as has already been suggested by Lipman. Whether or not phosphorylation of the vitamin occurs is now being investigated.

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Storage of Vitamin D in the Tissues of Growing Calves.*

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Numerous researches have been conducted with the view of determining the influence of varying intakes of vitamin D on the antirachitic potency of milk and eggs. Very few data are available, however, relative to the influence of vitamin D intake on the storage of this vitamin in the body tissues of various types of animals.

Metz and Coppens[1] have reported that some of the parenchymatous tissues from dogs contain considerable quantities of vitamin D. DeVaney and Munsell[2] have reported on the vitamin D content of ox, lamb, swine and calf livers, as purchased on the open market. Heymann[3] investigated the storage of vitamin D in various tissues of the rabbit, as resulting from feeding definite unitage of this vitamin in the form of viosterol in oil. According to this investigator, the amount of vitamin D storage was relatively small in all cases, the greatest storage being in the liver and in the blood, respectively.

It is highly probable that the lack of data concerning the ability of the larger animals to store vitamin D in their tissues is due to the amount of time and expense involved in carrying out any such investigations. Investigations of this type necessarily require an accurate measure of the total vitamin D intake of the particular animal while being maintained under carefully controlled conditions

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1 Metz, G. A., and Coppens, Ph. A., *Nederland. Tijdschr. v. Geneesk.*, 1934, **78**, 769.

2 DeVaney, G. M., and Munsell, H. E., *J. Home Ec.*, 1935, **27**, 240.

3 Heymann, Walter, *J. Biol. Chem.*, 1937, **118**, 371.

during a reasonable period previous to the time of slaughtering. Fortunately, in the investigation herein reported, an unusul opportunity for carrying out an investigation of this type presented itself when the Department of Dairy Husbandry completed some experiments which had been designed to determine the vitamin D requirement of growing calves.[4] During the above investigations a careful record had been kept of the total vitamin D intake and of the changes in body-weight of young calves from 2 weeks to 7 months of age. When the above animals were slaughtered at the end of the investigation, the tissues for the following studies were obtained.

Experimental. The calves from which the tissues used in these studies were taken were of the Holstein breed and were approximately 2 weeks of age when placed on experiment. These animals were maintained at all times in individual stalls in a darkened barn. Each calf was fed a diet consisting of a mixture of ground grains and skim milk powder and, in addition, received some roughage either in the form of night harvested dehydrated alfalfa hay or beet pulp. The various ingredients of the diet were so chosen as to formulate a mixture as low as possible in vitamin D but complete in all other known dietary respects. Each ingredient was subjected to biological assay for its vitamin D content previous to being incorporated into the ration.

In addition to this vitamin D-deficient basal diet, each calf, except those retained as negative controls, received carefully measured quantities of vitamin D as a dietary supplement. Some of the calves received the vitamin supplement in the form of a cod liver oil concentrate while other calves received the supplement in the form of irradiated yeast.

The length of time during which vitamin D supplements were fed before the animals were slaughtered varied somewhat with the different animals, ranging from 164 to 210 days. At the end of these periods the various animals were slaughtered in the usual manner and the desired samples taken for assay.

In preparing the samples for assay, the various tissues were taken from the animal body (in whole) as soon as possible, freed of connective tissue, weighed, macerated by passing through a meat chopper, immediately frozen and stored in this condition until ready for use. In the preparation of blood samples, an indefinite volume of the fluid (about 1000 ml) was collected in an oxalated glass container and stored at a low temperature until reduced to a suitable state for the feeding tests.

[4] *Research Bulletin* 364, Pennsylvania Agr. Exp. Station.

From calves selected from the first series of studies, the liver, heart, brain, kidneys and a part of the blood were retained for biological assay. At first, all of the above tissues except the blood were fed in the original state in sufficiently small daily portions to insure complete consumption. However, it was soon found to be impossible for the test animals to consume sufficient quantities of the fresh tissues to bring about a satisfactory antirachitic response. It was necessary, therefore, to desiccate these tissues in order to overcome this difficulty. Even after dehydrating the tissues, it was still impossible to make an accurate determination of the antirachitic value of brain, heart and kidneys because of the inadequate consumption of these tissues by the test animals. Therefore, from those calves selected from the second series of experiments, only the blood and liver were retained for biological assay.

In order to obtain the vitamin D from the blood and to preserve it in a suitable state for the feeding tests, a fat extraction according to the Rose-Gottlieb procedure was made and the combined ether extracts were reduced to a definite volume, which was proportional to the volume of the original blood used.

All biological assays were carried out according to the U. S. P. technic[5] (1936) of assaying for vitamin D.

The data obtained as the result of determining the antirachitic potency of the bloods and livers from the various calves are given in Table I.

The data presented in the table readily disclose the lack of efficient storage of vitamin D in certain tissues of the calf. In fact, variations in the daily vitamin D intake of as much as twenty-twofold resulted in only a threefold variation in the antirachitic potency of the bloods and a twofold variation in the antirachitic potency of the livers of the respective calves.

While a definite relationship between the vitamin D intake of the various calves and the amount of this substance found in the blood and in the liver of the respective calves does not appear to exist, the data obtained do indicate that, in most cases, those animals which received the greatest daily intake of vitamin D also stored the greatest amount of the antirachitic substance in their tissues. However, there appeared to be some exceptions to this generalization. In fact, those animals which were selected from the first series of experiments (Calves Nos. 367, 369, 381, 383, and 384) appear to have stored a lower percentage of the vitamin D in their tissues than did animals of the second series (Calves Nos. 409, 410, 411, 416, 419, 421, and 424). A possible explanation for this apparent difference

[5] *U. S. Pharmacopoeia*, Eleventh Decennial Revision, 1936, p. 482.

TABLE I.
Vitamin D Intakes of Calves During Experimental Periods and Storage of This Vitamin in Blood and Liver.

No. of calf	Duration of feeding test, days	Gain in wt, lb	Supplementary source of Vit. D	Total Vit. D intake, U.S.P. units	Vit. D per 100 ml blood, U.S.P. units	Vit. D per 100 g liver, U.S.P. units	Total Vit. D in blood,* U.S.P. units	Total Vit. D in liver, U.S.P. units
367	164	255	Oil	809,045	13	17	1481	386
369	174	175	"	110,770	7	15	612	265
381	164	255	None	129,695	11	13	1350	282
383	164	254	Yeast	805,810	15	19	1704	444
384	164	148	None	119,865	9	11	805	214
409	182	106	"	39,910	17	—	988	—
410	210	253	Yeast	249,095	21	22	2645	579
411	210	214	Oil	71,005	17	17	1678	361
416	210	200	Yeast	153,785	20	15	1988	328
419	210	166	"	77,495	15	20	1243	449
421	210	190	Oil	130,240	20	22	1750	296
424	203	180	None	48,700	—	17	—	352

*Total volume of blood calculated from the following equation:

$$\text{Vol. of blood in mls.} = \frac{\text{Wt. of calf (in lbs.)} \times 453.6 \times 0.0771}{1.059}$$

in the ability of the 2 groups of calves to store vitamin D in their tissues may be that the 2 groups of calves were born in slightly different seasons of the year. Those calves comprising the first series were born in late November, while those of the second series were born during the first half of September. If this difference in time of birth accounts for the variations in the response of the 2 groups of calves, it is possible that such a variation was due to a combination of the prenatal effect of the antirachitic condition of the cow on its offspring and to differences in the antirachitic potency of the liquid milk which the respective calves received during the first 2 weeks of life. As a whole, those calves comprising the second series received a lower intake of vitamin D during the experimental period than did those of the first series and, in turn, were able to store a larger percentage of this substance.

The 2 sources of vitamin D used in these investigations appear to be equally effective so far as storage of the antirachitic potency in the blood and in the liver of the calf is concerned.

An interesting point to be observed from the above data is the comparatively uniform concentration of vitamin D in the blood and liver of the calf. In fact, in the young of this species it appears that the liver is not an important storage organ for vitamin D. It seems, therefore, that unless there are other tissues of the calf which serve as more efficient storage organs for vitamin D than the liver or the blood, the ability of the calf to store vitamin D is rather limited. These data also indicate that the calf should be relatively susceptible to rickets if it were maintained for any extended period of time on a diet comparatively low in vitamin D and without access to ultraviolet radiation. However, serious outbreaks of rickets among calves maintained on the usual high grade rations have not been reported and in all probability have not been observed because of the appreciable antirachitic content of the sun-cured hays, roughage, and other constituents comprising a diet of this type.

Summary. A study was made of the effect of feeding young calves definite quantities of vitamin D, in the form of cod liver oil concentrate or irradiated yeast for periods of time ranging from 164 to 210 days, on the resultant storage of this substance in the blood and in the liver of the animal. From the data obtained in this study, it is concluded that only a relatively small percent of the antirachitic intake of the calf is stored in the blood and in the liver. The concentration of the antirachitic factor per unit weight was found to be approximately equal in the 2 tissues studied. Natural vitamin D as found in cod liver oil and activated ergosterol as found in irradiated yeast seem to be stored at about the same degree of efficiency.