomy about two weeks after last dose of gallium. Lung showed lesions, previously presumed to be metastases, to be necrotic nodules surrounded by fibrous capsule containing a few questionable neoplastic cells Patient well twelve months postoperatively
A.S. 010013 ♂04	Plasma-cell myeloma	166.0 mc. 662.1 mg. 12.9 mg./kg.	++	+	+	N.D.	N.D.	+	Death during course of therapy from acute nephrosis, mild subarachnoid hemorrhage, and cerebral edema complicating plasma-cell myeloma. Gallium of no benefit. Not considered cause of death Death four months after first tracer dose Ga^{72}. Autopsy
D.H. 010016 ♀12	Osteogenic sarcoma. Primary present	730.8 mc. 3125.1 mg. 80.2 mg./kg.	+++	+	0 to +	+++	+++	+++	No evidence that gallium influenced course of disease Death eight months after first tracer dose of Ga^{72}. Autopsy
L.K. 010019 ♂54	Osteogenic sarcoma	554.8 mc. 2400.4 mg. 31.0 mg./kg.	+++	+++	+++	+++	+	N.D.	Slight tenderness of tumor at end of treatment. No demonstrable effect of therapy
D.A. 010023 ♂17	Osteogenic sarcoma. Primary amputated. Pulmonary metastases	200.4 mc. 1595.5 mg. 30.1 mg./kg.	++	++	0	0	0	+	No effect of gallium on clinical course noted Death five weeks after first tracer dose Ga^{72}. Autopsy

(Table continued on pages 576 and 577)

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health temporarily. His blood picture failed to show the profound depression observed in patients given high doses of radiation with a similar total gallium dosage, but he did show a rather prominent anemia and a mild depression of the white count. A very definite skin rash developed.

In summary, it appears that the toxic reactions seen in the series of patients given large doses of gallium can be interpreted approximately as follows:

The profound bone marrow depression is characteristic of radiation damage and is probably chiefly caused by radiation, though an element of stable metal toxicity may also be contributory. The skin rash, on the other hand, seems undoubtedly a manifestation of toxicity of stable gallium. The gastrointestinal symptoms probably are due both to radiation damage and stable gallium toxicity.

LABORATORY DATA DURING AND AFTER THERAPY

Routine urinalysis, performed repeatedly in all cases, showed no distinct changes related to the Ga^{72}. Occasional one- to three-plus albuminuria was seen transiently during or immediately following treatment. In only 2 cases did this appear to be distinctly related to the Ga^{72} administration. Blood non-protein nitrogen determinations showed no change during and following therapy. A considerable number of patients had elevated blood alkaline phosphatase levels before treatment. Of 10 in this group for whom multiple determinations were available, none showed a consistent change which could be related to gallium treatment. Three patients with carcinoma of the prostate had repeated blood tests for acid phosphatase and, while there was considerable fluctuation in levels for each patient, there was no apparent relation to the course of gallium therapy. Liver function, in so far as it was reflected by thymol turbidity and cephalin cholesterol flocculation tests, showed no alteration.

The fact that many of the patients in this series had serious and increasing metabolic alterations due to progressive neoplastic growth made evaluation of gallium damage difficult. It appeared clear that during and immediately after treatment changes in kidney and liver function, if present, were slight in comparison with bone marrow injury.

EXTERNAL RADIATION MEASUREMENTS

In many instances, radiation measurements were made over various areas of the patients' bodies, usually twenty-four or forty-eight hours after Ga^{72} administration. The information gained from these studies correlated well with radioassays and autoradiographs made upon tissues obtained at biopsy or autopsy, but the external counting determinations were relatively insensitive and failed to indicate slight localized differences in concentration or to show the true magnitude of pronounced variations. Even over lesions which showed a local concentration of manyfold by biopsy or autopsy assays, it was unusual to obtain external counting levels more than three times those of the comparable uninvolved area.

GROUP OF PATIENTS SUBJECTED TO TRIAL OF THERAPY WITH LARGE DOSES OF GALLIUM[72]

Table XIII presents a brief summary of material on 21 patients who were given significant doses of radioactive gallium. The information is largely self-explanatory. The first column gives the patient's initials, his hospital number, sex, and age. The second column is the diagnosis based upon histologic or cytologic material. The third column includes the dosage of gallium in millicuries, total milligrams of gallium given, and the milligrams per kilogram of body weight. The next column indicates the severity of hematologic depression attributed to the gallium injected. The symbols +, ++, and +++ were used to indicate in a general way the severity of the hematologic depression. The patients recorded as showing a 3-plus blood change all had white cell counts below 1,000 per cubic millimeter for a significant period of time. The next column indicates the

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TABLE XIII: (*Continued*)

Case, Sex, and Age	Diagnosis	Mc. Ga^{72} Mg. Ga, and Mg./Kg.	Hematologic Depression	Anorexia Nausea Vomiting	Skin Rash	Evidence of Concentration in Tumor: External Counting	Operation or Biopsy	Autopsy	Clinical Impression
D.A.S. 010044 ♂72	Carcinoma of prostate, with bone metastases. Previous prostatectomy	462.9 mc. 1837.9 mg. 31.5 mg./kg.	++ to +++	++	++	++	++++	N.D.	Patient was on maintenance dose of stilbestrol, not changed for gallium therapy. Some subjective improvement following course of gallium, with decrease in bone pain
E.G. 110047 ♀57	Carcinoma of breast, with bone metastases. Previous mastectomy	517.1 mc. 2392.9 mg. 43.9 mg./kg.	++++	+	++	N.D.	++	N.D.	Patient was on testosterone throughout. Also received localized x-ray therapy during gallium course. Slight transient decrease in pain at end of course of gallium Death at home five months after first tracer dose Ga^{72}. No autopsy
J.F. 110049 ♂51	Adenocarcinoma of right kidney, with metastases to bone	490.7 mc. 2453.4 mg. 53.9 mg./kg.	++++	+++	++	N.D.	+	0	Patient died during severe hematologic depression. Gallium may have hastened death. No evidence of any benefit Death two months after first tracer dose of Ga^{72}. Autopsy
L.W. 110053 ♀17	Osteogenic sarcoma. Primary amputated. Pulmonary metastases	442.2 mc. 4601.8 mg. 66.7 mg./kg.	++ to +++	0	0	0	N.D.	N.D.	Pulmonary metastases enlarged during and following treatment. No evidence of any effect of treatment on course of the disease
J.D.P. 110054 ♂66	Carcinoma of prostate, with bone metastases. Previous prostatectomy and orchiectomy	443.6 mc. 2217.8 mg. 54.1 mg./kg.	+++	+++	0	++++	++++	No suitable bone samples at autopsy	Transient subjective benefit at end of course of gallium. Then rapid deterioration and death Death two months after first tracer dose Ga^{72}. Autopsy
J.M. 110055 ♂20	Chronic myelocytic leukemia. Late stage	571.8 mc. 2868.6 mg. 42.6 mg./kg.	++	0	0	N.D.	N.D.	(Autopsy assays for Au^{198})	Patient had unusual leukemic lesions, chiefly destructive, involving many bones. The gallium treatment caused some apparent decrease in bone pain temporarily Death two months after first tracer dose of Ga^{72}. Autopsy
E.E.R. 110057 ♂62	Carcinoma of prostate. Bone metastases	454.2 mc. 2266.9 mg. 31.2 mg./kg.	+++	+	+	++++	++++	(Autopsy, no assays)	This patient said that his pain greatly diminished during his treatment. There was no objective evidence of improvement. Severe bone marrow depression developed from the gallium and this was believed to contribute to his death Death two months after first tracer dose of Ga^{72}. Autopsy
B.D. 110061 ♂17	Osteogenic sarcoma. Lung metastases. Primary present	777.4 mc. 3702.1 mg. 70.9 mg./kg.	++	+	0	+	++++	0	Primary lesion (rt. leg) amputated after dose of gallium. Metastatic pulmonary lesions continued to grow during gallium therapy Death four months after first tracer dose Ga^{72}. Autopsy

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P.H.R. 110007 ♀ 64	Carcinoma of bladder. Bone and lung metastases	312.2 mc. 1665.2 mg. 23.0 mg./kg.	+++ to +++	+	0	N.D.	+++	N.D.	Patient stated that he had temporary definite relief of bone pain during gallium therapy. X-ray evidence of some recalcification of bone lesion in pelvis, but pulmonary metastases continued to grow during treatment. New bone lesions developed a few weeks after cessation of treatment. Death elsewhere six months after first tracer dose of Ga^{72}. No autopsy data
F.T. 110073 ♀ 13	Osteogenic sarcoma. Lung metastases. Primary present	272.7 mc. 1367.5 mg. 33.4 mg./kg.	++	+	0	+++	++++	0	Primary lesion amputated after test dose of gallium. Subsequent course of gallium treatment, during which the metastases grew rapidly. Death two and a half months after first tracer dose of Ga^{72}. Autopsy
H.H. 110070 ♀ 57	Carcinoma of breast. Bone metastases. Previous mastectomy	210.7 mc. 923.7 mg. 14.0 mg./kg.	++	++++	0	N.D.	0 to +	0	Patient tolerated only small amount of gallium. Rapid down-hill course apparently not influenced by gallium. (X-ray therapy given for local pain.) Estimyl throughout. Death one and a half months after first tracer dose Ga^{72}. Autopsy
P.J. 110087 ♀ 52	Carcinoma of breast. Bone metastases. Previous mastectomy	287.3 mc. 1278.3 mg. 19.5 mg./kg.	+++	++	0	0	+	N.D.	No change in bone lesions during or following gallium. No relief of bone pain

severity of anorexia, nausea, and vomiting, as they were attributed to the gallium treatment. The column indicating skin lesions needs no explanation. The next three columns indicate the degree of localized gallium uptake in the tumor. The concentration of gallium in each tumor is compared with that of available normal tissue.

PATIENTS STUDIED WITH TRACER DOSES

Another, entirely separate group of patients, 34 in number, were studied with tracer doses only of Ga^{72} (Table XIV). Some of these patients were being considered for a trial of therapy with this isotope. The remainder were patients admitted to the hospital for other forms of study or treatment, to whom it was possible to give the Ga^{72} tracer dose as an additional study.

THREE CASE REPORTS

Only the first of the following 3 case reports is presented as a typical example. It is of a patient with osteogenic sarcoma who showed concentration of gallium in the lesions but who failed to have apparent benefit from the treatment. The second is of an unusual case unlike any other in the series. The third case is that of the only patient given large doses of stable gallium.

CASE I: *History:* H. D. (No. 010016) was a 12-year-old colored school girl whose case is summarized in Figure 16. She was admitted on Aug. 23, 1950, with a history of pain and swelling of the left thigh beginning about one year prior to admission. In July 1950, upon the basis of x-ray studies, a diagnosis of osteogenic sarcoma of the femur with pulmonary metastases was made. The patient had received no radiation therapy or surgical treatment prior to admission.

Physical Examination: Temperature 37° C., pulse 70, respirations 22, blood pressure 104/78. The patient was slightly underweight but appeared to be in fairly good general condition. Her lungs were normal on physical examination. There was a large fusiform mass on the left thigh involving the distal half of the femur. The circumference of the thigh at the level of this mass was 37 cm., as compared with 27 cm. at the corresponding level on the normal side.

X-Ray Studies: Films showed a characteristic picture of osteogenic sarcoma of the femur. There were numerous pulmonary nodules

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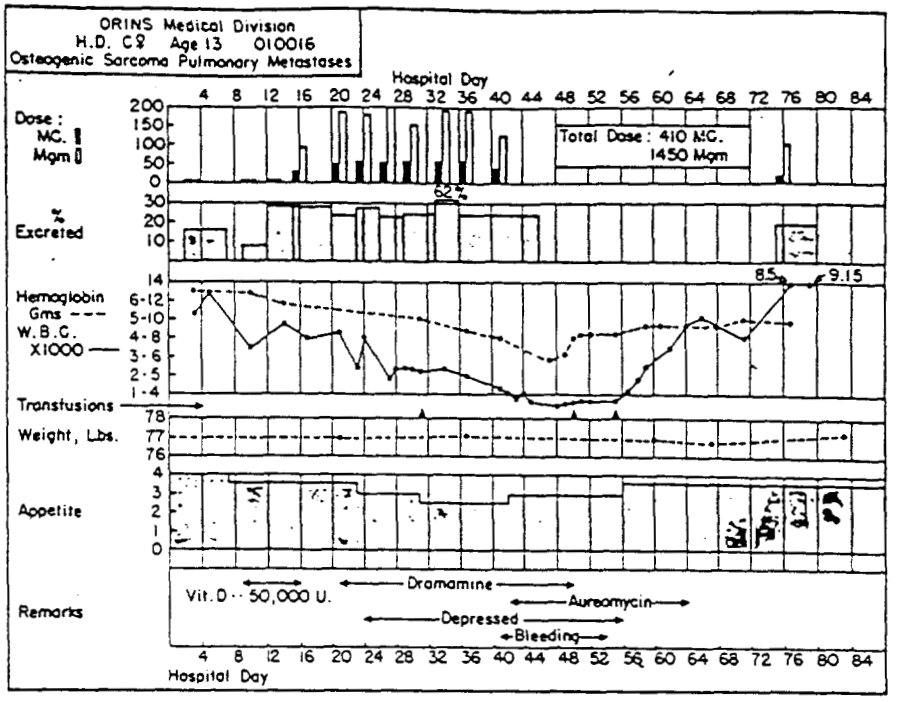

Fig. 16. Summary of Case No. 010016.

Course: Intravenous gallium citrate was given with 5–10 ml. of 10 per cent calcium gluconate intravenously before each dose.

Date	Dose (mc.)	Mg. of Gallium
8–18–50	1	9
8–25–50	1	9
8–28–50	1	5
8–31–50	29	90
9– 5–50	52	184
9– 7–50	54	179
9–11–50	49	204
9–14–50	54	155
9–18–50	56	195
9–21–50	53	199
9–25–50	41	123
9–30–50	22	102
TOTALS	413	1454

External counting studies showed counts over the lesion in the femur to be about three to four times the level on the normal side and two to three times the level over the trunk. No definite localization of the chest lesions could be shown by external counting. During this treatment, the patient suffered no severe nausea or vomiting, though she did complain of anorexia and malaise. During the period of greatest depression in the bone marrow, late in August and early in September, she had a distinct bleeding tendency, with some oozing from the gums and nose. She was given Aureomycin and blood transfusions during this period.

The laboratory findings during treatment were as shown on the following page.

During the gallium treatment the urine showed varying amounts of albumin up to 3+. On discharge it was negative.

After completion of the course of gallium, the patient rapidly improved and seemed to feel somewhat better than she had before the beginning of the treatment. She was discharged on Nov. 10, at which time the hemoglobin was 9.5 gm., white cell count 9,150. She was quite well and free from pain for several weeks. Early in 1951, she had increasing pain in the left femur and her general condition became poorer. She returned on Feb. 14, 1951.

Another extensive course of gallium[72] was given, bringing the total dose to 730 mc. Pronounced hematologic depression again occurred. Amputation of the leg with the primary lesion was done after the first dose of gallium and autograms and assays were obtained. Histologic sections were characteristic of a highly cellular osteogenic sarcoma. The patient died of extensive pulmonary metastases while she was showing toxic effects from her second course of gallium. She received a small dose of the isotope shortly before death for tissue radioactivity assays and autograms.

At *autopsy* there were found multiple pulmonary metastatic nodules, which practically replaced normal lung; these nodules showed a considerable amount of calcification.

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	Aug. 17, 1950	Sept. 25, 1950 (During gallium treatment)	Feb. 15, 1951 (Two and a half months after 1st course of Ga^{72})
R.B.C.	4.17 million	3.00 million	3.48 million
Hemoglobin	13.3 gm.	8.0 gm.	8.5 gm.
Hematocrit	41%	24%	28%
W.B.C.	5,200	1,350	9,250
Differential count			
Band neut.	2.0%	5.0%	4.5%
Segmented	44.0	41.0	76.0
Lymphocytes	45.0	45.0	8.0
Monocytes	7.5	7.0	10.0
Eosinophils	1.0	0	1.5
Basophils	0.5	(greatly	(slightly
Thrombocytes	(slightly increased)	decreased)	increased)
Red cells	N	N	
		Some "irritation" lymphocytes. Neutrophils with large granules or nuclear fragments	Moderate anisocytosis, poikilocytosis, and hypochromia. Some young monocytes

After Sept. 25, the white cell count fell still lower than on that date, reaching 500 per cu. mm. After about a week at very low levels, it gradually rose.

	Aug. 29–31, 1950 (Before treatment)	Oct. 19, 1950 (After treatment)
Alkaline phosphatase	6.3 Bodansky units	7.7 Bodansky units
Acid phosphatase	4.6 K.-A. units	2.3 K.-A. units
Cephalin cholesterol flocculation		
24 hours	+++	0
48 hours	++++	+++
Thymol turbidity	12.5 units	10.4 units

Comment: This is a case of osteogenic sarcoma with extensive pulmonary metastases in a young individual who received two extensive courses of Ga^{72}. The primary lesion and metastases continued to grow rapidly during and after treatment.

CASE II: *History:* D. H. (No. 010012) was a 19-year-old colored male whose case is summarized in Figure 17. He was admitted Aug. 3, 1950. He had a history of injury to his left femur about one year before admission, followed by pain and swelling, and an x-ray diagnosis of tumor involving the left femur was made in July 1949. Histologic studies showed on osteogenic sarcoma. A high thigh amputation was done in October 1949. In June 1950, there was x-ray evidence of a nodule in the left lung. This had enlarged on subsequent x-ray examination.

Physical Examination: Temperature 37° C., respirations 18, blood pressure 120/80. The patient was a well nourished, young Negro male, who was able to get around quite well with crutches. The left leg had been removed at the hip and the amputation wound was well healed. Physical examination revealed no further noteworthy abnormalities.

X-Ray Studies: 8-8-50: A postero-anterior chest film revealed a small rounded density in the left lower lung field, measuring approximately 1.5 cm. in greatest diameter, but with no evidence of calcification. A very small pneumothorax and pleural effusion were also seen in the left side.

8-24-50: Re-examination of the chest showed the previously described nodule in the left lower lung field to be approximately the same size as on the previous examination. The pneumothorax had increased and there was slightly more fluid present than on the previous examination. The heart size and shape had changed considerably, probably as the result of the pneumothorax.

10-3-50: Re-examination revealed some increase in the size of the nodule, which now measured approximately 3 cm. in greatest diameter. There were still some pneumothorax and a small amount of pleural fluid in the left costophrenic angle.

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TABLE XIV: SUMMARIES OF CASES IN WHICH Ga^{72} WAS GIVEN IN TRACER DOSES
(N.D., No data; 0, None; +, Little; ++, Moderate; +++, Pronounced)

Case, Sex, and Age	Diagnosis	Ga^{72} in mc.	Per cent Excreted	Evidence of Concentration in Tumor: External Measurement	Autopsy	Biopsy	Additional Data and Remarks
E.H.N. 010018 ♀46	Plasma-cell myeloma	(a) 1.0 (b) 23.5 (c) 23.5	49.2 24.9 14.7	N.D.	Autopsy but no gallium assay	N.D.	Sulfur studies done (S^{35} in yeast) after Ga^{72} test doses. Autopsy with S^{35} assays
T.C.R. 010020 ♀42	Lymphosarcoma	(a) 1.0	9.2	N.D.	Autopsy but no gallium assay	N.D.	Treated with P^{32} after trial gallium dose. Autopsy P^{32} studies
O.B. 010021 ♂43	Chronic lymphocytic leukemia	(a) 1.9 (b) 20.0	24.4 33.3	0	N.D.	N.D.	Patient treated with P^{32} and cortisone. No further gallium study
V.P.W. 010024 ♀52	Lymphosarcoma	(a) 1.5	4.6	N.D.	Autopsy but no gallium assay	N.D.	Single tracer dose for excretion studies. Later x-ray therapy. Autopsy. No isotope distribution study
A.G.W. 010025 ♀70	Undifferentiated carcinoma of thyroid	(a) 1.0	10.5		Autopsy but no gallium assay	N.D.	Studied chiefly with I^{131}. Given 1 mc. gallium for excretion. Tissue assayed for I^{131}
J.R.U. 010026 ♂56	Chondrosarcoma	(a) 2.2 (b) 27.9 (c) 22.0	7.4 20.6 21.0	0	N.D.		Biopsy twenty-four hours after 27.9 mc. Ga^{72} Tissues — cts./Gm. — μc./Gm Fat — 708 — 0.009 Skin — 1,736 — 0.022 Tumor — 855 — 0.011 Muscle — 731 — 0.009 Cartilage — 5,536 — 0.071
L.C. 010027 ♀35	Carcinoma of breast. Lung metastases with pleural effusion	(a) 1.9 (b) 22.3	6.0 16.4	0	N.D.	N.D.	Studied chiefly with Au^{198}. Given 1.4 mc. gallium for excretion and 22 mc. prior to external counting
M.T.V. 010028 ♀66	Plasma-cell myeloma	(a) 1.0 (b) 46.9	12.7 24.3	0	N.D.	N.D.	Treated with urethane after gallium study
J.M.S. 010029 ♀3	Neuroblastoma of adrenal	(a) 10.2	23.2	0	Autopsy but no gallium assay	N.D.	Studied chiefly with P^{32}. Autopsy distribution studies for I^{131}. Autopsy here. Tissue assay for P^{32}
M.T.M. 010030 ♂62	Carcinoma of prostate. Bone metastases	(a) 1.3 (b) 48.7	3.6 11.2	+	N.D.	++	Biopsy, trephine twenty-four hours after 48.7 mc. gallium Tissue — cts./Gm. — μc./Gm. Bone metastases — 29,000 — 0.373 Normal bone — 7,720 — 0.099
N.J.S. 010031 ♀53	Carcinoma of ovary	(a) 1.3 (b) 23.7 (c) 20.6 (d) 18.0	2.3 0.42 6.8 17.6	N.D.	Autopsy but no gallium assay	+	1. Biopsy twenty-four hours after 23.7 mc. Ga^{72} Tissue — cts./Gm. — μc./Gm. Cervical tumor — 13,400 — 0.172 Normal cervix — 1,025 — 0.013 Sigmoid tumor — 9,073 — 0.117 Normal sigmoid — 3,185 — 0.041 2. Biopsy twenty-four hours after 18 mc. Ga^{72} Tissue — μc./Gm. Liver — 0.17 Fascia, above umbilicus — 0.04 Tumor, necrotic — 0.14 Muscle, rectus — 0.02 Tumor retroperitoneal node — 0.11 Tumor retroperitoneal node — 0.13 Fat, from colon — 0.10 Abdominal fluid — 0.04 Subcutaneous tissue — 0.02 Skin — 0.05. Death three and a half months following first biopsy. Autopsy. Au^{198} distribution study

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TABLE XIV: (*Continued*)

Case, Sex, and Age	Diagnosis	Ga^{72} in mc.	Per cent Excreted	Evidence of Concentration in Tumor: External Measurement	Autopsy	Biopsy	Additional Data and Remarks
G.R.C. 010033 ♂44	Lymphosarcoma	(a) 22.2	14.8	N.D.	Autopsy but no gallium assay	0	Biopsy twenty-four hours after 22 mc. Ga^{72} Tissue — μc./Gm. Chest fluid — 0.051 Skin — 0.102 Muscle — 0.030 Lymph node — 0.089 Lymph node — 0.081 Autopsy five months later, with Au^{198} distribution study
J.D.L. 010034 ♂18	Reticulum-cell sarcoma	(a) 2.6	0.27	N.D.	Autopsy but no gallium assay	N.D.	Studied chiefly with P^{32}. Autopsy with P^{32} distribution study
P.J.F. 010036 ♂34	Sarcoma of ischium and thigh	(a) 22.0 (b) 23.5	12.8 14.9	0	N.D.	+	Biopsy twenty-four hours after 22 mc. Ga^{72} Tissue — cts./Gm. — μc./Gm. Tumor — 84,600 — 1.040 Muscle — 232 — 0.003 Tumor — 5,725 — 0.070
F.H. 010040 ♀61	Carcinoma of urinary bladder	(a) 1.3	4.6	N.D.	N.D.	N.D.	Studied chiefly with Au^{198}. Death at home. No autopsy
M.L.H. 010041 ♀37	Plasma-cell myeloma	(a) 1.3 (b) 23.0	2.5 12.4	N.D.	+	+	Biopsy twenty-four hours after 23 mc. Ga^{72} Tissue — μc./Gm. Skin scar — 0.05 Fat, subcutaneous — 0.03 Tumor — 0.14 Tumor — 0.48 Vertebra with tumor — 0.80 Vertebra without tumor — 0.53 Normal skin — 0.05
O.D. 010042 ♂40	Plasma-cell myeloma	(a) 1.0 (b) 20.8	1.2 12.5	0	N.D.	N.D.	Disease still responding to conventional therapy
P.E. 010050 ♂43	Plasma-cell myeloma	(a) 0.7 (b) 21.2	20.5 63.4	0	N.D.	N.D.	Patient not treated here. Discharged after the two tracer doses of gallium
N.M. 110060 ♀51	Hodgkin's disease	(a) 19.9	59.4	N.D.	Autopsy but no gallium assay	+	Biopsy twenty-four hours following 20 mc. Ga^{72} Tissue — μc./Gm. Tumor from neck — 1.627 Muscle — 0.113 Death one month after biopsy. Autopsy
C.L.C. 110064 ♂49	Small-cell sarcoma (Ewing's tumor) of sacrum	(a) 20.5	60.5	N.D.	0	0	Biopsy of lung after 20.5 mc. Ga^{72} Tissue — μc./Gm. Tumor 0.12 0.10 — 0.10 Lung — 0.16 Rib — 0.36 Muscle — 0.02 Plasma — 0.13 Skin — 0.10 Sudden death from pulmonary embolus one day after uneventful lung biopsy. Autopsy assays showed no significant concentration of gallium in tumor
W.B. 110065 ♂26	Hodgkin's disease	(a) 2.2 (b) 21.4	28.4 71.1	N.D.	N.D.	N.D.	Patient admitted for trial of gallium treatment but, after evaluation, it was decided that x-ray therapy would give him a better chance of improvement, and this has been the treatment.

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TABLE XIV: (*Continued*)

Case, Sex, and Age	Diagnosis	Ga^{72} in mc.	Per cent Excreted	Evidence of Concentration in Tumor: External Measurement	Autopsy	Biopsy	Additional Data and Remarks
L.S. 110068 ♀56	Plasma-cell myeloma	(*a*) 1.8 (*b*) 20.6	19.0 60.1	N.D.	Autopsy; no assay data	N.D.	Rib biopsy forty-five hours after 20.6 mc. Ga^{72} Tissue — μc./Gm. Rib — 0.56 Subcutaneous — 0.09 Skin — 0.11 Muscle — 0.09 Plasma — 0.21 Patient died suddenly. Autopsy. No tissue assay for radioactivity
G.H. 110071 ♂67	Chronic lymphocytic leukemia	(*a*) 22.0	53.4	N.D.	N.D.	0	Biopsy (lymph node) forty-eight hours after 22 mc. of Ga^{72} Tissue — μc./Gm. Lymph node — 0.11 Plasma — 0.33 W B C — 0.04 R B C — 0.09 Patient discharged after this biopsy study. X-ray therapy advised
C.I. 110074 ♂48	Plasma-cell myeloma	(*a*) 1.5 (*b*) 20.5	6.3 41.2	N.D.	N.D.	0	Biopsy of involved rib forty-eight hours after 20.5 mc. Ga^{72} Tissue — μc./Gm. Rib — 0.98 Muscle — 0.09 Skin and subcutaneous tissue — 0.14 Patient treated with x-rays and P^{32}. In fair condition five months following biopsy
R.P. 110076 ♂19	Giant-cell tumor of bone involving sacrum	(*a*) 2.8 (*b*) 22.4 (*c*) 58.7	13.2 51.7 58.7	0	N.D.	See assays	Biopsy forty-eight hours after 20.4 mc. Ga^{72} Tissue — μc./Gm. Soft tumor — 0.32 Bone and cartilage — 0.04 Thin layer of bone adjacent to tumor — 2.21 Bone and tumor — 1.67 Muscle — 0.05 Skin — 0.04 Plasma — 0.18 RBC — 0.04 Patient discharged for treatment elsewhere
E.E.N. 110083 ♂70	Carcinoma of prostate with extensive metastases	(*a*) 1.6 (*b*) 50.0	11.3 Death soon after 2nd dose	N.D.	+	N.D.	Patient, admitted in terminal condition, given Ga^{72} and P^{32} simultaneously for autogram studies at various stages of decay. Death five hours after administration of the two isotopes. Tissue assays for both isotopes
H.L. 110094 ♀34	Reticulum-cell sarcoma	(*a*) 21.9 (*b*) 17.8 (*c*) 21.2	53.6 48.8 65.8	+	N.D.	+	Biopsy of breast lesion forty-eight hours after 21.9 mc. Ga^{72} Tissue — μc./Gm. Tumor — 0.57 Skin — 0.11 Fat — 0.20 Plasma — 0.20 RBC — 0.07 Biopsy of right ilium forty-eight hours after 21.2 mc. Ga^{72} Tissue — μc./Gm. Bone with tumor — 2.20 Bone with less tumor — 1.39

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TABLE XIV: (*Continued*)

Case, and ♂	Diagnosis	Ga^{72} in mc.	Per cent Excreted	Evidence of Concentration in Tumor: External Measurement	Autopsy	Biopsy	Additional Data and Remarks
							Normal cortical bone 0.66 Muscle 0.04 Skin 0.17 Plasma 0.22 RBC 0.05 Patient discharged, x-ray therapy advised here, given near her home. Feeling fairly well three and a half months following biopsy
J.W.M. 110099 ♂40	Plasma-cell myeloma	(a) 2.4 (b) 21.5	9.1 39.9	0	N.D.	N.D.	Patient discharged after excretion study and external counting study had been done and after he had been started on urethane
W.R.M. 110101 ♂50	Plasma-cell myeloma	(a) 1.6 (b) 21.5	52.5 47.5	N.D.	N.D.	+	Biopsy of abdominal mass and ribs forty-eight hours after 21.5 mc. Ga^{72} Tissue — μc./Gm. Bone with ? tumor 0.87 Solid tumor 6.03 Bone with fibrosis 9.9 Bone with tumor 11.63 Muscle 0.06 Skin 0.10 Fat 0.03, 0.02 Plasma 0.19 RBC 0.04 Patient discharged on urethane, to continue under care of his personal physician
O.M. 110110 ♂7	Chronic osteomyelitis ? Squamous-cell carcinoma at site of inflammation	(a) 20.3	57.7	N.D.	N.D.	See assay data	No definite diagnosis of neoplasm. Biopsy forty-eight hours after 20.3 mc. Ga^{72} Tissue — μc./Gm. Granulation tissue and epithelium (2 samples) 0.18, 0.13 Epithelial, granulation tissue and bone spicules 0.51 Fat and subcutaneous tissue 0.42 Bone with epithelial and inflammatory tissue 0.92 Skin (normal) 0.14 Muscle 0.10 Plasma 0.21 RBC 0.05 Patient discharged. Advised to have care elsewhere for osteomyelitis. We could not decide surely about the malignancy of the hyperplastic epithelium near the draining sinus
E.M. 110111 ♂34	Round-cell sarcoma (Ewing's group) arising in os calcis	(a) 20.4	60.2	Slight evidence of concentration in one bone lesion	N.D.	0	Biopsy forty-five hours after 20.4 mc. Ga^{72} Tissue — μc./Gm. Bone and tumor of ilium (3 samples) 0.72, 0.59, 0.97 Normal bone 0.99 Muscle 0.01 Skin 0.04 Fat 0.03 Plasma 0.07 RBC 0.04

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TABLE XIV: (*Continued*)

Case, Sex, and Age	Diagnosis	Ga^{72} in mc.	Per cent Excreted	Evidence of Concentration in Tumor: External Measurement	Autopsy	Biopsy	Additional Data and Remarks
A.B. 110113 ♀43	Carcinoma of cervix with skeletal metastases	(*a*) 1.2 (*b*) 19.1	7.2 18.9	0	N.D.	N.D.	Patient discharged soon after test doses of gallium. X-ray therapy advised
R.M. 110116 ♀66	Plasma-cell myeloma	(*a*) 1.1 (*b*) 21.2	10.1 55.1	N.D.	N.D.	+	Biopsy forty-eight hours after 21.2 mc. Ga^{72} Tissue — mc. Ga Skin — 0.24 Subcutaneous tissue — 0.05 Muscle — 0.05 Involved rib with marrow — 1.94 Involved rib without marrow — 1.34 Plasma — 0.46 RBC — 0.12 Patient subsequently treated with cortisone and urethane.
A.A 110117 ♀56	Plasma-cell myeloma	(*a*) 0.93 (*b*) 21.2 (*c*) 16.6 (*d*) 5.7 (Some lost) (Died soon after dose)	16.9 54.3 19.9 11.7	N.D.	0	N.D.	Patient admitted in terminal condition. Death soon after last dose of Ga^{72}. Autopsy assays showed unusually high levels in kidney

11-2-50: A film of the chest made on re-admission showed the nodule to be approximately 2.5 cm. in diameter, definitely smaller than one month previously. The pneumothorax and effusion previously described were not seen on this film.

Course: On Aug. 4, 1950, the patient was given 0.9 mc. of Ga^{72}. Excretion and external counting studies were made. These were repeated on Aug. 10, 1950, following an additional 1 mc. dose of Ga^{72}. The external counting studies with a highly collimated Geiger-Müller tube were unsuccessful in demonstrating localized uptake of activity in the tumor nodule over the left chest, and survey study was unsuccessful in revealing any other areas of significant selective uptake of activity.

It was believed that the lesion was probably inoperable, because of the pleural fluid and pneumothorax, indicating probable pleural extension and perhaps implantation, and also because of the probability of multiple metastases not visualized roentgenographically rather than the single one shown on the film. It was decided, however, that if following the therapeutic trial of Ga^{72} no significant changes had occurred, an exploratory thoracotomy would be attempted and the lesion resected if possible.

The patient was given doses of Ga^{72} ranging from 20 to 78 mc. at approximately three- or four-day intervals until Sept. 25, 1950. At this time he had received a total of 578 mc. During the course of large doses of Ga^{72}, he manifested anorexia ranging from moderate to severe, with associated nausea and vomiting. Peripheral blood examinations revealed a decided drop in the white blood cell count to a low of 650 on Aug. 4, 1950, while the hemoglobin dropped to a level of 8.6 gm. on that date. The weight on admission was 175 pounds; on Oct. 9, it was 158 pounds. There were no manifestations of skin eruption, jaundice, or urinary abnormalities. The patient was discharged on Oct. 21, 1950, and returned to his home. During his stay there, he suffered no untoward complications. He was readmitted on Nov. 1, 1950. He was given 26 mc. of Ga^{72} on Nov. 2, 1950, and twenty-four hours later an exploratory thoracotomy was done.

The operation was performed by Dr. R. W. Postlethwait, of the Medical College of South Carolina. No pleural implants could be found. There was one nodule about 3 cm. in diameter in the interlobar fissure, partially in the lingula of the upper lobe. A second, but much smaller nodule, about 4 or 5 mm. in diameter, was present in the upper lobe. The hilus showed no evidence of extension of the disease. It was decided that a pneumonectomy should be done, and this was accomplished without difficulty.

Gross examination of the resected lung showed the larger nodule to be well below the pleural surface. This lesion consisted of an irregular zone of fibrosis within which was a dark red, firm blood clot. Two other similar but smaller lesions were also present. On histologic section the nodules were found to consist of a zone of hemorrhage and necrosis.

There were numerous hemosiderin-laden phagocytes throughout. The periphery was made up of proliferating capillaries and fibrocytes. Of real interest was the presence of several giant nuclei which were mono- or multinucleated, in the so-called capsule. These had a hyperchromatic appearance, with clumped chromatin and criteria sufficient to designate them as malignant cells.

The patient had a relatively uneventful postopera-

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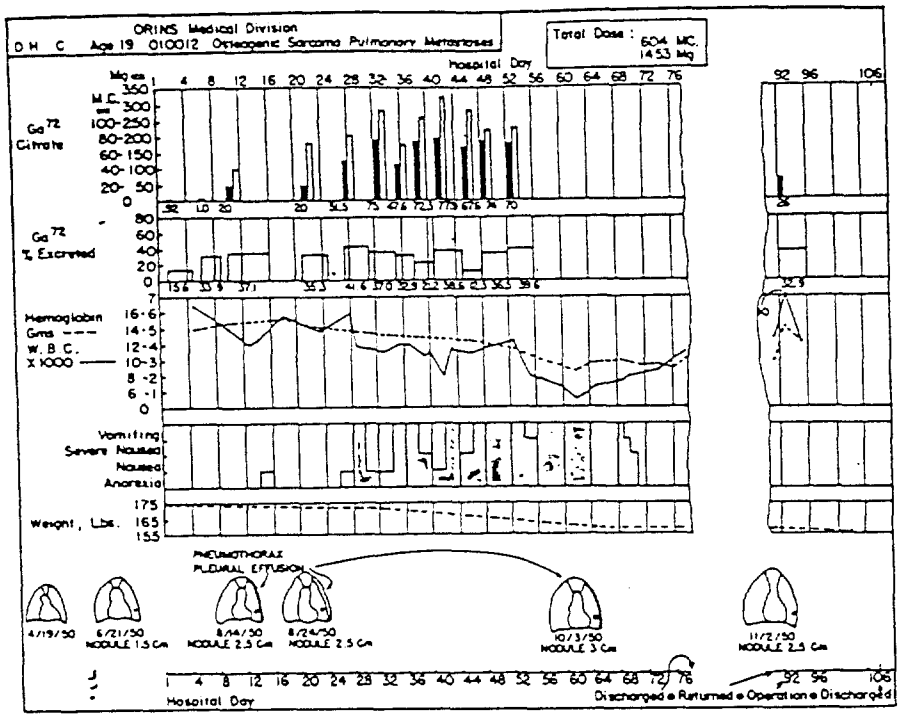

Fig. 17. Summary of Case No. 010012.

tive course and was finally discharged on Nov. 17, 1950, to return to his home. Hematology values v ormal.

Comment: This patient was thought to have typical pulmonary metastases from a primary osteogenic sarcoma. He received a large course of gallium[72], following which a pulmonary nodule, thought to be metastatic, first increased and then decreased in size. The lesions which were removed at pneumonectomy, instead of being typical metastases, were not characteristic of any specific lesion. Specific studies in reference to tuberculosis failed to bring out any supporting evidence for that disease. There seemed to be three possible explanations for the pulmonary lesions:

1. They may have represented pulmonary metastases of osteogenic sarcoma, which underwent spontaneous infarction for some reason.

2. They may have been pulmonary metastases of osteogenic sarcoma, which underwent necrosis because of treatment with radioactive gallium. If so, this is the only example of such an effect in the series, where there were many opportunities for it to occur.

3. These lesions may represent a pulmonary disease entirely unrelated to the primary osteogenic sarcoma, such as some form of chronic infectious granuloma or healing abscess due to aspiration. The serial chest films were much more compatible with the diagnosis of neoplasm.

CASE III: *History:* W. R. C. (No. 110088), a 27-year-old white male, was admitted in June 1951. A mass in the right leg had appeared in 1947. A supracondylar amputation was done in July 1950. The histologic diagnosis was fibrosarcoma. There was x-ray evidence of pulmonary metastases in June 1951. The patient had been asymptomatic and had not received any x-ray therapy.

Physical Examination: Temperature 37.2° C., pulse 92, respirations 20, blood pressure 145/100. The patient was moderately overweight, alert, and ambulatory with the aid of a prosthesis. Examination revealed no abnormality except for the absence of the right leg.

X-Ray Studies: On June 28, 1951, roentgenograms showed multiple nodular areas of increased density scattered throughout both lung fields. A complete bone survey showed no evidence of metastatic bone lesions. On Aug. 6, 1951, the x-ray findings were unchanged.

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Course: The patient was given 2.1 mc. of Ga^{72} intravenously on June 21, followed by external counting with a collimated Geiger tube twenty-four hours later, which revealed no localized areas of increased uptake. He was given stable gallium citrate intravenously with only tracer amounts of Ga^{72} twice weekly from June 21 through August 6. Approximately 250 mg. of stable gallium was given with each infusion, so that he received a total of 2,785 mg. or 35.8 mg./kg. The total Ga^{72} received was 13.7 mc. The stable gallium given equaled that administered as carrier to those patients who received Ga^{72} in large therapeutic amounts. There were some anorexia and occasional nausea toward the latter part of the treatment. Fever and a sore throat with ulcerated lesions on pharynx and gums developed midway through the course of gallium. Similar lesions had occurred on several occasions in the past. Fever and mouth lesions lasted about a week and disappeared under treatment with Aureomycin. The patient lost about 12 pounds of weight, but at the time of discharge, Aug. 17, 1951, his appetite was improving steadily. The eighteen-hour urine concentration test showed some change in that he was able to concentrate to 1.025 on June 21, but to 1.011 only on Aug. 16. On one occasion, July 18, there was +++ albuminuria, but none after that time; PSP renal function test, on Aug. 17, showed excretion of 42 per cent in one hour and 28 per cent in the second hour; non-protein nitrogen was not elevated.

At home, the patient's general strength improved, and he felt as well as before receiving the stable gallium. He had no cough or chest pain and his appetite was good. X-ray examination of the chest, in October 1951, showed that the pulmonary metastases had increased in size.

Laboratory Findings: Hematologic studies before administration of gallium (June 1951), those of Aug. 13, eleven days after the last injection of gallium, showing anemia and slight depression of lymphocytes, and those of Oct. 1, two months after gallium administration, were as shown on the next page.

Comment: This patient was given stable gallium citrate intravenously with only tracer amounts of Ga^{72}, in order to determine the toxic effects of the metal alone. The tracer amounts of Ga^{72} were given in order to obtain data on excretion. Toxic effects noted were (1) skin rash, (2) anorexia, (3) albuminuria on two occasions, and (4) decreased kidney function by concentration test. Because of a viral gingivostomatitis occurring during the middle of the course of gallium and lasting about a week, it was difficult to evaluate the gallium effects during that period. Nausea developed only twice. There was mild fluctuating depression of the white cell count and a definite depression of red cells. Thrombocytes were not affected.

DISCUSSION

The patterns of gallium localization were well demonstrated, especially in autoradiographic records, but the metabolic factors responsible for these localizations are not known. In normal bone the gallium was found to be just beneath the periosteum in a fine line. In growing bones, there was a striking local deposition at the epiphyseal lines. The lesions of osteogenic sarcoma showed great ability selectively to withdraw the gallium from blood. The distribution in these lesions was very uneven and could not be well correlated with any particular morphologic characteristic of the tumor. Two areas of the same lesion which appeared identical under the microscope sometimes showed a great difference in gallium content. However, there was a very pronounced tendency for the isotope to concentrate in and near areas of calcification in these lesions. Destructive lesions of bones showed a generally low concentration of gallium in their central areas, but sometimes a prominent deposition at areas where the neoplasm was invading normal bone. Thus there seemed to be a distinct tendency for the gallium to localize in areas of bone activity either osteoblastic or osteolytic.

Fairly high levels of radioactivity were seen in the normal liver, spleen, and bone marrow. It may be that this was in part related to the formation in the blood of a gallium colloid which was taken out by reticulo-endothelial cells. However, there is no evidence as yet for the existence of such a colloidal form of the isotope, and the facts that rather large amounts were excreted in the urine and that the kidneys and endocrine glands showed high levels of activity are somewhat against this hypothesis. Highly cellular soft-tissue tumors which showed no tendency to calcify sometimes had fairly high concentrations of the isotope, suggesting that it may be involved in nucleoprotein metabolism.

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Case No. 110088	June 1951	Aug. 13, 1951	Oct. 1, 1951
R.B.C.	6.10 million	3.88 million	5.85 million
Hemoglobin	16.5 gm.	10.6 gm.	13.9 gm.
Hematocrit	52%	32%	46%
Mean Corpuscular Volume	85	82	79
Mean Corpuscular Hemoglobin	27	26	24
Mean Corpuscular Hemoglobin Concentration	32	31	30
W.B.C.	6,650	5,500	5,750
Sedimentation rate	12 mm. in 1 hour (uncorrected)	20 mm. in 1 hour (corrected)	5 mm. in 1 hour (corrected)
Differential Count			
Myelocytes			0.5
Metamyelocytes			0.5
Band neutrophils	1.0	10	2.5
Segmented	51.5	54.5	45
Lymphocytes	31.5	15	30
Monocytes	9.0	16.5	4.5
Eosinophils	6.5	3.5	13.5
Basophils	0.5	0.5	2.5
Atypical cells (resembling lymphoid plasma cells)			1
Thrombocytes	Normal	Normal	Normal
"Toxic" granulation	++	++	+
Red cells	Normal	Moderate anisocytosis and poikilocytosis	Mild anisocytosis and poikilocytosis; some anisochromia. Occasional punctate basophilia of red cells. Reticulocytes slightly increased
	A few young lymphocytes		
	Toxic monocytes		
Bone marrow	Cellular, without definite abnormality		Essentially normal cellular marrow
Blood Chemistry			
Acid phosphatase	1.8 King-Armstrong units		0.28 m.m. units
Alkaline phosphatase	8.8 Bodansky units		2.1 Bodansky units
Thymol turbidity	9.4 units		20.8 units
Cephalin cholesterol flocculation	24 hr. 0		24 hr. 0
	48 hr. 0		48 hr. ++

Further information about the biochemical factors which influence the distribution of gallium may be forthcoming. If so, it may be possible to reinterpret the data obtained in the present study and to derive information about the nature of tumors involving bone.

As the study progressed, we were impressed with the almost complete lack of any clinical improvement following gallium treatment, even in patients who showed evidence of striking differential localization of gallium in tumor tissue. Most of the patients in whom gallium therapy was attempted were given maximum amounts of the isotope. Only the hopelessness of their prognoses justified a trial of doses so damaging to the hematopoietic tissues. A few evidences of apparent transient symptomatic benefit were seen in individuals with metastatic carcinoma. In one patient there was definite roentgen evidence of recalcification of osteolytic lesions following therapy. Patients with primary bone sarcoma showed no evidence whatever of any slowing of growth of primary or metastatic lesions, and in several cases lesions grew rapidly during high-dosage therapy.

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These findings are consistent with the familiar resistance of this neoplasm to conventional x-ray therapy.

In summary, it is our impression that Ga^{72} has been given a thorough clinical trial in many types of neoplasm involving bone and has been found of no therapeutic value. Gallium may be a valuable element for the study of biochemical and metabolic factors related to bone tumors.

VII. Studies of the Urinary Excretion of Gallium[72] in Man

H. D. BRUNER, M.D., JOE GRAY, SAMUEL W. ROOT, M.D., and MARSHALL BRUCER, M.D.

Studies of urinary and fecal excretion of the isotope were made routinely. Each patient was assigned an individual bedpan and urinal. Voided urine specimens were transferred to large bottles, and the original container was rinsed with small amounts of water. Collections were made over eight-hour and twenty-four-hour periods, and each total volume, including rinse water, was mixed, measured, and an aliquot was taken for radioassay. During the first few months of the study, separate eight-hour collections were made during the first three days after administration and twenty-four-hour collections subsequently as long as measurable activity remained or until the next dose was given. Since the major excretion was found to occur during the first twenty-four-hour period, eight-hour collections were not continued beyond the first day. Early studies showed minute fecal excretion of gallium[72] and fecal excretion studies were discontinued. Table XIV (Section VI) lists the detailed urinary excretion data obtained from some of the patients.

TABLE XV: STUDIES OF THE URINARY EXCRETION OF Ga^{72} IN MAN
("Tracer" dose of about 1 mc.)

Patient No.	Diagnosis	Dose (mc.)	Date	Per Cent of Dose Excreted							Remarks
				First Day (in 8 hr. periods)			Second Day	Third Day	Fourth Day	Total	
				1st	2nd	3rd					
7	Multiple myeloma	1.00	7/31	*	*	1.0	*	*		4.0	
13	Multiple myeloma	0.92	8/4	13.0	5.0	5.0	6.0	3.0	2.0	34.0	
18	Multiple myeloma	1.00	8/31	13.0	10.0	5.0	24.0	3.0		55.0	
16	Osteogenic sarcoma	1.00	8/18	18.0		3.0	3.0	2.0		26.0	Pre-Vit. D†
16	Osteogenic sarcoma	1.00	8/28	22.0		19.0	8.0			49.0	Post-Vit. D
14	Osteogenic sarcoma	1.00	8/7	2.0	8.0	3.0	5.0	2.0		20.0	
9	Osteogenic sarcoma	1.00	7/31	8.0	3.0	2.0	6.0	2.0		21.0	
8	Osteogenic sarcoma	1.00	7/31	8.0	3.0	2.0	6.0	2.0	2.0	23.0	Pre-Vit. D†
8	Osteogenic sarcoma	1.00	8/7	6.0	5.0	2.0	5.0	2.0		20.0	Post-Vit. D
12	Osteogenic sarcoma	0.92	8/4	10.0		8.0	5.0	3.0		26.0	Pre-Vit. D†
12	Osteogenic sarcoma	1.00	8/10	22.0	14.0	1.0	13.0	5.0		55.0	Post-Vit. D
19	Osteogenic sarcoma	2.30	9/5	13.0	3.0	3.0	3.0	5.0		27.0	Pre-Vit. D†
19	Osteogenic sarcoma	1.91	9/11	Less than 1% Total Excretion‡							Post-Vit. D
23	Osteogenic sarcoma	1.91	9/11	19.0	13.0	5.0	5.0	3.0		45.0	Pre-Vit. D†
23	Osteogenic sarcoma	1.00	9/18	13.0	5.0	2.0	2.0	2.0		24.0	Post-Vit. D
4	Metastatic thyroid carcinoma	1.00	7/17	10.0	3.0	6.0	10.0	5.0	6.0	40.0	
6	Metastatic thyroid carcinoma	1.00	7/31	5.0	5.0	2.0	5.0	2.0		19.0	
11	Chronic myelogenous leukemia	1.84	8/4	5.0	Lost	6.0	3.0	2.0	1.0	17.0	
17	Hodgkin's disease	1.00	8/25	13.0	3.0	2.0	3.0	2.0		23.0	
21	Chronic lymphatic leukemia	1.60	9/7	24.0	8.0	2.0	2.0	5.0	2.0	43.0	
20	Lymphosarcoma	1.60	9/7	5.0	5.0	2.0	3.0	1.0	1.0	17.0	

* Less than 1%.
† Prior to and after a weeks course of 150,000 I.U. of concentrated Vit. D, t.i.d.
‡ Probably due to injection in subcutaneous tissue.

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TABLE XVI: STUDIES OF THE URINARY EXCRETION OF GALLIUM IN MAN

(Doses larger than 1 mc.)

No.	Date	Diagnosis	Dose: Mc. Ga^{72}	Dose: Mc./-kg.	Dose: Mg. Ga	Dose: Mg./-Kg.	Per Cent Excreted of Initial Dose: First Day (in 8 hr. periods) 1st	2nd	3rd	Second Day	Third Day	Fourth Day	Total
7	8/4	Multiple myeloma	8.8	0.16	50	1.0	3	2	3	3	2		13
	8/7		8.4	0.15	88	1.4	3	2	3	4	2		14
	8/10		8.0	0.14	103	1.9	2	5	3	5	2	2	19
	8/14		10.0	0.17	50	1.0	5	2	3	5	2	2	19
	8/18		8.7	0.16	68	1.2	5	3	2	5	3		18
	8/21		9.4	0.17	75	1.4	5	6	3	5	1	1	21
	8/25		9.0	0.16	86	1.4	5	2	8	6	2		23
	8/28		8.0	0.14	43	0.8	11	5		3			19
	8/31		14.9	0.27	53	1.0	3	6	1	6	2		18
	9/5		12.3	0.23	39	0.7	11	3	3	5			22
	9/11		49.9	0.92	166	3.1	10		6	5	2		23
			147.4	2.51	821	14.9							
13	8/28	Multiple myeloma	20.0	0.34	107	1.8	19	6	3	6	3		37
	8/31		40.0	0.69	153	2.6	21	11	5	6	3	3	49
	9/5		52.7	0.91	184	3.2	23	6	3	6			38
	9/7		44.7	0.77	173	3.0	25	11	2	6	3	1	48
	9/11		20.6	0.36	64	1.1	Less than 1 per cent excreted by time of death fifty-five hours post-injection						
			178.0	3.07	681	11.7							
18	9/5	Multiple myeloma	23.5	0.57	78	1.9	19	11	6	5			41
	9/7		20.3	0.50	80	1.9	6	3	2	10	3	1	25
			43.8	1.07	158	3.8							
17	8/20	Hodgkin's disease	20.0	0.34	107	1.8	29	5	1	4	2	1	42
	8/31		51.5	0.83	200	3.3	35	5	5	3			48
			71.5	1.17	307	5.1							
	8/7	Osteogenic sarcoma	19.6	0.30	150	2.30	37	3	6	5	2		53
	8/10		28.6	0.44	250	3.85	42	8		5	2	1	58
	8/14		50.0	0.80	250	3.85	40	6	3	6	1		56
	8/22		37.6	0.58	300	4.60	38	5	6	5	1		55
	8/25		32.0	0.50	300	4.60	42		8	3	1		54
	8/28		20.0	0.30	107	1.65	35	10	2	5	1	2	55
	8/31		51.5	0.80	200	3.08	38	8	2	3	1		52
			239.3	3.72	1,557	23.93							
8	8/10	Osteogenic sarcoma	21.3	0.32	170	2.54	27	19	3	4	2	1	56
	8/14		50.0	0.75	250	3.73	46	10	3	5	2	1	67
	8/23		28.3	0.42	300	4.48	51	6	3	5	2		67
	8/28		50.0	0.75	270	4.03	48	6	2	4			60
	8/31		51.5	0.77	200	2.98	45	8	5	5	2	1	66
	9/5		70.6	1.05	271	4.04	42	6	2	3			53
	9/7		66.8	1.00	256	3.82	30	Lost	3	5	2		40
	9/11		92.8	1.38	342	5.10	37	6	5	5			53
			431.3	6.44	2,059	30.72							
12	8/14	Osteogenic sarcoma	20.0	0.30	100	1.50	34	10	5	11	1	2	63
	8/25		20.0	0.30	190	2.64	46	5	2	4	2		59
	8/31		51.5	0.72	200	2.78	50	8	3	3	2		66
	9/5		77.7	1.07	270	3.75	46	6	3	3			58
	9/7		47.6	0.66	157	2.18	37	8	5	2	2		54
	9/11		72.3	1.00	258	3.58	24		5	2	2		33
			289.1	4.05	1,175	16.43							
16	8/31	Osteogenic sarcoma	27.5	0.86	90	2.81	37		5	2	3		47
	9/5		52.0	1.62	84	5.75	34		3	3			40
	9/7		46.3	1.45	179	5.59	32	2	3	3	6		46
	9/11		49.0	1.53	166	5.19	30	3		3	3		39
			174.8	5.46	519	19.34							

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From excretion data some general conclusions may be drawn. Individual doses ranged from about 1 mc. (4.7 to 5.2 mg.) up to about 77 mc.

1. In general there was a tendency for higher doses to be associated with a higher excretion of gallium in man. This is similar to the excretion pattern observed in detailed studies of the rat.

2. Although a higher proportion of the metal was excreted with higher doses, the absolute amount of retention of metal was far greater with higher doses.

3. In some patients there was a tendency for much greater retention of metal, regardless of dose, than in other patients. This fact could not be correlated with the disease or other data.

4. No relationship was demonstrated between the sequence of doses. A build-up of the metal retained within the body did not affect the excretion of succeeding doses.

5. There was no evidence in the urine of damage to the genitourinary tract due to the passage through the kidney of large amounts of either metal or radiation. Such damage did occur and was an outstanding feature of the toxicity picture in dogs. It was completely absent in man.

Tables XV and XVI show urinary excretion data for patients receiving about 1 mc. and larger doses, respectively.

VIII. Autopsy Studies of Distribution of Gallium^{72}

MARSHALL BRUCER, M.D., GOULD A. ANDREWS, M.D., DONALD J. REHBOCK, M.D., SAMUEL W. ROOT, M.D., and JOE GRAY

ERRORS INHERENT IN AUTOPSY MEASUREMENTS

In Table XVII there is listed the *differential absorption ratio to heart* of Ga^{72} as it was retained in patients coming to autopsy. It is difficult to interpret these autopsy data since many errors are cumulative. The dosages and time of death could not be planned. The tissues were not perfused. The short half-life demanded rapid measurement, and with many samples to be counted, some with very low concentration, a simple measuring system had to be used.

Only small pieces of organs were measured. When a number of samples from the same organ were measured, the variation from sample to sample was considerable. This is not due to an error of measurement but represents a true variation in deposition within the organ. In measuring any of the gross autoradiograms of slices of tissue, it is easy to see how two 0.5-gm. samples could be selected from adjacent areas of the same tissue and give widely different results. In patient 010003, the lowest and the highest tumor assays from samples taken from the same metastasis read 13 and 254, different by a factor of 20. In another study, of samples selected from an autoradiogram in which one piece of tissue was taken from the area of least photographic exposure and one from the area of most photographic exposure, variations by a factor of 100 were demonstrated.

In a series of studies on adjacent samples taken from the femur, the areas were selected after looking at a thin-slice autoradiogram of the bone. The following are the measurements for 4 of 26 areas:

Area of Bone	Corrected Counts per minute per gram
Cortical bone	2.8×10^4
Trabecular marrow	7.4×10^4
Epiphyseal line	1.5×10^5
Subperiosteum	7.4×10^5

These thin-slice autograms range from 2 to 5 mm. thick, which is large in terms of the range of beta particles. When even thinner slices are prepared, the variation becomes more pronounced.

Another major error follows from the fact that all of these patients were in a rapidly developing terminal state. The distribution of the last dose of Ga^{72} may have little

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TABLE XVII: RADIOASSAY OF TISSUES FOLLOWING AUTOPSY OF THIRTEEN PATIENTS WHO RECEIVED Ga^{72}

Differential Absorption Ratio to Heart

Patient	110054	110061	110083	110073	110117	010023	010003	010013	010016	010041	110079	010049	110064
Last dose of Ga^{72} (mc.)	0.5	21.3	47.5	21.3	5.7	36.0	13.0	20.6	17.3	22.0	3.6	15.9	20.5
Time last dose to death hour	1.5	5	5	6	7	15	15	30	40	44	51	77	32
Disease	Prostate	Osteo	Prostate	Osteo	Plasmo	Osteo	Osteo	Plasmo	Osteo	Plasmo	Breast	Kidney	Ewing's
Heart	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0*
Plasma	8.2	1.3	2.0	2.1	2.9	†			1.1	3.0	0.4	0.1	4.0
Skin		3.4	5.7	3.7	3.8	3.2	1.7	2.9	1.5	4.0	2.0	1.7	3.5
Muscle		0.6	1.3	0.8	0.7	0.8	1.1	0.7	0.3	1.0	0.9	0.8	0.0
Fat		1.7	0.8	1.0	0.6				0.2	1.0			0.0
Liver	1.3	3.1	3.2		11.3	8.5	8.8	6.9	9.2	20.0	4.9	7.2	48.0
Bile	0.2	1.1		2.1		0.1	2.5	3.3		16.0	4.7	3.0	
Spleen	0.7	2.1	2.3	2.6	4.3	2.4	2.2	7.8	5.9	7.0	4.4	3.4	15.5
Gastrointestinal tract	0.9		37.3			3.6	2.5	2.7	3.6	7.0	1.9	1.7	11.1
Kidney	1.1	18.7	3.9	82.1	227.5	15.1	30.2	6.2	26.7	25.0	39.5	16.2	52.3
Lymph node	2.1	3.9	8.0	3.4			2.9	1.9	1.7	4.0	7.6		34.3
Pancreas	0.8			1.6	2.2	2.4	2.6	1.6	2.5	4.0	2.1	2.7	6.5
Prostate			2.4			1.2		1.2				4.2	4.7
Bladder	0.2			3.1		2.7	4.3	1.6	0.8	49.0	1.4		3.5
Adrenal	0.7	2.0	1.5	2.8	5.7	3.4	2.0	2.4	5.7	9.0	2.3	7.6	81.0
Testis or ovary		1.7					1.8	1.7	1.4			1.8	7.2
Brain	0.1			0.1	0.1		0.7	0.2		1.0		0.3	BG
Pituitary		1.5	2.8	1.2	1.2		1.7	1.8				5.4	8.8
Lung	0.2	1.8	3.4		2.6	2.4	15.6‡	2.8	1.9	6.0	2.9	8.0	17.6‡
Thyroid	1.1	1.5	3.0	1.6	1.7	2.0	1.6	1.2	1.2	3.0		3.1	6.4
Bone cortex		30.1	25.9	51.7	4.4‡	11.6	4.4	11.3‡		23.0‡	20.5		139.0
Bone medulla	0.5	19.5	55.4	43.6	6.4‡	37.8	22.5	5.2‡	27.5	24.0‡	16.1	9.4	41.0
Bone, mixed	0.5	20.2	37.4	30.1		4.5	20.0	7.0‡	21.0	20.0‡	12.4		40.3
Bone cartilage				3.8	4.4	3.7	1.7	4.6	2.8	8.0	4.3	3.4	8.5
Tumor low	1.0	1.6	1.4	1.4		0.5	13.0		1.9	8.0		1.4	0.0
Tumor high	1.8	6.4	3.6	2.5		55.8	253.7		86.5	34.0		10.2	17.4
Tumor in liver	0.4		2.4	2.0							1.5		

* Unreliable count.

† The blank spaces indicate either an unreliable count or no tissue was measured.

‡ Normal tissue mixed with tumor.

BG—Background count

Prostate—Carcinoma of the prostate with metastases to bone.

Osteo—Osteogenic sarcoma with pulmonary metastases.

Breast—Carcinoma of the breast with metastases to bone.

Kidney—Carcinoma of the kidney with metastases to bone.

Plasmo—Malignant plasmocytoma.

Ewing's—Ewing's sarcoma.

or no relation to the distribution of the first dose given weeks earlier. In patients No. 010023 and No. 010013 (Table XVIII), the radiation measurements of the last dose can be compared with measurements of the total accumulation of stable gallium in all doses.

The concentration of stable gallium, although highly influenced by the last dose in some organs, shows a different distribution than that expected from the Ga^{72} tracer on the last dose. In tissues such as skin and brain, where there seems to be an early saturation deposit, the stable and radioactive concentrations are the same. In the kidney, the radioactive dose is in the excretion phase and is higher, as might be expected. The lung appears to accumulate a significant amount of material. The liver shows a very high deposition in the plasmocytoma case, very low in the sarcoma case. These differences illustrate the fact that all of the autopsy measurements are of an element in only the early part of its metabolic distribution.

TABLE XVIII: RELATIVE AMOUNT OF STABLE AND RADIOACTIVE GALLIUM IN TISSUES

(Normalized to Skin Concentration)

Tissue	No. 010023 Osteogenic Sarcoma		No. 010013 Malignant Plasmocytoma	
	Ga^{72}	Stable Ga	Ga^{72}	Stable Ga
Skin	1.0	1.0	1.0	1.0
Brain	Trace	Trace	0.1	Trace
Liver	2.3	1.9	2.4	13.0
Kidney	4.8	0.6	2.1	0.6
Lung	0.8	1.6	1.0	1.6
Tumor (least)	0.2	2.1	1.0	0.6
Tumor (most)	17.6	16.0	3.3	3.2

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Only one autopsy case is presented in detail, that of patient G. P. (010003).

A 14-year-old white school girl fell in the autumn of 1949 and scraped the skin of the left leg from the ankle to knee. After a few weeks, she noticed pain and swelling over the left mid-thigh. Following a diagnosis and a series of twelve x-ray treatments at a local hospital, she was referred to the Bowman-Gray School of Medicine in March 1950. There, after a biopsy and diagnosis of osteogenic sclerosis sarcoma, a disarticulation of her left leg was done on April 1, 1950. She was discharged on April 13 in good condition. There were no signs of metastases.

On May 7, 1950, the patient noticed a tight feeling in the chest, which she described as a deep chest cold. Readmission examination revealed pleural effusion and metastatic lesions in both lungs. It was felt that nothing further could be done with standard therapy and that because of the rapidly growing lesions the patient was a logical candidate for experimental therapy with radiogallium. She was therefore referred to the Medical Division of the Oak Ridge Institute of Nuclear Studies at Oak Ridge.

Upon admission, the patient was in reasonably good condition. Physical examination showed the findings which would be expected in the presence of rapidly growing pulmonary metastases and bilateral pleural effusion. Chest films obtained just prior to admission revealed extensive nodular lesions in both lung fields, with evidence of calcification in some. The left side was almost obliterated, but there was an area of probable tumor with calcification just over the diaphragm.

Figure 18 shows, in summary form, the patient's general course during the thirty-four days of hospitalization. As noted in the general progress notes, there appeared to be a relatively steady and rapid decline after the fifteenth day. This is confirmed by another estimate of general condition—the nurse's estimate of the patient's appetite. Following the fifteenth day there was a marked apathy toward food, with difficulty in eating, and on the twenty-fourth day a tube was inserted in the stomach and forced feedings were instituted. Pain of increasing severity developed in the region of the left chest. This was not a typically pleuritic sporadic pain but was described as steady and annoying and seemed to be located especially in the posterior left chest wall. There was tenderness to pressure over some areas. The daily estimate of the amount of pain which the patient seemed to experience is shown in Figure 18. Along with this is a record of the narcotics used, their kind, and the number of administrations. The patient was first given codeine, which was replaced by Demerol in increasing dosage. Morphine in 8-mg. doses was started on the nineteenth hospital day and changed to 6-mg. doses on the twenty-ninth day.

About midway during hospitalization a mild

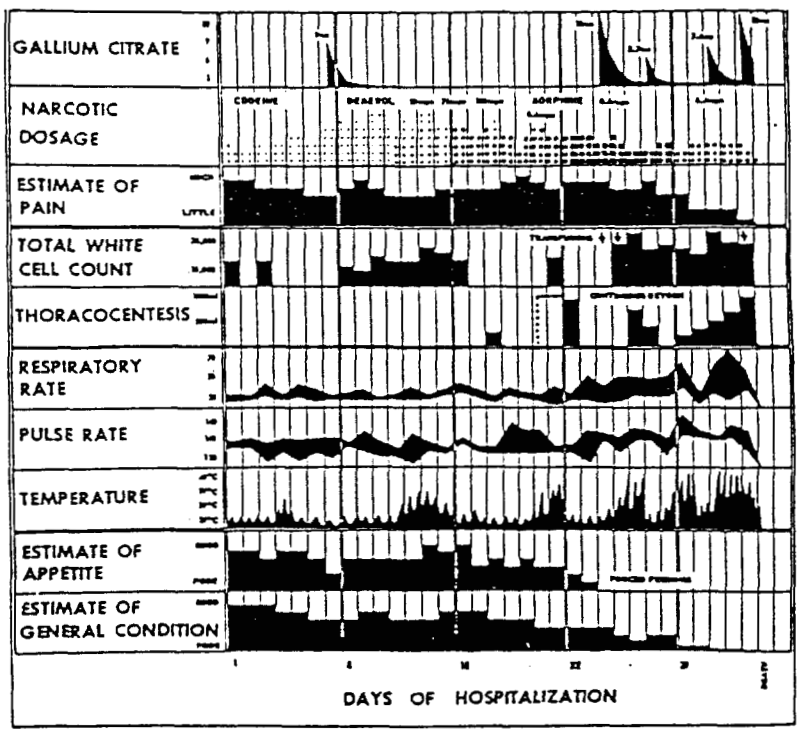

Fig. 18. Summary chart of Case No. 010003.

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TABLE XIX: EXCRETION OF RADIOGALLIUM INTO PLEURAL FLUID COMPARED WITH LEVELS IN PLASMA WITHDRAWN SIMULTANEOUSLY

Patient G.P. 010003: Osteogenic Sarcoma (Primary Removed) Metastatic to Lungs

Time of Withdrawal of Pleural Fluid	Volume Withdrawn (ml.)	Counts/min./ml. at "0" Time $\times 10^3$		Approximate* μc./ml. $\times 10^{-4}$	
		Pleural Fluid	Plasma	Pleural Fluid	Plasma
After 15 mc. dose					
48 hr.	750	3,500	8	9,270	21
After 5.7 mc. dose					
9 hr.	425	1,200	5	3,200	13
53 hr.	450	800	1	2,120	0.5
93 hr.	120	60	1	159	0.5
After 7.4 mc. dose					
5 hr.	500	2,400	8	6,350	21
22 hr.	550	500	4	1,330	11
32 hr.	625	800	3	2,120	8
47 hr.	750	1,000	1	2,660	0.5

* Measured 1.7 per cent beta counting efficiency.

shortness of breath developed, and it was apparent that pleural fluid was collecting at an increasing rate. A thoracentesis was attempted on the seventeenth hospital day. Only about 150 c.c. of fluid could be obtained. The fluid clotted promptly but incompletely. The sediment showed many red cells but no recognizable neoplastic cells. During the next few days the patient became progressively weaker and more dyspneic. On the twentieth hospital day continuous nasal oxygen therapy was started. Except for a few short periods in a tent, al oxygen was continued to death. Weakness dyspnea were progressive. Pleural effusion developed on the right side. Frequent thoracenteses were attempted. The material withdrawn became more viscous and, especially on the left, seemed to be pocketed in small thick-walled cavities. Because of the rapidly increasing respiratory embarrassment an attempt was made to dissolve the fibrin material with an enzyme preparation supplied by Lederle Laboratories. This preparation contained Streptokinase, 250,000 units, and Streptodormase, 200,000 units. Two ampules were injected on the left side twenty-four hours apart. Beginning on the thirtieth day of hospitalization, within forty-eight hours there was a striking change in character of the pleural fluid. It became possible to withdraw a large amount of hemorrhagic but free flowing fluid. Beginning on about the twenty-ninth day the patient became increasingly moribund. She died early on the thirty-fourth day of hospitalization.

Radiation Data: On the seventh day of hospitalization, a 9-mc. dose of gallium citrate was injected intravenously, preceded by a prophylactic dose of calcium gluconate. This was followed by 15 mc. on the twenty-fourth day, 5.7 mc. on the twenty-seventh day, 7.4 mc. on the thirty-first day, and 13 mc. fifteen hours before death. Radiation surveys with a highly collimated counting system revealed significant localized deposition of gallium in the epiphyseal regions and more activity over bone than over soft tissue in general. One area corresponding to the probably calcified tumor over the left diaphragm showed almost twice the activity of any other area.

About six blood samples were taken after each dose for radioassay. The gallium[72] curve for disappearance from the blood stream followed an almost exponential form. Up to about 50 per cent of the initial level was obtained in twenty-four hours: to 20 per cent in forty-eight hours. After ninety-six hours the amount was below efficient counting levels.

All urine samples were assayed. Approximately 60 per cent of the total dose was excreted *via* the kidneys. Of this amount, two-thirds was excreted during the first eight hours following injection; nine-tenths the first day after injection. An insignificant proportion of the excreted gallium was found in the feces. Attempts were made to study excretion in sweat and saliva, but the samples were at background levels.

An interesting accumulation of gallium occurred in the pleural fluid. In Table XIX, the pleural fluid counting levels are compared with levels in the plasma withdrawn at about the same time. There was a significantly greater amount of gallium retained in the pleural fluid than in the plasma.

Autopsy Report: The body appeared extremely emaciated. There was very little subcutaneous fat. There were no abnormalities in the head, neck, abdomen, and extremities. The left leg had been removed by a disarticulation amputation.

The right pleural cavity was almost obliterated by extensive fibrous adhesions which were readily separated. The left pleural cavity contained a large quantity of turbid brown fluid with many masses of fibrin-like material. The right lung was fully expanded but was studded with numerous flat tumor nodules measuring up to 2 cm. in diameter. The left lung was collapsed except in the upper third. The periphery of this upper third was formed by a thick layer of hard yellow tumor with multiple areas

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TABLE XX: RADIOASSAY OF AUTOPSY SAMPLES. Patient G.P., 010003

	ACT/gm.*	DARH†	DARCB‡
MISCELLANEOUS ORGANS			
Thyroid	3,375	1.62	
Pituitary	3,545	1.66	
Right adrenal	3,431	1.65	
Left adrenal	4,882	2.35	
Right ovary	3,668	1.62	
Left ovary	3,955	1.90	
Breast	2,575	1.24	
Uterus	3,213	1.54	
Urinary bladder	8,888	4.28	
Kidneys	6.27×10^4	30.16	
Heart	2,079	1.00	
Brain	1,409	0.68	
Larynx	4,333	2.08	
Trachea	4,487	2.16	
Left lung	2.7×10^4	13.00	
Right lung	3.24×10^4	15.58	
Skeletal muscle	2,332	1.12	
Skin	3,474	1.67	
Spleen	4,599	2.21	
Cervical lymph node	5,977	2.91	
Mediastinal lymph node	5,600	2.69	
Small bowel mesentery plus nodes	3,901	1.88	
DIGESTIVE TRACT			
Tongue	3,859	1.86	
Salivary glands	3,092	1.49	
Esophagus	5,327	2.56	
Stomach	2,688	1.29	
Jejunum	1.08×10^4	5.19	
Ileum	8,072	3.88	
Cecum	5,212	2.51	
Transverse colon	4,672	2.25	
Rectum	3,119	1.50	
Liver	1.82×10^4	8.75	
Gallbladder	6,993	3.36	
Pancreas	5,372	2.58	
Bile	5,111	2.46	
BONE			
Sternum	2.21×10^4	10.64	2.42
Fifth left vertebra	4.68×10^4	22.52	5.14
Shaft of femur	9,113	4.38	1.00
Trochanter of femur	2.69×10^4	12.95	2.96
Calvarium	1.9×10^4	9.14	2.09
Mandible	3.0×10^4	14.44	3.30
Distal metaphysis of femur	2.99×10^4	14.38	3.28
Distal epiphysis of femur	3.38×10^4	16.26	3.71
Distal epiphysis line area, femur	6.5×10^4	31.29	7.14
Fatty marrow, femur	6,492	3.12	0.71
Tooth (1st molar)	9,719	4.68	1.07
Articular cartilage, distal femur	3,550	1.71	0.39
OSTEOGENIC METASTASES			
Left diaphragm	5.26×10^4	25.32	5.78
Thoracic aorta area	1.85×10^5	89.24	20.37
Thoracic aorta area	5.27×10^5	253.68	57.92
Right thorax, retropleural	1.84×10^5	88.56	20.22
Apex, left lung	2.7×10^4	13.00	2.97
Adjacent to left adrenal	1.24×10^4	5.96	1.36
Upper lobe, right lung	3.24×10^4	15.58	3.56
Bony nodule, right lung	1.64×10^5	78.88	18.00
Bony nodule, right lung	8.86×10^4	42.62	9.73
Bony nodule, right lung	1.41×10^5	67.82	15.48
Bony area, left lung	2.85×10^5	137.18	31.32
Left diaphragm	2.23×10^5	107.26	24.49

* ACT/gm. Counts per minute per gram tissue (c./m./g.) with standard corrections.
† DARH. Ratio of c./m./g. tissue to c./m./g. heart.
‡ DARCB. Ratio of c./m./g. tissue to c./m./g. cortical bone (shaft of femur).

of calcification. The lower two-thirds were completely collapsed and mostly replaced by firm tumor with patchy areas of calcification. The diaphragm on the left side was infiltrated with tumor. The heart appeared normal.

Microscopically the tumor was characterized by cells of the osteoblastic type interspersed with much osteoid tissue. There were patchy areas of calcification.

The final pathologic diagnosis was osteogenic sarcoma metastatic to the lungs, primary in the left femur.

In Table XX a detailed autopsy radioassay for this patient is presented. The distribution is remarkably like that shown in the rat. A few exceptions can be explained. The very high counts in the lung are an artifact, since no really normal lung was obtainable. Some tumor was present even in the very small pieces. The very high counts in the kidney samples can be explained on the basis of the time of death after the last dose. The patient died just after the height of the excretory phase. There is some indirect evidence of excretion through the liver into bile. The low count in the stomach, high in the jejunum, and decreasing down the intestine would suggest the possibility of reabsorption but probably indicates insufficient time for the activity to progress down the gastrointestinal tract. The patient was in a terminal state and normal peristaltic movement cannot be assumed.

The wide variations in the bone and tumor samples do not represent the degree of variation which may have actually existed. These samples were taken without reference to gross autoradiograms and may have missed the areas of lowest and highest deposition.

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Table XVII lists the differential absorption ratios of various tissues as they are normalized to a heart value. This differential absorption ratio (H) value is the only valid comparative figure to use because of the variation in dosage, time of administration, and type of patient. The values were calculated from the average number of microcuries per gram of tissue as estimated from one to seven samples of similar tissues. The radiation values were corrected back to a "0" time, which was defined as the time of administration of the last dose of Ga^{72} received.

IX. Autoradiographic Studies

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During the clinical studies on $gallium^{72}$ a program of autoradiography was in the process of development. This was the first group of patients on whom the Medical Division staff had prepared autoradiograms, and the methods were undergoing change during the course of the study. Consequently, there is a great deal of variability in the technic used and the re-

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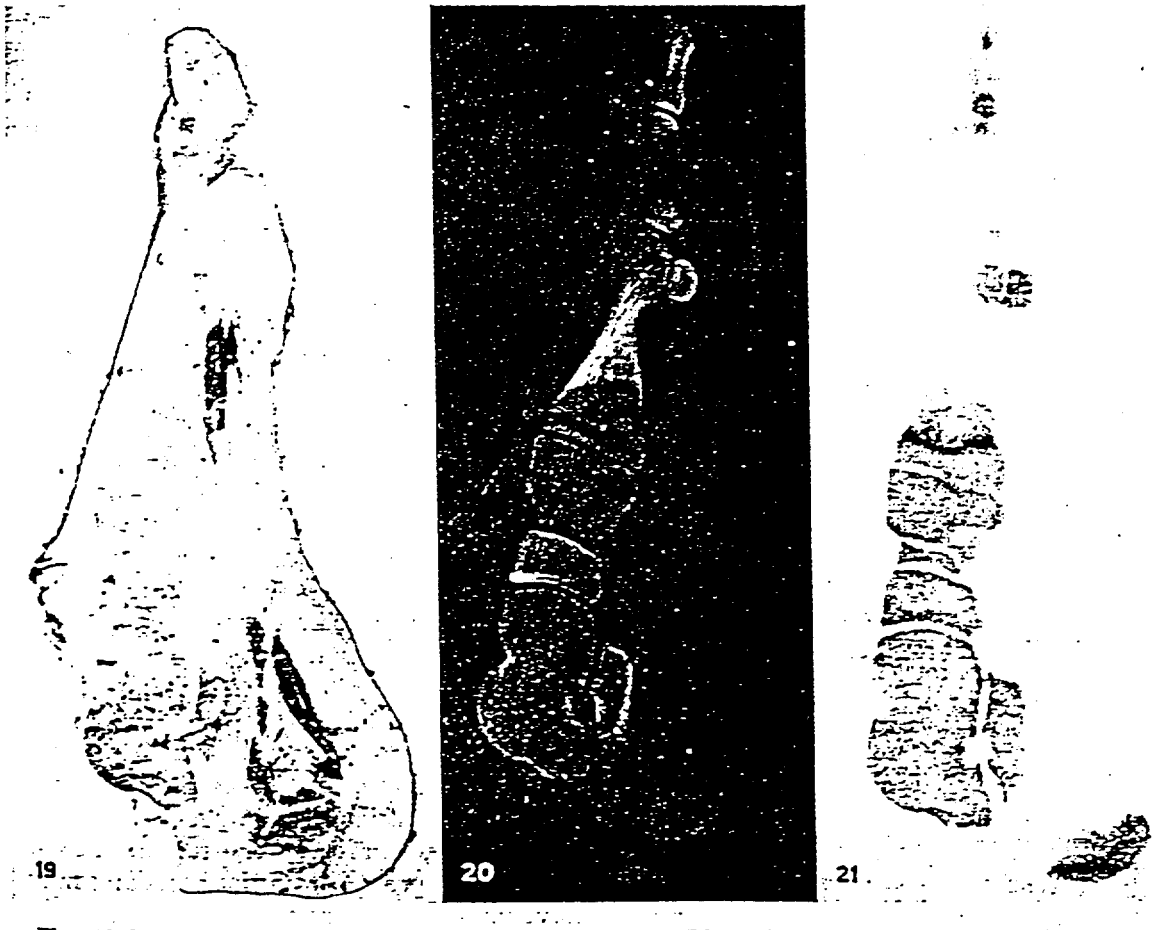

Figs. 19–21. Patient H. D., No. 010016, 12-year-old female. Diagnosis: Osteogenic sarcoma, primary present. (Detailed history given in Section VI.) For detailed legends, see following page.

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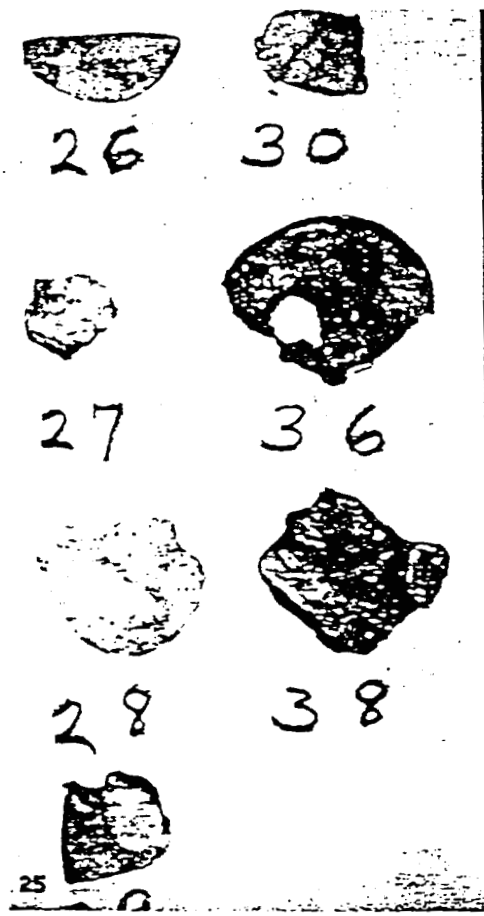

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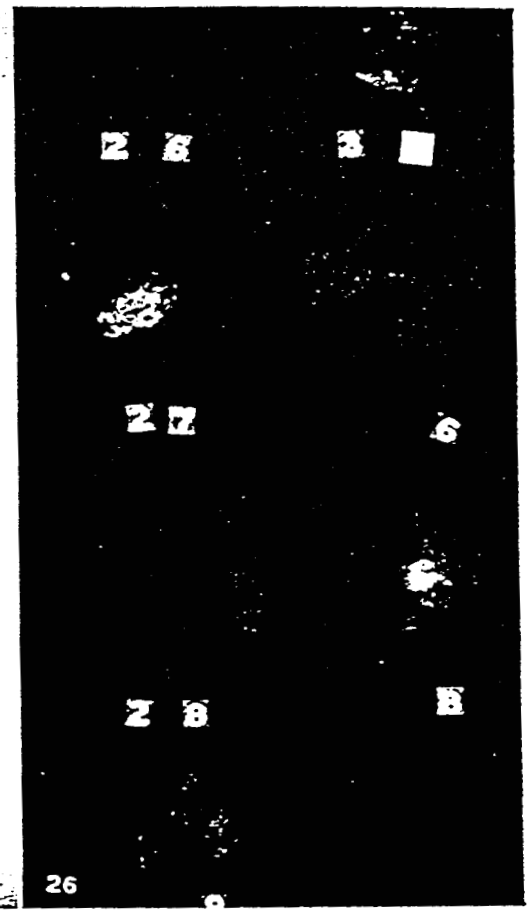

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Patient H. D., No. 010016 (see also preceding page).

Fig. 19. Photograph of a gross specimen prepared after a high thigh amputation had been performed. The leg was frozen in dry ice and cut with a band saw. The specimen is a longitudinal section of the foot 2 mm. thick. No neoplasm or abnormality. The pliofilm which is used to separate the specimen from the film is apparent in the photograph.

Fig. 20. Roentgenogram of the specimen, showing the normal bone detail in a thin section of the foot.

Fig. 21. Autoradiogram prepared from the specimen shown in Fig. 20. The patient was given 22 mc. of Ga^{72} forty-eight hours before operation. The autoradiogram was exposed for twelve hours. It shows the normal distribution of gallium in bone. There is a prominent concentration at the epiphyseal line at the upper end of the metatarsal.

Fig. 22. Photograph showing a section through the thigh. This shows the osteogenic sarcoma involving the femur.

For legends of Figs. 23–26, see opposite page.

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sults are not as suitable for a comparative s 'vsis as they might otherwise have b

Two methods for improving the usefulness of gross autoradiograms are apparent and are now being developed. One of these is the establishment of quantitative data so that, on the basis of the autoradiogram, it will be possible to determine the amount of the isotope in a given small volume of tissue. The other improvement is a better correlation of the histologic changes with the autoradiographic findings.

In spite of the limitations of these studies, they have contributed information not obtainable by other methods in the gallium study. They have shown in detail the distribution of gallium in some situations in which routine tissue assay methods are not sufficiently discriminating. They have served to emphasize the extremely uneven distribution of gallium in neoplastic metastases.

The autograms were prepared from specimens of tissue a few millimeters thick. Many of these were frozen in dry ice before c ng. They were cut by several methods. Most of the bone specimens were cut with a band saw. The exposure was made on Kodak x-ray, no-screen film; a sheet of pliofilm was used to separate the tissue from the film.

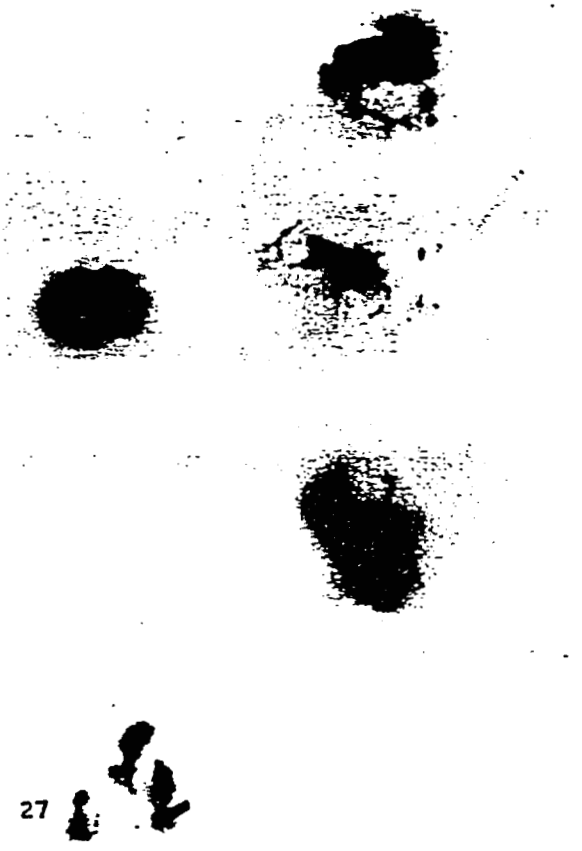

Fig. 27. Patient H. D., No. 010016. See also Figures 19–26. Autoradiogram prepared from the group of pulmonary metastases shown in Figs. 25 and 26. The patient had received 17.3 mc. of gallium[72] about two days before death. Comparison with the roentgenogram shows that the gallium distribution parallels calcification to a striking degree.

Figures 19–49 show photographs of gross specimens, roentgenograms of these specimens, and the autoradiograms.

Patient H. D., No. 010016.

Fig. 23. Roentgenogram of the specimen shown in Fig. 22, showing the striking abnormal bone formation by the neoplasm.

Fig. 24. Autoradiogram, exposed for twelve hours, and therefore comparable quantitatively with the one of the foot from the same patient (Fig. 21). It is apparent that there is a heavy concentration of gallium in the neoplasm and that the concentration seems largely related to calcification in the tumor. Remnants of the normal cortex of the femur show very little gallium uptake, as is usual in normal bone. The usual periosteal and endosteal uptake is absent because of the presence of neoplasm.

This patient was subsequently treated with radioactive gallium and died several months later.

Fig. 25 shows a group of pulmonary metastases which had been separated from the lung parenchyma and arranged for section and autoradiogram.

Fig. 26 is a roentgenogram of these metastases. In many of them calcification is prominent.

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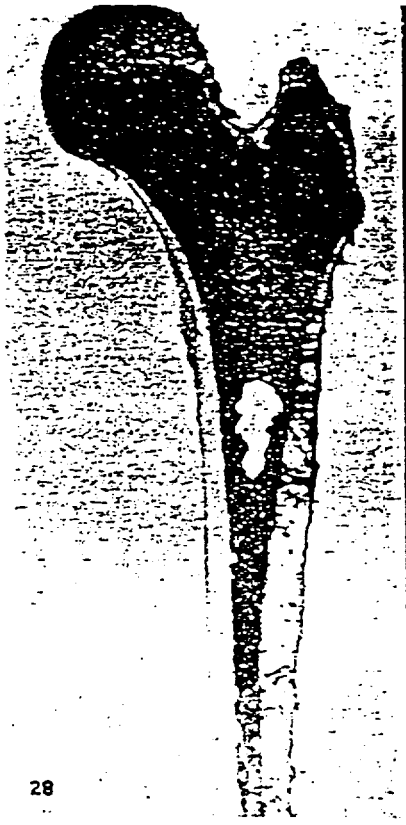

Figs. 28–30. Patient D. A. (No. 010023), 17-year-old male. Diagnosis: Osteogenic sarcoma.

Fig. 28. Photograph of femur obtained at autopsy, after a course of gallium treatment. It does not contain any neoplasm. The patient had pulmonary metastases from a previousy amputated primary lesion of the opposite femur. He had a striking pulmonary osteoarthropathy believed to be associated with the metastases.

Fig. 29. Roentgenogram of specimen in Fig. 28. Periosteal reaction with new bone formation is quite apparent.

Fig. 30. Autoradiogram from the same specimen. This patient had received 36.0 mc. of Ga^{72} approximately two days before death. The autoradiogram was exposed for twelve hours. There is a striking concentration of gallium associated with the periosteal proliferation.

Figs. 31–33. For legends, see top opposite page.

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Figs. 31–34. Patient M. L. H. (No. 010041), 37-year-old male. Diagnosis: Malignant plasmocytoma. Death after a dose of gallium had been given for excretion and autopsy studies.

Fig. 31. Photograph showing the upper end of a femur and the head of the femur which were broken during autopsy in spite of care in manipulation. It is apparent that the upper end of the femur is extensively involved by the plasmocytoma.

Fig. 32. Roentgenogram of specimen shown in Fig. 31. Extensive bone destruction due to malignant plasmocytoma is apparent.

Fig. 33. Autoradiogram from same specimen. The patient had received 23 mc. of Ga^{72} approximately two and a half days before death. The exposure was for twenty hours. There is a very distinct concentration of the gallium related to the lesion. It appears that the concentration is greatest at the edge of the tumor, where there is contact with residual normal bone.

Fig. 34. Roentgenogram of skull, revealing destructive lesions.

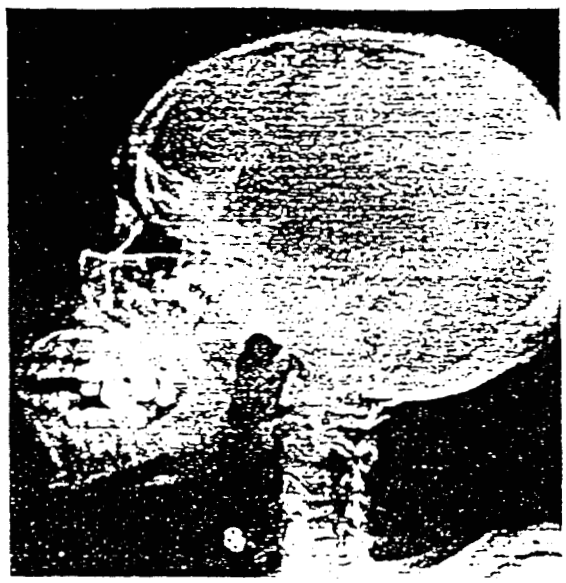

Fig. 35. Autoradiograms from the patient shown in Figs. 31–34. This is reproduced chiefly for the skull lesion, which appears in the form of a rectangular specimen at the left. This was a characteristic "punched out" lesion of malignant plasmocytoma in the skull. A section of bone was cut so as to include the lesion. The center consisted of well preserved malignant plasmocytic cells. It is apparent that the gallium concentrated at the periphery of the lesion, where normal bone was involved. This specimen was also exposed for twenty hours.

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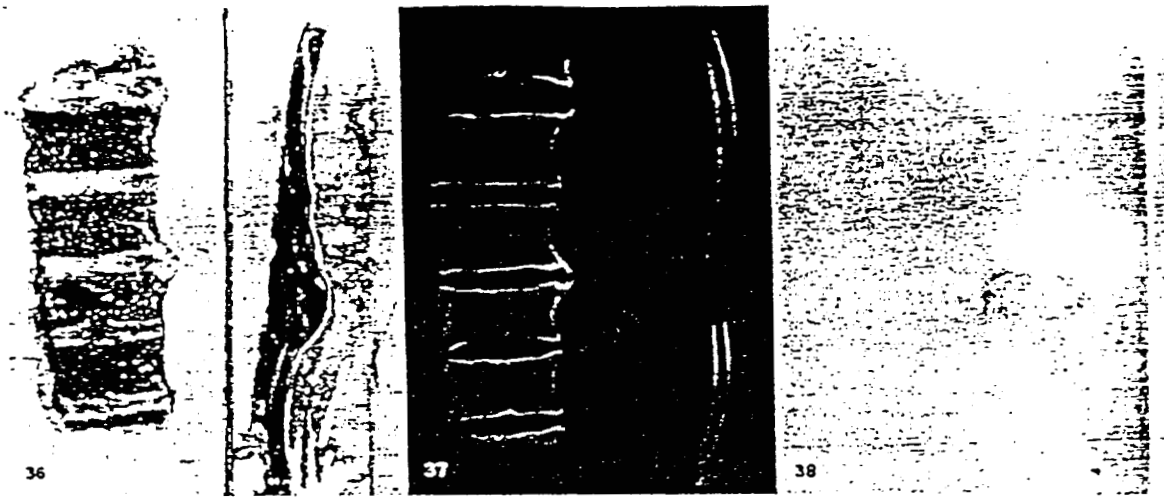

Fig. 36–38. Patient J. F. (No. 010049), 51-year-old male. Diagnosis: Adenocarcinoma of right kidney with metastases to bone. This patient had destructive bone metastases and died during a course of gallium treatment.

Fig. 36. Photograph of gross specimens imbedded in plaster for autoradiography. It shows a rib with an extensive destructive lesion and a section of spine, with a vertebral body invaded by neoplasm.

Fig. 37. Roentgenogram of specimens shown in Fig. 36. The left specimen has been inverted so that it is a mirror image of the one shown on the preceding photograph. The bone destruction caused by neoplasm and some hypertrophic lipping of the vertebral bodies are shown.

Fig. 38. Autoradiogram prepared from the same specimens. The patient had received 15.8 mc. of gallium about three days before death. The autograms were exposed for four days. The section of vertebral bodies is on the left and the section of rib on the right. The section of rib is in the same position as on the photograph and is opposite to its position shown in the roentgenogram. The rib shows only a few scattered spots of radioactivity. The vertebral body shows a distinct concentration of the gallium in the area involved by neoplasm.

Figs. 39 and 40. Photograph and autoradiogram of kidney of patient shown in Figs. 36–38.

Fig. 39. Kidney containing primary lesion. The more uniform appearing tissue is residual normal kidney.

Fig. 40. The small amount of gallium present is almost exclusively in the normal kidney and is absent in the neoplasm.

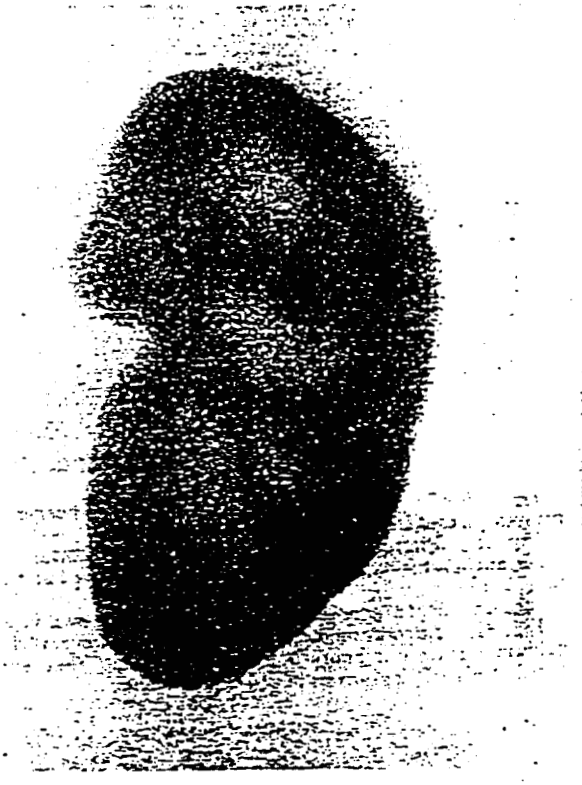

Fig. 41. Patient B. D. (No. 110061), 16-year-old male. Diagnosis: Osteogenic sarcoma. This patient received 21 mc. of gallium approximately two days before death. The above autogram is made from a section of normal kidney. It was exposed for three hours.

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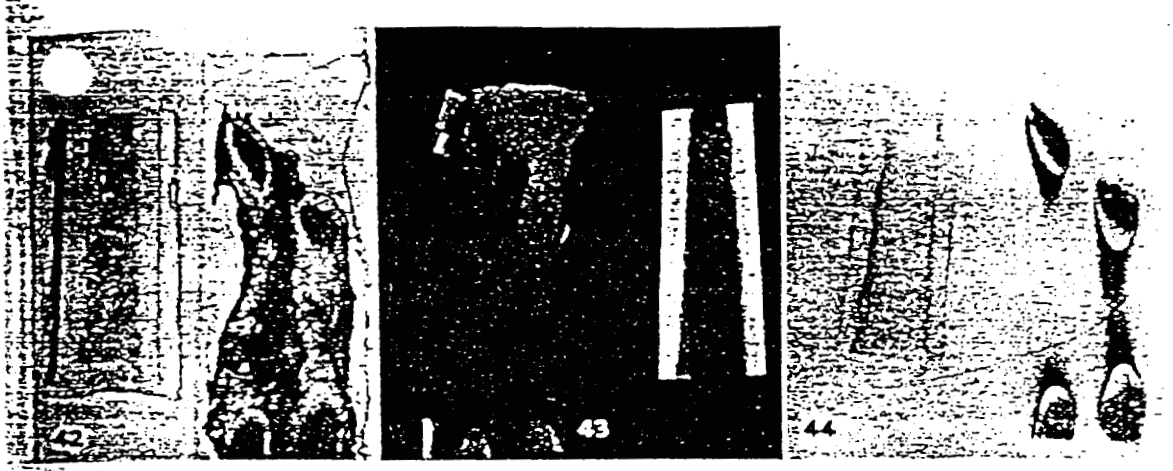
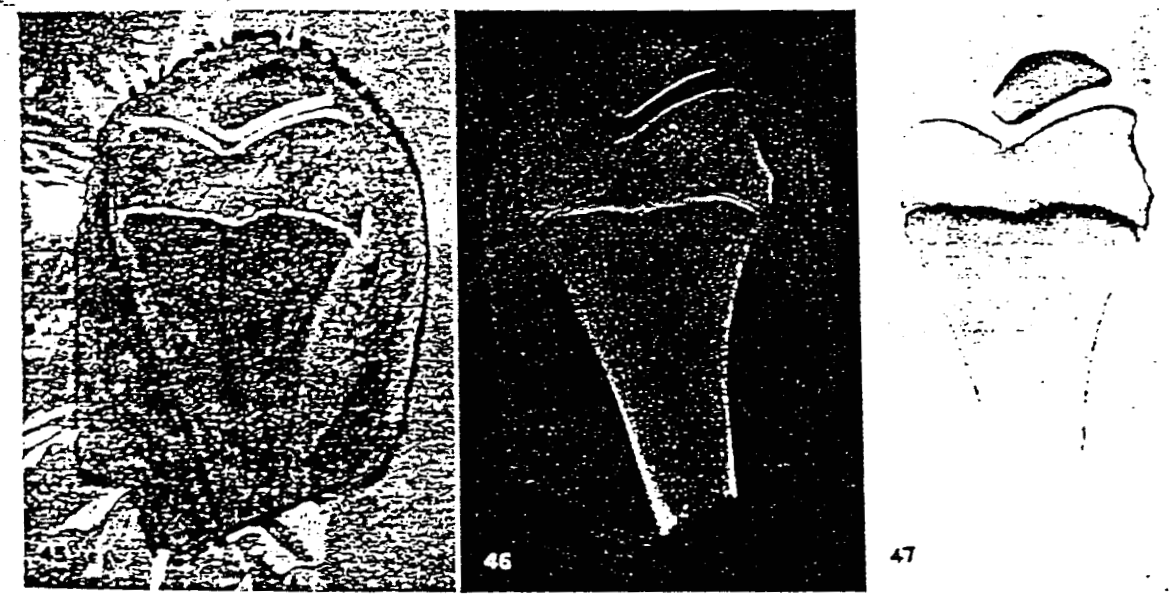


Figs. 42–44. Patient B. D. (No. 110061), with osteogenic sarcoma.
Fig. 42. Photograph showing gross specimen of ribs and shaft of femur mounted in plaster for autogram preparation.
Fig. 43. Roentgenograms of specimen showing bone detail.
Fig. 44. Gross autogram. Exposure was for three hours. There is a striking concentration of gallium in the periosteum of the ribs. The femur shows a delicate pattern of gallium along the endosteum, periosteum, and trabecular structures.



Figs. 45–47. Patient F. T. (No. 110073), 13-year-old male. Diagnosis: Osteogenic sarcoma; lung metastases; primary present.
Fig. 45. Photograph of the gross specimen obtained at amputation. The pliofilm covering is visible. Many other autograms were prepared from this patient but the one presented here is shown to indicate normal bone detail.
Fig. 46. Roentgenogram of the specimen shown in Fig. 45.
Fig. 47. Autoradiogram from specimen shown in Figs. 45 and 46. The patient had been given 20.4 mc. of gallium forty-eight hours before amputation. The exposure was for six hours. This shows the concentration of gallium at the epiphysis in a young patient.

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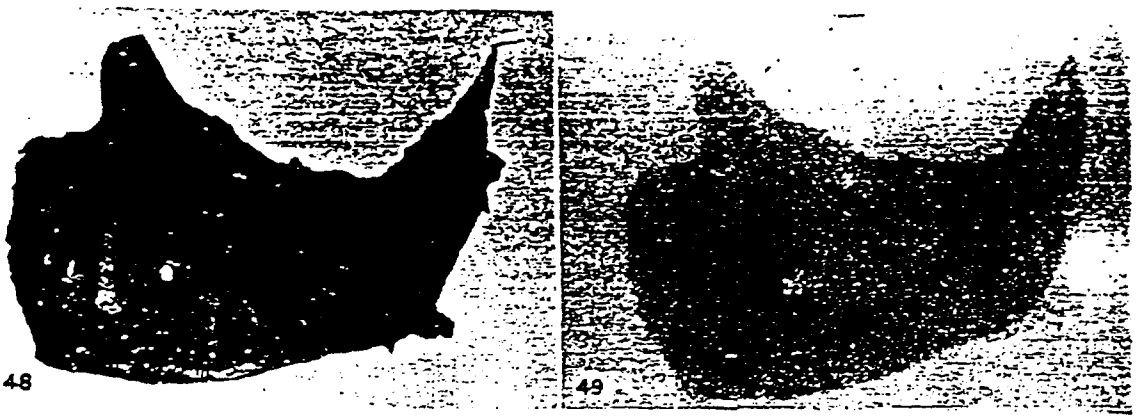

Figs. 48 and 49. Patient A. A. (No. 110117), 56-year-old female. Diagnosis: Malignant plasmocytoma.
Fig. 48. Gross photograph showing section of liver prepared for autoradiography.
Fig. 49. Autoradiogram prepared from the liver slice shown in Fig. 48. The patient had received 5.7 mc. of gallium five and a half hours before death. Exposure was approximately nineteen hours. There is a rather uniform distribution of gallium in the liver.

X. Preliminary Data on Gallium^{67}

H. D. BRUNER, M.D., RAYMOND L. HAYES, PH.D., and JESSE D. PERKINSON, JR., PH.D.

These studies on Ga^{67} were carried out in the belief that the metal's bone-seeking properties had not been fully exploited by the low specific activity Ga^{72} available from the Oak Ridge reactor. The short half-life of Ga^{72}, 14.3 hours, became a further disadvantage when it developed that the biochemical processes giving the desired differential localization were slower than the physical decay. Thus, much of the radiation was expended before the isotope had been differentially localized. Finally, the metal poisoning from the unavoidably present stable gallium limited the amount of radioactive Ga^{72} which could be administered.

All these very important practical objections apparently could be circumvented by use of Ga^{67}, provided it behaved like Ga^{72} in the body. The following basic experiments were designed to check this point, using for comparison the data reported in Section V of this series.

1. PHYSICAL DATA

Ga^{67} is prepared by a $(p, 2n)$ reaction with very high purity zinc in a 25-mev cyclotron. Except for Ga^{67}, all of the gallium radioisotopes also formed are relatively short-lived, so that after ninety-six hours, with a loss of slightly more than half the original Ga^{67} content, the target drops to a radiation level reasonably safe for processing. The extraction of gallium by isopropyl ether from 7 N HCl is remarkably complete and yields a carrier-free preparation (26). The term "carrier-free" is used in the sense that the concentration of the stable gallium isotopes, Ga^{69} and Ga^{71}, was below the limit of spectrographic detectability, which was 2×10^{-7} gm. of gallium per milliliter on the instrument available. Actually, there must have been present some stable gallium which originated from impurities in the target and from subsidiary nuclear reactions giving chiefly Ga^{69}. Since 1 mc. of Ga^{67} weighs 1.67×10^{-9} gm., the total gallium content of the solutions was between 2×10^{-7} and 2×10^{-8} gm. per milliliter.

The partial decay schemes of Ga^{67} stated in source books (27) were inadequate for calculating the probable tissue radiation dose. This necessary datum was provided by Doctors A. R. Brosi and B. H. Ketelle of the Chemistry Division of the Oak Ridge

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National Laboratory, who carried out original studies upon our request. They have suggested the decay scheme shown in Figure 50, using portions of three of the four runs of Ga^{67} prepared for the animal experiments reported below. The scheme and values are provisional and subject to modification on publication of the complete data, but such revision will be minor and will not significantly change the values in this report. It will be noted that there is one 8.6 kev *K* capture x-ray per disintegration, followed by a gamma ray, or, in some cases, a cascade of gamma rays. The weakest gamma ray, 94 kev, was found to undergo internal conversion in 40 per cent of cases. For dosage purposes, this is the equivalent *per disintegration* of one electron with a range of approximately 5 mm. plus 0.264 essentially monochromatic internal conversion electrons having a range of 0.13 mm. Thus, some of the energy of disintegration is expressed as electrons of high specific ionization and very short range.

The remaining gamma rays are therapeutically undesirable in that they give rise to a cross-fire effect on distant tissues which amounts to whole-body irradiation. Nevertheless, the inverse-square law favors the deposition of energy in the immediate vicinity of a local concentration of such a gamma-ray-emitting isotope.

The half-lives of these four samples of Ga^{67} as determined by a liquid counting system and a bismuth-wall gamma-counter tube was 3.24 days and ranged between 3.17 and 3.28 days (76.1 to 78.7 hours) by least-squares computation. This contrasts with values of 83 hours observed by Dudley (18) in samples of Ga^{67} produced by (*d*, *n*) and extracted differently. The half-lives obtained by Brosi and Ketelle with their refined instruments correspond to the seventy-eight-hour half-life. In this report, the 3.24-day half-life and 4.67-day (112.1 hours) mean-life will be used.

Using the formulations of Marinelli, Quimby, and Hine (3) and the above decay data, the radiation doses given in Table XXI were calculated on the basis of the

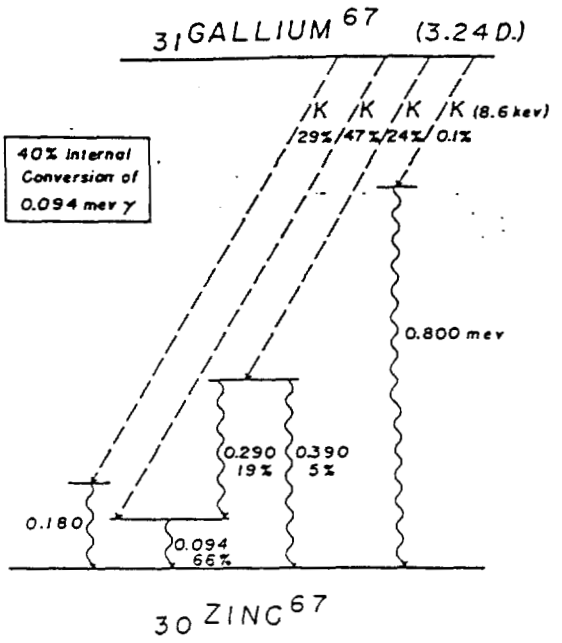

Fig. 50. A simplified decay scheme of Ga^{67} with a half-life of 3.24 days.

TABLE XXI: RADIATION DOSAGE COMPUTED FOR 1 MICROCURIE OF Ga^{67} OR Ga^{72} COMPLETELY DECAYING IN 1 GRAM OF TISSUE LOCATED AT THE CENTER OF THE THEORETICAL CYLINDER OF THE BODY (4)

		Total Roentgens	Beta Component	Gamma Component
Ga^{67}	Man	28.97	9.53	19.44
($T^1/_2$ = 3.24 days)	Rat	14.47	9.53	4.94
Ga^{72}	Man	79.00	20.60	58.40
($T^1/_2$ = 14.3 hr.)	Rat	35.05	20.60	14.45

roentgens or rep arising from the complete disintegration of 1 mc. in 1 gm. of tissue. The analogous data for Ga^{72} are added for comparison.

The I gamma value weighted for the frequency and energy of the various gamma-ray components was computed as 0.975 r/mc.-hr. at 1 cm.; this is 11 per cent of the 8.4 r/mc.-hr. for radium.

2. DISTRIBUTION OF CARRIER-FREE GA^{67} IN THE RAT

Procedure: Thirty-two male white rats weighing 240 ± 30 gm. received carrier-free Ga^{67} by the intravenous route. This solution contained 0.15 mg. of citric acid per milliliter to insure complexing and was adjusted to *p*H 7.4. The original Ga^{67} solu-

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TABLE XXII: DISTRIBUTION OF Ga^{67} IN SELECTED ORGANS OF RATS AT VARIOUS TIMES AFTER INTRAVENOUS ADMINISTRATION OF THE CARRIER-FREE Ga^{67} CITRATE COMPLEX

Day sacrificed Number of rats	1 6	3 6	5 6	10 5	15 5	20 4
Estimated cumulative dose in roentgens	0.22–1.08	0.80–2.14	1.85–8.10	11.12–18.25	55.20–144.0	165.0–284.0
A. Per cent retained	78.6	65.6	60.7	52.5	48.3	45.1
Per cent excreted	21.4	34.4	39.3	47.5	51.7	54.9
Fraction in:						
Urine	0.70	0.55	0.52	0.49	0.49	0.48
Feces	0.30	0.45	0.48	0.51	0.51	0.52
B. Total organ content in per cent of original dose						
Blood plasma	1.56	0.55	0.21	0.10	0.08	0.15
Blood cells	0.15	0.18	0.08	*	*	*
Liver	21.39	13.97	12.40	4.28	2.76	2.87
Spleen	1.27	1.17	1.19	0.99	0.87	1.23
Kidney	0.89	0.89	0.77	0.66	0.56	0.63
Heart	0.07	0.07	0.07	0.06	0.05	0.05
Femur	1.21	1.12	1.01	0.72	0.91	1.05
Skeleton†	26.25	26.07	25.32	16.19	21.79	23.43
Remainder	25.15	21.65	19.67	29.45	21.25	15.69

* Negligible.
† Calculated as 7.06 per cent of the body weight times the activity per gram of femur.

tions were diluted with blank citrate of the same concentrations and *p*H, since the rats sacrified on the first day required only 1/130 as much Ga^{67} as those sacrified on the twentieth day. Thus, all animals received identical injections except for the content of Ga^{67}, for an excess of radioactivity was experimentally undesirable. The largest amount of gallium was below spectrographic detectability.

The rats were housed in individual cages which permitted collection of urine and feces without contamination of each other (24). At one, three, five, ten, fifteen, or twenty days after injection, the rats were nembutalized, viviperfused with 200 ml. of saline, and the tissue was analyzed for Ga^{67} after being digested with HNO_3. A bismuth-wall Geiger-Müller tube mounted vertically in a lead shield counted the gamma rays according to the method described by Marinelli (28). The samples were counted to obtain a standard deviation of 2 per cent or less, or 5 per cent or less when the net count was very low, *i.e.*, less than twice background. In other respects, the experimental procedures were as described in Sections III and V of this series, except that the prophylactic dose of calcium was omitted.

Results: At the top of Table XXII are shown the numbers of animals and the estimated minimum and maximum whole-body radiation doses accumulated at time of sacrifice. Below, in order, are: (A) The average cumulative percentage of the total dose which was excreted or retained and the partition of the excreted fraction between urine and feces; (B) the average percentage of the injected dose retained by the organs or tissues listed. No great error was introduced by neglecting the weight of the organs, since the animals were of essentially the same size and the dose was calculated relative to weight. Each value in the table is the average for the number of animals indicated at the top of the table. The standard deviations, corrected for the small samples, ranged between 10 and 30 per cent of their respective means in most cases, but the liver and blood-cell fractions in some instances had standard deviations as much as 50 per cent of their means. In contrast, those for the femur were regularly about 10 per cent, except for the ten-day animals, 2 of which unaccountably gave distinctly lower values to break an otherwise smooth trend. On the whole, however, these means represented the central tendency reasonably well.

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The data in Table XXII show that the liver lost most of its initial Ga^{67} content, while the bone and sixteen other organs (some not shown in the table) retained most of their initial uptake during the twenty-day period. The continued presence of small amounts of Ga^{67} in the plasma suggests that plasma was acting as a transport-equilibration agent, as it does for iron.

The tissue distribution of the isotope retained at each interval is expressed in Table XXIII in terms of Marinelli's differential absorption ratio (3), which is computed as:

$$\frac{\text{Activity per gram of tissue}}{\text{Total retained activity} \div \text{body weight}}$$

This ratio permits inter-comparison of the relative Ga^{67} concentrations in the various tissues of the different groups of animals by normalizing initial dose, body weight, excretion, and variations of organ weights. During the first half-life of Ga^{67}, the first three days after injection, the liver, spleen, and femur showed approximately equal retention at a level about three times higher than the next tissue, the kidney. After the third day, the spleen had the highest relative concentration, not because it picked up additional amounts, but because it lost relatively less than the other tissues, as can be seen from Table XXII. The liver differential absorption ratio decreased sharply between the fifth and fifteenth day and thereafter held a low level about equal to the kidney, lung, and lymph nodes. The femur showed a mild increase after the first day, the figure remaining at about 0.75 the spleen value. Of the several types of osseous tissue analyzed, the incisor teeth, including the pulpy roots, gave the highest values, followed closely by the shaft of the femur. Sixteen additional tissues (most are omitted from the table) showed differential absorption ratios of 1.0 or less, although for some tissues a distinct trend could be made out, as for the lymph nodes in Table XXIII.

The urinary excretion during the first twenty-four hours following injection was 2.5 times that in the feces, but thereafter the feces contained 1.5 to 3.0 times as much activity as the urine per daily collection period (Fig. 51). Forty per cent of

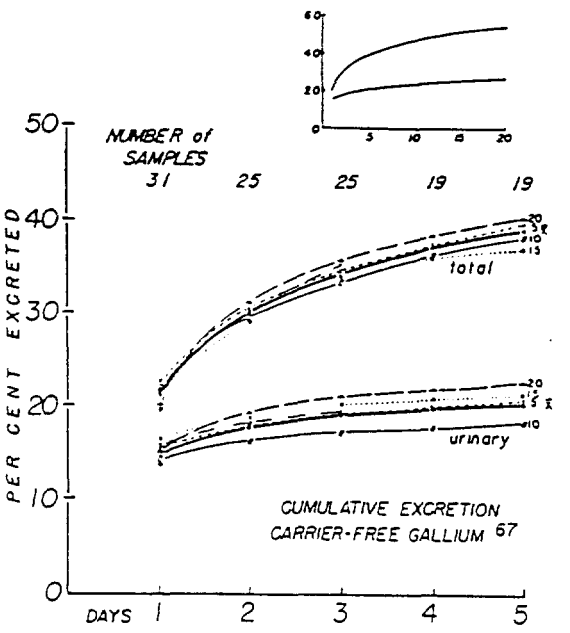

Fig. 51. The total and urinary excretion of Ga^{67} during the first five days after injection of the carrier-free Ga^{67} stabilized at pH 7.4 with citrate. The average values for all groups are shown by the heavy lines. The light lines represent the averages of each group separated according to time of sacrifice, which was associated with size of dose and total radiation received (Table XXII). The mean excretion values for the entire twenty days are shown in the upper right-hand box.

TABLE XXIII: AVERAGES OF THE DIFFERENTIAL ABSORPTION RATIOS OF TISSUES OF RATS SACRIFICED AT THE INDICATED TIMES AFTER INTRAVENOUS ADMINISTRATION OF CARRIER-FREE Ga^{67} CITRATE COMPLEX

Day of sacrifice Number of rats	1 6	3 6	5 6	10 5	15 5	20 4
Liver	7.1	5.8	5.5	2.4	1.7	1.8
Spleen	5.1	6.9	8.1	8.5	8.0	10.3
Kidney	1.3	1.7	1.5	1.5	1.3	1.8
Heart	0.3	0.3	0.3	0.3	0.3	0.4
Lung	0.9	0.7	0.8	2.1	0.8	1.7
Lymph nodes	1.4	1.6	1.8	1.8	2.9	1.4
Blood plasma	0.7	0.2	0.1	0.1	0.1	0.1
Blood cells	0.1	0.1	0.1	*	*	*
Skeletal muscle	0.3	0.2	0.2	0.5	0.5	1.0
Skin	0.4	0.3	0.3	0.3	0.3	0.4
Teeth	2.8	4.4	4.9	6.5	6.3	8.2
Mandible	4.0	5.0	4.8	4.7	6.5	7.4
Calvarium	2.3	2.8	3.1	2.6	4.1	4.2
Femur	4.7	5.6	5.9	4.4	6.5	7.4
Shaft	5.0	6.1	6.2	4.4	7.3	7.7
Epiphyses	4.3	4.9	5.4	4.5	5.6	7.1

* See footnote to Table XXII.

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the total dose was excreted by the fifth day and 57 per cent (range 51.9 to 67.3 per cent) by the twentieth day. The average 50 per cent excretion point, the biological half-life (3), occurred on the thirteenth day. The separate average excretion patterns for the various groups of rats (Fig. 51) were essentially identical, despite the range of radiation and metal dosage. Although the level of metal dosage is so small that a hundredfold variation is not detectable, such a range in radiation dosage might well have affected body water-excretion processes. For example, the twenty-day rats received about 100 times as much radiation over the first twenty-four hours as the rats which were sacrificed at twenty-four hours, but the excretion patterns of the two were similar.

Discussion: These results differed significantly from those in Section V, which dealt with the distribution of Ga^{72}-labeled gallium citrate administered at the mg./kg. dose level. In those experiments, the differential absorption ratio of the femur was about four times greater than that of the liver, the next most active tissue at twenty-four hours (3.3 hours beyond the mean-life of Ga^{72}). At ninety-six hours, the femur was twelve to fourteen times more active than the spleen; during that time the liver had lost much of its gallium, while the femur and spleen had not. The cumulative excretion over ninety-six hours totaled 76 per cent, with the urine contributing 8.5 times as much as the feces. Also, at this mg./kg. level of carrier gallium, a fifty-fold variation in dose of metal produced significant differences in the total amount excreted during the first twenty-four hours and in the urine/feces ratio. The above carrier-free results, therefore, differed in: (*a*) percentage of the dose excreted, (*b*) the pattern of excretion, (*c*) the absence of changes in excretion pattern when the concentration of metal was varied more than a hundredfold, and (*d*) the time changes and inter-relations of the differen-

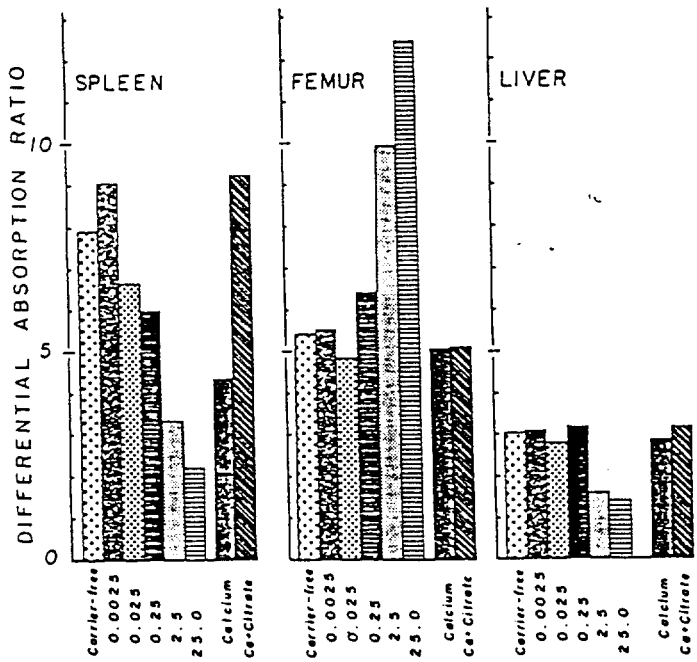

Fig. 52. Histogram of the data of Table XXV, showing the relation of the differential absorption ratio to dose level of carrier gallium in milligrams per kilogram. The metal was injected intravenously five days previously as the citrate complex. The two control groups received calcium plus tracer Ga^{67}, and calcium plus tracer Ga^{67} plus Na citrate in the amounts used at the 25 mg./kg. dose level.

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TABLE XXIV: DISTRIBUTION OF GALLIUM IN THE ORGANS OF RATS SACRIFICED FIVE DAYS AFTER INTRAVENOUS ADMINISTRATION OF INCREASING DOSES OF CARRIER GALLIUM CITRATE COMPLEX TRACED BY Ga^{67}

Dose in mg. Ga/kg.	0.00	0.0025	0.025	0.25	2.5	25.0	Ca Control	Ca + Cit Control
Number of rats	4	4	4	4	4	4	4	4
A. Per cent retained	56.1	59.2	59.7	47.9	31.1	18.5	62.0	57.4
Per cent excreted	43.9	40.8	40.3	52.1	68.9	81.5	38.0	42.6
Fraction in:								
Urine	0.51	0.49	0.52	0.67	0.81	0.91	0.53	0.55
Feces	0.49	0.51	0.48	0.33	0.19	0.09	0.47	0.45
B. Total organ content in per cent of original dose								
Blood plasma	0.16	0.24	0.18	0.25	0.15	0.07	0.23	0.22
Blood cells	0.07	0.07	0.05	0.06	*	*	0.07	0.03
Liver	5.81	6.52	6.37	5.88	1.83	0.98	6.64	6.09
Spleen	1.66	1.46	0.85	1.00	0.31	0.13	1.10	1.86
Kidney	0.92	0.95	0.81	0.85	0.38	0.19	1.10	0.83
Heart	0.06	0.07	0.06	0.05	*	*	0.07	0.06
Skeleton†	21.57	23.19	23.63	21.58	21.65	15.72	22.14	20.07
Remainder	25.88	26.73	27.71	18.19	6.78	1.41	30.66	28.23

* and † See footnotes to Table XXII.

tial absorption ratios of femur, liver, spleen, and other organs to one another.

Because of the following additional variables, it cannot be assumed that the presence or absence of carrier gallium was solely responsible for these differences: (1) Ga^{67} was used for the carrier-free experiment, whereas Ga^{72} acted as the "tracer" for the stable $Ga^{69, 71}$, which was unavoidably mixed with Ga^{72}. The conventional assumption is that body mechanisms cannot distinguish between the isotopes of elements above carbon but, in the absence of data to the contrary, it should be regarded solely as an assumption. (2) The associated radiation doses differed both as to total amount, timing, and, in consequence of the different differential absorptions, the distribution over the body. (3) In the carrier-free experiments, the total amount of citrate injected was approximately 10 μg. and was not preceded by calcium.

The following experiment, therefore, was planned in order to minimize or control these variables while bringing together the preceding experiments which had dealt with the extremes of carrier dosage.

3. EFFECT OF THE QUANTITY OF GALLIUM ON ITS DISTRIBUTION

The essential design of this experiment was to use carrier-free Ga^{67} to "trace" stable $Ga^{69, 71}$, which was varied by a factor of 10 over 5 orders of magnitude, holding constant the mol ratio of gallium to citrate to calcium ions and the total volume of injections.

Procedure: Thirty-two male white rats weighing 250 ± 20 gm. were divided at random into eight groups of 4 animals each. For each rat a constant amount of carrier-free Ga^{67} was taken, to which was added carrier $Ga^{69, 71}$ citrate in proportion to body weight plus saline to make 1 ml. The quantity of carrier was adjusted to give dose levels of 0.00 and 0.0025, 0.025, 0.25, 2.5 and 25.0 mg. of gallium per kilogram of body weight for six of the eight groups. These mixtures, preceded by the requisite amount of calcium gluconate, were injected intravenously *via* the tail vein, as described in detail in Section IV. The mol ratios of gallium to citrate to prophylactic calcium ion were maintained in all groups at 1.0 to 1.2 to 2.4. Sterile physiological saline was used to dilute the concentrated solutions appropriately and to wash in the injections.

The two remaining groups served as controls against the possible effects of the calcium or the calcium-plus-citrate injections. One group of 4 rats received calcium gluconate followed by carrier-free Ga^{67} and saline in the amounts used at the 25 mg./

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TABLE XXV: AVERAGES OF THE DIFFERENTIAL ABSORPTION RATIOS OF TISSUES OF WHITE RATS SACRIFICED FIVE DAYS AFTER INTRAVENOUS ADMINISTRATION OF INCREASING AMOUNTS OF CARRIER GALLIUM CITRATE TRACED BY Ga^{67}

Dose in mg. Ga/kg.	0.00	0.0025	0.025	0.25	2.5	25.0	Ca Control	Ca + Cit Control
Plasma	0.08	0.12	0.09	0.14	0.13	0.09	0.20	0.10
Blood cells	0.03	0.03	0.02	0.04	0.02	0.02	0.04	0.02
Liver	3.06	3.08	2.76	3.21	1.61	1.40	2.83	3.17
Spleen	7.91	9.03	6.66	6.01	3.29	2.17	4.29	9.21
Kidney	2.10	1.94	1.60	1.94	1.51	1.33	1.98	1.67
Heart	0.30	0.33	0.28	0.27	0.15	0.11	0.31	0.27
Femur	5.45	5.55	5.34	6.39	9.89	12.42	5.06	5.13
Remainder	0.88	0.86	0.85	0.86	0.92	0.91	0.85	0.87

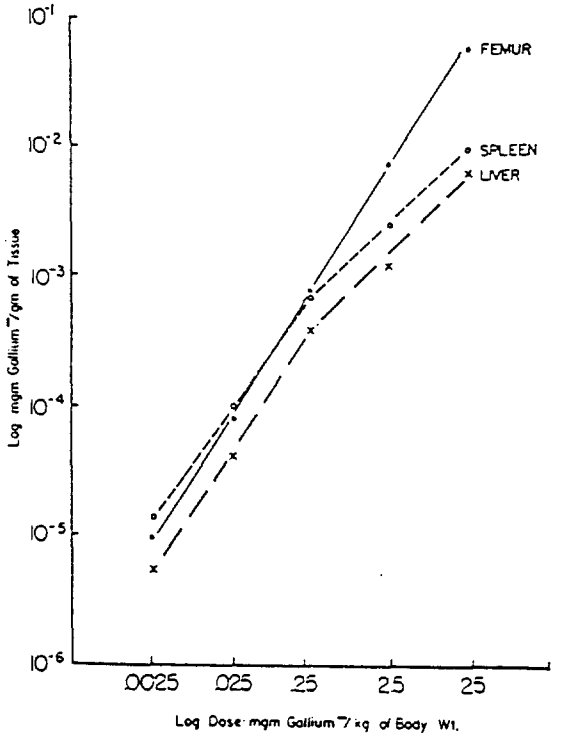

Fig. 53. The log of the absolute milligrams of gallium retained per gram of spleen, femur, and liver was plotted against the log of the dose in mg./kg. of body weight.

kg. dose level of carrier gallium. The other received calcium gluconate followed by carrier-free Ga^{67} plus sodium citrate adjusted to *p*H 7.4 and saline in amounts used for the 25 mg./kg. dose level. All animals were sacrified one hundred and twenty hours after injection. They were viviperfused, and the activities of the tissues were determined as described above (p. 604). These experiments were comparable to the others of the series in all other respects.

Results: The distribution and excretion patterns of gallium in the carrier-free and low-dose groups (Tables XXIV and XXV) matched the analogous data for rats killed at five days in the preceding experiment (Tables XXII and XXIII). On the other hand, the results for the 2.5 and 25 mg./kg. groups agreed with the four-day data of the Ga^{72} tracer experiments described in Section V. Thus, the metabolic processes of the rat apparently were not able to distinguish between Ga^{67} and Ga^{72} and, hence, between Ga^{69} and Ga^{71}, the stable isotopes. An isotope effect did not appear in these experiments.

The distribution of gallium seems not to have been affected by differences in the radiation emitted by these two isotopes. The differences were both in the absolute radiation dose, as shown in Table XXI, and in the rate of delivery of the dose to tissues due to the differences in half-life and shifts about the body relative to time. Thus, the five-day cumulative whole-body dose for the Ga^{67} rats was estimated to be about 5 r received more or less exponentially over the five days, whereas, the 25 mg./kg. Ga^{72} rats received about 80 r exponentially over the first day.

With one exception, the data of the two control groups coincided closely with the corresponding carrier-free and low-carrier-dose groups (Tables XXIV and XXV and Fig. 52). The exception, the spleen of the calcium control, was the result of one anomalous low value. Otherwise, the data indicate that the calcium alone, or the calcium plus citrate, did not significantly influence the distribution of the element. On the other hand, because calcium and

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citrate are present in blood and tissues, a re good experimental control cannot be obtained until another salt satisfactory for intravenous injection is found. Thus, it cannot be absolutely denied that calcium or calcium plus citrate was necessary for the observed changes. Nevertheless, the abrupt shift within a continuous series which was designed on constant mol proportion procedures, identified gallium as the important variable.

Figure 52, prepared from the data of Table XXV, shows that a change in the relative distribution of Ga^{67} occurred when the amount of carrier $Ga^{69,71}$ exceeded the 0.25 mg./kg. level. Above this dose, the five-day differential absorption ratio of the femur sharply increased. At the same time that of the spleen and liver, and to a lesser extent that of the lung, heart, and lymph nodes, decreased significantly. Thus, the relative concentration of gallium was higher in the spleen than in the femur at low carrier doses, but this relation was inverted at the high levels. The point of inversion was between 0.25 and 2.5 mg. of gallium pe ogram.

Figure 53 and Table XXIV indicate that this differential in favor of the femur was not due to the bone retaining a relatively larger fraction of the higher doses of gallium; instead, the other organs retained less. By plotting the log of the absolute milligrams of gallium retained per gram of a tissue against the log of the dose in milligrams per kilogram of body weight, linear relationships were obtained. These retention curves for the spleen and liver were inflected at the 0.25 mg./kg. dose and, in so far as three points warrant, the slopes were essentially linear both below and above the point of inflection. The retention by the femur, on the other hand, was essentially linear over five orders of magnitude on both axes; however, the somewhat lower value for the femur at the 25 mg./kg. dose suggests that this tissue, too, was beginning to shade off. Such linear relationships correspond to the formula $Y = a \cdot X^b$, but in view of the small numbers of animals no attempt was made

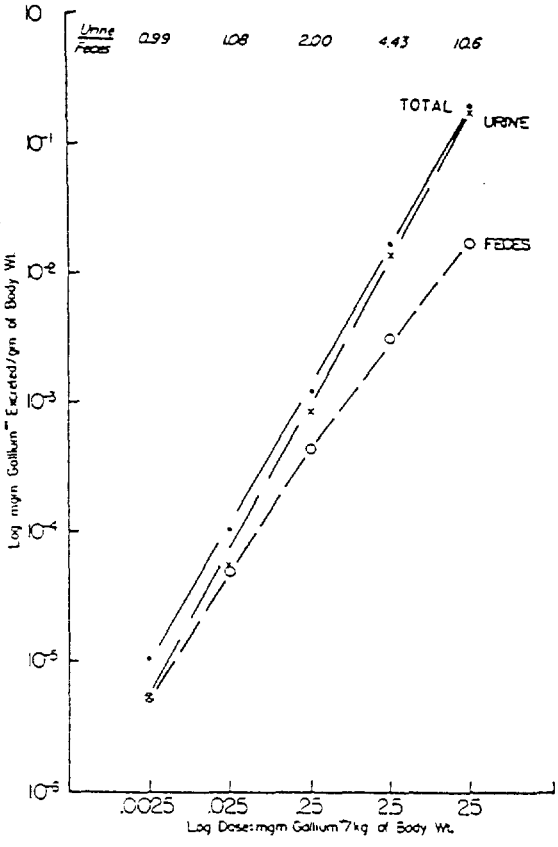

Fig. 54. The log of the absolute milligrams of gallium per kilogram excreted in the urine or feces, and the total excretion, was plotted against the log of the dose of gallium in milligrams per kilogram of body weight. The urine to feces ratio at the various dose levels is given across the top.

to obtain the numerical values of the constants a and b.

The excretory pattern altered as the dose of carrier gallium increased. As shown in Table XXIV and at the top of Figure 54, the urine and feces contributed about equally to the five-day total excretion at doses of carrier between 0.00 and 0.25 mg./kg. Above this level the urine/feces ratio increased, finally reaching 10.5 at the 25 mg./kg. dose. In all instances, the bulk of the urinary excretion occurred during the first twenty-four hours after injection; thereafter, the feces carried as much or more gallium than the urine per twenty-four-hour collection period. Figure 54, which is a log-log plot of the absolute amount of gallium in the urine and feces relative to dose of carrier, makes it clear

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that this seeming predominance of the urinary route was the result of decreased fecal excretion. Urinary excretion proceeded linearly over this 10^5 dosage range, while the fecal excretion showed a point of inflection at the 0.25 mg./kg. of carrier level of the liver and spleen in Figure 53. The data in Table XXV and Figure 54 make clear the sequences in the transition from the carrier-free pattern in section 2 above to that seen in the large carrier dose experiments using Ga^{72} in Section V.

Discussion: These data show that the quantity of gallium administered influenced the manner in which the metal was distributed and excreted by the rat. So far as is known, this point has not been studied in detail with reference to a substance essentially foreign to animal metabolic processes. A carrier effect on iodine uptake by the thyroid is now well recognized and more recently a carrier effect has been described for Ca^{45} (29), but in both instances the isotopes were added to an already existing metabolic pool of the element. The added labeled atoms were, therefore, competing with the rest of the pool for the metabolic processes responsible for the uptake. How successful they were would depend upon the relative number of labeled and stable atoms in the pool. In the case of calcium, the number of atoms in the blood pool is very large, 5.4×10^{21} compared to the 7×10^{14} in a millicurie of carrier-free Ca^{45}. The absolute size of the iodine pool (1.2×10^{18}) is much smaller than that of the calcium, but still very large compared with the 3.7×10^{13} I^{131} atoms in a 1-mc. carrier-free dose. The addition of carrier has the effect of enlarging the pool, diluting the radioisotope, and decreasing the chance that a given radioactive atom will be metabolized in a particular way and time.

Gallium presents a different problem in that it is normally absent from body tissues except for traces in bone (11), *i.e.*, less than 1 μg. per gram or 1 part per million, the limit of chemical analysis. However, because gallium occurs in all materials containing aluminum and zinc, it would be expected that some gallium must be present in blood. The 25, 2.5, and 0.25 mg./kg. doses of carrier would produce initial average concentrations of 25, 2.5, and 0.25 μg./gm. respectively. Such concentrations are well above the concentrations which might normally exist in blood, but the still lower doses would give concentrations in the same range as blood. A ratio of injected gallium to normal blood content of possibly 10^{-1} to 10^{2} is, however, quite different from the ratios for Ca^{45} and I^{131}, which would be in the range of 10^{-5} to 10^{-7}. Thus, except at the carrier-free dose, it is difficult to think that the injected gallium competes for the biological processes in the sense that I^{131} or Ca^{45} does.

The data in Figures 53 and 54 suggest a different possibility. Such linear relationships on log-log paper, corresponding to $Y = a \cdot X^b$, occur regularly in many varied physiologic processes and especially in interspecies relationships (30). Therefore, it is possible that the metabolic processing of the injected gallium was orderly and took place in a physiologic manner. This need not imply that the mode of retention was absorption, ion exchange, or other mechanism, or that the excretion process in the kidney involved a renal threshold, was a secretory process, or damaged the tubules. On the other hand, it is unlikely that there are processes in the body specifically designed to deal with gallium.

Probably only the highest dose of gallium was in the pharmacologic or toxic range. The 25 mg. of gallium carrier per kilogram is about 10 per cent of the beginning of the lethal dose range, which is not too far above 220 mg. of gallium per kilogram (Section IV). No chemical signs of toxicity were observed except that this group of rats remained quiet for a day. Histologic evidence of damage to the viscera was absent.

According to the data on rats given large doses of gallium labeled with Ga^{72} (Sectior V), a rather good differential localization i bone was obtained by the fourth day aft injection. The experiments in this rep demonstrate that the differential was tained by default of the other organs:

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tissues other than bone *retained* relatively less of the metal at the higher doses of carrier. Prior to these experiments, it had been more or less tacitly assumed that the bone exhibited a special metabolic affinity for gallium. These data indicate that the process(es) responsible for the uptake by the liver, spleen, and similar organs had less capacity to handle high loads than did that for bone. The similarity of the slopes of liver, spleen, and femur at the low dose levels suggests that the process(es) of uptake, whatever it was, was not too different in these tissues. Shubert (31) has observed that non-specific biochemical reactions occur more frequently than is suspected, particularly when the material is present in concentrations of 10^{-6} molar or lower. This may apply to the uptake of gallium.

While it does not follow that the liver, spleen, and bone of man will show this carrier effect, the regularity of these rat data suggests that it is not restricted to the rat but is a physiological generality. Therefore, the clinical studies must be planned and conducted with this possibility in mind. The practical application of these experiments is that this factor, which may prove to be of critical importance for successful use of Ga^{67} in man, would probably not have been suspected and identified in even the best clinical study.

In using Ga^{67} to treat osteogenic and related neoplasms, an additional complication must be recognized. The neoplasm may or may not share in this segregating process, and it is possible that a given tumor may exhibit completely independent mechanisms for picking up or rejecting gallium and other substances.

In short, whether Ga^{67} is differentially retained by osteogenic neoplasms can be determined only by assay in patients with tumors, but the assay must be planned with provision for the carrier effect.

SUMMARY

Preliminary data necessary for biological use of radioactive Ga^{67} are presented.

In a conventional tracer experiment on 32 rats with carrier-free Ga^{67}, the urine and feces contributed about equally to the 39 per cent excreted in five days. The femur, representative of bone, had a differential absorption ratio about 0.75 that of the spleen, while the liver lost much of the Ga^{67} activity it had picked up initially. These data differed in several respects from those of comparable experiments in which the rats received milligram amounts of stable gallium[69, 71] labeled with Ga^{72} (reported in Section V of this series).

In a succeeding experiment, the factor responsible for these differences was identified as the quantity of carrier or stable gallium administered to the animals. By controlling or keeping constant all factors except quantity of carrier gallium, it was found that the carrier-free pattern occurred when the dose of carrier was below 0.25 mg. of gallium per kilogram; above this level the pattern associated with high doses of carrier was obtained. A therapeutically desirable part of the latter experiments was a differential absorption ratio for bone which was ten to fifteen times higher than the second highest tissue. This difference was caused by a decreased percentage retention of the activity by the liver and spleen as the dose was increased, while the percentage retention of activity by the bone remained approximately constant.

Thus, these experiments suggest that stable $Ga^{69, 71}$ may have to be added to the carrier-free Ga^{67} preparations in order to secure the high differential concentrations of the radioisotopes in or around osteogenic neoplastic growth, but the question will have to be answered by experiments on patients suffering from such neoplasms.

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SUMARIO

Estudio del Galio

Con todo pormenor preséntase este minucioso estudio del galio radioactivo (Ga^{72}), llevado a cabo por el personal directivo de la División Médica del Instituto Oak Ridge de Estudios Nucleares, de Oak Ridge, Tenn., E. U. A.

Estudiáronse las características físicas e irradiatorias del isotopo e investigáro cuando poseían valor biológico, los asp químicos. La hemi-vida es de 14.3 h que se aproxima al límite inferior factibilidad para empleo clínico. una gran cantidad de compuestos, el de galio resultó ser el más ac

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Usáronse varias técnicas de preparación para adaptar el complejo citratado para empleo animal y humano.

Los experimentos de toxicidad general en ratas y perros revelaron una sensibilidad muy distinta conforme a especies. La DL 50 de diez días para ratas consecutivamente a la administración endovenosa fué de 220 mg. de galio por kilogramo de peso, comparado con una DL 50 en quince días de 18.2 mg. por kilogramo en los perros que recibieron las mismas preparaciones.

Los estudios en las ratas mostraron que el estado físicoquímico del compuesto de galio afectaba la distribución del metal consecutivamente a la inyección intravenosa. Al inyectarse en forma de complejo citratado soluble a un pH de 7.4, el hueso reveló la máxima absorción diferencial, aumentando esto durante un período de tres días. Ese diferencial favorable se debió a que el hueso absorbió inicialmente una fracción mayor de la dosis y la retuvo más firmemente que los tres tejidos que le siguieron en absorción, a saber, el hígado, el bazo y el riñón.

Consideróse que esas observaciones justificaban un ensayo clínico del complejo en el hombre, y más en particular en los tumores óseos, aunque no aportaban información relativa a qué dosis suministraría la absorción específica máxima compatible con la mínima toxicidad metálica e irradiación orgánica total.

En general, estudiáronse dos grupos de enfermos. En uno, tratóse abiertamente de producir un efecto terapéutico con Ga^{72}. En el otro, no se hizo ninguna prueba terapéutica, sino que se estudió la localización o absorción en los varios tumores o sitios. El radiogalio fué administrado intravenosamente a varias dosis a plazos de tres o cuatro días. Durante el tratamiento y después se ejecutaron frecuentes estudios hematológicos, se hicieron valoraciones de la radioactividad, se obtuvieron autorradiografías de los tejidos extirpados en la biopsia o autopsia y se verificaron los estudios histológicos de rigor. También se midió la excreción de galio en la orina y las heces.

Las manifestaciones más uniformes de toxicidad se relacionaron con el tubo gastrointestinal: anorexia, náuseas y vómito. Se observó una profunda depresión de la médula ósea, debida probablemente a la irradiación. Las reacciones cutáneas se imputaron a la toxicidad del estable galio. La distribución de éste en las lesiones óseas fué desigual, pero parecía existir tendencia a la localización en zonas de osteoactividad, ya osteoblástica u osteolítica. En general, aunque se administraron cantidades máximas del isotopo, hubo falta casi absoluta de toda mejoría clínica, si bien se observaron algunos signos de pasajero beneficio sintomático en enfermos que tenían metástasis carcinomatosas. No hubo ningún indicio del menor retardo del desarrollo de los sarcomas osteógenos, ya primarios o metastáticos.

Acompáñanse relatos de los estudios de la excreción urinaria en el hombre y de los estudios autorradiograficos.

Completa el trabajo una reseña preliminar de los estudios experimentales realizados con el galio[67], que tiene una demivida de 3.17 a 3.28 días.

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