heparin every 12 hr. Group 1 received 5 USP units, and Group 2, 15 USP units per injection. The first dose of heparin was administered just prior to the subcutaneous inoculation of the tumor. Group 3, the untreated control, was inoculated with tumor at the same time as Groups 1 and 2. Inoculation of the tumor was made by trocar, under aseptic conditions. The course of tumor growth was followed by manual palpation. At the end of the seventh day, there were no differences in size of graft among the three groups. The tumors were large, and the animals debilitated.

Previous determinations of blood-clotting time in C3H/Jax mice showed the average normal time to be 49 sec. This increased to 7.8 min, 30 min after an injection of 5 USP units of heparin. Ten hr after the injection, the time dropped to 2.6 min. Blood samples taken 30 min after an injection of 10 units of heparin failed to clot 4½ hr later. Ten hr after the 10-unit dose, clotting time dropped to 1.8 min.

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Influence of Vitamin D on Deposition of Gallium in Bone of the Rat[1]

H. C. Dudley and Leo Friedman

Naval Medical Research Institute, Bethesda, Maryland, and U. S. Food and Drug Administration, Washington, D. C.

Previous studies in various species have demonstrated that gallium and radiogallium (Ga^{72}), upon parenteral administration as the citrate, are rapidly and preferentially deposited in bone (*1–3*). Clinical studies of the isotope (Ga^{72}) have indicated that it may be useful in diagnostic procedures for the localization of osteogenic and metastatic bone lesions (*4*). To extend our understanding of the behavior of gallium in the normal processes of bone formation and repair, studies were made of the effect of vitamin D upon the deposition of this element in the bones of rats.

Preliminary studies indicated that the oral administration of vitamin D to rachitic rats and chicks, several days before the subcutaneous administration of Ga^{72}, resulted in an increased uptake of gallium by the bones when compared with the rachitic controls, which received no vitamin D.

After these preliminary findings, more detailed studies of rats were carried out. These were designed to show (*a*) effect of age and body weight on gallium uptake in the bones, (*b*) effect of vitamin D on the uptake of gallium in bones of animals raised and maintained on an adequate diet, and (*c*) the effect of vitamin D on deposition of gallium in the bones of rachitic rats.

The test animals were injected subcutaneously with gallium citrate containing 210–240 μc Ga^{72}/mg carrier Ga. The citrate complex (pH 6.5) was prepared as described previously (*5*). The concentration of the Ga^{72} was adjusted so that an injection of 1–2 ml was sufficient to give a dose of 400–600 μc Ga^{72}/kg body weight. The animals were killed 18–24 hr after subcutaneous injection, and the long bones of both hind legs were removed and cleaned free of muscle tissue. The bones were sectioned to separate the shaft from the end portions, and the resulting samples were placed in weighed planchets for radiochemical counting. Gamma counting was done by means of a shielded end window Geiger tube, using a 1750 mg/cm² adsorber to eliminate the β-particles and backscatter. A sufficient number of counts was made (in duplicate) to give a statistical accuracy of at least ±4%. All counts were calculated to the time of injection, allowing for decay time ($t\frac{1}{2} = 14.3$ hr).

A suitable aliquot of a standard sample of Ga^{72} was counted under the same conditions and used to calculate the quantity of Ga^{72} as μc/g dry bone. The initial standardization of this sample was by means of an ionization chamber meter, standardized against a known radium source (*3*).

After completion of counting, the planchets were dried at 110°–120° C for 16 hr, cooled, and weighed. The dried sample was then transferred to a weighed porcelain dish, heated to 1000° C, cooled, and again weighed, and the percentage ash in the dry bone was calculated.

Although efforts were made to standardize the conditions of feeding, sex, age, and weight of the animals of any one series, it must be emphasized that comparison of results of different series must be made with caution. The difference in the dose of Ga^{72}, differences in strain of animals, their age, and diet, are important factors that may contribute to differences between the series. Each series must therefore be considered essentially as a unit.

The series A albino rats were reared and maintained on a commercial diet (Purina Rat Chow), supplemented with fresh vegetables. The immature rats (108 g) were of the same stock as were the young adult animals (237 g).

The series B albino rats were young adults (205 g) of a different stock colony than series A. Littermates were selected at weaning and raised to maturity on an adequate diet. Five of these animals were fed (by stomach tube) 25 USP units of vitamin D daily for 4 days prior to administration of the gallium. The five littermates were given no vitamin D other than that contained in the basal diet.

Series C animals were rachitic rats. These rats were selected from two litters, so that each of the treated and untreated groups contained an equal number of animals from each litter. The young rats were maintained from time of weaning on a rachitogenic diet

[1] The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Navy Department.

consisting of yellow corn 76, wheat gluten 20, $CaCO_3$ 3, NaCl 1. Severe rickets was observed after 3–4 weeks on this ration. Fifty USP units of vitamin D was fed by mouth daily for 4 days before the administration of gallium. Healing of rickets was proceeding rapidly in the rats fed vitamin D, as evidenced by increased

TABLE 1

RADIOGALLIUM (Ga^{72}) CONTENT OF LONG BONES OF RATS 18–24 HR FOLLOWING SUBCUTANEOUS INJECTION OF GALLIUM (Ga^{72}) CITRATE

Treatment	Av body wt	No. of animals	Dose (mc/kg)	μc Ga^{72}/g dried bone		E/S† ratio
				Shaft	Ends	
Series A*						
Immature	108	5	.62	2.9	7.9	2.7
Adult	237	5	.56	2.6	7.1	2.7
Series B						
Adults	205	5	.46	1.4	3.8	2.7
Adults fed 100 units vitamin D	205	5	.46	1.4	3.6	2.6
Series C						
Rachitic rats	67	6	.56	3.7	5.3	1.4
Rachitic rats fed 200 units vitamin D	67	6	.56	3.9	7.5	1.9

* Albino rats. Series A of a different stock colony from series B and C.

† E/S ratio calculated from

$$\frac{Ga^{72} \text{ content of dried bone ends}}{Ga^{72} \text{ content of dried bone of shaft}}$$

bone ash and by a line test performed (*6*) on a part of the group. Those not receiving vitamin D remained severely rachitic.

Table 1 gives a résumé of the results of the studies outlined above. In series A, in which the animals were maintained on a good commercial stock diet, the gallium deposition in immature and adult rats was found to be essentially the same. The small difference observed is readily accounted for by the difference in dosage. These results indicate that neither age nor body weight of rats is a significant factor influencing the amount of gallium deposited in the long bones of the rat.

The animals used in series B were maintained on a stock diet compounded to contain a minimal amount of vitamin D necessary for normal calcification and growth. Normal calcification was indicated in the bone ash data (not shown here). In these rats, the administration of a large supplemental dose of vitamin D over a 4-day period prior to the injection of gallium failed to influence the deposition of this element in bone.

The data presented for series C demonstrate that subcutaneously injected gallium is readily deposited in the bones of severely rachitic rats. It is noteworthy that this deposition of gallium has occurred in bones where there was little or no concurrent deposition of calcium salts as indicated by bone ash determinations.

A marked difference in the distribution of gallium laid down in rachitic bone compared with its deposition in normal bone is clear, first from the amount of gallium in the shaft of the rachitic bone and, second, from the ratio of the gallium in the ends of the bone to that in the shaft (E/S ratio). In experiments not detailed here, it was invariably observed that the gallium content of rachitic shafts was higher than that of the shafts of comparable normal control animals. In normal animals the E/S ratio is 2.6 to 2.7, whereas in the rachitic femur it falls to 1.4.

When vitamin D was administered to rachitic rats for 4 days prior to the gallium injection, calcification was observed both by line test and by increased bone ash. The increase in gallium content of the shafts of these bones, over that of the untreated controls, was small and of questionable significance, but the increase in the ends of the bones was greater than 40%. This resulted in an increased E/S ratio, approaching that of the normal animals.

Other studies were designed to test the usefulness of Ga^{72} as a tool in the bioassay of vitamin D. The results indicate that the quantitative estimation of radiogallium in the bones of rachitic rats is not a reliable or sensitive indication of vitamin D dosage.

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On the Mechanism of Formation of Higher Alcohols During Alcoholic Fermentation

J. G. B. Castor and J. F. Guymon

Enology Laboratory, Division of Viticulture, University of California, Davis

It has long been accepted that the higher aliphatic alcohol fraction, so-called fusel oil, present in yeast-fermented media is formed by deamination and decarboxylation of amino acids (*1, 2*). This has been referred to as the Ehrlich mechanism by Thorne (*3*). It was further pointed out by Ehrlich (*1*) and Neuberg and Hildescheimer (*4*) that a requirement for higher alcohol formation from amino acids by yeasts was the simultaneous conversion of appreciable amounts of sugar to ethanol. However, fusel oil is not formed during fermentation of sugar by cell-free yeast preparations (*5, 6*). Although the mechanism of the conversion of carbohydrates to ethanol has been intensively studied, the nature of the relationship between it, the Ehrlich mechanism, and other biochemical processes during alcoholic fermentation is not clear.