Distribution of Vitamin D_3-4-C^{14} in Rachitic and Vitamin D_3 Treated Chicks.* (30435)

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The tissue distribution of radioactively labelled vitamins D_2 and D_3, administered orally or injected, has been studied primarily in rachitic and normal (or vitamin D-treated) rats(1,2,3), although limited data are available for Rhesus monkeys(2) and infants(4).

These reports indicate that both forms of vit. D are widely distributed in the animal, with a major fraction of the administered dose going to liver tissue. Furthermore, it was found that a large fraction was excreted in the feces as presumably inactive polar breakdown products of the original vit. D molecule. Finally, it was stated that rachitic rats absorb a higher portion of an orally administered dose than normal rats(1,2).

In view of the importance of vit. D in the dietary of the chick and the interesting ability of the chick to distinguish by its physiologic response between vit. D_2 and D_3 (differing in both respects from the rat), a simple distribution study was performed on the fate of orally administered vit. D_3-4-C^{14} in rachitic and vit. D_3-treated chicks with the purpose of assessing whether any major species differences existed. The data obtained, while limited, indicate that wide tissue distribution obtains in the chick as in rats.

Experimental procedure. The chicks used in this study were male White Leghorns (Mt. Hope Queens), which were fed a commercial "rachitogenic chick test diet"† from the day after hatching. Three vitamin D_3-treated chicks were daily orally intubated with 6 IU vit. D_3 in 0.10 ml sesame oil and weighed 230, 236, and 255 g on day 26. The 3 rachitic chicks were intubated with the sesame oil alone and weighed 172, 181, and 190 g on day 26. Food was withdrawn on the 26th day and each chick was intubated with 0.3 ml sesame oil containing 0.427 μc (28.5 μg) of vit. D_3-4-C^{14} (Sp. act. 15 μc/mg).‡ Thus, each group of 3 chicks received a total of 2.82×10^6 dpm.

Twenty-four hours after administration of the labelled vit. D_3, each chick was decapitated and its blood collected from the severed neck. Internal organs were dissected and the gut contents flushed out with water and pooled with the 24-hour excreta. The samples from the 3 chicks of each group were pooled for extraction and counting. Each sample was refluxed with 10 parts of 15% KOH in methanol, extracted with 20 volumes dichloromethane, and the vacuum-dried extract partitioned between 15-ml volumes of iso-octane and 67% methanol as previously described(5). All the vit. D_3 plus some breakdown products partition into iso-octane. All the radioactivity in the 67% methanol consisted of polar breakdown products.

Radioactivity in the skin and muscle was estimated by analyzing 1-gram samples from each chick and calculating the total for the group on the basis that skin was 25% and muscle was 40% of the total body weights. One tibia from each chick was analyzed and the results for total skeleton estimated on the basis that bones were 15% of the body weight. The estimated percentages of muscle and bone correspond to values given by Latimer(6); the estimate for skin is somewhat higher than the sum of his values for integument plus feathers, to account for the total weights of our chicks.

The partition phases were dried, dissolved into 10 ml POP/toluene counting fluid or in certain instances into Brays solution(7), and

* This paper is based on work performed under contract with U. S. Atomic Energy Commission at Univ. of Rochester Atomic Energy Project, Rochester, N. Y.

† General Biochemicals Inc., Chagrin Falls, Ohio. Contained 1.43% calcium and 1.10% phosphorus (Ca/P ratio of 1.3) by analysis.

‡ Manufactured by N. V. Philips-Duphar, Weesp, Netherlands.

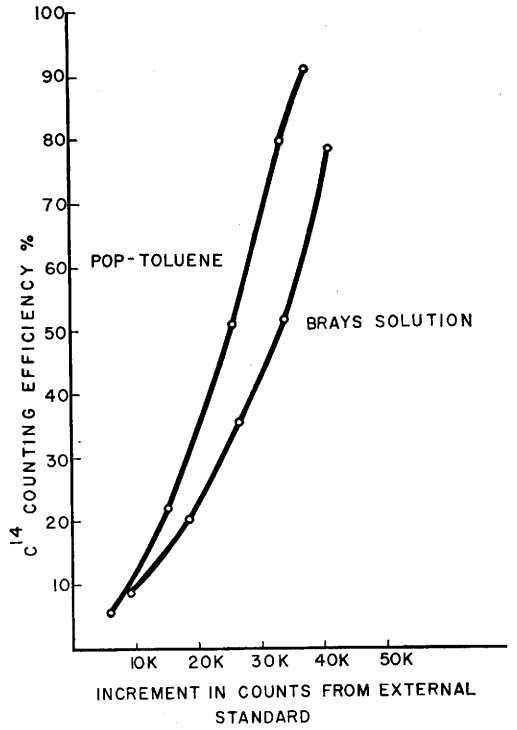

FIG. 1. Quench-Correction Curve for Colored Samples in ANSitron Liquid Scintillation Counter. Auramine O added as yellow quenching agent to 10 ml toluene-POP or Brays solution. Toluene-C^{14} employed as absolute standard.

counted in the ANSitron liquid scintillation counter. The absolute disintegration rate for each sample was evaluated using the external standardization method(8) based on a quenching curve for colored samples. The quenching curve was prepared using a series of standards in which the color of certain tissue extracts, *e.g.*, liver, spleen, red blood cells, was simulated with yellow dye, auramine O. A smooth quenching curve was obtained over the entire counting range of the instrument (Fig. 1).

It should be noted that this curve is for colored samples. The standard counts for test samples quenched by the presence of added alcohol (and water) fell a bit to the right of the curve drawn, *i.e.*, a higher external standard count for the same efficiency. Our samples, however, were dried prior to addition of POP/toluene or Bray's counting solution.

Results and discussion. The overall recovery of administered radioactivity was excellent, in view of the necessity of estimating the total amounts in muscle, bone and skin from representative samples.

Use of the external standard quench correction curve (Fig. 1) was of great value in correcting the measured counts/min back to an absolute disintegration rate. It can be pointed out that the counting efficiency varied considerably from one tissue to another depending on color, etc., and in one case, liver, was as low as 11 and 13% compared to 82.5% for a standard. Most extracts counted at between 40-70% efficiency.

It is evident from the data shown in Table I, that orally administered vit. D_3-4-C^{14} was widely distributed throughout the body of the chick. Considering first the vit. D_3-treated (and presumably "normal") chicks, several points may be brought out. (1) Relatively large fractions of radioactivity were in the excreta and intestinal tract contents (16.7%) and in the tissues of the digestive tract. The liver contained over 11%, but owing to their mass, even greater amounts were present in muscle, bone, and skin. (2) On a concentration per gram basis, the organs fell into 3 general groups, depending on whether they were lower than, roughly equivalent to, or higher than the calculated average concentration assuming even distribution throughout the body (0.174%/g for rachitic and 0.147%/g for vit. D_3-treated). Muscle and pancreas were low. Bones, skin, lungs, heart and red blood cells contained between 0.07 and 0.16%/g, a bit less than the estimated "average" distribution. Serum and the following soft tissues had "greater than average" concentrations: Liver, kidneys, adrenal glands, spleen and gall bladder, and the entire intestinal tract. (3) From the relative ratio of radioactivity which partitioned into 67% methanol, a rough idea of the extent of breakdown to polar material can be drawn. By this criterion, only a small fraction of the small intestinal tract activity was degraded vit. D_3, while in contrast *most* of the activity in skin, and spleen and gall bladder was polar breakdown products and muscle and bone also contained relatively large fractions as polar material.

Comparison of the rachitic chicks with the vit. D_3-treated group reveals several differences. Although rachitic chicks excreted somewhat more than normal chicks (21% *vs* 16.7%), the concentration of activity in the small intestinal tissue was strikingly higher in the rachitic group (compare middle and lower thirds of small intestine). Yet the livers, ceca, and large intestines contained much less of the administered dose in the rachitic animals. Other tissues were quite comparable—muscle, bone, skin, adrenal glands, etc. In this respect, the results differ from those obtained in rats, where liver, bones and certain other organs of rachitic animals took up greater fractions of the administered dose than the organs of normal rats(1,2).

Recently, presumptive evidence for a role of adrenal glands in the physiologic action of vit. D was given by the finding that an

TABLE I. Distribution of Vitamin D_3-4-C^{14} in Chicks (% in Organ).

	Rachitic				Vitamin D_3-treated				
	Wet wt	Iso-oct.	67% MeOH	Total	% per g	Wet wt	Iso-oct.	67% MeOH	Total	% per g
Red blood cells	3.32	.34	.08	.42	.13	5.29	.41	.10	.51	.10
Serum	7.74	1.25	.64	1.89	.24	5.88	1.65	1.32	2.97	.51
Liver	13.03	5.02	.60	5.62	.51	15.24	10.34	.89	11.23	.74
Excrement*	29.32	18.17	3.29	21.46	.73	29.99	13.10	3.61	16.71	.56
Cloaca and large bowel	4.71	3.66	.16	3.82	.81	6.00	6.73	.16	6.89	1.15
Small bowel—Upper ⅓	3.43	2.22	.10	2.32	.68	4.07	1.66	.13	1.79	.44
" " —Middle ⅓	3.32	7.26	.13	7.39	2.22	3.95	4.11	.18	4.29	1.09
" " —Lower ⅓	2.38	2.89	.07	2.96	1.24	3.40	.72	.09	.81	.24
Cecum	9.05	1.52	.39	1.91	.21	14.12	5.10	1.24	6.34	.45
Crop and gizzard	27.61	7.12	2.44	9.56	.35	27.41	4.74	2.04	6.78	.28
Kidneys	7.14	1.63	.20	1.83	.26	6.00	.80	.34	1.14	.19
Heart	3.26	.34	.07	.41	.13	4.15	.11	.16	.27	.07
Pancreas	2.72	.16	.04	.20	.07	2.67	.10	.06	.16	.06
Adrenal glands	.13	.076	.005	.081	.62	.09	.041	.013	.054	.60
Lungs	4.29	.56	.12	.68	.16	3.18	.25	.10	.35	.11
Spleen and gall bladder	2.98	.51	.39	.90	.30	3.55	.68	1.14	1.82	.51
Muscle	(218)	5.67	4.01	9.68	.044	(288)	6.52	6.08	12.60	.044
Bones	(81.5)	5.53	2.81	8.34	.10	(108)	7.77	3.99	11.76	.11
Skin	(135)	6.45	8.44	14.89	.11	(180)	6.87	12.80	19.67	.11
Total body	543	70.38	23.98	94.36	.174	721	71.70	34.44	106.14	.147

* Includes intestinal tract contents.

adrenal cortex inhibitor (o, p-DDD) was capable of blocking the stimulatory effect of vit. D_3 on absorption of Ca^{45} by *in vivo* intestinal loops in rachitic chicks(9). However, there is no evidence from our results that the chick adrenal glands were selectively concentrating vit. D at least at 24 hours. The uptake by chick adrenals was small, only 0.08% being present in the rachitic and 0.05% in D_3-treated groups. The adrenal concentration (on wet weight basis) similarly was not distinctive, only 0.6% per gram for both groups.

Summary. The distribution of vit. D_3-4-C^{14} in rachitic and vit. D_3-treated chicks was assessed following oral intubation into chicks fed rachitogenic diet alone (rachitic) or treated with 6 IU/day of vit. D_3. At 24 hours, the chicks were sacrificed and the radioactive material in each tissue was extracted from saponified tissue, then partitioned into a nonpolar fraction (iso-octane) containing primarily vit. D_3 and a polar fraction (67% methanol) containing only breakdown products. Radioactivity was assayed in the ANSitron liquid scintillation counter using automatic external standardization for correction of counting efficiency.

Radioactivity was widely distributed in all tissues of the chicks with high uptake, on a concentration basis, by intestinal tract, liver, kidneys, adrenal glands, spleen and gall bladder and in serum. Muscle and pancreas were low in localized uptake. However, bone, muscle and skin each quantitatively accounted for a greater percentage than liver. From the relative proportion of activity in the polar partition phase (67% methanol) compared to that in the iso-octane partition phase, it can be inferred that the intestinal tract activity was mainly vit. D, in contrast to skin, spleen and gall bladder, muscle, and bone where considerable breakdown products were present.

The small intestines of the rachitic animals contained considerably more activity than corresponding tissue in the vit. D_3-treated chicks, whereas livers, ceca and large intestines contained less activity. Rachitic chicks excreted more activity than the vit. D-treated animals. Other tissues (muscle, bone, skin, adrenal glands) were quite similar in uptake in both groups.

1. Kodicek, E., in Transfer of Calcium and Strontium Across Biological Membranes, Ed., R. H. Wasserman, Academic Press, N. Y., 1963, p185.

2. Blumberg, A., Aebi, H., Hurni, H., Schönholzer, G., Helv. Physiol. Pharmacol. Acta, 1960, v18, 56.

3. Norman, A. W., DeLuca, H. F., Biochemistry, 1963, v2, 1160.

4. Kodicek, E., in Drugs Affecting Lipid Metabolism, S. Garattini, R. Paoletti, Eds., Elsevier, Amsterdam, 1961, p515.

5. Chen, P. S., Jr., Terepka, A. R., Lane, K., Marsh, A., Anal. Biochem., 1965, v10, 421.

6. Latimer, H. B., J. Agri. Res., 1924, v29, 363.

7. Bray, G. A., Anal. Biochem., 1960, v1, 279.

8. Higashimura, T., Yamada, O., Shidei, T., Int. J. Appl. Rad. Isotopes, 1962, v13, 308.

9. Sallis, J. D., Holdsworth, E. S., Am. J. Physiol., 1962, v203, 506.

Received April 19, 1965. P.S.E.B.M., 1965, v120.

Temperature Acclimation Effects on Monkey Liver RIBO- and Deoxyribonucleic Acid and on Ribonucleic Acid of Other Organs.* (30436)

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In the squirrel monkey (*Saimiri sciurea*) it has been found(1), as in other animals(2-6), that there is a growth of internal organs in cold acclimation. The heart, liver, pancreas, and kidneys of the monkey grow in response

* Supported in part by USPHS Grant CA 4271-05 from Nat. Cancer Inst., N.I.H.; from the U. S. Army, Grant DA-49-193-MD-2558; and from the Kaiser Foundation, Grant 50.

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