

Urinary EXCRETION OF GALLIUM

NMRI-531 ✓
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NAV1.954611.002

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RESEARCH REPORT
Project NM 007 081.06.11

NAVAL MEDICAL RESEARCH INSTITUTE
NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND

17 December 1951

ABSTRACT

A method is described for the separation of gallium from urine by diethyl ether extraction of 6N HCl solution. Colorimetric or fluorometric estimation of 8-hydroxyquinoline-gallium complex in chloroform is the basis for quantitative determination.

In the rabbit and dog, intravenous injection resulted in lower urinary excretion of the administered gallium citrate, comparable with animals similarly treated by subcutaneous injection. The urinary excretion of gallium during the first 24 hours after its intravenous injection varied widely (4 - 78 per cent) in patients receiving radiogallium (Ga^{72}). No correlation was observed between the clinical manifestations and the rate or amount of Ga excreted.

Studies of the rate of urinary excretion of Ga^{72} in man and dog indicate that in both the rate is initially high, but reaches a low level within 18 hours. Blood gallium levels fall rapidly after intravenous injection and approach low constant values within six hours.

Published in part in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE 37: 676, 1951. Additional data on the urinary excretion of gallium by man and animals are included.

Issued by The Naval Medical Research Institute

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INTRODUCTION

In previous studies it was demonstrated that parenterally administered gallium was rapidly deposited in osseous structures (1,2). It thus appeared that the element might prove useful in the study of bone metabolism. Studies with the radioactive isotope (Ga^{72}) both in the laboratory (3,4) and clinically (5,6) indicate the radioactive isotopes of gallium may have medical significance. Since urinary excretion was shown to be the chief means of clearance of this element (7), more detailed studies of this phase of the metabolism of gallium were indicated.

The present report deals with (a) a chemical method for the determination of gallium in urine, (b) a study of the influence of dose and route of injection on the urinary excretion of gallium by the rabbit and the dog, and (c) a study of the gallium excretion of patients receiving single doses of gallium citrate intravenously. The gallium was the carrier which accompanied Ga^{72} used in the clinical study of persons having neoplastic lesions involving bone.

METHODS

The chemical procedure for the determination of gallium in urine is based on the fact that gallium may be quantitatively extracted from 6N HCl solutions by diethyl ether (8). After separation the gallium is converted to an 8-hydroxyquinoline complex in chloroform solution (1,9). In the range of 0.05 to 1.5 mg., the yellow color of the complex is measured in a photocolorimeter. In the range of one to ten micrograms, the fluorescence produced by ultraviolet light is used to estimate the gallium content of unknowns.

Analytic procedure.- To 20 cc. of freshly voided urine are added ten drops of concentrated (37 per cent) HCl to dissolve all precipitates. Add 4 cc. of 40 per cent hydroxylamine hydrochloride solution and 1 cc. of 10 per cent sodium acetate solution. Let stand in a stoppered container for at least ten minutes. Add 20 cc. of concentrated HCl, cool, and extract 10 cc. of this mixture twice with 10 cc. portions of acidified ethyl ether. The ether should have been previously shaken with twice its volume of 6N HCl.

The ether extracts are combined and extracted twice with 10 cc. portions of distilled water. The gallium chloride passes into the aqueous layer.

To the aqueous gallium solution, add 2 cc. of 40 per cent hydroxylamine hydrochloride and 1 cc. of 10 per cent sodium acetate solution. Adjust the pH of this mixture to 2.5 to 2.8. The control of the pH at this point is of greatest importance to insure specificity. Add 2 cc. of 5 per cent 8-hydroxyquinoline sulfate (previously adjusted to pH 2.6).

Extract the hydroxyquinoline complex with two 5 cc. portions of redistilled chloroform. Adjust the final volume to 10 cc. with a small amount of chloroform;

cover with about 0.5 cc. of the water phase. Let stand for at least 15 minutes and compare with standards. In the range 0.05 to 1.5 mgms., the yellow color is a measure of the gallium in the chloroform solution. In the range two to ten micrograms, fluorescence may be used to estimate the gallium content in a suitable photofluorometer having an ultraviolet source.

Notes on analytic procedure.-

(a) Prepare hydroxyquinoline and hydroxylamine solutions fresh weekly and store under refrigeration.

(b) In the colorimetric procedure recovery at 0.005 mg. is 70 per cent of the added gallium. In the range 0.01 to 1.0 mgs., recovery is 96 to 98 per cent of added Ga. Using the fluorometric technique, at 1 to 2 micrograms, gallium recovery is 80 per cent of added standard amounts, while in the range of 3 to 10 micrograms, recovery is essentially quantitative. Details of the recoveries are shown in table 1.

The specificity of this method depends on the reduction of iron by hydroxylamine in the presence of sodium acetate. In the ferrous state iron is not extracted by ether from 6N HCl. Such iron as may normally be carried into the final 8-hydroxyquinoline solution will offer no difficulties at a pH of 2.5 to 2.8. Dark color in the chloroform solution indicates the presence of excessive amounts of iron and a probable pH of 3 or more in the final extraction procedure. Low values in the fluorescent range will be obtained if the final chloroform extraction is at pH less than 2.5. Indium may interfere, but has not been found in the urine samples studied. No normal constituents of animal or human urine have been noted in routine sampling which give fluorescence under the conditions of this procedure. High blanks can be expected if the conditions are not controlled, and particularly if the 8-hydroxyquinoline solution is not prepared weekly.

(c) Prepare standards of 5 gamma and 0.5 mgms. content and carry through the entire extraction procedure. Use these solutions as reference standards since this method will correct for unavoidable losses through extraction and will quickly indicate spurious results due to contamination of reagents.

(d) In figure 1 are shown the color characteristics of the 8-hydroxyquinoline-gallium complex in chloroform, and the light transmission of the filter used. The fluorescence produced by ultraviolet light is a continuum in the yellow-green range. Filters available with most photofluorometers are satisfactory for this emission.

(e) In figure 2 are shown the curves indicating the range in which the light transmission or emission is a straight-line function of the concentration of gallium-quinolate in 10 cc. of chloroform.

(f) The final chloroform extracts if covered with a small amount of the aqueous phase (reducing atmosphere) are stable for 24 hours or longer if stored at 5 to 10° C.

For the study of the influence of dose and route of administration on the amount of gallium excreted in the urine, albino rabbits were injected in the ear vein or subcutaneously over the lumbar region of the back, with a solution of gallium citrate containing 20 mg Ga/cc, at pH 6.5 to 7.0. The animals were housed in individual metabolism cages. Urine was collected at the end of each 24-hour period, and the gallium content of a suitable aliquot was determined by the procedure described above. Dogs were similarly treated but in the case of this species the intravenous gallium was given by way of a superficial femoral vein.

The radiogallium (Ga^{72}) citrate used in the excretion and blood gallium studies of the patients was injected into one of the superficial veins of the forearm. Blood samples were drawn at suitable intervals from a vein of the opposite arm. The dogs used in the radiogallium studies were similarly treated, except that one of the superficial femoral veins was used as the portal of entry, and site of withdrawal of blood.

The preparation and standardization of the gallium and radiogallium (Ga^{72}) citrate solutions have been described in detail elsewhere (4,10). The radiogallium (Ga^{72}) citrate was contained in carrier gallium and was administered to the patients in the tracer studies at dosages ranging from 3 to 9 mg carrier gallium, which contained 300 to 700 microcuries Ga^{72} . In the case of those patients receiving the larger doses (60 - 70 mc Ga^{72}), the gallium was immediately preceded by an intravenous dose of 500 to 800 mg calcium chloride ($CaCl_2$). In the dogs given 12 mc Ga^{72} , 500 mg of intravenous calcium chloride was also required to prevent the onset of hypocalcemic shock. Under all circumstances the counts obtained from Ga^{72} samples were calculated to a reference time, usually time of injection, correcting for decay ($T_{1/2}$, 14.3 hrs.).

RESULTS

In table 2 are shown the results of a study of the influence of the route of parenteral administration (intravenous or subcutaneous) on the urinary excretion of gallium by the rabbit. Also shown are the effects on excretion of varying the subcutaneous dosage. These results indicate that at a moderate (non-toxic) dose of gallium citrate the rabbit excretes a significantly greater quantity of gallium when it is administered subcutaneously than if the gallium is introduced intravenously. If the toxic range (15-45 mg Ga/kg) is given subcutaneously the proportion of gallium excreted is significantly lower than in the case of the smaller subcutaneous dose (5 mg Ga/kg).

In table 3 are shown the results of a study of the influence of the route of administration and of dosage on the urinary excretion of gallium by the dog. These results indicate that, as in the case of the rabbit, intravenous injection tends to decrease the excretion of gallium by the dog. The influence of dosage is not so clear in the case of the dog, probably because the two dose levels are relatively close together (1 and 5 mg Ga/kg). The LD_{50} for dogs is approximately 15 mg Ga/kg.

In table 4 are shown the results of studies of the urinary excretion of gallium by man (male and female patients) receiving intravenous doses of gallium citrate ranging from 3 to 71 mg Ga. These persons exhibited clinical manifestations of neoplastic conditions, many of which involved bone. These patients received the indicated quantities of nonradioactive carrier gallium, and the results of urine examination shown in part 1 of table 4 were carried out by the chemical technique reported above.

In part 2 of table 4 are shown the results of the studies on urinary excretion of gallium as determined by radiochemical counting techniques. These results confirm and augment the results of the chemical determinations. In the 60 patients no correlation between the clinical picture and the rate of excretion of gallium has been observed. The wide variation in the 24-hour specimens (4-78 per cent of injected Ga) carries through to the 48-hour specimens. In general, the major fraction of gallium is excreted within the first 24 hours after injection. The patients who excreted a small fraction during the first 24 hours usually continued the low rate of excretion during the next 24 hours.

Details of radiogallium excretion.- In figure 3 are shown typical results of detailed studies on the urinary rate of excretion of Ga^{72} by the dog, following intravenous injection of gallium citrate (total, four dogs studied). These findings indicate early excretion, followed by negligible amounts. These results are explained by the rapidly decreasing blood concentration of gallium (Ga^{72}) (fig. 4) which reached a low, relatively constant value within six hours after intravenous injection.

In figure 5 are shown the detailed results obtained from the study of a catheterized patient who received a tracer dose (0.4 mc) of Ga^{72} and later a larger dose (12 mc). This case is typical of three other patients similarly studied, and indicates marked early urinary excretion with decreasing amounts to 24 hours after injection. The findings are explained by the rapid fall in the Ga^{72} blood concentrations following intravenous injection (fig. 6). From these results it appears that after four hours the blood-gallium level has reached a near constant value.

DISCUSSION

Previous studies in this laboratory indicated that urinary excretion of gallium, following parenteral administration, is the chief means of clearance by rabbits and dogs (Feces Ga/Urine Ga 1/20 to 1/50) (7). On the basis of this information and the preliminary clinical findings, it was hoped that a study of the urinary excretion of gallium might prove a useful adjunct in diagnostic studies utilizing radiogallium (5). However, the studies herein reported indicate that this is not the case. The diuretic effect of citrate, differences in threshold of the kidneys, as well as conditions of polyuria, may all contribute to a greater urinary clearance of gallium. These variables must be considered when evaluating the results of gallium excretion studies.

No explanation can be offered at this time for the decreased excretion of gallium following intravenous administration of gallium citrate. It would first appear that this might result from greater retention by the skeletal structure. Other work in progress here indicates that the uptake in bone is a function of the dose but not the route of administration. Thus it appears that intravenous injection of gallium may induce greater deposition in the soft tissues.

The rapid clearance of the citrate from the blood stream might be explained on the assumption that this compound may be a colloid, and its disappearance resulted from phagocytic action. To determine if gallium citrate (at pH 7.4) was a macromolecule, or colloidal in nature, water solutions of the compound were placed in cellophane bags, with and without horse serum. In all cases the gallium rapidly dialyzed through the cellophane. Thus it appears that gallium is in a true solution. Slight dissociation of the gallium citrate is indicated, since the usual soluble gallium salts $[Ga(NO_3)_3, GaCl_3]$ produce a curdlike precipitate with proteins.

SUMMARY AND CONCLUSIONS

1. A method is described for the separation of gallium from urine by ether extraction of 6 N HCl solution. Colorimetric or fluorometric estimation of the 8-hydroxyquinoline-gallium complex in chloroform is the basis for quantitative determination.
2. In the rabbit and dog, intravenous injection resulted in lower urinary excretion of the administered gallium citrate, comparable with animals similarly treated by subcutaneous injection.
3. The urinary excretion of gallium during the first 24 hours after its intravenous injection varied widely (4 - 78 per cent) in 60 patients receiving radiogallium (Ga^{72}). No correlation was observed between the clinical manifestations and the rate or amount of Ga excreted.
4. Studies of the rate of urinary excretion of Ga^{72} in man and in dog indicate that in both the initial excretion rate is high and reaches a low level in less than 18 hours. Blood gallium levels fall rapidly after intravenous injection and approach a low value within six hours.

ACKNOWLEDGMENTS

The authors wish to acknowledge the support and assistance of Commander W. C. Mulry, MC, USN, and Commander E. R. King, MC, USN, and for their permission to use the clinical data referred to in this paper.

The methods of standardization of Ga^{72} using radium as a reference was developed by Commander F. W. Chambers, MSC, USN.

The application of this standardization to the biological studies was made by LT(JG) J. W. Duckworth, MSC, USNR, and Ens. R. Sharp, MSC, USN, who have also carried out the radiological counting of Ga^{72} in tissues and excreta.

The radiogallium (Ga^{72}) used throughout these studies has been made available by the Isotopes Division, U. S. Atomic Energy Commission.

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Table 1.- Result of analysis of standard samples of gallium.

COLORIMETRIC DETERMINATION		
Mg. Ga Present	Mg. Ga Found*	Per cent Recovered
0.005	0.0035	70
0.010	0.0096	96
0.050	0.048	96
0.080	0.079	98
0.100	0.097	97
0.150	0.146	97
PHOTOFLUOROMETRIC DETERMINATION		
μ g. Ga Present	μ g. Ga Found*	Per cent Recovered
2.0	1.6	80
3.0	3.0	100
5.0	5.4	108
7.0	7.4	106
9.0	9.2	102

*Mean of 5

Table 2.- Urinary Excretion of Gallium by the Rabbit

Mg. Ga injected	Mg. Ga excreted in:			% Gallium excreted in 48 hr.
	0-24 hr.	24-48 hr.	48-72 hr.	
	Dosage: 5 mg. Ga/kg intravenously*			
13	1.4	0.4	.08	13.8
10.8	0.6	0.1	.04	6.5
14	0.3	0.2	.19	3.6
11.6	1.3	0.3	.12	13.8
12.8	1.3	0.3	.64	12.5
8	1.3	0.1	.07	17.5
17.0	2.3	0.7	.14	13.0
17.5	3.3	0.6	.11	22.3
16.5	2.7	1.4	.57	24.9
	Dosage: 5 mg. Ga/kg subcutaneously*			
11.6	9.3	0.8	.01	87.0
11.0	5.6	0.6	.02	56.4
9.2	2.0	0.2	.03	24.0
11.6	6.6	0.3	.03	59.4
10.2	9.0	0.7	.05	95.1
11.0	9.3	0.3	.01	94.1
19.0	3.7	2.3	0.2	31.6
19.0	7.6	1.1	0.2	45.8
	Dosage: 15 mg. Ga/kg subcutaneously*			
63.0	0.0	10.9	0.8	17.3
61.5	18.7	7.9	0.6	43.2
51.0	19.2	1.8	0.7	41.2
78.0	14.3	8.6	1.7	29.3
69.0	8.3	2.8	0.9	16.1
60.0	4.3	13.3	1.6	29.3
	Dosage: 45 mg. Ga/kg subcutaneously**			
126	19.9	3.0	0.2	18.0
108	23.4	11.7	0.4	32.5
126	29.5	6.5	N.D.	28.6
68	21.0	3.3	Died	35.7
126	18.8	19.4	3.0	30.3
104	31.2	2.3	0.4	32.2

*This dosage produces no significant changes in the urine.

**This dosage is the LD₅₀, and produces within 24 hours marked changes in kidney function. Albuminuria and glucosuria are common.

Note: Gallium administered as the citrate, in solution containing 20 mg. Ga/cc, at pH 6.5.

Table 3.- Urinary Excretion of Gallium by the Dog

Mg. Ga injected*	Mg. Ga excreted in:			% injected Ga excreted in urine in 48 hr.
	0-24 hr.	24-48 hr.	48-72 hr.	
	Dosage: 5 mg. Ga/kg subcutaneously**			
50	35.6	None	4.0	71.2
46	36.4	4.9	0.9	90.0
64	27.4	10.8	1.1	59.7
82	35.1	6.0	1.2	50.1
98	43.9	0.6	1.5	45.4
96	42.0	1.7	2.8	45.5
	Dosage: 5 mg. Ga/kg intravenously**			
59	16.2	1.5	2.5	30.0
65	8.3	1.2	2.5	14.6
60	13.8	0.8	2.6	24.3
75	11.2	4.1	0.0	20.4
85	36.1	4.4	0.0	47.6
51	13.3	1.1	0.0	28.2
	Dosage: 1.0 mg. Ga/kg intravenously			
12	4.3	1.6	0.1	49.1
12	4.5	0.8	0.1	44.1
16	8.2	1.0	0.1	57.5
20	14.8	1.8	0.6	83.0
19	11.0	0.5	0.1	60.5

* Gallium administered as the citrate, in solution containing 20 mg. Ga/cc, at pH 6.5.

** The LD₅₀ for the dog is approximately 15 mg. Ga/kg. The dose of 5 mg. Ga/kg produces minimal kidney changes, which are often accompanied by albuminuria.

Table 4.- Urinary Excretion of Gallium by Man following intravenous administration of gallium citrate

Number of patients*	Dosage (intravenous) (mg. of Ga)**		Excretion of Gallium as % of dose	
9 7 6	Determined*** chemically		<u>0 - 24 hr.</u> 4 - 43% 16 - 33% 25 - 37%	
22 38	Determined by radio- chemically counting of Ga ⁷² ****		<u>0 - 24 hr.</u> 4 - 33% (mean 15)	<u>24 - 48 hr.</u> not deter- mined 0.4 - 27% (mean 10)

*These patients (male and female) exhibited clinical signs of neoplastic involvement.

**This was the carrier gallium which accompanied the Ga⁷² used in diagnostic studies (5).

***Determined by the chemical method described above.

****The gallium excretion was determined by radiochemical counting technique (4,5).

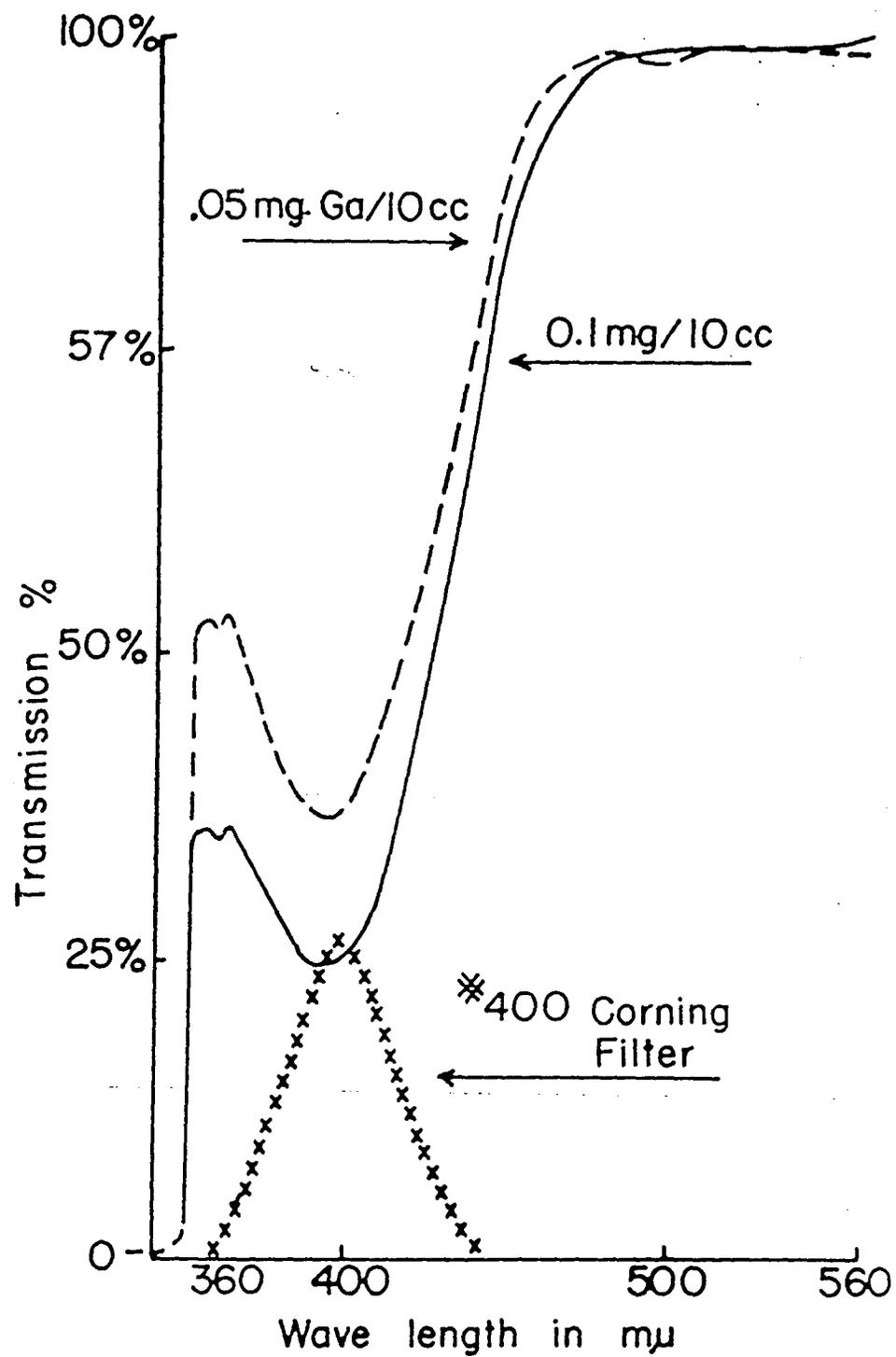


FIGURE 1. Color characteristics of 8-hydroxyquinoline - Gallium complex.

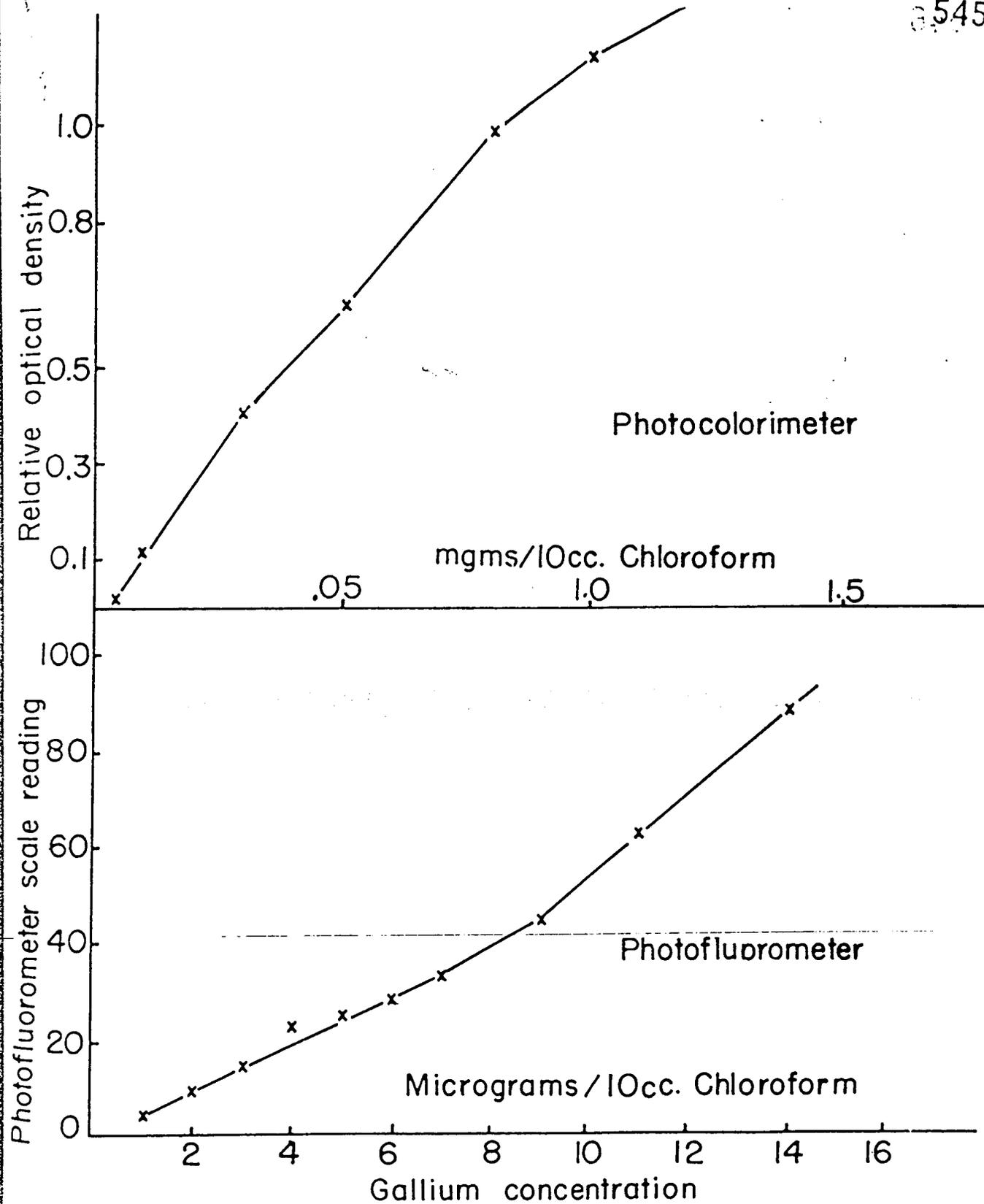


FIGURE 2. Relationship of color density or emission. With charge of Ga-hydroxyquinoline concentration in chloroform.

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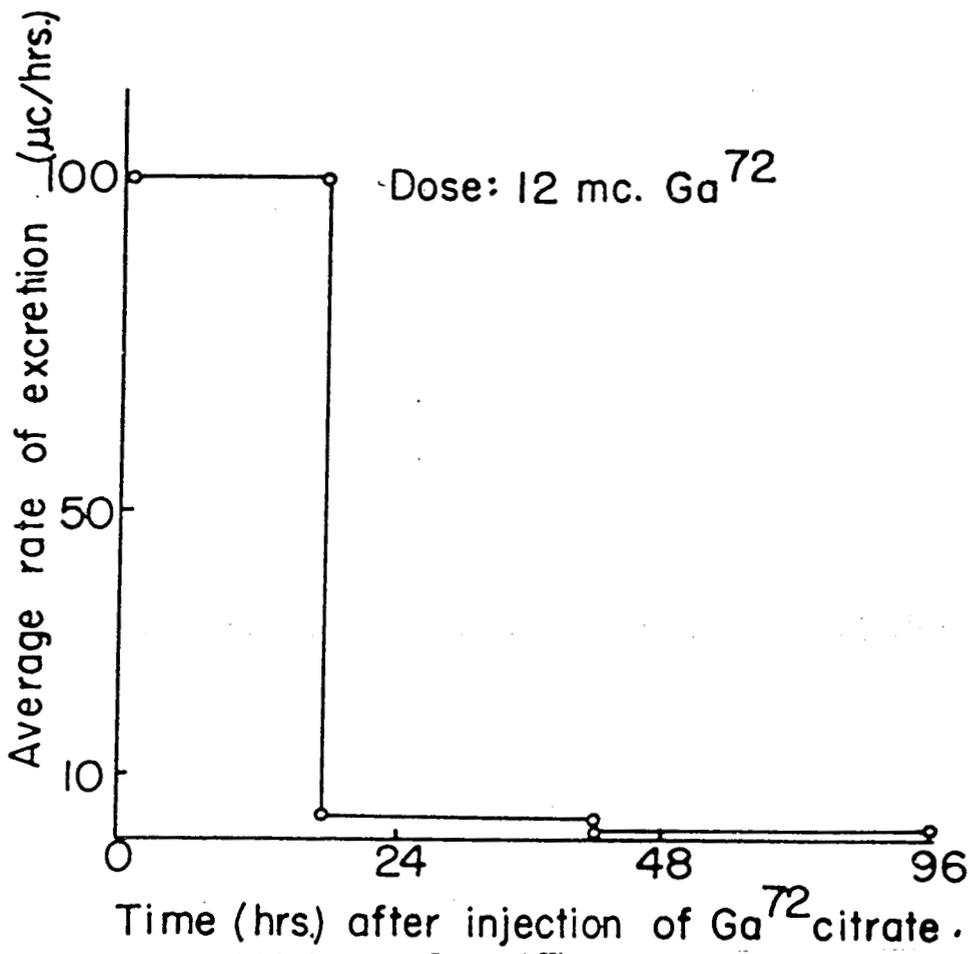


FIGURE 3. Urinary excretion of Ga⁷² by dog.

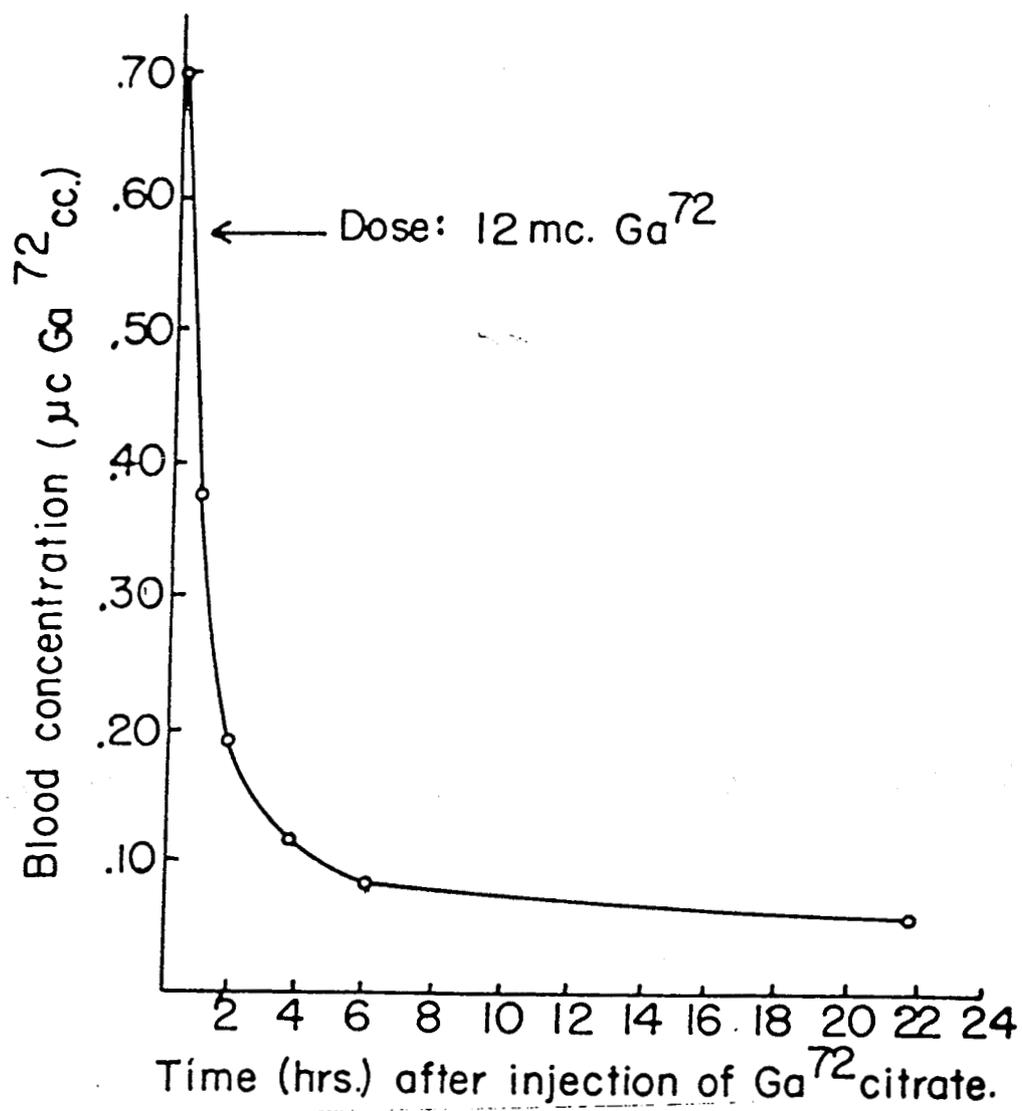


FIGURE 4. Ga^{72} content of blood of dog.

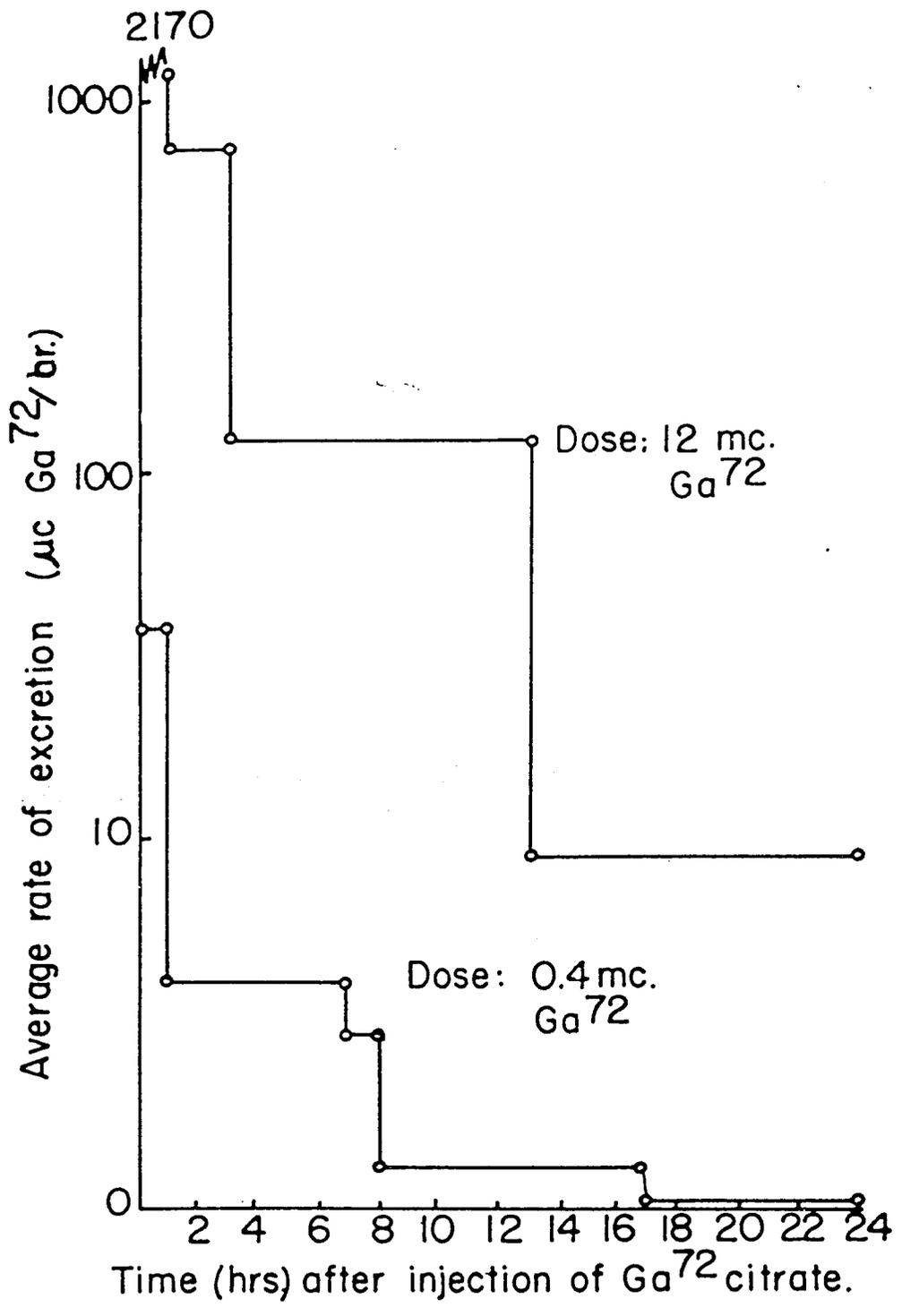


FIGURE 5. Urinary excretion of Ga⁷² by man.

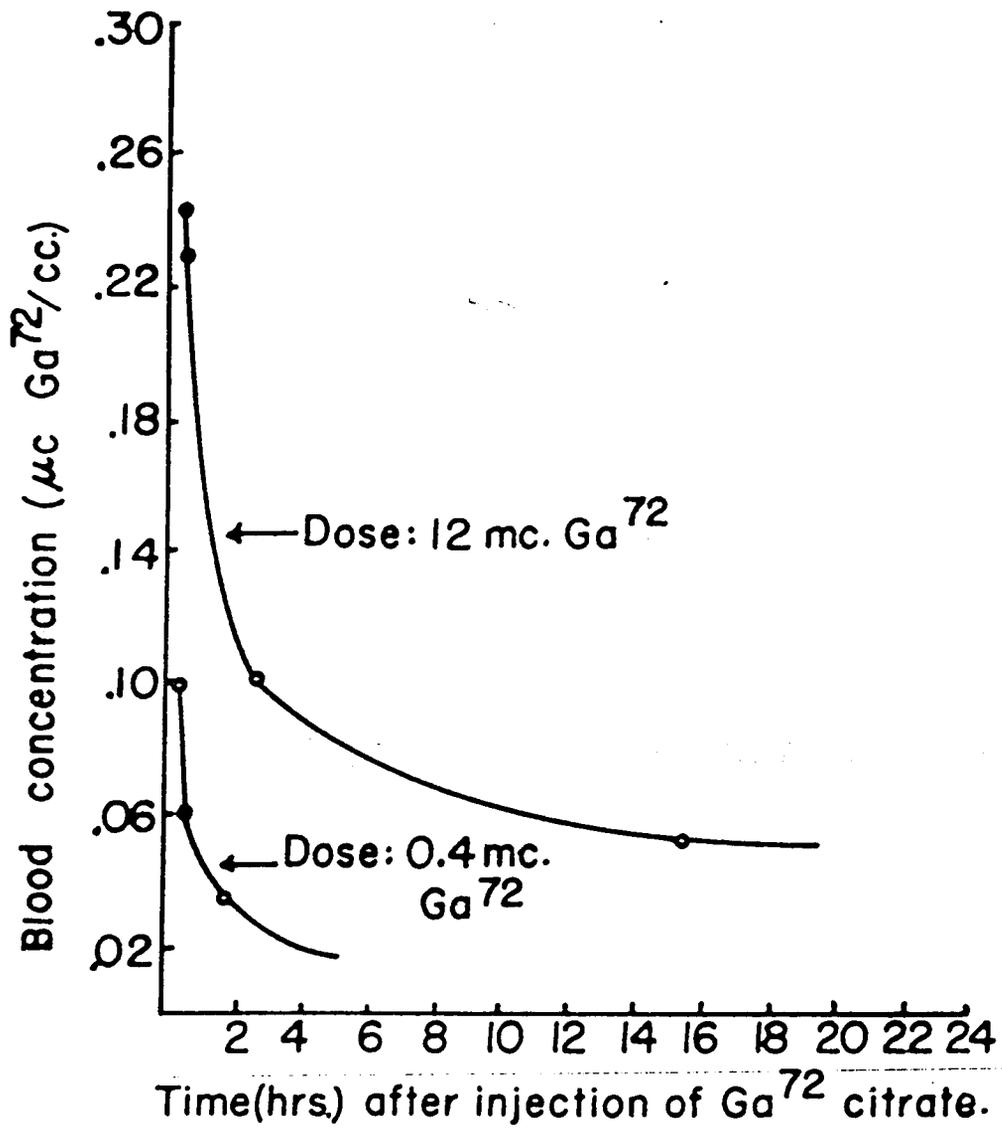


FIGURE 6. Ga^{72} content of blood of man.