Summary. Three all-dystrophic litters of mice from *dydy* matings were produced by artificial insemination of juvenile or young adult dystrophic females with sperm collected from dystrophic males, following induced ovulation in the female. Fertility and litter size of inbred and hybrid dystrophic (*dydy*) mice are compared to inbred and hybrid normal (*Dy-*) mice. It was found that hybrid dystrophic mice were far superior to inbred dystrophic mice and provided a practical means to obtain all-dystrophic litters. The insemination procedure makes it possible to obtain known dystrophic tissue at prenatal and early postnatal stages for histological and biochemical analyses.

1. Michelson, A. M., Russell, E. S., Harman, P. J., *Proc. Nat. Acad. Sci.*, 1955, v41, 1079.
2. Loosli, R., Russell, E. S., Silvers, W. K., Southard, J. L., *Genetics*, 1961, v46, 347.
3. Russell, E. S., Silvers, W. K., Loosli, R., Wolfe, H. G., Southard, J. L., to be published.
4. Dziuk, P. J., Runner, M. N., *J. Reprod. Fertil.*, 1960, v1, 321.
5. Melrose, D. R., *Proc. IIIrd Int. Congr. Anim. Reprod., Cambridge*, 1956, sec. 3, p68.
6. Stevens, L. C., Russell, E. S., Southard, J. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 161.

Received December 11, 1961. P.S.E.B.M., 1962, v109.

Intestinal pH and Absorption and Deposition of Ca^{47} in the Rachitic Chick.* (27292)

R. H. WASSERMAN AND A. N. TAYLOR
Department of Physical Biology, N. Y. State Veterinary College, Cornell University, Ithaca, N. Y.

It has been generally assumed that radiocalcium after intestinal absorption behaves the same as if introduced parenterally and, therefore, the amount of ingested radiocalcium deposited in the skeleton would reflect absorption(1-4). The validity of this assumption is usually tested by comparing the amount of radionuclide deposited after parenteral administration under a given set of conditions; if (as usually observed) no differences are noted, it would then appear that the experimental situation was without effect on the skeleton *per se* and differences in the radionuclide content of skeleton after oral administration would reflect relative differences in intestinal absorption.

Recently, it was reported that lactose injected with Ca^{45} into the ileal lumen of the rat increased the relative deposition of the absorbed Ca^{45}(5); earlier observations by Vaughn and Filer(6) suggested that Ca^{45} absorbed from a solution containing glucose also was deposited to a greater degree in the femur of rats than that absorbed in absence of the carbohydrate. Thus, it became apparent that, for certain comparisons, the amount of Ca^{45} found in bone was not strictly a reflection of the degree of Ca^{45} absorption.

The effect of vit. D on absorption of Ca^{47} from ligated duodenal segments and the deposition of this radionuclide into the tibia was recently determined in the rachitic chick (7); it was found that duodenal absorption of Ca^{47} with or without vit. D-treatment was directly related with the tibia content of radionuclide. In these studies, the dosing solution containing Ca^{47} was injected into the duodenal lumen at a relatively low pH because, it was reasoned, ingesta normally passed into the duodenum from the gizzard would have a pH of about 2.6(8). Upon further experimentation, it was noted that, at a near neutral pH, proportionally more of the absorbed Ca^{47} was deposited in the tibia of the vit. D-treated chick than in that of the rachitic chick. These and related observations are reported here.

Experimental. One-day-old chicks were placed on a complete growing mash for 5 days, then were given a rachitogenic diet (General Biochemicals, Inc.) *ad lib* for 3 to 4 weeks. After this period, the chicks showed severe signs of rickets. Twenty-four hours before experiment, one-half of the group was given 500 I.U. vit. D_3 orally in Wesson oil and the rachitic controls, Wesson oil alone.

* Supported by Nat. Inst. Health Grant.

TABLE I. Effect of pH of Dosing Solution on Duodenal Absorption and Tibia Deposition of Ca^{47}.*

Exp.	Time after Ca^{47} admin. (min.)	pH of dosing solution	Duodenal absorption (% dose Ca^{47}) Rachitic	Vit. D	Tibia deposition (% dose Ca^{47}) Rachitic	Vit. D_3	% absorbed Ca^{47} in tibia Rach.	Vit. D	Vit. D/ rach.
I	10	1.9	4.8 ± .1	27 ± 3	.18 ±.01	.85 ± .09	2.8 ±.1	3.2 ±.2	1.1
I	10	6.0	7.7 ± 1.0	32 ± 3	.13 ±.01	1.14 ± .03	1.9 ±.3	3.4 ±.2	1.8†
II	30	2.0	11 ± 1	50 ± 5	.52 ±.06	2.1 ± .3	4.9 ±.6	4.2 ±.2	.9
II	30	4.5	16 ± 1	58 ± 4	.37 ±.03	2.1 ± .3	2.3 ±.1	3.6 ±.4	1.6†
II	30	7.0	11 ± 1	55 ± 1	.36 ±.02	2.4 ± .3	3.2 ±.1	4.5 ±.3	1.4†

* Values represent mean ± stand. error of mean of 5 birds/group.
† Significantly different from unity at $p < .01$.

After an overnight fast, the chicks were anesthetized with ether, a laporatomy performed and the duodenum removed from the body cavity. The segment was ligated proximal to the pancreatic and bile ducts and 1 ml of the dosing solution injected through a ligature into the proximal duodenal segment; length of the segment averaged about 8 cm. The duodenum was then returned to the abdominal cavity. The dosing solution contained 1 mg Ca (as the chloride) and 1 μc Ca^{47} (Oak Ridge National Lab.) per ml; pH adjustments were made with dilute HCl and NaOH using a Beckman Zeromatic pH meter. At various times after Ca^{47} administration the chicks were killed with Nembutal and duodenum and tibia excised. The residual Ca^{47} in the duodenum was determined by counting the intact segment directly or by transferring the lumenal solution with saline into a large counting vial; the gamma activities in the duodenum (or duodenal solution) and tibia were determined in a well-type scintillation detector. Ca^{47} activity in the wall of the intestine would contribute negligibly to the measurement.

Results. In Table I, data on the effect of initial intestinal pH on the duodenal absorption and tibia deposition of Ca^{47} over 2 time periods are given. Vit. D, as expected, greatly increased duodenal absorption of Ca^{47} by factors of between 4 and 6. The pH of the dosing solution *per se* did not produce any consistent effects on the amount of Ca^{47} transferred across the intestinal barrier.

To assess the relative deposition of absorbed Ca^{47} into tibia, the "percent absorbed Ca^{47} in tibia" was calculated for the rachitic and vit. D_3-treated chicks (last columns, Table I). At 10 minutes, proportionately less Ca^{47} was deposited in the rachitic tibia at pH 6.0 than at pH 1.9. In the vit. D-treated groups, pH had no significant effect on the deposition of absorbed Ca^{47}; also, at pH 1.9, there were no differences between Ca^{47} uptake by tibia of rachitic and vit. D-treated chicks. At the later time period (30 minutes), the same trends were observed; at the higher pH, less of the Ca^{47} absorbed from the duodenum was deposited in the tibia of the rachitic chicks whereas the pH of the dosing solution had little effect on Ca^{47} deposition in the vit. D-treated groups.

The time pattern of tibia deposition of Ca^{47} absorbed from the duodenum at pH 7.0 was next investigated in rachitic and vit. D-treated chicks (Table II). As before, significantly more of the absorbed Ca^{47} was accumulated by the tibia of the vit. D-repleted group than in the rachitic group. This was seen at 2.5 min. after introducing the Ca^{47} solution into the duodenum and at all later time periods. Of interest was the apparent downward trend of the vit. D/rachitic ratios (Table II, last column) with time, indicating that an increasing portion of absorbed Ca^{47} was moving into rachitic tibia at the later time periods; this could be brought about by a change in the manner by which Ca^{47} was handled by the intestine or would suggest that Ca^{47} re-distribution within the chick after absorption was not influenced by the aforementioned variables of pH and vit. D.

In the third study, the movement of Ca^{47} into tibia after intravenous injection (femoral vein) was determined in rachitic and vit. D-repleted chicks at 2 time periods, 5 and 15 min. As can be seen from Table III, vit. D deficiency did not significantly alter the amount of Ca^{47} accumulated by tibia. Thus,

TABLE II. Duodenal Absorption and Tibia Deposition of Ca^{47} in the Rachitic Chick.*

Time after Ca^{47} administration† (min.)	Duodenal absorption		Tibia deposition		% absorbed Ca^{47} in tibia		
	Rachitic	Vit. D	Rachitic	Vit. D	Rachitic	Vit. D	Vit. D/rach.
	(% dose)						
2.5	15 ± 2	21 ± 2	.02 ±.004	.09 ±.01	.12 ±.01	.41 ±.04	3.4§
5.0	24 ± 2	32 ± 2	.07 ±.01	.26 ±.05	.30 ±.06	.82 ±.02	2.7§
10.0	24 ± 2	44 ± 4	.12 ±.005	.57 ±.07	.52 ±.04	1.28 ±.01	2.5§
30.0‡	30 ± 2	66 ± 5	.38 ±.02	1.72 ±.16	1.28 ±.05	2.60 ±.08	2.0§

* Values represent mean ± stand. error of mean of 6 birds/group.
† pH of dosing solution was pH 7.
‡ 30 min. group run under same conditions but on a different day.
§ Significantly different from unity at $p < .01$.

these results strongly suggested that the effect of pH and vit. D on skeletal deposition of absorbed Ca^{47} was associated with the intestinal absorptive process and not with ability of the tibia to accumulate radiocalcium.

Discussion. The above data emphasize that caution should be used when skeletal uptake of a bone-seeking radionuclide is used as an index of relative absorption; this was also shown by Lengemann and Comar(5). Plasma levels of radiocalcium are often employed as an indicator of radiocalcium absorption; since treatment in the present study caused a change in distribution of Ca^{47} between skeletal and non-skeletal compartments, it would seem that assessing plasma levels alone would yield the same errors as measuring skeletal radiocalcium. It is insufficient to determine the effect of an experimental variable on skeletal deposition of labeled calcium after intravenous injection and, in seeing little or no differences, assume that absorbed radiocalcium would also be unaffected by the variable. It becomes apparent that the relationship between radiocalcium absorption and the deposition of that absorbed radiocalcium must be more thoroughly scrutinized under a given set of circumstances before skeletal Ca^{47} can be used as an index of Ca^{47} absorption.

TABLE III. Deposition of Ca^{47} into Chick Tibia after Intravenous Injection.*

Time after Ca^{47} admin. (min.)	Tibia uptake of Ca^{47}		
	Rachitic	Vit. D_3	Vit. D_3/rach.
	(% dose Ca^{47})		
5	2.1 ± .2	2.4 ± .1	1.1
15	3.0 ± .2	2.8 ± .1	.9

* Values represent mean ± stand. error of mean of 6 birds/group.

The reasons for the different rates of uptake by tibia of absorbed Ca^{47} at various acidities of dosing solution and in the presence or absence of vit. D are not apparent from the present data. Consideration was given to the effect of different rates of absolute Ca^{++} absorption on percent of radionuclide deposited in tibia. This was tested in a preliminary experiment in which either 0.2 mg Ca^{++} or 0.7 mg Ca^{++} (labeled with Ca^{47}) was infused intravenously into rachitic and vit. D-treated chicks over a 15-minute period; this would correspond to 20% and 70% absorption from a dosing solution containing 1 mg Ca^{++}. The results showed that there were no differences in deposition of Ca^{47} in tibia due to rate of Ca^{++} infusion or to the vit. D-status of the chick. Further, from Table I (30-minute values), it was shown that in the rachitic chick significantly less of the absorbed Ca^{47} was deposited in tibia at pH 7.0 than at pH 2.0 although Ca^{47} absorption *per se* was unaffected by pH. Therefore, it appeared unlikely that rate of Ca^{++} absorption was a pertinent variable influencing Ca^{47} deposition.

An obvious working hypothesis for future experiment would involve some type of complex formation between calcium and an unknown ligand. Such a ligand could either enhance Ca^{47} deposition in vit. D-treated chicks or decrease Ca^{47} uptake by tibia in the rachitic birds. The effect of pH on the relative accumulation of absorbed Ca^{47} by the skeleton would be as theoretically predicted if a bound form of calcium were involved;

that is, at a low pH, calcium would be essentially ionized in the presence of strong chelates and perhaps could then move across the intestinal barrier in that form and no differences would be seen. At a higher pH, calcium would begin to form strong associations with anions, such as citrate and phosphate; the behavior of the absorbed Ca^{++} would then be dependent upon the modifying effect of the complexing agent. However, considerably more data are required to establish a relationship between the above observations and a hypothetical complexing agent. Also, the role of plasma citrate, known to be elevated by vit. D(9), must be evaluated in respect to the present findings.

Summary. The effect of pH of the dosing solution on the relative tibia deposition of Ca^{47} absorbed from the duodenum of rachitic and vit. D-treated chicks was examined. Vit. D had its usual enhancing effect on Ca^{47} absorption; however, it was observed that the percent absorbed Ca^{47} deposited in tibia varied with intraduodenal pH and vit. D-status of the chick. At low pH values (1.9, 2.0), there were no differences in the percent of duodenally absorbed Ca^{47} accumulated by tibia in rachitic or vit. D-treated chicks whereas, at high pH values, proportionally less of the absorbed Ca^{47} was deposited in rachitic tibia; pH was without effect on uptake of Ca^{47} by tibia in the vit. D-treated birds.

The technical assistance of Harold Vunk and John Sherrod is gratefully acknowledged.

1. Migicovsky, B. B., Nielson, A. M., *Arch. Biochem. Biophys.,* 1951, v34, 105.

2. Worker, N. A., Migicovsky, B. B., *J. Nutrition,* 1961, v74, 490.

3. Wasserman, R. H., Comar, C. L., Nold, M. M., *ibid.,* 1956, v59, 371.

4. Wasserman, R. H., Comar, C. L., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 314.

5. Lengemann, F. W., Comar, C. L., *Am. J. Physiol.,* 1961, v200, 1051.

6. Vaughn, O. W., Filer, J. R., Jr., *J. Nutrition,* 1960, v71, 10.

7. Wasserman, R. H., *ibid.,* 1962, in press.

8. Sturkie, P. D., *Avian Physiology,* Comstock Publishing Assn., Ithaca, N. Y., 1954, p178.

9. Harrison, H. E., *Helvitica Paed. Acta,* 1959, v14, 434.

Received December 12, 1961. P.S.E.B.M., 1962, v109.

Effect of Several Anti-Leprosy Drugs on Multiplication of Human Leprosy Bacilli in Foot-Pads of Mice. (27293)

CHARLES C. SHEPARD AND Y. T. CHANG*

Communicable Disease Center, U. S. Public Health Service, Atlanta 22, Ga., and National Institute of Arthritis and Metabolic Diseases, NIH, Bethesda, Md.

When inoculated into the foot-pads of mice leprosy bacilli multiply slowly (generation time 20 to 30 days) to a level of 10^5 to 10^7 bacilli per foot-pad(1,2). The bacilli are then detectable in histologic sections. The incubation period (time from inoculation until development of significant lesion in the sections) varies with the number of bacilli inoculated; with an inoculum of 5×10^3 bacilli, the incubation period is 5 to 10 months.

This system, though slow, enables one to work experimentally with the multiplication of leprosy bacilli outside of the human patient. We wish here to report on its suitability to drug testing. The experimental design was one thought likely to produce clear-cut results; the drugs were fed at a high (but sub-toxic) level, and the bacilli were inoculated at the lowest dose that seemed likely to give consistently positive results in the controls.

Materials and methods. The inoculum was from the second passage of a strain (B2409) that was derived from a skin specimen removed at biopsy of a patient with typical lepromatous leprosy. The number of bacilli in-

* Leonard Wood Memorial (American Leprosy Foundation).