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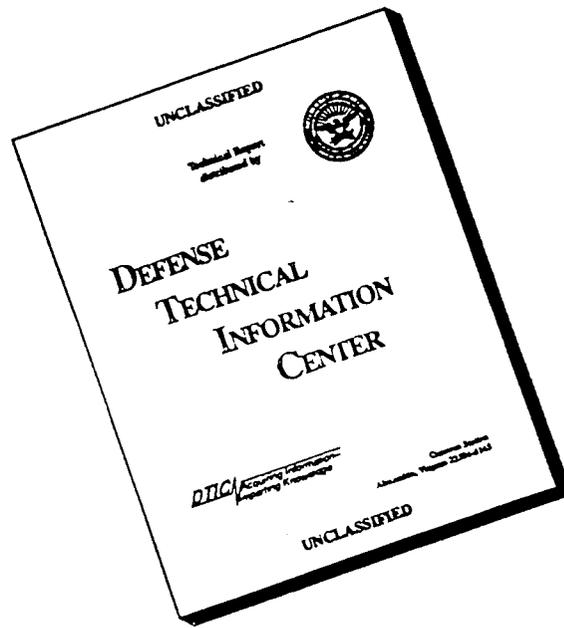
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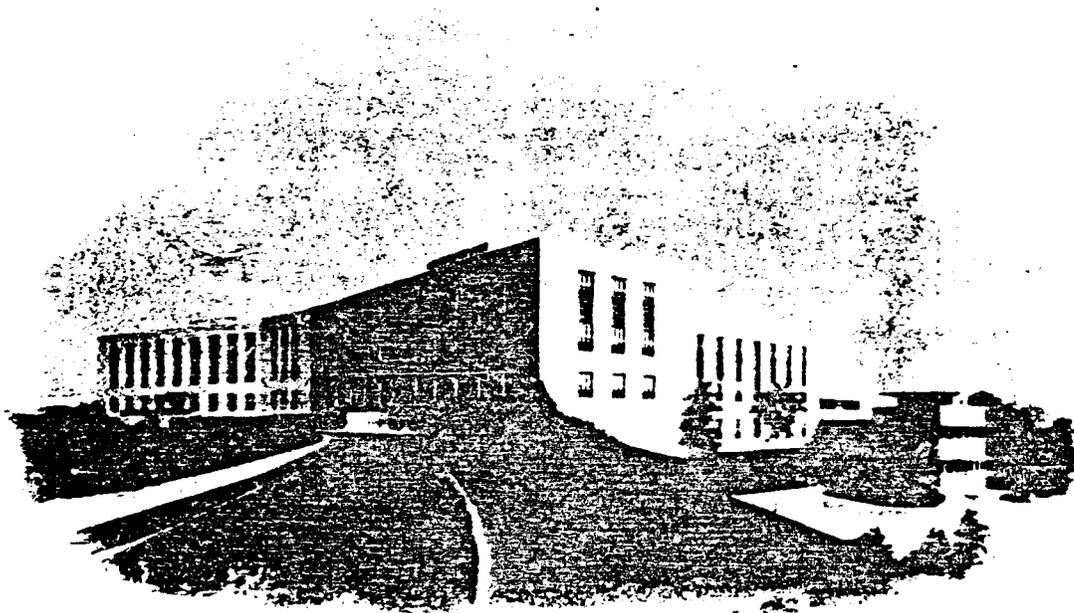


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GALLIUM: STUDIES OF ITS DEPOSITION IN AND CLEARANCE FROM BONE

RESEARCH REPORT  
Project NM 007 081.06.12

GALLIUM: STUDIES OF ITS DEPOSITION IN AND CLEARANCE FROM BONE

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Vol. 10

pp. 319-330

RESEARCH REPORT  
Project NM 007 081.06.12

NAVAL MEDICAL RESEARCH INSTITUTE  
NATIONAL NAVAL MEDICAL CENTER  
BETHESDA, MARYLAND

25 June 1952

## ABSTRACT

Previously it has been shown that gallium, administered parenterally as the lactate or citrate, is rapidly deposited in osteoid structures, particularly in the teeth and in areas of osteoblastic activity. Radiogallium ( $Ga^{72}$ ) has shown promise as a diagnostic agent for the localization of malignancies involving bone.

Laboratory studies indicate that in the rabbit the route of administration, intravenous or subcutaneous, does not significantly influence the amount of Ga contained in the bones 24 hours after injection. By varying the dose from low to toxic levels, it has been shown in rats and rabbits that at low levels (1 - 15 mg. Ga/kg) the amount of Ga deposited in the femur is directly proportional to the dose. As the dosage approaches the toxic level (30 - 45 mg. Ga) the amount of Ga deposited reaches a relatively constant value.

Studies in dogs and rabbits of modes of clearance of Ga from bone by producing an acidosis, or by the administration of chelating agents, indicate that gallium is not significantly affected by these procedures. Normally it remains in the bone for six months or longer following administration of gallium citrate.

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## INTRODUCTION

In previous studies it was demonstrated that gallium is an element which rapidly enters osteoid tissue, particularly areas of proliferation, irrespective of whether this growth is normal or pathologic in nature (Dudley et al., 1949, '50) (King and Perkinson, 1950). The laboratory findings have been confirmed in part by clinical studies (Mulry, 1951, King 1952, Brucer 1952) utilizing radiogallium ( $Ga^{72}$ ).

For many years administration of ammonium chloride, vitamins, and varied Ca/P mixtures have been used for the removal of lead from viable bone. These and other methods have been studied (Aub et al., 1938)(Copp et al., 1947) in an attempt to promote the elimination of radioactive materials. Concurrently with the study reported here, Forman and Hamilton (1951) have shown the effect of various chelating agents on the urinary excretion of  $Ce^{144}$  and  $Pu^{239}$ .

The experiments herein described utilized experimental animals for the following purposes:

- (a) to determine the influence of route of administration (intravenous or subcutaneous) of gallium citrate on the uptake of gallium by bone of the rabbit;
- (b) to determine the influence of the dose of gallium citrate on the amount of gallium deposited in bone of the rat and rabbit;
- (c) study of influence of time on the clearance of gallium from bone of the rat and rabbit;
- (d) study of the methods for promoting urinary clearance of gallium by the rabbit and dog.

## METHODS

Gallium citrate was prepared as described previously (Dudley, 1950) and a solution of this salt at pH 6.5 containing 20 mg. Ga/cc. was injected either intravenously or subcutaneously. When subcutaneously administered, the gallium citrate solution contained one per cent procaine hydrochloride in order to prevent undue pain at time of injection. At the higher doses of gallium the intravenous injection was preceded by an intravenous dose of 200 mg.  $CaCl_2$  in order to prevent a hypocalcemic type crisis (Dudley et al., 1950).

In order to study the influence of dose and route of administration, adult albino rabbits (3.2-3.8 kg.) were given gallium citrate either intravenously or subcutaneously at doses from 1 to 45 mg. Ga/kg. Adult albino rats (225 gm.) also were injected subcutaneously at dosages of 1 to 100 mg. Ga/kg. Twenty-four hours after injection, the animals were killed and both femurs were removed. The bones were freed of all muscle and connective tissue as well as marrow and the entire femurs of three or more animals were used at each concentration and route of administration. These bones were crushed, pooled and chemical determinations were made (in duplicate) of the gallium content, by the methods previously described (Dudley 1949, Munn 1951).

To determine the influence of time on the clearance of gallium from the bones, male albino rabbits, of equal size, age and general condition, were injected intravenously with gallium citrate at 30 mg. Ga/kg. Male albino rats were also subcutaneously injected with gallium citrate at a dosage of 30 mg. Ga/kg. Three animals of each species were killed at each suitable time from 4 hours to 12 months after injection. The femurs of the three animals were cleaned as shown above, crushed, pooled, and the gallium determined chemically.

Studies of the urinary excretion of gallium (1,10) indicate that 48 hours after injection of gallium citrate, the urinary gallium drops to a low level, and often continues at trace level for an extended period thereafter. A study has been made of the influence of various complexing agents, citrate, and an acidosis-like condition, in promoting the increased urinary excretion of gallium. These studies have been made after the organism had attained an essentially equilibrium condition with respect to the rate of excretion of gallium. This phase of the investigation was prompted by the findings that the possible therapeutic application of radiogallium ( $Ga^{72}$ ) in malignancies involving bone was seriously limited by the toxicity of the repeated doses of non-radioactive gallium ( $Ga^{69,71}$ ) which accompanied the  $Ga^{72}$  (6,7).

Rabbits were injected intravenously with gallium citrate (15 mg. Ga/kg) and urine collected daily for three to five days, until the gallium excretion had reached low levels. After this time the animals (six in each series) were given daily, for three days, (a) 2 gm.  $NH_4Cl$  by mouth, (b) 100 mg. sodium citrate/kg. (intrav.). (c) 10 mg/kg. disodium ethylene diamine tetraacetic acid (intrav.), (d) 35 mg/kg. 8-hydroxyquinoline, (intrav.) (e) 40 mg/kg. aluminon (ammonium surin tricarboxylic) (intrav.) or (f) 10 mg/kg., intramuscularly, dimercaptolpropanol (BAL). After these treatments the urine of each animal was collected daily for three days, and the gallium excretion determined chemically (1,10).

Two groups of dogs, three in each group, were intravenously injected with gallium citrate (5 mg. Ga/kg.), the urine collected daily for three days, and the gallium excretion determined. Three days after administration of the gallium, each dog was administered by mouth 5 grams  $NH_4Cl$ , in enteric tablets daily for three days in order to produce an acidosis-like condition. The urine was collected daily during this time and the amount of gallium excreted determined.

One of these groups of dogs, following this treatment with  $NH_4Cl$ , was injected intramuscularly with dimercaptolpropanol (BAL) (10 mg/kg.) and the urinary excretion of gallium determined daily for an additional three days. Following this study, these dogs were killed and the gallium content of the liver, kidney, spleen and femur determined.

## RESULTS

In figure 1 is presented a resumé of results of studies of the influence of dose and route of administration (intravenous or subcutaneous) of gallium citrate on the uptake of gallium by the bones of rabbits and rats. These findings indicate that in the rabbit, at a dosage of 1 to 45 mg. Ga/kg., the route of administration has no significant influence on the amount of gallium deposited in the normal femur 24 hours after injection. It is also indicated that in the dosage range 1 to 15 mg. Ga/kg., the amount of gallium deposited in the femur of the rat

and rabbit is directly proportional to the amount of gallium administered. However, above this range, in both rats and rabbits, the amount of gallium deposited per mg. of gallium injected falls off rapidly.

The 24-hour gallium content of the bone shown in figure 1 following a dose of 30 mg. Ga/kg., was found to be relatively constant from 24 hours to six months after injection. The gallium content of the femur in the rabbit over this period averaged 65 $\gamma$ /gram of fresh bone, and in the rat 53 $\gamma$ /gram. During the 6 to 12 months period the gallium decreased in the rabbit femur to 19 $\gamma$ /gram and in the rat femur 22 $\gamma$ /gram. These findings indicate that the deposits of gallium in bone, resulting from intravenous injection of gallium citrate, are relatively stable. This conclusion is further strengthened by the results given below on the slight effects of rather drastic efforts to induce increased urinary output of gallium.

Studies of the influence of various agents on the urinary excretion of gallium have given the following results:

*Rabbits.* Intravenous injection of sodium citrate three days following gallium citrate produced lethal hypocalcemic-type shock at moderate levels of the Na citrate. Therefore, this salt was useless in the study of gallium clearance.

In doses up to 2-gm.  $\text{NH}_4\text{Cl}$  for three days, no significant rise in the urinary gallium was observed (fig. 2). Intravenous injection of the chelating agents, disodium ethylene diamine tetraacetic acid and 8-hydroxyquinoline produced no significant change in the rate of urinary excretion of gallium in the rabbit. The intravenous injection of aluminon and the intramuscular injection of BAL four days after injection of 15 mg. Ga/kg. (as citrate) produced a transitory rise in the urinary gallium (11 of 12 animals) which lasted but for 24 hours. This effect may be due to a combination of the reagents with gallium, but may also be due to simple diuretic effect of the agent.

Examination of the tissues of six rabbits receiving BAL and six controls which received the same dose of gallium but no BAL, indicated that no significant reduction in gallium content of the liver, spleen, kidneys, or femur occurred following administration of 10 mg. BAL/kg. daily for three days.

*Dogs.* The daily feeding of 5 gm.  $\text{NH}_4\text{Cl}$  for three days (in enteric capsules) to two groups of grown dogs. (11-19 kg.) produced a slight rise in the urinary gallium in six of six which lasted for only the first 24 hours. When three of these dogs were treated daily for three days with BAL (10 mg/kg. intramuscularly), the urinary gallium again rose (three of three) and remained at a moderate level during the three days in which the BAL was administered (fig. 3).

The tissues which normally concentrate gallium were removed at autopsy of the three dogs which had received both the  $\text{NH}_4\text{Cl}$  and BAL treatment. These yielded the following mean gallium values in micrograms Ga/gram fresh tissue: Liver 29, spleen 26, kidney 17, and whole femur 13. There are no control values with which to compare these findings (see rabbit results above). However, these findings are given here to indicate that the gallium was not cleared entirely from the tissues of the dogs by rather drastic treatment.

## DISCUSSION

The results of the studies on the length of time necessary to effect even partial clearance (with time) of gallium from bone indicate that once this element is deposited in the bone structure it is relatively immobile. However, this conclusion must be considered in the light of other findings that the soft tissues (liver, kidney, spleen) which at first contain high concentrations of gallium lose it more rapidly than does bone (1). Therefore, the gallium in the bone may represent the most stable deposits, which are cleared only after the soft tissues lose the gallium deposited therein. It may also be postulated that the gallium in bone is in an equilibrium state such that its concentration appears to remain constant. This condition would continue as long as there was gallium available in the soft tissues to replace that given off by the bone during normal metabolic processes.

In the study of five species of animals over an extended period, we have observed no delayed effects of gallium, either grossly or on microscopic examination of selected tissues. Thus it appears that the concentrations of gallium laid down in bone are not a toxic hazard, as in the case of lead. Repeated doses of gallium citrate (11) in dogs and rabbits have shown this metal to be cumulative in its toxic effects but no evidence of marked replacement of calcium in the bone has been observed, as in strontium rickets.

Throughout our studies of the metabolism of gallium and its predisposition for bone, it has been found that there are many factors which may influence the amount of gallium deposited in normal bone. These factors include species, age, diet, general condition, water intake, and perhaps of major importance, individual kidney threshold. Therefore, comparison of results from different groups or stains of any one species, must be done with realization of the limitations imposed by these normal variations.

The deposition of many metallic elements which tend to localize in bone have been described in the literature for the past half century. It was through classical chemical procedures that these results were obtained. The recent development of autoradiographic techniques have made possible the precise localization of these metals, such definition now being possible down to the particular tissue in question, and often down to the individual cell.

By means of autoradiographic techniques summarized by Yagoda (1949), it has been shown that  $\text{Ca}^{45}$ ,  $\text{Sr}^{89}$ ,  $\text{Y}^{90}$ ,  $\text{Ce}^{144}$ , and  $\text{Am}^{241}$  are deposited in the bones of experimental animals in many respects similar to gallium (Copp et al. 1951, 1947, Hamilton 1948). These studies, when viewed in the light of the earlier findings, indicate that many metals tend to form insoluble complexes in viable bone. The advantages of radiogallium in the possible application to clinical studies is that of a short half life beta gamma emitter, ( $t_{1/2}$ ;  $\text{Ga}^{67}$  80 hrs.,  $\text{Ga}^{72}$  14.3 hrs.). With these short half lives, these isotopes lie well within the safety zone (max.  $t_{1/2}$  30 days) suggested by the U. S. Atomic Energy Commission.

## SUMMARY

Studies of the effect of dose and route of administration on experimental animals indicate that the quantity of gallium deposited in normal bone, following administration of gallium citrate, is independent of the route of administration but is directly proportional to the dose between 1 and 15 mg. Ga/kg.

Gallium content of normal bone showed little decrease from 24 hours to 8 months following administration of gallium citrate.

No significant increase in the urinary excretion of gallium was occasioned by administration of various complexing agents,  $\text{NH}_4\text{Cl}$ , and BAL.

## REFERENCES

1. Dudley, H. C., *J. Pharm. Exper. Therap.*, 95: 482, 1949; 96: 224, 1949.
2. Dudley, H. C., Imirie, G. W., and Istock, J. T., *Radiology* 55: 571, 1950.
3. King, E. R. and Perkinson, J. D., *Texas Reports, Biol. and Med.* 8: No. 4, Winter 1950.
4. Mulry, W. C. and Dudley, H. C., *J. Lab. Clin. Med.*, 37: 239, 1951.
5. King, E. R. et al. *Radiology* (in press) 1952.
6. King, E. R. and Dudley, H. C., *J. Lab. Clin. Med.*, (in press) 1952.
7. Brucer, M., Bruner, H. D. and Andrews, G. A., *Radiology* (in press) 1952. (presented before Radiological Society of N. Amer. Dec 1951).
8. Dudley, H. C., *J. Am. Chem. Soc.*, 72: 3822, 1950.
9. Dudley, H. C., Henry, K. E., and Lindsley, B. F., *J. Pharm. Exper. Therap.*, 98: 409, 1950.
10. Munn, J. I., Walters, N. H. and Dudley, H. C., *J. Lab. Clin. Med.*, 37: 676, 1951.
11. Dudley, H. C., *Nucleonics*, 1952 (in press).
12. Yagoda, H. J., *Radioactive Measurement with Nuclear Emulsions*, John W. Wiley & Sons, New York, 1949.
13. Copp, D. H. et al., *Metabolic Interrelations*, page 226, Edit. E. C. Reifensens. John Macy Foundation, 1951.
14. Copp, D. H., Axelrod, D. J., and Hamilton, J. G., *Amer. J. Roentg.* 58: 10, 1947.
15. Hamilton, J. G., *Rev. Modern Physics*, 20: 718, 1948.
16. Aub, J. C. et al., *Ann. Int. Med.* 11: 1443, 1938.
17. Copp, D. H., Axelrod, D. J. and Hamilton, J. G., *Am. J. Roentg. Rad. Therap.* 58: 110, 1947.
18. Foreman, Harry, and Hamilton, J. G., *Univ. of Calif. Radiation Lab.*, UCRL 1351, June 14, 1951.

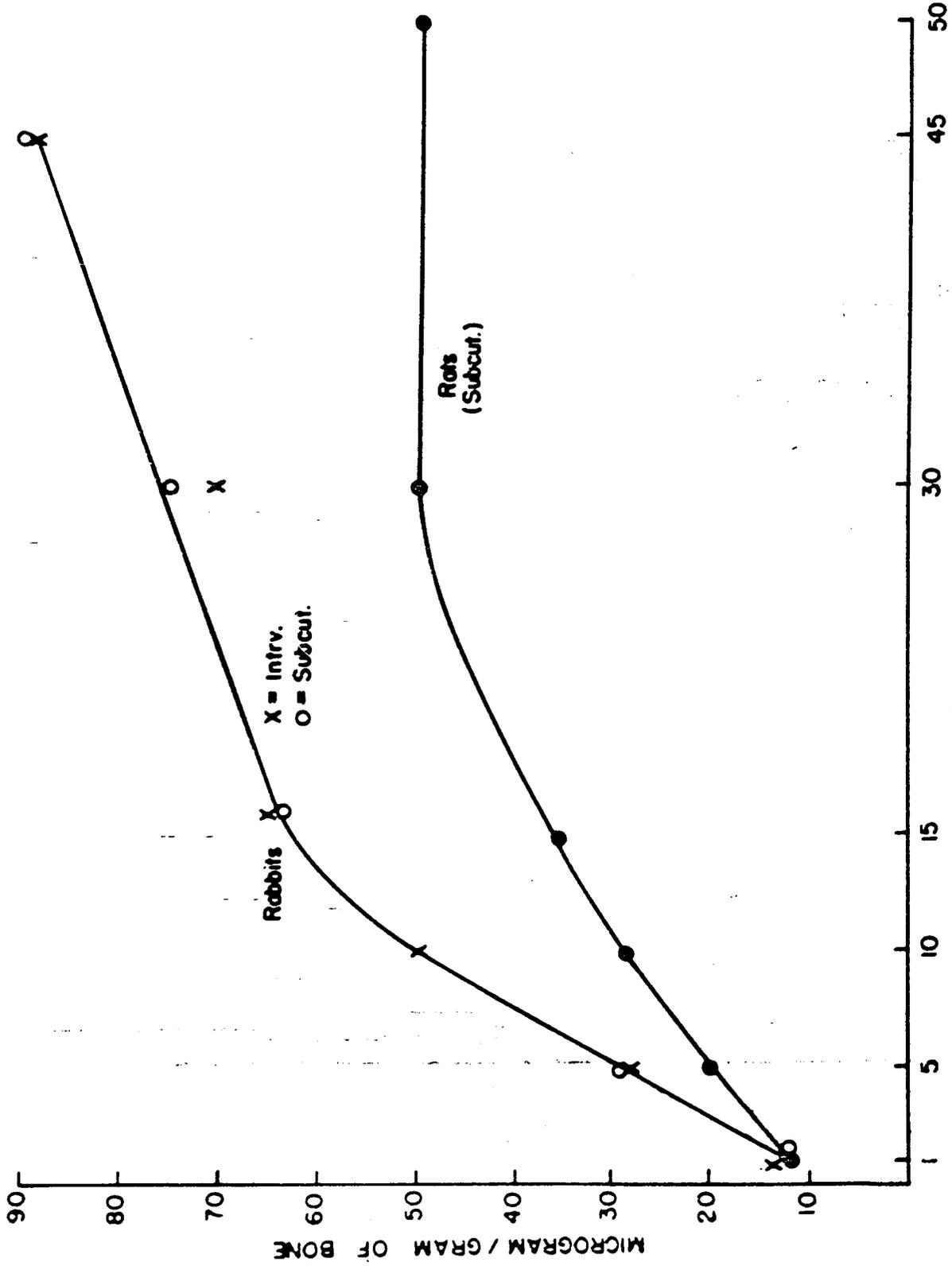


Figure 1. Influence of dose and route of administration on uptake of gallium in bone.

RABBIT  
Dosage 15 Mg. Ga/kg.

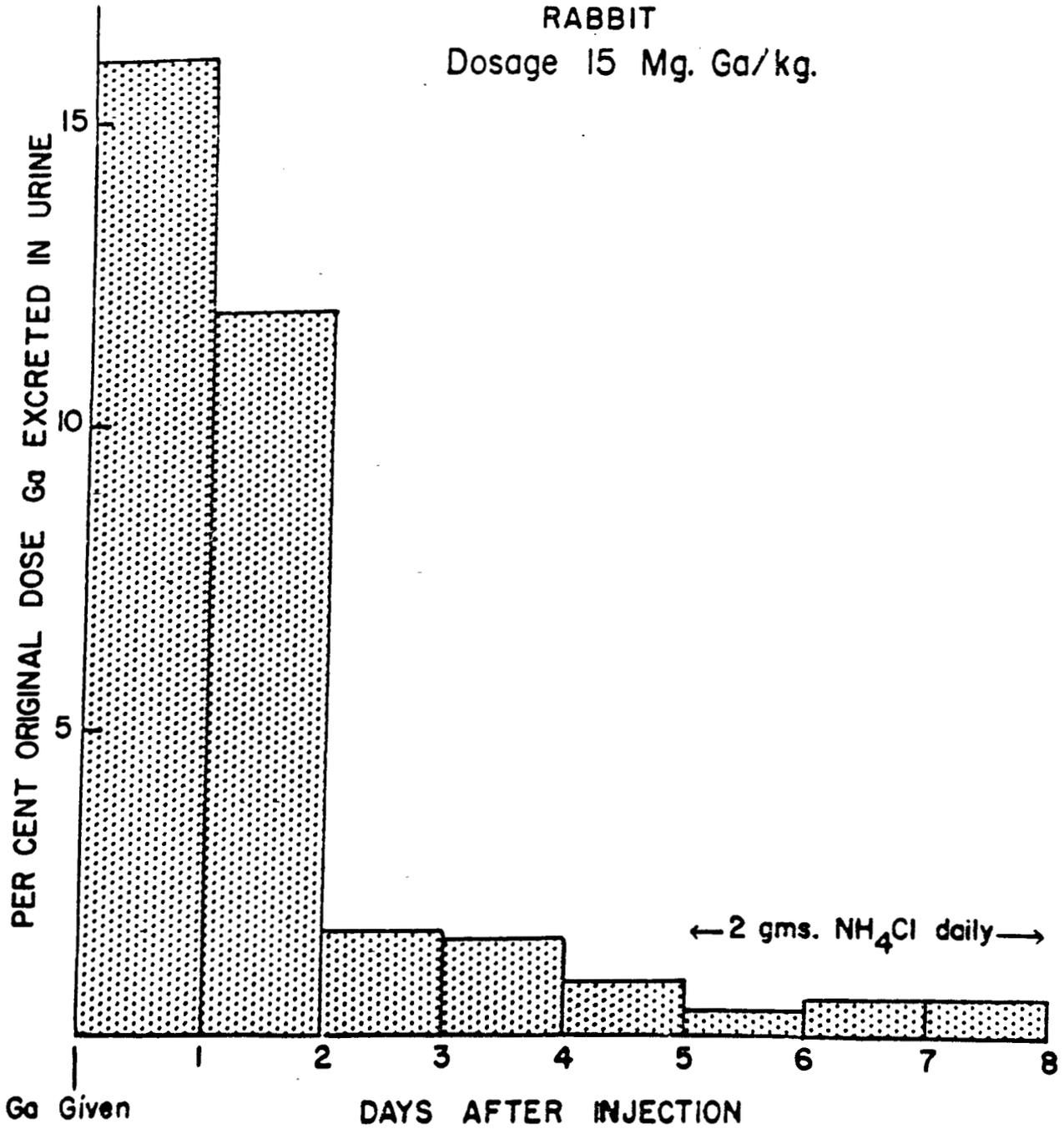


Figure 2. Urinary excretion of gallium by the rabbit following feeding of NH<sub>4</sub>Cl.

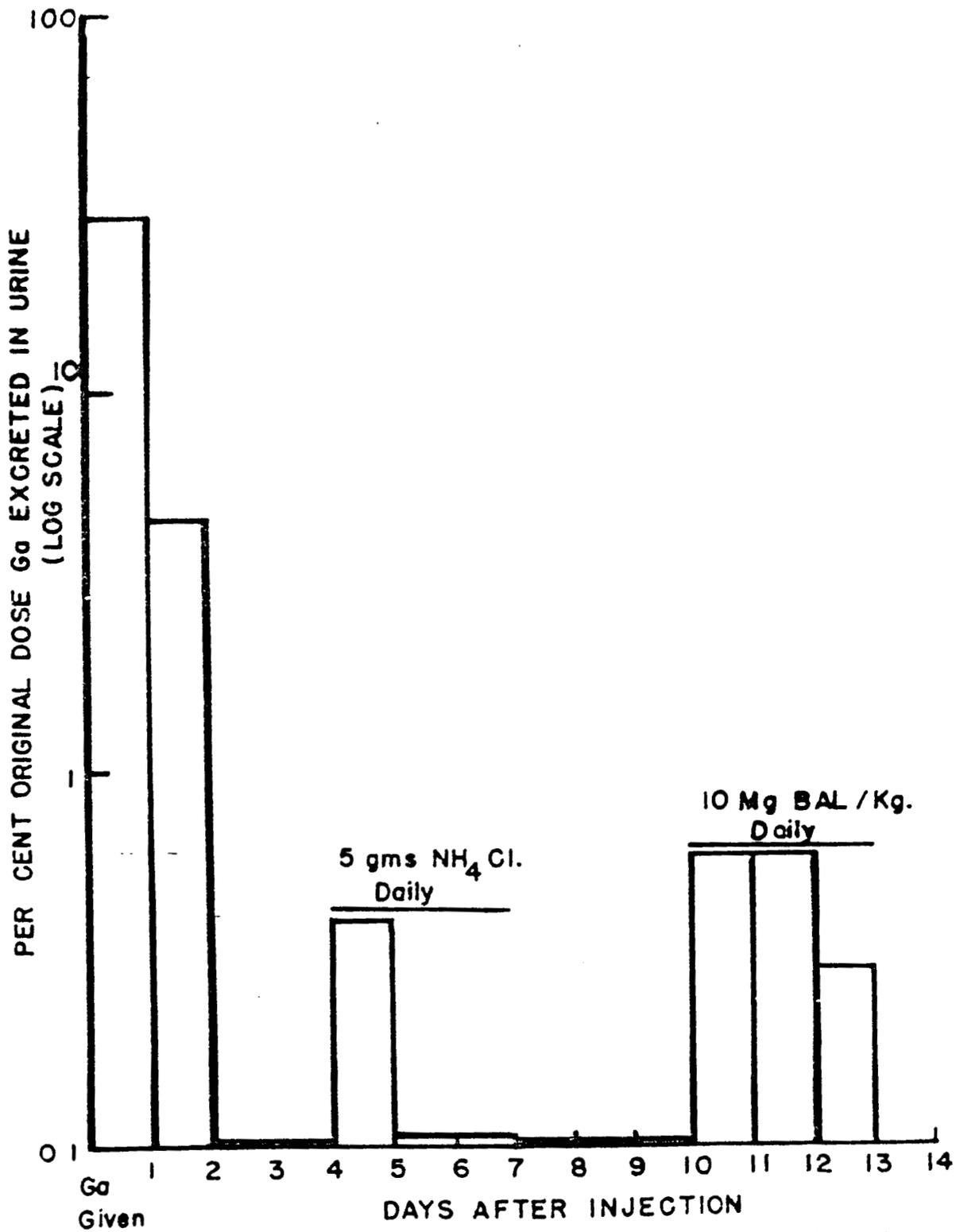


Figure 3. Effects of NH Cl and BAL on urinary excretion of gallium by the dog (6 Animals).

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