Vitamin D Studies and Rickets

By

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A THESIS

Presented to the Graduate School of Michigan State College of Agriculture
and Applied Science in Partial Fulfillment of Requirements
for the Degree of Doctor of Philosophy

Chemistry Department
East Lansing, Michigan
1935

Reprinted from THE JOURNAL OF NUTRITION
Vol. 11, No. 6 and Supplement, June, 1936

A STUDY OF THE SEASONAL VARIATION OF VITAMIN D IN NORMAL COW'S MILK [1]

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THREE FIGURES

(Received for publication February 7, 1936)

In recent years a great deal of attention has been given to the importance of vitamin D in the human dietary and particularly to the desirability and means of enhancing the milk supply in this factor. The methods by which the latter has been achieved are well known and relatively little thought is now given to the antirachitic potency of milk produced under ordinary farming conditions. Although many studies have been made of the vitamin D content of cow's milk, none of them has been of a comprehensive nature. Table 1 summarizes the results of some of these investigations.

Similar trends in variations of the vitamin D potency of milk are indicated by the work of other investigators. Luce ('24) found milk from pastured cows definitely richer in vitamin D than that from stall-fed cows kept in the dark. Luce concluded that the concentration of the antirachitic factor in milk depended on the ration and possibly also on the degree of illumination received by the cow. Chick and Roscoe ('26) found 0.25 gm. of milk fat from pastured cows equal to 0.60 gm. milk fat from cows fed green feed in a dark stall. Exposure of the cow to outdoor light without a change in ration resulted in a twofold increase in the vitamin D content of

[1] The data in this paper were taken from the thesis presented by H. Ernest Bechtel to the faculty of the Graduate School of Michigan State College in 1935 in partial fulfillment of the requirements for the degree of doctor of philosophy.

the milk fat. Dutcher and Honeywell ('27) examined some Kansas butter samples and found that milk fat from cows exposed to sunshine was superior in vitamin D potency to fat produced by cows fed in the dark.

These and other studies which might be mentioned indicate in a general way the variations that occur in the vitamin D potency of milk and the factors which contribute to this variability. However, most of the work done has been of a rather fragmentary nature so that it seemed desirable to make a more extended study of the subject.

TABLE 1

SOURCE OF DATA	VITAMIN D CONTENT OF NORMAL MILK UNITS PER QUART		
		U.S.P.[1]	Steenbock
Steenbock and associates (Wisconsin) ('30)	June 1925	21.3	7.9
	September 1925	36.5	13.5
Mitchell and associates (Pennsylvania) ('32)	Summer milk	16.2	6.0
Krauss (Ohio) ('33)	Winter milk	7.6	2.8
	Summer milk	16.2	6.0
Russell (New Jersey) ('33)	Summer milk		
	Always less than	43.2	16.0

[1] The values in this column were obtained by multiplying the number of Steenbock units by 2.7.

MATERIALS AND METHODS

This study was begun in 1932, the milk being derived from various sources. Milk from the Holstein and Guernsey herds at Michigan State College was assayed monthly over a period of 2 years. Only the higher producers, namely, cows that were being milked three times daily were used, the average number of Holstein and Guernsey cows being fourteen and eight, respectively, for the 2-year period. From these cows 100-pound portions of composite 24-hour samples were saved monthly.

Monthly samples of a similar nature were derived from a few of the highest producing cows in the Holstein herd of the Michigan Experiment Station. These cows were kept on a ration of alfalfa hay, silage and corn.

From July 1933 to July 1934 monthly samples of Michigan State College Creamery butter, made from milk produced by local Michigan dairymen, were also put aside for vitamin D assays. Approximately fifteen herds, consisting largely of grade Holstein cows were represented in this group of samples.

In the case of the milk a small portion was used for a fat determination, the remainder being run through a cream separator. The butter obtained by churning the cream was packed in paper cartons and stored at 0°F. until the samples were to be assayed. At this time the butter was heated on a steam bath for about an hour and the relatively pure milk fat upon which the assays were conducted was suctioned off. Except when needed for assay, the fats were kept at 0°F.

The vitamin D determinations were carried out by the curative feeding technic, several changes being made in the official procedure. The Steenbock basal ration was slightly modified to obtain more consistent and somewhat better growth of the rats during the preliminary period. The rachitogenic diet used throughout this study was composed of the following:

	Per cent
Yellow cornmeal	38.0
Oatmeal	37.5
Wheat gluten	20.0
Calcium carbonate	3.0
Sodium chloride	1.0
Yeast powder	0.5

Instead of feeding the fat as a daily supplement during the first 8 days of the test period, the entire amount was mixed with 40 gm. of the basal diet. This mixture was found to be consumed in 6 to 8 days after which the basal diet was fed to finish the 10-day period. Control rats receiving 29 mg. of Official Reference Oil equivalent to 2.7 U.S.P. units were used for comparison. The usual staining technic was applied to the radii and ulnae.

In carrying out the assays a preliminary test was made to determine the approximate vitamin D content of the various samples. The confirmatory tests were then conducted at three levels using three to five rats at each level.

It became apparent early in this investigation that fats of low potency could not be assayed because of the limited capacity of the rats to consume fat. Amounts up to 6 and 8 gm. were consumed fairly consistently. However, when the dosage was increased to 10 gm. approximately only half of the test animals consumed the fat-basal diet mixture in 8 days. Attempts were therefore made to effect a concentration of the vitamin D so that fats of lower potency might be assayed. Although the work of Kon and Booth ('34) indicated that at least a part of the vitamin D of butter fat was unstable and could not be recovered quantitatively in the non-saponifiable matter, their method as well as several modifications were given a trial. Sometimes the recovery of vitamin D was quantitative, but more often it was not, so that this method of concentration was abandoned.

Inasmuch as the concentration of vitamin D in cod liver oil may be accomplished by extraction of the oil with alcohol, this method was next tried and proved to be satisfactory. The fats of low potency were therefore treated in the following manner: 100 gm. of melted milk fat was placed in a separatory funnel previously warmed in a 37°C. oven and 100 cc. of hot ethyl alcohol (95 per cent) was added. The mixture was then shaken fairly vigorously and the funnel placed into the 37°C. oven until the layers had separated. The fat was then drawn off into a warm beaker and the alcohol layer into a 500 cc. volumetric flask. The fat was returned to the separatory funnel and the beaker rinsed with 50 cc. of hot alcohol which was then added to the fat. The mixture was again shaken and the layers allowed to separate in the oven. The separations were made as before and three additional extractions carried out with 50 cc. portions of hot alcohol. By this process approximately 20 per cent of the fat was removed and this fat contained all of the vitamin D. The combined extracts were brought to a volume of 500 cc. with ether in order that the fat would be kept in solution. It was observed that the antirachitic value was retained for at least 2 months when the extract was stored at 0°C. Aliquots of this solution were poured on 40 gm. portions of the rachitogenic diet in evaporating dishes and the ether and alcohol allowed to evaporate spontaneously. These mixtures were then fed in the usual manner.

TABLE 2

Antirachitic potency of milk fat obtained from the Holstein herd[1]

DATE	FAT-CONTAINING 1 U.S.P. VITAMIN D UNIT	AVERAGE DAILY PRODUCTION PER COW		FAT IN MILK	VITAMIN D PER QUART OF MILK		AVERAGE DAILY AMOUNT OF SUNSHINE
		Fat	Vitamin D		U.S.P.	St.[2]	
	gm.	*gm.*	*U.S.P.*	*per cent*			*hours*[3]
1932							
July	1.5	817	545	3.26	17.8	6.6	11.5
August	1.1	717	701	2.88	20.2	7.5	10.0
September	1.1	862	784	3.53	27.7	10.3	7.8
October	3.3	748	227	2.78	6.3	2.3	3.3
November	—	—	—	—	—	—	4.0
December	—	—	—	—	—	—	3.0
1933							
January	5.6	826	148	3.15	4.6	1.7	4.1
February	9.3	875	94	3.26	3.1	1.1	7.1
March	3.7	835	226	2.97	6.7	2.5	4.4
April	3.7	748	202	2.93	5.9	2.2	6.8
May	3.0	730	243	2.89	7.0	2.6	8.5
June	—	—	—	—	—	—	12.7
July	1.5	776	517	3.20	16.6	6.1	12.8
August	1.9	862	454	3.28	14.9	5.5	10.8
September	1.5	780	520	2.98	15.5	5.7	7.1
October	3.7	690	186	2.96	5.5	2.0	6.0
November	7.4	753	102	3.19	3.2	1.2	2.0
December	—	—	—	—	—	—	1.4
1934							
January	—	—	—	—	—	—	2.3
February	7.4	844	114	3.17	3.6	1.3	5.9
March	7.4	939	127	3.29	4.2	1.6	5.0
April	4.4	871	198	3.06	6.1	2.3	6.0
May	3.0	898	299	3.31	9.9	3.7	11.3

[1] Average of fourteen cows per month.

[2] Steenbock.

[3] Average amount of available sunshine according to the East Lansing Weather Bureau.

The above method permitted practically complete recovery of the vitamin D from samples of milk fat of which 2 to 10 gm. had to be fed of the original fat to get the typical narrow continuous line of calcification. Inasmuch as there was no way of checking the fats of lower potency directly, it had to be assumed that the method was also satisfactory for such

TABLE 3

Antirachitic potency of milk fat obtained from the Guernsey herd[1]

DATE	FAT-CONTAINING 1 U.S.P. VITAMIN D UNIT	AVERAGE DAILY PRODUCTION PER COW		FAT IN MILK	VITAMIN D PER QUART OF MILK		AVERAGE DAILY AMOUNT OF SUNSHINE
		Fat	Vitamin D		U.S.P.	St.[2]	
	gm.	*gm.*	*U.S.P.*	*per cent*			*hours*[3]
1932							
July	1.1	649	590	4.93	43.8	16.2	11.5
August	1.1	685	623	4.86	43.1	16.0	10.0
September	1.3	708	545	5.15	38.7	14.3	7.8
October	3.0	699	233	4.52	14.7	5.4	3.3
November	3.7	708	191	4.02	10.6	3.9	4.0
December	3.7	930	251	4.76	12.6	4.7	3.0
1933							
January	4.4	907	206	4.42	9.8	3.6	4.1
February	9.3	835	90	4.57	4.8	1.8	7.1
March	3.7	762	206	4.33	11.4	4.2	4.4
April	3.0	658	219	4.34	14.1	5.2	6.8
May	3.0	694	231	4.42	14.4	5.3	8.5
June	—	—	—	—	—	—	12.7
July	1.5	744	496	4.43	28.8	10.7	12.8
August	1.9	853	449	5.09	26.2	9.7	10.8
September	1.9	694	365	4.61	23.7	8.8	7.1
October	2.6	721	277	4.76	17.9	6.6	6.0
November	3.7	912	246	4.86	12.8	4.7	2.0
December	—	—	—	—	—	—	1.4
1934							
January	—	—	—	—	—	—	2.3
February	7.4	866	117	4.44	5.9	2.2	5.9
March	5.6	835	149	4.50	7.8	2.9	5.0
April	3.7	826	223	4.52	11.9	4.4	6.0
May	3.0	871	290	4.90	15.9	5.9	11.3

[1] Average of eight cows per month.

[2] Steenbock.

[3] Average amount of available sunshine according to the East Lansing Weather Bureau.

TABLE 4

Antirachitic potency of milk fat obtained from the experiment station herd[1]

DATE	AVERAGE DAILY FEED INTAKE			FAT CONTAINING 1 U.S.P. VITAMIN D UNIT	AVERAGE DAILY PRODUCTION PER COW		FAT IN MILK	VITAMIN D PER QUART MILK		DAILY AMOUNT SUNSHINE
	Alfalfa	Silicon	Corn		Fat	Vitamin D		U.S.P.	St.[2]	
	lb.	*lb.*	*lb.*	*gm.*	*gm.*	*U.S.P.*	*per cent*			*hours*[3]
1933										
July	24.2	—	15.1	1.3	544	418	2.30	17.3	6.4	12.8
August	26.3	—	13.8	1.5	617	411	2.64	17.2	6.4	10.8
September	26.4	5.5	8.3	1.5	581	387	3.18	20.7	7.7	7.1
October	23.1	25.9	4.4	2.6	508	195	3.34	12.5	4.6	6.0
November	26.0	20.9	3.5	3.7	662	179	4.02	10.6	3.9	2.0
December	24.9	24.0	4.1	3.7	435	118	3.29	8.7	3.2	1.4
1934										
January	—	—	—	—	—	—	—	—	—	2.3
February	26.1	26.0	0.3	4.4	327	74	3.40	7.5	2.8	5.9
March	21.2	19.7	3.2	4.4	531	121	3.73	8.3	3.1	5.0
April	23.8	13.0	9.3	3.7	535	145	2.84	7.5	2.8	6.0
May	26.0	3.6	12.6	2.2	794	361	3.13	13.9	5.1	11.3

[1] Average of five cows per month.

[2] Steenbock.

[3] Average amount of available sunshine according to the East Lansing Weather Bureau.

TABLE 5

Antirachitic potency of creamery milk fat[1]

DATE	FAT CONTAINING 1 U.S.P. VITAMIN D UNIT	FAT IN MILK	VITAMIN D PER QUART OF MILK		AVERAGE DAILY AMOUNT OF SUNSHINE
			U.S.P.	Steenbock	
	gm.	*per cent*			*hours*[2]
1933					
July	1.3	3.5	26.3	9.7	12.8
August	1.9	3.5	18.0	6.7	10.8
September	1.3	3.5	26.3	9.7	7.1
October	2.6	3.5	13.1	4.9	6.0
November	4.4	3.5	7.8	2.9	2.0
December	3.0	3.5	11.4	4.2	1.4
1934					
January	—	—	—	—	2.3
February	3.7	3.5	9.2	3.4	5.9
March	—	—	—	—	5.0
April	—	—	—	—	6.0
May	3.0	3.5	11.4	4.2	11.3
June	1.9	3.5	18.0	6.7	11.8

[1] Average of twelve herds of cows per month.

[2] Average amount of available sunshine according to the East Lansing Weather Bureau.

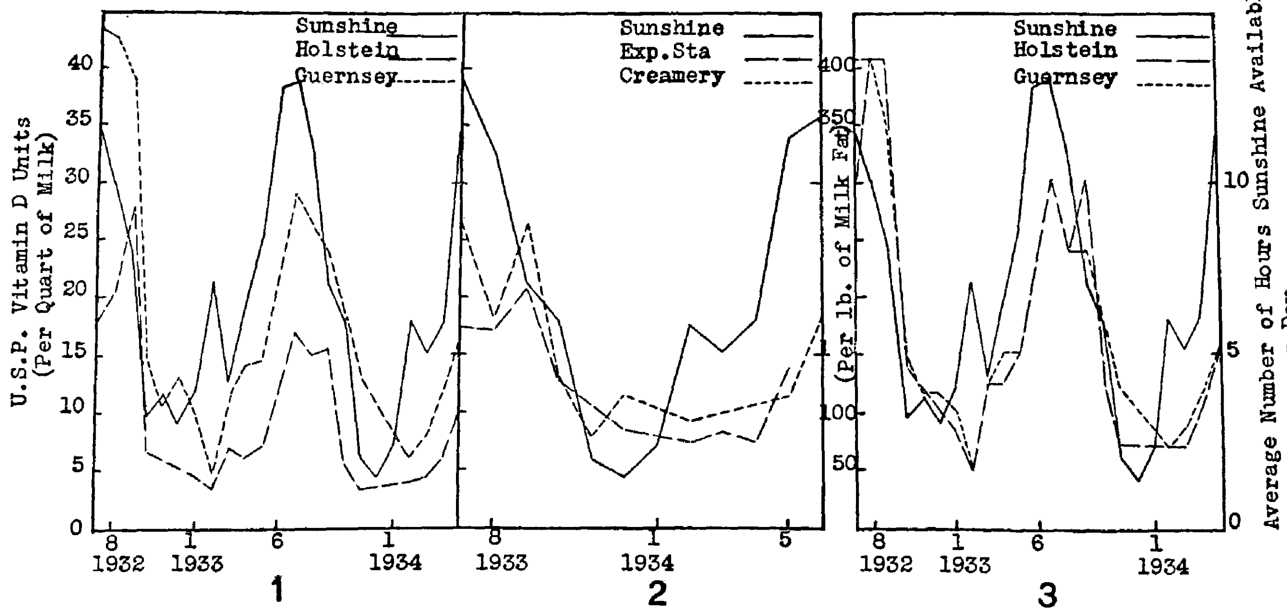

Fig. 1 Antirachitic potency of milk from the Holstein and Guernsey herds, and available sunshine.
Fig. 2 Antirachitic potency of milk from the Experiment Station herd and M. S. C. Creamery, and available sunshine.
Fig. 3 Antirachitic potency of milk fat from Holstein and Guernsey herds, and available sunshine.

fats. Quantitative recovery was also obtained when a definite amount of vitamin D from the official reference oil was added to milk fat and subjected to hot alcohol extraction.

The results of this study of the seasonal variation in the vitamin D content of cows' milk are presented in tables 2, 3, 4 and 5 and portions of the data are shown graphically in figures 1, 2 and 3. The data include not only the results of the bioassays but also the average daily production of milk fat and the number of vitamin D units in the milk fat. The results were also calculated in terms of U.S.P. units per quart and these values are presented in figures 1 and 2 with the average daily hours of sunshine available each month. To simplify the comparison of these results with those given in the older literature the antirachitic potency is also expressed in terms of Steenbock units.

Inasmuch as exposure of the cows to sunlight and the ingestion of sun cured roughages are two important factors which influence the vitamin D potency of the milk, a brief reference to the general management of the several dairy herds is appropriate. The main Holstein and Guernsey herds of the college were kept under parallel conditions at all times. From May to September, inclusive, these animals were pastured an average of 8 hours daily and received no hay or corn silage. During October they were pastured an average of 5 hours daily and received about 1 pound of hay per 100 pounds of body weight. From November to April, inclusive, the animals were put out doors in dry lot for an average of 2 hours daily. During this period in 1932–1933 they received besides their allowance of grain an average of approximately 2 pounds of hay per 100 pounds of body weight, no corn silage being included. During the corresponding period in 1933–1934 the animals received $\frac{3}{4}$ pound of hay and 3 pounds of corn silage per 100 pounds of body weight in addition to grain. The average weight of the Guernsey cows was 1150 and that of the Holsteins 1400 pounds.

The Holstein cows in the experiment station herd were out of doors in dry lot an average of 7 hours daily from May to September, inclusive, and about 2 hours daily during the other months. These cows were kept on a ration of alfalfa hay, corn silage and corn as shown in table 4.

The general management of the local Michigan dairy herds which served as the source of the college creamery butter samples was typical of that practiced in this state. The cows were fed chiefly home grown feeds consisting largely of alfalfa and cereal grains and were usually pastured as early and as late as conditions permitted. They were probably exposed to sunshine for a longer time than the cows in the college herds.

Regarding the assaying of the various samples of butter, there was a considerable interval between the time the samples were obtained and the time the bioassays were made. This delay was due chiefly to the fact that a satisfactory method had to be developed before the samples of low vitamin D potency could be assayed. However, there appeared to be no danger of a loss of antirachitic potency because some of the older samples were assayed 30 months after the first test was completed, the results indicating that vitamin D in milk fat is stable for at least 30 months when the samples are stored at 0°F. in the dark.

Practically all of the results given in tables 2, 3, 4 and 5 were obtained by using the alcohol extraction method, although most of the summer samples and a few of the more potent winter samples were also assayed by feeding the original fat.

DISCUSSION

In this investigation two assumptions were made which appear to be justifiable. It was assumed that all of the antirachitic potency of cows' milk is present in the milk fat and that there was no significant loss in potency incidental to the separating and churning of the cream.

The standard curative feeding technic was selected for the bioassays because this method has a number of definite advantages over the prophylactic procedure. Besides the fact that the former is much more widely used, it permits the feeding of relatively large amounts of fat without interfering with the test itself. In the prophylactic method the addition of vitamin D free fat to the basal rachitogenic diet will of itself cause a definite increase in the ash content of the bones, the increase depending on the amount of fat added. In this connection the slight modification of the rachitogenic diet seems justified because the rats attained a slightly larger size at the end of the preliminary period and had somewhat better appetites. This permitted the feeding of larger amounts of fat which was necessary in the case of the samples of lower potency. Nevertheless there were limitations in the capacity of the rachitic rats to consume fat and this necessitated concentration of the vitamin D. The alcohol extraction method described above seemed to solve this difficulty.

Inasmuch as Kon and Booth ('34) had felt that the vitamin D in winter milk might be different from that in summer milk because of the difference in stability to saponification, some of the more potent winter samples were assayed both by feeding the original fat and an equivalent amount of the extract. Although many of the rats failed to consume the larger doses of fat during the first 8 days of the experimental period, a sufficiently large number was used so that an assay at the 10-gm. level was made possible. The results indicated that there was no apparent loss in vitamin D in making the alcohol extractions of the winter samples tested, and served as the basis for the assumption that the assays of fats of still lower vitamin D potency by this method of concentration might be reliable.

The results obtained demonstrate that milk produced by cows managed under practical farming conditions varied as much as 900 per cent in antirachitic potency, reaching a maximum from June to September and beginning with October, declining rapidly to a minimum which usually occurred in February. From the assays made on the milk fats it was calculated that the maximal potency of the milks examined in this study was 43.8 U.S.P. units per quart. Values of 20 to 30 units per quart were not uncommon during the summer months whereas values of 8 units and less were frequently observed during the winter months. These results in a general way corroborate those of other investigators.

Regarding the factors which contribute to the variability in the vitamin D content of milk, the amount of exposure of the cows to sunlight probably plays the major role. This is strikingly indicated by the excellent correlation between the vitamin D potency of the milk and the amount of available sunshine as shown in figures 1, 2 and 3. Undoubtedly even better correlation might have been obtained if a record had been kept of the hours of actual exposure to sunlight as well as of the ultraviolet intensity of the sunlight. The lack of agreement during February is to be explained on this basis.

It follows from the above that the vitamin D contained in ordinary dairy feeds, particularly roughages and silage, however important this source may be to the general well being and productiveness of the dairy cow, contributes relatively little to the vitamin D content of the milk. Furthermore the rapid drop in the antirachitic potency of milk which follows the decrease in exposure of the cows to sunlight suggests that under ordinary conditions of management and feeding the dairy cow has practically no opportunity to build up a reserve of vitamin D during lactation.

In comparing the Holstein and Guernsey samples as shown in tables 2 and 3 it is interesting to note that there was little difference in the antirachitic potency of the milk fat. However, because of the higher per cent of fat in the milk of the latter breed, the calculated vitamin D content of the milk was greater.

SUMMARY

1. A method is presented for the concentration of the antirachitic factors in milk fat thus making possible the biological assay of fats of low potency.

2. The monthly assay of milk fats from several sources over a period of 2 years shows that milk may vary as much as 900 per cent in antirachitic potency. Highest values were obtained during July, August or September and lowest usually in February. Vitamin D values ranging from 4.8 to 43.8 U.S.P. units per quart of milk were observed in the case of Guernsey milk whereas the extreme values for Holstein milk were 3.1 to 27.7 U.S.P. units per quart.

3. The close correlation between the antirachitic potency of milk and the amount of available sunshine indicates that the exposure of cows to sunlight is the major factor contributing to the vitamin D content of milk.

4. Apparently the cow has little or no opportunity to store vitamin D during lactation under ordinary dairy management conditions.

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THE ANTIRACHITIC ACTIVITY OF VARIOUS PARTS OF THE CORN PLANT AT THE TIME OF ENSILING

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SECTIONS OF DAIRY HUSBANDRY AND CHEMISTRY

Previous work at this Station demonstrated that corn silage has antirachitic properties when fed to dairy calves, and to rats (1). The present report is a continuation of this work, and is concerned with the vitamin D content of several parts of the corn plant at the time of ensiling.

As indicated elsewhere (1), corn is usually harvested for silage when some parts of the plant have become dry in the field, while other portions of the plant remain green. The available data suggest that corn silage may derive its antirachitic properties from the sun-dried portions of the corn plant which are present at the time of ensiling.

Experimental

In 1933, several parts of the corn plant were collected from a field of corn on the day that the material was being ensiled. Among these were tassels, silk, some of the upper green leaves, and some of the lower dry leaves. Each of these materials was placed immediately in a drying room at 50°—60° C. after collection, and after about one week the dried material was ground and stored in stoppered bottles at room temperatures until needed for assaying.

The vitamin D determinations were made by the biological assay method. The following was used as the basal rachitogenic diet:

Yellow corn meal	38.0 per cent
Oat meal	37.5 per cent
Wheat gluten	20.0 per cent
Calcium carbonate	3.0 per cent
Sodium Chloride	1.0 per cent
Dried yeast	0.5 per cent

The curative technique was used in accordance with standard line-test procedure and vitamin potencies were determined in terms of U. S. P. rat units of vitamin D. The materials to be assayed were incorporated at various levels in 40 gm. of the basal rachitogenic diet and fed to standard rachitic rats. The results of all assays were compared with those from rats which received a known amount of vitamin D in the form of international reference cod liver oil.

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Reprint from Michigan Agricultural Experiment Station Quarterly Bulletin, Vol. 18, No. 3, February, 1936.

Results

In Table 1 are given the antirachitic values of the various parts of the corn plant assayed.

Table I.

Portion of the Corn Plant Assayed	Vitamin D Units per Pound of Dry Matter*
Tassels	1226
Silk	2449
Dry Leaves	2449
Green Leaves**	—

*Air dry basis.
**Ten grams had no antirachitic action when fed to a standard rachitic rat.

Discussion and Summary

Biological assays of the corn plant collected at the time of ensiling demonstrated that those parts of the plant which were sun-dried in the field were potent sources of vitamin D. Of practical importance were the dry leaves on the lower portions of the plant. It was found that 0.5 gm. of the dry leaves produced a narrow continuous line of calcification across the metaphyses of the radius and ulna in a standard rachitic rat. However, as much as 10 gm. of dried material prepared from the green leaves possessed no demonstrable quantity of vitamin D.

These findings are in agreement with other work in the literature which shows that fresh, green plant materials are ordinarily devoid of antirachitic activity, whereas plant materials allowed to dry in direct sunshine develop antirachitic qualities.

The data presented in Table I. indicate that corn silage derives antirachitic qualities from those parts of the corn plant which were sun-dried in the field prior to ensiling.

Conclusion

The antirachitic activity of corn silage is derived chiefly from those portions of the corn plant which were sun-dried in the field prior to ensiling.

Reprinted from JOURNAL OF DAIRY SCIENCE, June, 1936, Vol. XIX, No. 6

VITAMIN D STUDIES IN CATTLE

IV. CORN SILAGE AS A SOURCE OF VITAMIN D FOR DAIRY CATTLE*

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In previous reports from this Station, experimental data have been submitted upon the antirachitic value of sun-cured hay for dairy cattle (1), the vitamin D sparing action of certain magnesium compounds when only small amounts of this vitamin were present in the dairy ration (2), and the essential nature of radiant energy in the dietary régime of the dairy calf (3). The present report is concerned with the antirachitic value of corn silage for dairy cattle.

Hess and Weinstock (4) reported that green plants grown in the dark contained no vitamin D but became antirachitic after ultraviolet irradiation. Bethke, Kennard and Kik (5) failed to prevent leg weakness in chicks when green, fresh red-clover was fed as 50 per cent of the ration. Steenbock and associates (6) found that clover hay cured in the dark was inactive antirachitically. Mellanby and Killick (7) reported that summer-grown grass contained more of the calcifying factor than cabbage. Green spinach grown in midsummer has been reported by Chick and Roscoe (8) to have a slight but appreciable antirachitic value. While an investigation regarding the actual feeding of fresh, green plants as a source of vitamin D to dairy cattle is lacking, the indications are that fresh, green pasture grasses, in general, are poor sources of antirachitic substances.

As previously indicated (1), the antirachitic potency of hays is related to their exposure to solar ultraviolet rays. It is the usual practice to harvest corn for silage when many of the ears have become dented, the bottom three or four leaves and a portion of the husk have become dry while the remainder of the plant is still green. Corn at this stage of maturity is mostly green plant substance and for this reason has been considered a poor source of vitamin D, although this point has not been specifically investigated. Because of the presence of sun-dried leaves, however, and other material

* Published with the permission of the Director of the Experiment Station as Journal Article No. 250 (n.s.).

** This manuscript is part of a dissertation presented to the Faculty of the Graduate School of Michigan State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Received for publication January 27, 1936.

on the plant at the time of ensiling, it was thought possible that corn silage might have an appreciable antirachitic value.

The object of this investigation was to determine the antirachitic value of corn silage for dairy cows.

EXPERIMENTAL

The corn silage was made from dent corn which was cut when the kernels were dented and the lower leaves were dry. One sample of silage was taken for each of the following years, 1931–1934, inclusive, for biological assay with rats. Each sample of silage was immediately put in a drying room at 50°–60° C. after collection and after about one week, the dried material was ground and stored in stoppered bottles at room temperatures until needed for assaying. The vitamin D content of the silage was determined by the curative feeding technique with rats according to a standard line-test procedure. The vitamin D contents of the silage, in terms of U. S. P. rat units, are shown in Table 1.

TABLE 1

Vitamin D content of silage, U. S. P. rat units

CURATIVE MATERIAL	UNITS PER POUND DRY MATTER*
Corn silage 1931	165
Corn silage 1932	122 –
Corn silage 1933	122
Corn silage 1934	165

* Air dried basis.

The nineteen grade-Holstein dairy calves of either sex which were used in this experiment were divided into 5 groups. The calves in the first 4 groups were placed on experiment at birth but the calves in group 5 were several months of age when placed on this experiment. The management of the calves and the composition of the rachitogenic calf ration were similar to that previously reported (1–3). Blood samples were obtained from each of the experimental calves every two weeks and the plasma from each sample was analyzed for calcium, inorganic phosphorus (9) and magnesium (10) by methods already recorded. At the time of post-mortem examination, certain bones were saved from each animal and studied histologically (11). The chemical analysis of the various feeds used in this investigation are given in Table 2.

The calves in Group I, C–140, C–161 and C–164, were fed the unsupplemented basal rachitogenic ration. The calves in Group II, C–135, C–139 and C–141, each received the ash from one pound of dry silage per day in addition to the basal ration. The calves in Group III, C–132, C–136, C–137,

TABLE 2

Chemical analysis of feeds and water

MATERIAL	MOISTURE	Ca	P	Mg
	per cent	*per cent*	*per cent*	*per cent*
Whole milk	....	0.120	0.093	0.012
Skim milk	....	0.122	0.096	0.012
Corn and oats	11.80	0.128	0.321	0.156
Grain mixture	11.30	0.494	0.364	0.199
Corn silage 1931–32*	76.61	0.175	0.062	0.122
Corn silage 1932–33	78.02	0.101	0.054	0.067
Corn silage 1933–34	70.51	0.103	0.054	0.088
Corn silage 1934–35	69.00	0.126	0.064	0.115
Corn silage ash	....	7.190	2.950	6.770
Water	....	0.0082	....	0.0027

* Each year's analysis began with September.

C–138 and C–145, received from one to six pounds of silage per day per animal. Following the above curative feeding trials, the calves in Group IV, C–156, C–159 and C–160, were used in a preventive trial. The calves in this group received the basal ration but corn silage was added as the sole source of vitamin D when the calves were 30 days of age. The calves in the above groups which survived were slaughtered at approximately 190 days of age, with the exception of C–159 which was changed to another experiment.

Calves C–168, C–169, C–188, C–195 and C–167 were subsequently added to this experiment as Group V. The first four calves were rachitic at the time when silage was added to their basal rachitogenic ration. Calf C–168 was 269 days of age and had been suffering from rickets for about 50 days, calf C–169 was 348 days of age and had been suffering from rickets for about 100 days, calf C–188 was 428 days of age and had had rickets for more than 200 days and calf C–195 was 395 days of age and had had rickets for at least 60 days when silage was added to their rations. Calves C–168 and C–169 were subsequently slaughtered, C–188 died while on experiment and C–195 was continued on experiment to determine the effect of the ration upon growth and reproduction. Calf C–167 was normal when silage was added to the ration.

RESULTS

The results obtained from the experiment are presented in Tables 3–7, inclusive. Table 3 summarizes the data pertaining to the age when the calves were placed on experiment, the age when the supplement was added, the first evidence of rickets, the age of the calves at the termination of the experiment and the terminal plasma calcium, magnesium and inorganic phosphorus values. Table 4 gives the data for a representative animal in Groups I–IV, inclusive.

TABLE 3

Summary of data pertaining to calves which received the rachitogenic ration

CALF	EXPER. STARTED. AGE	SUPPLEMENT ADDED. AGE	RACHITIC. AGE	EXPER. ENDED. AGE	TERMINAL PLASMA Ca	P	Mg
no.	*days*	*days*	*days*	*days*	*mg. per 100 cc.*		
Group I. Basal ration							
C–140	1	none	73	88 D[1]	9.4	13.80	2.33
C–161	1	”	127	162 D	9.3	9.06	2.19
C–164	1	”	108	158 D	8.8	6.69	1.87
Group II. Basal ration plus silage ash							
C–135	1	93	72	108 D	6.8	6.51	2.80
C–139	1	90	71	114 D	7.3	8.62	1.68
C–141	1	79	73	193 S[2]	8.4	5.84	2.43
Group III. Basal ration plus silage							
C–132	1	93	93	193 S	8.2	3.81	2.00
C–136	1	95	81	191 S	8.6	3.68	2.45
C–137	1	92	67	192 S	8.6	3.50	....
C–138	1	86	86	193 S	10.0	7.35	2.82
C–145	1	92	92	190 S	10.0	2.27	2.45
Group IV. Basal ration plus silage before the onset of rickets							
C–156	1	32	161	186 S	7.5	3.63	1.56
C–159	1	33	116	190[3]	–	–	–
C–160	1	30	95	193 S	8.0	3.01	1.80
Group V. Basal ration plus silage (older calves)							
C–167	192	192	none	1140[4]	....	....	....
C–168	269	269	210	371 S	11.9	7.27	3.12
C–169	348	348	248	414 S	12.3	6.79	2.45
C–188	428	428	225	520 S	8.5	4.13	2.33
C–195	366	366	300	813[5]	......	....	....

[1] D denotes died. [2] S denotes slaughtered. [3] Changed to another experiment. [4] Still on experiment. [5] Changed to another experiment, cow still alive.

Group I.—The three calves in this group received the unsupplemented basal ration. Anorexia was manifested by C–140 and C–161 after the onset of rickets and C–161 also had a convulsion at 162 days of age. C–164 had a severe convulsion at 128 days of age at which time 5 cc. of parathormone was injected subcutaneously. A mild convulsion was again noted at 157 days of age and the calf was found dead on the following day. The condition of the pen indicated that the calf had died in a convulsion.

Group II.—The three calves in this group had their basal rachitogenic ration supplemented with silage ash. C–135 contracted bilateral pneumonia

Growth data, mineral and silage intakes and blood plasma values of representative calves

AGE	WT.	HT.	DAILY SILAGE INTAKE				AV. DAILY INTAKE			BLOOD PLASMA		
			Silage	D.M.	D.M. per kilo	Vit. D units	Ca	P	Mg	Ca	P	Mg
days	*lb.*	*cm.*	*lb.*	*lb.*	*gm.*	*U.S.P.*	*gm.*	*gm.*	*gm.*	*mg. per 100 cc.*		
Group I. C–161. Basal ration												
30	88	79					4.3	3.3	0.4	13.1	6.53	2.21
60	122	82					8.8	6.1	1.4	12.6	6.25	2.62
90	144	86					9.8	9.3	2.0	10.6	7.95	2.36
120	163	89					12.7	9.8	2.4	10.7	7.42	2.16
150	169	90					8.9	6.7	3.4	8.5	5.37	1.80
162[1]	......	......					......	......	......	8.8	7.28	2.13
Group II. C–141. Basal ration plus silage ash												
70[2]	132	78					8.3	7.6	1.5	9.2	5.63	2.80
100	162	83					14.8	9.4	3.2	8.4	6.89	1.95
130[3]	192	87					14.2	10.1	4.8	7.1	6.38	1.85
160[3]	199	90					11.1	7.3	5.3	6.9	5.80	1.71
190[3]	202	91					11.0	7.3	5.3	7.8	5.11	2.03
193[4]	201	......					......	......	......	8.4	5.84	2.43
Group III. C–145. Basal ration plus silage												
100	188	85	2.1	0.5	2.6	60	14.2	10.2	3.7	9.5	4.66	2.58
130	239	89	5.6	1.3	5.7	159	19.3	12.8	7.1	10.3	3.10	2.29
160	277	92	6.0	1.4	5.4	171	17.1	9.9	7.8	11.4	3.11	2.60
190[4]	280	96	5.8	1.4	4.8	166	16.6	9.2	7.4	10.7	2.99	2.44
Group IV. C–159. Basal ration plus silage before the onset of rickets												
30[5]	102	77	......	......	......	......	4.6	3.5	0.5	12.1	6.55	2.41
60	123	80	......	......	......	......	8.5	7.0	1.1	11.9	6.60	2.36
90	152	84	0.7	0.2	1.3	19	10.0	9.1	2.2	11.7	5.82	2.21
120	184	88	1.0	0.2	1.4	27	13.7	10.3	3.0	9.9	7.25	2.37
150[6]	202	90	1.7	0.4	1.9	46	9.3	6.1	3.9	8.5	5.46	1.98
180	228	92	2.5	0.6	2.4	67	11.3	7.7	4.8	8.0	6.82	1.99
210[7]	229	......	3.1	0.7	3.0	83	11.8	7.9	5.1	6.6	5.48	1.65

[1] Convulsion, died. [2] Added silage at 78 days of age. [3] Convulsions 129, 167, 183, and 184 days of age. [4] Slaughtered. [5] Added silage at 32 days of age. [6] Stiff at 166 days of age. [7] Convulsions at 198 days of age, injected 5 cc. of parathormone subcutaneously. Removed from experiment at 210 days of age.

and died at 108 days of age. C–139 had a convulsion at 88 days of age and died at 114 days of age. C–141 had convulsions at 129 and 167 days of age. Parathormone was injected after each convulsion. Convulsions were again noted at 183 and 184 days of age. The calf was slaughtered at 193 days of age.

Group III.—After 90 days of age, the five calves in this group received the basal ration supplemented with small amounts of silage. All of the calves showed the clinical evidences of rickets from 62 to 93 days of age. C–137 had convulsions at 83 and at 90 days of age. The corn silage supplement was started at 92 days of age and convulsions were observed at 128 days of age. The calves in this group were slaughtered at approximately 190 days of age. C–145 was representative of this group, therefore, the calcium, phosphorus and magnesium intakes and the concentration of these constituents in the blood plasma are presented in Table 4.

Group IV.—The three calves in this group had their ration supplemented with corn silage at about 30 days of age and before the onset of rickets. The calves did not develop rickets until they were from 95 to 161 days of age. They did not consume enough silage at an earlier age to prevent rickets but the silage consumption tended to delay the symptoms. As they became older an insufficient amount of silage was consumed to prevent rickets because of anorexia. C–156 and C–160 were slaughtered at about 190 days of age and C–159 was changed to another experiment at 210 days of age. The representative experimental data secured from C–159 are presented in Table 4.

Group V.—With the exception of C–167, the older calves which were used in this group to determine the antirachitic effect produced by the ingestion of large amounts of silage were rachitic when placed on experiment.

Calf C–167. This calf had previously been used to determine the efficacy of solar ultraviolet radiation in preventing the manifestations of rickets (3). Table 5 presents the growth data, feed consumption and blood plasma values from 210 days of age until the termination of the experiment. She received corn silage as the sole source of vitamin D at 192 days of age and ate it with avidity from the very beginning, grew normally and maintained a sleek appearance at all times. C–167 was first bred at 467 days of age and again at 487 and 515 days of age. She aborted at 601 days of age and was again bred at 662 and 765 days of age. Beginning at 920 days of age, all grain was withheld from the ration and corn silage was the sole source of food other than wood shavings, salt and water. This heifer gave birth to a normal 85-pound heifer calf at 1032 days of age and had a retained placenta. C–167 averaged approximately 45 pounds of milk per day, containing 3.5 per cent fat, during the first 100 days of lactation on a ration which consisted of the rachitogenic grain mixture, corn silage, wood shavings, salt and water. Pre-

AGE	WT.	HT.	DAILY SILAGE INTAKE				AV. DAILY INTAKE			BLOOD PLASMA		
			Silage	D.M.	D.M. per kilo	Vit. D units	Ca	P	Mg	Ca	P	Mg
days	*lb.*	*cm.*	*lb.*	*lb.*	*gm.*	*U.S.P.*	*gm.*	*gm.*	*gm.*	*mg. per 100 cc.*		
210	314	98	3.0	1.1	3.3	129	14.9	9.9	6.5	10.4	8.23	2.47
240	345	102	11.7	2.6	7.4	315	16.1	10.1	7.8	11.7	7.60	2.68
270	401	103	16.5	3.6	9.0	445	19.3	12.0	9.7	12.2	7.81	3.12
300	453	108	18.7	4.1	9.0	502	22.0	13.8	11.0	11.4	7.36	2.48
330	486	109	16.3	3.6	7.4	440	20.2	12.6	10.0	11.7	7.75	3.40
360	546	111	19.3	4.3	7.8	521	23.3	14.5	11.6	11.4	6.85	3.47
390	607	114	20.0	4.4	7.2	539	23.7	14.8	11.9	12.3	7.60	3.09
420	645	117	20.0	4.4	6.8	539	23.8	14.8	11.9	11.9	7.24	3.33
450[2]	708	119	20.0	4.4	6.2	539	25.4	15.9	12.5	10.9	7.25	3.11
480	739	120	20.0	4.4	5.9	539	30.4	19.3	14.5	11.3	6.99	2.67
510	813	122	20.0	5.9	7.2	723	34.5	21.4	17.9	11.3	6.92	3.03
540	847	123	20.0	5.9	7.0	723	34.1	21.4	17.7	11.6	6.16	3.03
570	852	123	20.0	5.9	6.9	723	33.0	21.4	17.3	12.5	6.16	3.19
600[3]	850	124	14.1	4.1	4.8	506	22.1	13.9	11.7	12.9	5.63	3.16
630	858	126	16.6	4.9	5.7	600	21.7	13.0	12.1	13.0	6.05	2.88
660[4]	887	127	14.8	4.4	4.9	534	22.7	14.1	12.2	11.9	5.85	2.61
690	924	126	19.5	5.8	6.2	705	26.1	16.6	14.6	11.4	5.00	2.82
720	973	129	20.0	5.9	6.1	723	28.5	18.1	15.5	10.6	5.73	2.78
750[4]	1031	129	22.3	6.6	6.4	808	27.7	17.1	15.8	10.8	5.68	2.77
780	1070	129	30.7	9.0	8.4	1107	25.8	14.1	16.7	11.0	5.86	2.53
810	1103	130	30.0	8.9	8.0	1084	26.2	14.0	16.6	11.6	6.94	2.26
840	1150	131	32.2	9.5	8.2	1161	24.2	13.1	16.4	11.1	6.65	2.45
870	1186	132	35.0	10.9	9.1	1329	27.3	13.5	21.0	11.6	6.63	3.00
900	1208	133	35.0	10.9	9.0	1329	28.2	13.5	21.3	10.8	6.87	2.76
930	1233	133	38.7	12.0	9.7	1467	26.7	13.3	21.9	10.4	6.16	3.14
960	1251	133	45.0	14.0	11.1	1709	26.9	13.1	23.8	10.9	6.56	3.49
990	1254	132	40.6	12.6	10.0	1547	24.7	11.8	21.7	10.6	6.07	2.71
1020[5]	1187	132	41.8	13.0	10.9	1587	24.8	12.1	21.8	10.5	5.72	2.63
1050	1044	133	32.9	10.2	9.8	1250	33.8	19.2	23.1	9.8	4.45	3.06
1080	1090	133	40.7	12.6	11.6	1546	67.0	41.5	38.6	10.9	4.99	2.57
1110	1124	132	44.6	13.8	12.3	1695	76.1	47.4	43.6	11.0	5.13	2.76
1140	1149	.....	45.0	14.0	12.1	1708	76.3	47.7	43.6	10.5	3.91	3.22

[1] Data from birth to 190 days of age appeared in Paper III, (3) this series.
[2] Bred at 467, 487 and 515 days of age.
[3] Aborted at 601 days of age.
[4] Bred at 662 and 765 days of age.
[5] Calved at 1032 days of age.

liminary results indicate that the milk produced by C–167 contained considerably less than 2.7 U. S. P. vitamin D units per quart. The blood plasma data fail to reveal any significant variations from normal. However, there were two gradual drops in the inorganic phosphorus values. The first drop was associated with the natural physiological disturbance due to abortion and the second was due to parturition.

Calf C–168. This calf received from 15 to 20 pounds of corn silage per day as the sole source of vitamin D from 269 days of age until the end of the experiment. The calf was suffering from severe rickets when first placed on experiment and the blood calcium and inorganic phosphorus values were 7.6 and 9.84 mg. per 100 cc. of plasma, respectively. The silage was readily consumed from the beginning and at 303 days of age the calf was able to rise to its feet with much less difficulty than a week earlier. The plasma calcium and inorganic phosphorus values were 9.3 and 5.48 mg. at 303 days of age and by the following week they had returned to normal, 10.9 and 6.76 mg., respectively. The gains in body weight were also greater from this time on until the animal was cured of rickets and slaughtered at 371 days of age. The terminal calcium and inorganic phosphorus values were 11.9 and 7.27 mg. The kidneys showed extensive areas of scar tissue.

Calf C–169. This calf received 15 pounds of corn silage per day as the sole source of vitamin D beginning at 348 days of age. It was severely rachitic and had difficulty in rising to its feet. The blood calcium and inorganic phosphorus values were 8.0 and 5.84 mg. per 100 cc. of plasma. The corn silage was readily consumed and 10 days later the calf was able to rise to its feet with much less difficulty. The blood values were approaching their normal concentrations by 370 days of age and the calf began to gain in body weight. C–169 was cured from rickets when slaughtered at 414 days of age and the terminal calcium and inorganic phosphorus values were 12.3 and 6.79 mg. The kidneys showed more extensive areas of scar tissue than observed in C–168. The data are tabulated in Table 6.

Calf C–188. This calf was 428 days of age and had been suffering from rickets for more than 200 days when corn silage was added to the ration as the sole source of vitamin D. The animal was severely rachitic at this time and was only maintaining a constant body weight. The blood calcium and inorganic phosphorus values were 7.2 and 7.06 mg. per 100 cc. of plasma. The silage was readily eaten but the grain was refused part of the time. By 472 days of age the animal was extremely stiff and lame and was rarely seen standing so that it became necessary to place the feed in a basket on the floor of the stall. The plasma calcium had now increased to 7.8 mg. but the inorganic phosphorus had declined to 4.51 mg. Placing the feed before the animal resulted in an increase in silage consumption but no improvement was noticed in its well-being. At 504 days of age the animal was observed

TABLE 6

C–169. Growth data, mineral and silage intakes and blood plasma values

AGE	WT.	HT.	DAILY SILAGE INTAKE				AV. DAILY INTAKE			BLOOD PLASMA		
			Silage	D.M.	D.M. per kilo	Vit. D units	Ca	P	Mg	Ca	P	Mg
days	*lb.*	*cm.*	*lb.*	*lb.*	*gm.*	*U.S.P.*	*gm.*	*gm.*	*gm.*	*mg. per 100 cc.*		
340[1]	364	98					15.7	10.9	6.3	8.0	5.84	2.62
347[2]	360						16.6	11.6	5.6			
350	360		6.7	1.5	4.1	180	18.2	12.1	8.1	9.6	4.22	3.31
360	367		14.5	3.2	8.7	391	18.9	11.8	9.3	10.6	4.70	2.28
370	377		15.0	3.3	8.7	404	19.2	11.9	9.5	10.5	5.25	3.43
380	372	103	15.0	3.3	8.9	404	19.2	11.9	9.5	11.0	5.65	2.90
390	387		15.0	3.3	8.5	404	19.2	11.9	9.5			
400	402		15.0	3.3	8.2	404	19.2	11.9	9.5	11.6	6.25	3.02
410	421	105	15.0	3.3	7.8	404	19.4	11.9	9.5		6.07	
414[3]	447		15.0	3.3	7.4	404	19.4	11.9	9.6	12.3	6.79	2.45

[1] Convulsion at 344 days of age.
[2] Started on silage at 348 days of age.
[3] Slaughtered.

struggling to its feet, breathing hard and with nostrils distended. The following week C–188 was emaciated, unable to rise to its feet and refused all feed and water. The plasma calcium and inorganic phosphorus values at this time had declined to 7.1 and 2.44 mg. The animal lapsed into a comatose condition at 518 days of age and failed to respond appreciably either to intravenous injections of solutions of calcium gluconate, glucose and magnesium sulfate or to a subcutaneous injection of viosterol. Death occurred at 520 days of age. There were patches of scar tissue in the kidneys, the muscle tissue showed considerable edema and there were evidences of muscle injury around the leg joints. The most characteristic autopsy finding was the generalized condition of pitting and erosion of the articular surfaces of the long bones. Illustrations and a detailed description of the histological findings are given elsewhere (11).

Calf C–195. This calf was 366 days of age and had suffered from rickets for more than 60 days when corn silage was added to the rachitogenic ration as the sole source of vitamin D. The animal's legs were stiff and the knees were bowed markedly. The plasma calcium and inorganic phosphorus values were 9.5 and 3.01 mg. per 100 cc. at this time. The appetite for silage was only fair but it gradually improved and by the following month the calf was able to rise to its feet with much less effort. The addition of silage had little effect upon the blood constituents during the first two months but during the next two months, the concentration of calcium returned to normal and the inorganic phosphorus manifested a slow but steady rise. Estrus was first noted at 413 days of age, at which time the bowing of the knees had become less marked. Improvement was rapid after that time. C–195 had recovered from rickets by 543 days of age, was bred and thereafter made normal gains in body weight. The heifer gave birth to a normal 82-pound bull calf at 813 days of age. The data secured from this animal are summarized in Table 7.

DISCUSSION

When calves less than 190 days of age were used as test animals it was impossible to either cure or prevent rickets by supplementing the basal rachitogenic ration with corn silage. A larger percentage of the calves survived until 190 days of age, however, when silage supplemented the basal ration (Table 3). This suggested that silage exerted some antirachitic activity although the amount ingested was insufficient to maintain or to restore health. Higher intakes of silage were precluded by anorexia. Calf C–145 had the best appetite for silage but the average consumption was less than six pounds per day. This amount of silage was equivalent to a daily intake of approximately 5 grams of silage dry-matter per kilo of body weight and was ineffective in curing rickets. The ingestion of three pounds of corn silage per day failed to prevent rickets in young calves.

TABLE 7

C–195. Growth data, mineral and silage intakes and blood plasma values

AGE	WT.	HT.	DAILY SILAGE INTAKE				AV. DAILY INTAKE			BLOOD PLASMA		
			Silage	D.M.	D.M. per kilo	Vit. D units	Ca	P	Mg	Ca	P	Mg
days	*lb.*	*cm.*	*lb.*	*lb.*	*gm.*	*U.S.P.*	*gm.*	*gm.*	*gm.*	*mg. per 100 cc.*		
240	471	110								12.0	6.45	2.68
270	514	112					19.2	13.2	7.6	10.0	7.30	3.15
300	527	115					19.5	13.2	7.8	10.3	5.74	2.53
330	536	116					18.8	13.2	7.5	9.7	4.98	2.52
360	545	117					18.5	13.2	7.4	10.1	4.37	2.60
365[1]	549	....					18.5	13.2	7.4	9.5	3.01	2.50
390	547	118	16.1	4.3	7.9	532	25.2	16.6	13.5	9.7	2.49	2.43
420[2]	532	120	18.5	5.5	10.2	669	24.8	16.1	13.9	9.8	2.99	2.28
450	560	121	17.8	5.3	9.4	643	24.7	15.9	13.7	10.3	3.54	2.35
480	577	122	15.7	4.6	8.0	567	25.1	16.0	13.3	11.1	5.61	2.15
510	620	125	19.5	5.8	9.3	705	25.1	15.6	14.1	10.9	5.56	2.09
540[3]	655	125	23.8	7.4	11.2	903	26.2	15.2	17.4	11.6	5.63	2.50
570	670	123	19.5	6.1	9.0	741	23.8	14.9	16.2	11.6	5.98	2.35
600	705	126	24.2	7.5	10.6	919	26.8	18.0	19.1	11.7	6.03	2.98
630	724	127	24.8	7.7	10.6	941	26.7	15.5	17.9	11.4	5.78	2.89
660	770	126	25.9	7.0	9.1	857	26.1	14.8	17.0	11.8	6.00	2.55
690	823	126	27.2	8.4	10.2	1034	27.4	16.2	18.9	11.3	6.78	2.57
720	842	127	28.9	9.0	10.6	1099	28.0	16.4	19.7	11.7	6.83	2.87
750	873	127	27.2	8.4	9.6	1034	28.6	16.2	19.3	11.0	6.39	2.77
780	911		26.9	8.3	9.1	1020	27.3	16.6	18.8	11.5	5.93	2.59
810	921		25.5	7.9	8.6	971	26.8	15.7	18.2	10.6	5.56	2.62
813[4]										10.0	4.19	2.58

[1] Started on silage at 366 days of age.
[2] Estrus noted at 413, 457, 520 and 543 days of age.
[3] Bred at 543 days of age.
[4] Calved at 813 days of age.

When older calves were used, the clinical manifestations of rickets were alleviated in three out of four cases by the daily ingestion of 15 to 20 pounds of silage (Tables 6–7). Rickets was also prevented in C–167 from six months to three years of age by the ingestion of corn silage (Table 5). The abortion of C–167 was probably not associated with a vitamin D deficiency in view of the results reported by Moore and associates (12). The decrease in the concentration of inorganic phosphorus in the plasma of C–167 and C–195 at the time of parturition and the abortion of C–167 was associated with the physiologic disturbances caused by these acts rather than by a deficiency of the antirachitic factor. It has been shown (9) that such a change in the composition of the blood plasma is not uncommon in dairy cattle at the time of parturition.

In order to compare the results obtained with the various animals it seems convenient to express the intake of corn silage in terms of grams of silage dry-matter per kilo of body weight. On this basis, 7 to 10 gm. of silage dry-matter per day was not only effective in curing and preventing rickets but also supplied a sufficient amount of the antirachitic factor for normal growth and reproduction.

Calf C–188 ingested from 15 to 20 pounds of silage per day which was equivalent to 8 to 12 gm. of silage dry-matter per kilo of body weight, yet failed to recover from rickets. This was the only one of the older calves which failed to respond favorably to silage feeding. This calf is regarded as an atypical case. The inability to cure C–188 is ascribed to the severity of the rachitic condition which had developed before curative measures were begun. Failure to utilize its feed properly, together with its emaciated appearance and autopsy findings in the joints, indicate that the animal was suffering from far-advanced rickets which had given rise to other nutritional disturbances refractory to ordinary vitamin D therapy. Hutyra and Marek (13) state that if rickets is far advanced and has given rise to emphatic nutritional disturbances, death always follows, either through exhaustion or through some complication. The ultimate healing of the process is frequently prevented by ulcers formed in the course of the disease in the articular cartilages.

Bioassays made with rats indicated that 7.5 to 10.0 gm. of dry corn silage contained an equivalent of 2.7 U. S. P. units of vitamin D (Table 1). Assuming that 10.0 gm. of dry silage contained 2.7 U. S. P. rat units, during the period 1931–1934 inclusive, the daily intake of vitamin D units was calculated for each of the experimental calves (Tables 4–7).

The representative tables included in this paper show that the addition of corn silage to the rachitogenic ration appreciably increased the intake of magnesium. In view of the results of a recent investigation (2), it is possible that the presence of magnesium in silage may augment the efficacy of the antirachitic material in corn silage.

SUMMARY AND CONCLUSIONS

1. It was the purpose of this investigation to determine the antirachitic value of corn silage for dairy calves by the use of both curative and preventive feeding trials.

2. Samples of corn silage for the years 1931–1934 contained from 122 to 165 U. S. P. vitamin D units per pound of dry matter.

3. Calves less than 190 days of age were unsuitable test animals for both curative and preventive trials because of anorexia and failure to ingest adequate amounts of silage. In one instance, the feeding of corn silage to a yearling calf failed to cure severe rickets complicated with muscle atrophy and erosion of the articular surfaces.

4. The daily ingestion of an equivalent of 7.0 to 10.0 gm. of dry corn silage per kilo of body weight was effective in curing and preventing rickets in yearling calves and also supplied sufficient antirachitic material for normal growth and reproduction in dairy cows.

5. When corn is cut at the usual stage of maturity for corn silage it possesses definite antirachitic qualities when fed to dairy cows.

The writers are indebted to Mr. C. C. Lightfoot for technical assistance in the determination of the blood values and to Mr. O. B. Winter and Miss Lillian I Butler for the chemical analyses of the feeds.

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Technical Bulletin No. 150 May, 1936

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Of Agriculture and Applied Science

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PATHOLOGY, AND CHEMISTRY

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INTRODUCTION

Recognition of the importance of vitamin D in dairy calf nutrition has resulted in considerable work on the production and prevention of rickets in calves by the Michigan (1-6), the Pennsylvania (7) and the Wisconsin Agricultural Experiment Stations (8). This report primarily concerns results of a study of the pathology of ricketic bones produced in calves at the Michigan Agricultural Experiment Station.

Few histological data are available which deal with rickets in calves. Hutyra and Marek (9) presented a good clinical picture of calf rickets but reported nothing concerning the histological findings, although they included several general anatomical changes which were due to rickets. These investigators also noted that the bone development of a one-year-old child corresponded to the bone development of a calf during fetal life. From this observation, they concluded that calf rickets and late rickets in children were similar. Marek and Wellman (10) reported considerable data upon the histology of rickets in sheep and swine but relatively few data upon calves. The Pennsylvania station (7) presented a brief description of a photomicrograph of the ninth costochondral junction taken from a ricketic calf. No histological study of calf rickets was included in the report from the Wisconsin station (8). Theiler's (11) work in South Africa was concerned with aphosphorosis in heifers between two and three years of age, so that his photomicrographs are not directly applicable to the present study because of the age of his animals. Harris (12) has published extensively on human rickets. Maxwell, Hu and Turnbull (13) have described in detail the histology of a case of fetal rickets in the human being. The histology of rickets in the rat has been studied by Pappenheimer (14) and Dodds and Cameron (15) who have stated that the histological pictures in human and in rat rickets are fundamentally identical. The work of the latter group of investigators has been a helpful guide in the present histological study.

Paucity of fundamental data in the literature concerning the histology of rickets in dairy calves is the justification for reporting the present study in detail.

MATERIALS AND METHODS

More than 100 dairy calves have been used in the experiments on low vitamin D rickets which have made ricketic bones available for this work. From this group, five normal and eleven ricketic calves were selected for the detailed histological study which is presented in this bulletin. The majority of the selected cases were male calves of grade Holstein breeding and varied from 151 to 520 days of age at death. The duration of rickets, as indicated by the blood plasma analyses for calcium and inorganic phosphorus, varied from 38 to 239 days so that this group represents many stages in the severity of the disease.

Early in the investigation it became apparent that the costochondral junction at the ventral end of the rib was the best index to the severity of rickets because it showed to a greater degree the same changes displayed by the humerus, femur, metacarpus and metatarsus. Mid-frontal sections of about 4 mm. in thickness were taken from the last three inches of the ventral end of the left eighth rib of each calf. These sections were studied by the comparison of roentgenograms, photographs of specimens stained in silver nitrate solution, and histological sections.

After considerable preliminary work the following technique was adopted in the preparation of the sections for histological studies: The specimens were fixed in 10 per cent formalin, washed a few hours in running water, hardened in 80 per cent alcohol for at least 24 hours, washed a few hours in water, and decalcified at 37° C. in a 5 per cent aqueous solution of potassium dichromate until the tissues were soft. This procedure usually required from four to six weeks when the decalcifying solution was renewed every week. This method of decalcification, which was far superior to the nitric acid method and also several other decalcifying solutions, resulted in excellent staining differentiation between osteoid and osseous tissues. The paraffin imbedding technique was used almost exclusively. Celloidin imbedding was time-consuming, laborious, and much difficulty was encountered in getting the celloidin sections to lie perfectly flat on the slides. The celloidin sections also stained less permanently. Most of the sections were cut at a thickness of eight microns and were regressively stained in Harris' acidulated hemotoxylin solution, followed by staining for three seconds in a one-half per cent eosin solution. The eosin was made up in a 70 per cent alcohol solution. Each 50 cc. of solution contained five drops of 2 per cent glacial acetic acid solution. Such tissues as liver, kidney and spleen were fixed in Zenker's solution, followed by the paraffin technique and were stained in the hemotoxylin and eosin solutions in the usual manner.

RESULTS

The ages of the calves at death, the duration of the ricketic condition and other data pertaining to the animals used in the study of bone histology are summarized in Table 1. The growth data and the concentrations of calcium, inorganic phosphorus and magnesium of the blood plasma of each individual calf are presented in Tables 2-9, inclusive.

Characteristic Symptoms of Vitamin D Deficiency in Calves

In this investigation the decrease in the concentration of calcium and/or inorganic phosphorus of the blood plasma was among the first signs of low vitamin D rickets. The most conspicuous post mortem findings, however, were those which apparently accompanied the blood changes and which occurred in the bones. Clinically, the skeletal changes included bowing of the forelegs either forward or to the side, swelling of the knee and hock joints, straightening of the pasterns, occasional ring-like swellings on the pasterns, and humping of the back (Fig. 1). Posterior paralysis occurred in cases of fractured vertebrae. Fractured femora sometimes occurred. Other symptoms frequently observed were stiffness of gait, dragging of the rear feet, standing with the rear legs crossed, irritability, tetany, rapid respiration, bloat, anorexia for grain and roughages but not for milk, weakness and inability to stand for any length of time, and finally the retardation or complete cessation of growth in body weight.

The post mortem examinations of the ricketic calves showed that the principal alterations were confined to the skeleton. The liver, kidneys and spleen were negative, but the gall bladders frequently contained as much as 500 cc. of a viscous (sometimes ropy) orange to yellow colored bile. The gall bladders of normal calves contained less than 100 cc. of amber to olive-green colored bile. Bile stained ingesta occurred in the upper part of the small intestine in a few cases. Enteritis was also occasionally seen. Excessive accumulations of synovial fluid in the joints were frequently observed in the more ricketic calves. This material was especially abundant in the larger joints and varied from a thick, viscous fluid to a heavy, jelly-like substance.

The alterations in the skeletons may be more readily understood by first studying the process of osteogenesis as discussed by Maximow and Bloom (16) and by Leriche and Policard (17). An understanding of the origin and importance of the epiphyseal or intermediary cartilage is especially desirable.

The Normal Costochondral Junction

The following detailed discussion of the normal costochondral junction will serve as a working basis in interpreting the histological picture of ricketic bone. This description is based on material from five normal dairy calves which were between 161 and 317 days of age at the time of slaughter.

For convenience of description the ventral-epiphyseal end of the rib, beginning at the union of the rib with the sternum and proceeding vertebrally, was divided into seven zones. The first five of these zones are according to the divisions suggested by Dodds and Cameron (15) for the epiphyseal cartilage (Fig. 3).

1. Zone of Reserve Cells—This area comprised the last several millimeters of the ventral end of the rib and usually terminated in a moderately concave fashion where it joined the second zone. The tissue was typical hyaline cartilage and was traversed in several places by vascular bundles, the diameters of which were sometimes as great as 0.75 mm. (Fig. 3). The number of vascular bundles was greater when the second zone was approached. There were indications, as referred to below, that the blood vessels in the cartilagenous end of the rib anastomosed with the vascular system in the diaphysis.

Cartilage cell lacunae were irregularly scattered, usually only partly filled by the contained cells, and became more numerous as the second zone was approached. They varied from oval or crescent forms to more spherical shapes which characterized the mature cells (Fig. 4). Lacunae adjacent to the larger vascular bundles were frequently flattened with their long axes transverse to those of the bundles. The young, crescent-shaped cells were usually grouped in pairs, except in the ventral-peripheral regions of Zone 1 where as many as eight cells occurred in one group. When arranged in isogenous pairs—and this seemed to be more frequent near the second zone—the crescent shaped cells were situated with their concave surfaces directly apposed to each other. In this part of Zone 1, the interstitial substance between isogenous pairs of cells was sometimes invisible when viewed in one plane, as in a section, and two immature cells appeared to be in one oval-shaped cavity (Fig. 4).

Individual cartilage cells, when not lost or shrunken, conformed in shape to their respective lacunae. The mature resting cell, when it filled its lacuna, averaged about 20 microns in longest diameter. The cytoplasm was reticular, moderately basophilic, and contained several vacuoles. The nucleus, which was basophilic and reticular, was about six microns in diameter and contained several chromatin granules. Cartilage cells were occasionally found which suggested stages in mitosis, while other cartilage cells appeared to be in various stages of degeneration.

2. Zone of Cell Proliferation—Proceeding from Zone 1 toward the diaphysis there were many pairs of isogenous cells arranged predominately at first and later exclusively with their long axes perpendicular to the long axis of the bone. This marked the beginning of Zone 2 (Fig. 3).

Daughter cells, when they originated in this area, appeared to undergo immediate division and gave rise to four cells. This process usually continued and produced 8, 16, or more cells in one isogenous group. For the eighth costochondral junction of the dairy calf, the number of such cells in one group which appeared most frequently, approximated 32 cells—indicating that each parent cell had divided five times. This number was variable, however, and it was difficult to determine accurately when the cells were so closely apposed to each other. They frequently failed even to approximate a multiple of two. This may have been caused by the plane of sectioning, or possibly the group was not fully developed at the time of tissue fixation or that certain cells in such a group divided more actively than others.

Cells in these isogenous groups continued to arrange themselves during proliferation with their long axes perpendicular to the long axis of the bone and piled one above the other so that the long axis of the group was parallel to that of the bone. Thus, a group or column of cells arose from one parent cell. These columns extended across the bone with each column separated from its neighbor by homogeneous interstitial substance. Additional columns arose and arranged in more or less tandem fashion to form a row consisting of groups or columns of cartilage cells. As new groups arose the older ones were seemingly pushed on toward the diaphysis of the bone. Groups within rows were separated by a thick mass of interstitial matrix in contrast to the thin transverse walls between cells within any one group.

Cartilage cells in this zone were approximately of equal size and stage of immaturity. In the axial part of the bone this zone terminated on its vertebral side in a concave manner as it merged into the next zone. This concavity sometimes continued across the entire bone, in which case Zone 2 had a uniform depth, but in most cases this zone became deeper as it extended from the axial area toward the peripheral part of the bone and usually attained maximum depth at 1.5 to 4.0 mm. beneath the perichondrium. Zone 2 averaged about 600 microns in depth in the axial region of the bone and occasionally became as deep as 1,300 microns in the peripheral part of the rib.

3. Zone of Cell Growth—This zone was identified in two ways, first, by the gradation in size of cells, and second, by the gradual thinning of the interstitial matrix (Fig. 3). Growth of the cells in this zone was apparently limited at first to thickening in a plane parallel with the long axis of the bone. Later, when their thickness equaled their width, these cells seemed to enlarge in all directions and ceased growing when they attained an average diameter of more than 30 microns.

Growth resulted in crowding and mutual pressure. The cells assumed multangular shapes, while the matrix between the cells within a group gradually disappeared and the columnar arrangement within the isogenous groups became less perfect. There was lateral expansion of these groups which involved a thinning of the interstitial matrix between the rows.

Mature cells in this zone, except for their larger size and absence of immature forms, were similar to those in Zone 1. In fixed section, in about the last 100 microns as Zone 4 was approached, the cartilage cells occupied a decreasing percentage of the areas in their lacunae. The nuclei appeared shrunken, the cytoplasm increased in density, particularly in the region of the endoplasm where a condensation was sometimes observed, definitely outlining and surrounding the nucleus. Several small vacuoles appeared in the cytoplasm. As these degenerative processes continued, the outline of the nucleus disappeared and finally more than the usual number of empty lacunae became visible. Thus, while growth was the predominating feature of this zone, it was nevertheless superseded in many, but not all, of the cells by what has been called hypertrophic degeneration.

4. Zone of Mature Cells—In the normal rib, the most characteristic feature of this zone was calcification of the interstitial matrix. This calcified matrix was later removed in its entirety. For this reason, this is sometimes called the zone of temporary or provisional calcification. This zone followed the outline of the previous area and showed a continuation of the same degenerative changes which had not gone to completion even in this zone (Figs. 3, 7, and 9).

In proceeding vertebrally through this zone, coarse granules of a basophilic substance were observed in the interstitial matrix. As these granules increased in number, they produced a stippled effect. Later they became obscured as they increased in number and became more closely packed. This basophilic substance marked the location of inorganic salts. The salts were deposited in all of the longitudinal walls between the rows of cells and between the groups of cells within the rows, but never between the cells within the isogenous groups because here the interstitial matrix appeared to be absent. This point is intentionally emphasized because of some debate over it in the literature. Calcification was often more complete in the margins than in the axial areas of the longitudinal walls of cartilage matrix (Fig. 9).

This zone averaged about 100 microns in depth. In about half the cases studied, it was much deeper just beneath the perichondrium where the cartilage rows were prolonged. Here calcification, in exceptional cases, extended for 1,000 microns between the rows, but the density of mineral deposition was less and the process was usually not continuous from the vertebral margin of Zone 4. The reason for this extended distribution of calcified matrix is unknown. Possibly it adds strength to the rib in the region where it occurs.

5. Zone of Cartilage Removal—Zone 4 was bordered on its vertebral margin by a series of sac-like structures which formed the zone of cartilage removal (Fig. 7). Zone 5 was 50-75 microns deep. The sac-like structures were variable in size and extended in the direction of the long axis of the bone. They were formed by the union of two or more cartilage cell lacunae following the destruction of the cell capsules by the embryonic marrow. The marrow elements were slower in destroying the calcified substance, so that marrow could be seen advancing from the diaphysis toward the ventral end of the rib in the form of narrow tongues between the walls of calcified interstitial substance which acted as guides in the process. The sac-like structures were bordered, therefore, on either side by calcified matrix, while their vertebral ends were exposed to embryonic marrow. They may be partially filled by degenerating cartilage cells and by marrow elements (Figs. 7 and 8).

6. Zone of Ossification—Exclusive of Zone 1, which varied in depth depending upon the manner of dissecting the rib from the sternum, the ventral-epiphyseal end of the rib averaged about 1,350 microns (with variations from 1,150 to 1,450 microns) in depth in the axial region of the bone and about 2,100 microns (with variations from 1,150 to 4,350 microns) in the peripheral region. As previously mentioned, vascular bundles extended from Zone 1 through the remainder of the epiphysis and appeared to anastomose with the vascular system in the diaphysis.

Zone 6 constituted the beginning of the diaphysis. The zone usually had a convex outline on its ventral margin (Fig. 11). Embryonic marrow was observed advancing from the diaphysis toward the ventral end of the bone. In advance of this, erythrocytes, fibrin and sometimes serous material, and occasional endothelial cells were frequently observed, closely followed by large numbers of embryonic connective tissue cells (Fig. 7). Degenerating cartilage cells frequently appeared to be revived at this point, because of a new source of nutritive material from the diaphysis.

Calcified tissues resisted removal by the marrow. About 50 microns vertebral to Zone 5, cells were occasionally seen similar in size to the primitive wandering cells of the embryonic connective tissue lining the persisting bars of calcified matrix (Figs. 7 and 13). These were osteoblasts, the cells associated with the production of acidophilic layers of osteoid tissue occasionally observed on these trabeculae. Osteoblasts averaged about 16 microns in diameter and were mononuclear. The nucleus measured about seven microns in diameter and contained one or more large nucleoli eccentrically placed near a pale attraction sphere. The cytoplasm was reticular, intensely basic, and contained small vacuoles. The calcified bars of matrix appeared to serve at least three purposes. They strengthened the area in which they occurred, served as guides for the advancing marrow in cartilage removal, and formed bases for the deposition of osteoid tissue. Most of these bars persisted into the diaphysis to about 700 microns vertebral from Zone 5 and then many of them were gradually removed by osteoclasts. Osteoclasts appeared to function specifically in the removal of calcified cartilage and osseous tissue, and in this way aided in the advancement of the embryonic marrow and at the same time assisted in the internal reconstruction of bone. Osteoclasts were most noticeable on the vertebral ends of persisting bars of calcified matrix where they caused terminal erosion of the bars (Figs. 5, 15, and 16). These cells, however, were also observed about as far ventrally in the rib as were osteoblasts. Osteoclasts were variable in size and contained from two to 25 or more nuclei. Each nucleus was about five microns in diameter and contained several coarse granules. The cytoplasm was vacuolated, took a less basic stain than that of the osteoblast, and frequently showed pseudopodal projections. The means by which osteoclasts erode calcified matrix and bone have not been included in this study. Whether the process of erosion is one of osteolysis or of phagocytosis is a debated question.

Persisting trabeculae of calcified tissue eventually lost their calcified cartilage cores by chondrolysis, and the remaining trabeculae of bone became thicker further in the diaphysis by peripheral additions of osteoid tissue. The presence of narrow margins of osteoid tissue at any one time indicate early ossification in the normal rib. The line of demarcation between osseous and osteoid tissues was a gradual one marked by an increase in the number of basophilic granules so that the stippled effect gradually became more dense in passing from osteoid to osseous tissue. These granules sometimes appeared to be deposited in striations (Fig. 5). Osteoid tissue was always accompanied by osteoblasts but the converse was not always true. Frequently many osteoblasts, instead of a single layer, were seen bordering trabeculae, and whole clumps of these cells occurred in crotch-like areas where two trabeculae had united (Fig. 14). Individual osteoblasts were commonly noticed in the process of being surrounded by osteoid tissue (Fig. 13). After incorporation in the bone these osteoblasts gradually shrank and assumed the flattened character of osteocytes (bone cells). In stained preparations the osseous material in the trabeculae exhibited various shades of basophilia. This fact has suggested the possibility that different degrees of calcification were represented in the bone.

Decrease in the number of persisting trabeculae was accompanied by larger marrow spaces between the trabeculae. At about 15 mm. vertebral from Zone 5 the embryonic marrow had disappeared and hemopoietic foci, along with vacuoles (probably of fatty origin) appeared and eventually completely masked the loose connective tissue further in the diaphysis. Hemocytoblasts, erythroblasts, and neutrophil myelocytes constituted the majority of the cells. Many small foci of lymphoid cells were scattered in the red marrow. Here osteoblasts and osteoid tissue, as well as osteoclasts, were limited largely to the peripheral regions of the diaphysis on trabeculae closely beneath the periosteum.

7. Zone of Compact Substance—Two questions are considered under this zone which has been called, for want of a better name, the zone of compact substance. First, what accounts for the narrowing of the rib bone in passing from the ventral-epiphyseal end toward the vertebral end? And second, what sort of covering does the rib possess to separate it from surrounding tissues?

The cartilagenous end of the rib was covered by a moderately dense connective tissue (the perichondrium) which varied from 150 to 500 microns in thickness. At the junction of Zones 1 and 2 the perichondrium usually extended as a less dense connective tissue inward to a distance sometimes as great as 800 microns. When viewed in one plane, as in a section, it gave the impression of a cone with the apex directed toward the center of the bone (Fig. 11). Blood vessels frequently covered the crest as well as the lateral borders of this area. The perichondrium extended over the zones of cell proliferation and cell growth as a denser connective tissue of 200-300 microns in thickness. Beginning with Zone 5 it continued as the periosteum. Rarely, a narrow strip within the perichondrium over Zone 4 appeared to be calcified. At 1.5 mm. vertebral from Zone 5 the periosteum thickened by extending as a looser connective tissue inward between the persisting trabeculae to a distance of 1.5-2.0 mm. and then gradually decreased to 150 microns or less at 25 mm. vertebral from Zone 5 where it only extended in between a few of the peripheral trabeculae. This decrease in thickness of the periosteum occurred at a point of increasing size of peripheral trabeculae, which later grew thicker and assumed the character of a more compact material than the trabeculae in the marrow cavity.

Gross measurements of mid-frontal sections indicate that the rib was widest somewhere near the junction of Zones 5 and 6 where it varied from 20 to 30 mm. in width in normal calves of the ages under consideration. Through a process of narrowing, sometimes referred to as tubulation, the rib gradually decreased to a minimum width of 4-8 mm. at about 50 mm. vertebral from Zone 5. This tubulation occurred by terminal erosion of the persisting trabeculae of bone which occur in the extreme peripheral areas and which extend parallel to the long axis of the bone and therefore join the periosteum at an oblique angle. Evidence of this erosion was that one or more osteoclasts were observed adjacent to the vertebral ends of these trabeculae (Fig. 6). This erosion, which in all probability weakened the bone, was compensated by the extension of the periosteum into the interior of the bone in the manner described above.

The Costochondral Junction in Rickets

This description is based on material from 11 calves which were suffering from vitamin D deficiency and which were, except for one animal which died from complications of rickets at 520 days, between

151 and 330 days of age at the time of slaughter. The duration of the ricketic condition varied from 38 to 239 days. The cases are about equally divided between (a) low blood plasma calcium rickets and (b) low blood plasma calcium-low inorganic phosphorus rickets. No cases of low plasma inorganic phosphorus rickets, uncomplicated by low calcium, are included in this study.

The alterations which were observed in the ricketic bones will be considered according to the zones in the normal rib. The histological sections will be arranged in sequence of events as they occurred during the development of the ricketic rib from the normal rib.

1. Zone of Resting Cartilage—This zone usually terminated in a moderately concave manner on its vertebral side similar to that in the normal rib. In prolonged rickets it varied to the extent of meeting Zone 2 in a convex manner. In the cases of prolonged rickets there was an increase in the number of oval-shaped cavities apparently formed by the union of two lacunae, irregularly arranged adjacent to Zone 2 and similar to those noted in the normal bone (Figs. 17 and 18). This alteration was most pronounced in severe rickets when it involved as much as the last two mm. adjacent to Zone 2. In rickets of shorter duration, these enlarged cavities appeared only in isolated patches adjacent to Zone 2. In such areas the matrix between isogenous pairs of cells was reduced to a thin network. The mature cartilage cells were fewer in number and the impression was obtained that the decrease in the rate of growth had resulted in the accumulation of immature cartilage cells.

2. Zone of Cell Proliferation—At the junction with Zone 1, the outline of this area at first became moderately undulated. As the severity of rickets increased, this general outline became more convex on its ventral margin in the axial region of the bone, whereas in prolonged disease it even extended into the resting cartilage in the axial part of the rib in a wedge-shape manner. The apex of the wedge was sometimes rather broad, while the sides extended out from the base toward the peripheral part of the bone where they either terminated in the usual manner or curved ventrally for some distance just beneath the perichondrium. In addition to the altered outline, Zone 2 revealed an irregular decrease in depth in rickets and was as shallow as 100 microns in certain places while of normal dimensions in others. There was some evidence that the average number of cells per isogenous group was reduced in rickets (Fig. 17).

3. Zone of Cell Growth—This area followed the outline of the previous zone in the same bone. It was unaffected in depth, except in rare instances when it was partially obliterated in places on its vertebral side by sinusoidal cavities containing embryonic marrow (Fig. 17).

4. Zone of Mature Cells—Whereas the changes thus far noted in the cartilagenous end of the rib were not always conspicuous, especially in mild rickets of short duration, the alterations in Zone 4 were more pronounced and were therefore more consistently observed. These changes may be followed with considerable satisfaction when the sequence of events is traced in the costochondral junctions of the ricketic ribs when they are empirically arranged in order of increasing severity of rickets.

Retarded provisional calcification was the alteration first observed (Fig. 9). Here, Zone 4 was of normal depth but the area of provisional calcification extended irregularly into the matrix, from the vertebral side of this zone, and averaged less than 50 microns in depth, while in places it measured zero when it failed to extend ventrally beyond the fifth zone. As the severity of rickets increased, only occasional longitudinal walls of cartilage matrix between the rows of mature cells were calcified. In more advanced rickets calcification was entirely absent in this area and frequently only involved a relatively small amount of the matrix in Zone 5 adjacent to Zone 4.

Lack of calcification in Zone 4 was soon followed by lengthening of the rows of mature cartilage cells. At first, cartilage tongues, each consisting of a group of cell rows and their intervening matrix, projected vertebrally for a short distance into Zone 5 and gave an undulating outline of the vertebral margin of Zone 4 (Fig. 10). Lengthening of the rows became more generalized in more advanced rickets and this zone became deeper across the entire width of the bone (Fig. 19). The number of mature cells in rows was greater in prolonged rickets when this zone reached a depth as great as 800 microns in the axial region of the bone and as great as six mm. in the more peripheral part of the bone (Fig. 20). The matrix between these lengthened columns of cells, while homogeneous in appearance and free of granules of calcified substance, tended toward a deeper basic stain than that in Zone 3. The mature cartilage cells in this zone in prolonged rickets averaged less than 20 microns in diameter. This decrease in cell size was probably a result of pressure, exaggerated by dystrophic conditions arising from the increase in size of the vegetative cartilage mass. Several stages of degeneration were observed and cells were dislodged from many lacunae. Cartilage cell rejuvenation, however, was common adjacent to Zone 5 (Fig. 22).

Zone 4 followed on its ventral side the outline of Zone 3. On its vertebral margin two factors helped to produce an extremely irregular outline at the junction of Zones 4 and 5. One of these was the crowding of the vertebral ends of the longitudinal rows of cells which caused whole tongues of cartilage to bend so that the rows of cells in these tongues no longer extended parallel to the long axis of the bone. This buckling of cell rows was favored by the lack of provisional calcification (Figs. 21 and 22). The second factor, which operated to produce an irregular outline at the junction of Zones 4 and 5, was the uneven advancement of the embryonic marrow from the diaphysis (Fig. 12).

Zones 2, 3, and 4 in the ricketic bone may contribute as much as two mm. in the axial area, and as much as seven mm. in the peripheral region, to the length of the rib. This thickened cartilage at the ventral end of the rib was traversed, as in the normal bone, by vascular bundles which appeared to anastomose with vessels in the diaphysis.

5. Zone of Cartilage Removal—This zone probably showed more pronounced alterations than any other part of the bone in rickets (Figs. 21 and 23). In early rickets, cartilage removal continued at a relatively more rapid rate than provisional calcification so that cartilage removal, while occurring in the usual manner, advanced ventrally to a position abreast with, but never ventral to, the line of provisional calcification. Apparently the embryonic marrow does not normally remove cartilage when the matrix between the cells is uncalcified, which accounted for the accumulation of mature cartilage cells in Zone 4.

In more severe rickets there was a failure of calcification in Zone 4 and also in most of the matrix in Zone 5. The cessation of normal cartilage removal halted growth-involving ossification. Such cessation in growth, however, was in some way compensated, as illustrated by height-at-withers measurements of severely ricketic calves which continued to gain in height although at a reduced rate, even when body weight was stationary or decreasing. The rib shared in this skeletal growth in rickets. At least partial compensation for lack of calcification occurred as follows: In rickets of long duration the rows of mature cartilage cells lengthened appreciably, which sometimes amounted to as much as six mm., and a new method of cartilage removal was apparent. The embryonic marrow which advanced ventrally from the diaphysis was still the active agent of cartilage removal, but instead of progressing evenly in narrow tongues, it was unguided by calcified walls of matrix and advanced unevenly and in a more or less oblique manner in broad tongues into Zone 4. This advancement destroyed wide areas of cartilage, including cells and matrix—both of which appeared to be destroyed with equal ease and rapidity (Figs. 12 and 20). Extremely large and irregularly shaped sac-like structures occurred adjacent to Zone 4. These contained in addition to the elements seen in normal sac-like areas, numerous erythroblasts and hemocytoblasts enmeshed in loose connective tissue (Figs. 9, 12, and 20). Osteoblasts were observed in advance of osteoclasts, generally, although the reverse sometimes occurred (Fig. 9). The cartilage matrix which partially surrounded the sac-like areas assumed an acidophilic reaction.

The marrow invariably advanced further ventrally, sometimes by as much as four mm., in the axial region of the bone (Fig. 12). The great unevenness of the marrow advancement gave the appearance of what has been referred to as "irregular thickening of the cartilage" and was occasionally exaggerated by actual splitting of the vertebral part of Zone 5 (Fig. 21). Irregular advancement of marrow led in some places to sac-like areas of embryonic bone marrow which appeared, in one plane as in a section, to be isolated in the zone of mature cells (Fig. 20), and in other places it led to isolated masses of cartilage in the diaphysis.

Just vertebral to the zone of sac-like areas, there occurred patches of connective tissue in which the central parts had taken a deeper acidophilic stain identical with that of osteoid tissue (Figs. 23 and 24). Imbedded in such tissue were one or several cells that resembled osteoblasts, suggesting that certain of the local hemocytoblasts had differentiated into osteoblasts which had, in turn, elaborated osteoid tissue. These new patches of osteoid tissue served as bases for additional osteoblast activity. Occasionally, isolated masses of cartilage with acidophilic matrix appeared in which the cells had not only been rejuvenated but had also been transformed to osteoblast-like cells, suggesting the possibility that cartilage can be changed *en masse* into osteoid tissue. Isogenous pairs of cells were observed in the larger masses of cartilage along with degenerating cartilage cells. The net result of all of this cell activity was a zone consisting largely of osteoid tissue. Because of the manner in which the osteoblasts appeared to develop, the characteristic arrangement of these cells bordering on trabeculae, as revealed in normal ribs, was inconspicuous in spite of the large amount of osteoid tissue that was present (Fig. 23). The osteoblasts in this region of the bone have been called "Osteoidoblasts" by some workers on the basis that these cells produce tissue which does not ossify and therefore, the cells were regarded as degenerate forms of osteoblasts. Such a distinction is unjustified in this study because these cells appeared to be identical in their adult form with the osteoblasts in normal ribs. It does not seem illogical, therefore, to ascribe the failure of ossification in rickets to any one of several causes —such as a deficiency in vitamin D—rather than to an inherent defect in the osteoblasts. Osteoclasts were few in number in this region. They seemed to have no specific function in this area but appeared because of their proximity to the diaphysis.

The osteoid tissue varied considerably in depth in any one bone because of irregular marrow advancement, but it measured as much as six mm. in depth in more prolonged rickets. In milder rickets the normal and abnormal types of cartilage removal were observed in the same bone (Fig. 19).

The vertebral outline of Zone 5 was strongly concave where it merged into Zone 6. This transition in zones was a gradual one in which basophilic granules appeared in isolated areas and surrounded individual cells in osteoid tissue. Such cells were conspicuous because of the characteristic halo about each one (Fig. 23). The halo became narrower with increase in the number of granules and increased ossification nearer the diaphysis. There also appeared small trabeculae of bone, surrounded on all sides by broad layers of osteoid tissue. Osteoblasts soon bordered these trabeculae in the usual manner as Zone 6 was approached.

6. Zone of Ossification—Reorganization of material between the patches of osteoid tissue occurred soon after the first signs of ossification were observed, and it was here that Zone 6 began (Fig. 23). Proceeding vertebrally, the spaces between trabeculae enlarge, and the embryonic marrow was soon replaced by loose connective tissue. Several millimeters vertebral to Zone 5, hemopoietic elements and vacuoles began to mask the loose connective tissue in the marrow spaces. Neutrophil myelocytes were as numerous here as in the normal red marrow.

In the extreme ventral area of Zone 6 the trabeculae were short and stubby and were bordered by broad osteoid tissue margins. They also revealed frequent transverse anastomoses (Fig. 20). More vertebrally in the diaphysis these trabeculae assumed a more normal shape and distribution as the osteoid tissue margins gradually became narrower. In the red marrow region, trabeculae continued to show abnormally broad margins of osteoid tissue, which sometimes appeared to be more sharply demarcated from osseous tissue in rickets than in normal bone. Chondrolysis was an unimportant growth feature here because the appearance of calcified cartilage cores was inversely proportional to the severity of rickets.

7. Zone of Compact Substance—At the junction of Zones 1 and 2 the perichondrium was thickened and sometimes extended inward as far as 2.5 mm. It continued in a moderately thickened form over Zones 2, 3, and 4 and in this location trabeculae of bone, mediary between the external and internal surfaces, were usually seen. These trabeculae appeared to have the same origin as those in the vertebral part of Zone 5. The reorganization of the material between trabeculae occurred in a similar manner and resulted in the formation of marrow spaces which continued further vertebrally as a part of the marrow cavity. The perichondrium continued as periosteum of about normal thickness.

Tubulation appeared to occur in the normal manner. The beading or enlargement of the ventral end of the rib, which was commonly observed in gross at the time of slaughter, was an inconspicuous feature of the bone when studied with the microscope. Gross measurements of mid-frontal sections showed that the diameter of ricketic ribs varied from 20 to 35 mm. whereas the range of normal bones was from 20 to 30 mm. The average diameter was about six mm. greater in the ricketic bone. Microscopic observations showed that the greatest diameter was through the area of osteoid tissue in Zone 5, in the region just vertebral to the cartilagenous end of the rib. An excess of osteoid tissue appeared to be the cause of this increase in size of the rib end. This enlargement was more apparent than real, however, because the curvature of the medial border of the ventral end of the rib was greater in rickets Figs. 25, 26, 27, and 28). The curvature of the lateral border increased in the same direction. This change in curvature was greatest at about 20 mm. vertebral to Zone 1 and could be the result of a weakened rib responding to normal physiological demands such as respiration and/or support of the diaphragm. This inward curving of the ventral end of the rib exaggerated any enlargement that did occur, especially when viewed from the medial border of the bone.

The Costochondral Junction in Healing Rickets

Calf C-224 was the only animal included in this phase of the study. This calf developed low plasma calcium rickets at 110 days of age. The daily amount of whole milk fed was increased at a later date, and at 140 days of age the plasma calcium began to increase and was nearly normal when the animal was killed at 163 days of age (Table 6).

This case is of interest because it appeared to resemble the epiphyseal type of healing rickets usually observed in the rat. This was best illustrated in the roentgenogram which showed at about six mm. vertebral to the cartilagenous end of the bone, a narrow region extending half way across the bone from the medial side and which also showed a lack of ossification (Fig. 26). Histologically, a more or less normal costochondral junction was observed except in the narrow uncalcified area in Zone 6, where the trabeculae were small and scattered in an area consisting mostly of connective tissue.

Roentgenographic Appearance of Normal and Ricketic Ribs

Roentgenograms of normal ribs showed a sharp line of demarcation at the junction of the diaphysis with the cartilagenous end of the bone (Figs. 25, 26, and 28). The diaphysis usually ended in a predominately convex manner where it joined the cartilage. Trabeculae of osseous tissue were conspicuous and indicated a distribution of inorganic salts grossly identical with that described above in the histological study of the normal rib. The ventral-epiphyseal end of the rib curved in a medial direction. The walls of the diaphysis became thicker in proceeding vertebrally from the costochondral junction.

Osteoid tissue has the same homogeneous appearance as cartilage in the roentgenogram so that certain definite abnormalities were visible in rickets. The line of junction of cartilage and diaphysis was irregular and indefinite, and consisted of an area, varying in depth, in which incomplete calcification of tissues had occurred. The so-called "cupping," or development of a concave outline of the ventral end of the diaphysis, was not a characteristic feature in the ricketic calf rib in the specimens studied. In many cases, the end of the diaphysis became more convex in rickets, due to the advancement of embryonic marrow in the axial region of the bone.

Other than an increase in medial curvature of the rib, the roentgenographic evidences of rickets in the specimens studied were confined largely to the area of excess osteoid tissue which separated the ventral end of the diaphysis from the cartilagenous end of the bone. The importance of this localization of ricketic changes is discussed below.

Growth As a Modifying Factor in Rickets

The effect of growth upon rickets is illustrated when calves C-237 and C-238 are compared (Tables 8 and 9, Figs. 19 and 20). These animals received about the same amount of vitamin D in the form of whole milk, and both developed low plasma calcium rickets by 90 days of age. These calves were slaughtered at about the same age. The rate of gain in body weight was much slower for C-237. Histological studies show very definitely that the ricketic alterations in the rib were much less extensive in the slower growing calf.

A comparison of calves C-170 and C-232 may be used to illustrate the influence of age in rickets (Tables 5 and 7, Figs. 10 and 29). C-232 developed low plasma calcium rickets by 100 days of age, exhibited low inorganic phosphorus values at 140 days, and was slaughtered at 161 days of age. C-170 developed low plasma calcium rickets by 170 days of age, the inorganic phosphorus fluctuated considerably at this time but was not definitely subnormal until after 280 days of age. This animal was slaughtered at 330 days of age. Both calves gained subnormally in body weight. Keeping in mind the age difference and the fact that the older calf had rickets for 160 days, while the younger calf had about the same severity of rickets for only 61 days, as judged by the blood plasma analyses, it is significant that in roentgenographical as well as in histological studies, the younger calf (C-232) manifested the more extensive ricketic alterations, although it suffered from the disease for a much shorter time.

Complications in Rickets

Calf C-188 (Table 5) developed rickets at 380 days of age and in addition to the usual symptoms the animal showed muscle atrophy and extreme emaciation. C-188 died at 520 days of age, following a comatose condition and failure to respond to subcutaneous injections of viosterol and intravenous injections of calcium gluconate and magnesium sulphate solutions. It lost more than a pound of body weight daily during the last 100 days of life.

On post mortem examination, the marked pitting and erosion of the articulating surfaces were most conspicuous, particularly at the distal end of the femur and the proximal end of the humerus (Fig. 33). This condition existed in the peripheral as well as the central parts of the articular areas. Where the cartilage surface was not destroyed, it was very loose and easily removed by the fingers. Bone of a porous-plate appearance was exposed. Spongy pads of protein-like material covered these areas. Roentgenograms of thin sections of bone removed from such areas showed an irregular rarefaction of the bone beneath the eroded or pitted surfaces (Fig. 32). Histological studies indicate that fibrous connective tissue occupied these rarefied, calcium-free areas.

In the ventral end of the rib a few faint indications of arrested-growth lines, marking areas of increased numbers of transverse trabeculae, were observed in the roentgenogram vertebral to the cartilagenous end of the rib (Fig. 25). Histologically, provisional calcification was somewhat retarded and incomplete. A shallow area of calcium-free osteoid tissue was vertebral to Zone 4. Following this area were short trabeculae of bone with broad margins of osteoid tissue and more than the normal number of transverse anastomoses. The lack of extensive changes in this ricketic rib was attributed partly to the age of the animal, and partly to the severity of the disease and its halting effect upon growth.

Type of Rickets and Histological Alterations

When observed in prepared sections, the histological alterations in the rib appear to be identical in low plasma calcium rickets and in rickets associated with low calcium and low inorganic phosphorus.

DISCUSSION

Rickets in dairy calves suffering from a deficiency of vitamin D was characterized by changes in the bones (Figs. 1-34), which were apparently accompanied by decreased concentrations of the plasma calcium and/or inorganic phosphorus (Tables 2-9). Other changes, more inconsistently found, were the accumulation of bile in the gall bladders and the presence of bile stained ingesta in the upper region of the small intestine. The costochondral junction at the ventral end of the rib was selected as the best and most convenient index to the degree of rickets in calves (Figs. 1 and 12).

When compared with the normal cases, the ricketic costochondral junctions were characterized fundamentally by retarded and incomplete provisional calcification of the cartilage matrix, which in turn caused temporary failure of cartilage removal by the embryonic marrow (Figs. 9 and 10). Mature cartilage cells then accumulated up to a point where a ricketic type of cartilage removal was initiated by the embryonic marrow. It was this latter process which caused the most conspicuous alterations in the rib (Figs. 12, 19, 20, 21, and 29).

If the theory is accepted that retarded provisional calcification is the fundamental change in the bone in rickets—and there is every reason to believe that such is the case in rickets caused by a vitamin D deficiency—then the histological alterations in the ricketic bones may be explained in what appears to be a very satisfactory manner. Thus, in the absence of the longitudinal trabeculae of calcified cartilage matrix to act as guides, the embryonic marrow in the ricketic type of cartilage removal advanced irregularly and obliquely in broad tongues which caused an uneven decrease in depth of the zone of mature cells (Fig. 12). The irregular manner of marrow advancement led to sac-like areas which appeared, in one plane as in a section, to be isolated in the zone of mature cells. In other instances it resulted in what appeared to be isolated masses of cartilage vertebral to the zone of mature cells. Vertebrally, in the region adjacent to the large sac-like areas, were seen patches of connective tissue which occupied most of that part of the rib (Fig. 23). In these patches hemocytoblasts appeared to change to osteoblasts which produced areas of osteoid tissue in the patches of fibrous connective tissue (Fig. 24). Eventually most of this zone was occupied by osteoid tissue, the accumulation of which was probably of a compensatory nature in the absence of bone formation. Thus, in reality cartilage removal in rickets was fundamentally the same as that in the normal bone, but a different appearance was produced because of the lack of provisional calcification. Changes were less extensive further vertebrally in the diaphysis as the bone approached a more normal appearance.

The beading or swelling commonly observed in the ventral ends of ribs from ricketic calves was not a conspicuous change when viewed in the microscope. There was, however, a moderate swelling of the ventral end of the bone, the origin of which appeared to be due to the accumulation of osteoid tissue. This enlargement was exaggerated by the increased curvature of the medial border of the rib (Figs. 25, 26, and 28).

Rickets affected the growing ends of bones (Figs. 25, 26, and 28). The alterations were less severe in the slower growing calves (Figs. 19 and 20) and also in the older animals when the rate of growth had decreased (Figs. 10 and 29). Under the conditions of this study growth occurred at a more rapid rate in the ventral end of the rib than in the limb bones. This is also indicated in the new born calf which is up on its feet within a few hours after birth, which indicates that at birth the leg bones are relatively more mature than the ribs. The localization of the ricketic changes at the end of the rib is a fundamental point of some importance. In the first place, it is additional evidence that rickets occurred only where there was growth, and secondly, it has a practical application in making ash determinations on ricketic bones. Obviously, even though there may be a large excess of osteoid tissue at the costochondral junction it will not be truly reflected in the ash analysis of the entire bone since the ricketic changes are confined to a very small part of an otherwise well ossified rib.

Fragmentary evidence indicates that the epiphyseal type of healing rickets occurred in one calf in this study (Fig. 26).

With the exception of the blood plasma and possibly the lymph, the changes which occurred in the soft tissues of the body were nonspecific and were probably the result of general impairment of body health. In one case of advanced rickets, however, muscle atrophy and erosion of the articular surfaces were observed in addition to the usual symptoms of rickets in calves. Pitting and erosion of the joints was seen in several of the ricketic calves in the present investigation but never to such a marked degree as in the one calf described above (Figs. 33 and 34). Hutyra and Marek (9) included ulcerous destruction of the joint cartilages along with inflammation of the joints in their list of general anatomical changes of rickets in cattle. They also noted that severe rickets was accompanied by increasing emaciation and that prognosis depended upon nervous disturbances and complications.

Gullickson and co-workers (18) did not make histological studies of the rickets-like disease which they encountered in calves. Their general observations, however, regarding gain in body weight, composition of the blood plasma and skeletal alterations, including erosion of the articulating surfaces, are fundamentally in agreement with the findings of our work on rickets in dairy calves.

The two types of low vitamin D rickets observed in this study appear to be histologically identical. Perhaps one should not expect to find histological differences in bones from cases of low blood calcium rickets and cases in which the concentrations of both calcium and inorganic phosphorus are low.

A comparison of the results which were obtained in this investigation with the reports in the literature indicates that the alterations in ricketic bone are fundamentally the same in the calf as those described for the young rat and for the infant. This similarity in response to a deficiency of the antiricketic factor demonstrates the suitability of the dairy calf for problems of fundamental research in the nutrition of vitamin D.

SUMMARY

This investigation was designed to determine the histological alterations that occur in the tissues of dairy calves suffering from a deficiency of vitamin D. The study was based on 16 grade Holstein calves which were selected from a group of more than 100 animals available for this work. The ages of the calves varied from 151 to 520 days at the time of death. Five of these calves were normal, while the remaining 11 calves represented several stages in the severity of rickets. The duration of the disease varied from 38 to 212 days. One case of healing rickets was included in the study and also one case of rickets in which muscle atrophy and erosion of the joint cartilage had occurred.

Low vitamin D rickets in dairy calves was characterized by changes in the bones which were apparently accompanied by decreased concentrations of calcium and/or inorganic phosphorus in the blood plasma. The costochondral junction at the ventral end of the rib was found to be the best index to ricketic changes in the skeleton. Mid-frontal sections of about four mm. in thickness were taken from the last three inches of the ventral end of each rib and studied by the comparison of roentgenograms, photographs of specimens stained in silver nitrate solution, and histological sections.

Roentgenograms were superior to the specimens stained in silver nitrate for this study. Sections were prepared for histological study by decalcification in 5 per cent aqueous solution of potassium dichromate followed by the paraffin imbedding technique and were regressively stained in hematoxylin and eosin solutions.

Detailed descriptions were made of the costochondral junctions of both normal and ricketic ribs. In the specimens studied, the histological changes were largely confined to a relatively small portion of the bone at the costochondral junction.

Retarded provisional calcification of the cartilage matrix appeared to be the fundamental change in rickets. The most conspicuous changes in the microscopical study, however, were the irregular removal of cartilage by the embryonic marrow and the accumulation of excess osteoid tissue. The beading of ricketic ribs appeared to be caused by the accumulation of osteoid tissue and was exaggerated in appearance on the medial border by increased curvature of the rib in a medial direction. "Cupping" of the ventral-epihyseal end of the diaphysis was not a prominent feature in roentgenograms of the costochondral junctions of ricketic calves used in this study.

Growth was an important modifying factor in rickets. More severe rickets was associated with more rapid growth. Within a given period of time, younger calves developed more florid rickets than did older calves.

Low plasma calcium rickets appeared to be histologically identical to low calcium-low inorganic phosphorus rickets.

Fragmentary evidence suggested that the epiphyseal type of healing rickets occurs in dairy calves. Emaciation of the body and erosion of the articular cartilages occurred as complications in one case of rickets.

Histological alterations of rickets in calves, as observed in this investigation, appear to be fundamentally the same as those recorded in the literature for the infant and for the young rat.

Abnormal accumulations of bile of an orange to yellow color and of viscous character were observed in several cases of rickets at post mortem. Bile stained ingesta occasionally occurred in the upper part of the small intestine. Enteritis was noted in a few cases.

CONCLUSIONS

In the specimens studied, the following conclusions seem to be justified:

1. At autopsy, low vitamin D rickets in dairy calves is characterized principally by changes in the bones. These changes may be conveniently studied in the ventral end of the eighth rib.
2. Retarded provisional calcification of cartilage matrix appears to be the fundamental change in rickets. The most conspicuous histological changes in the disease, however, are irregular removal of cartilage and accumulation of excess osteoid tissue.
3. Accumulations of osteoid tissue are responsible for the beading of ricketic ribs. This beading is typically exaggerated in gross appearance on the medial border of the rib by increased curvature of the rib in a medial direction.
4. Growth is an important modifying factor in rickets. The ricketic condition is confined largely to the growing area at the end of the bone. More severe rickets occurs in more rapidly growing dairy calves. Age, because of its relation to growth, is also a modifying factor in that younger calves develop more florid rickets than older calves within a given period of time when maintained under similar conditions.
5. Histological alterations of rickets in calves are fundamentally the same as those recorded in the literature for the infant and for the young rat.

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Table 1. Calves selected for studies on bone histology.

Calf No.	Age at death (days)	Duration of rickets (days)	Remarks
C-151	194	Normal	Exposed to summer sun.
C-176	317	"	Received 5 cc. cod liver oil and 15 gm. of MgO daily.
C-229	163	"	Received 4 lb. of irradiated milk daily.
C-231	161	"	Received 4 lb. of irradiated milk daily.
C-233	161	"	Received 4 lb. of irradiated milk daily.
C-148	232	212	Ate 1 lb. sun-cured hay daily. Low P—low Ca rickets.
C-155	195	145	Ate 1.5 lb. sun-cured hay daily. Low Ca rickets.
C-170	326	160	Received 5 cc. cod liver oil daily. Low Ca—low P rickets.
C-171	327	185	Rubbed 2 cc. 250D viosterol daily upon the skin. Low Ca—low P rickets. Unusually poor gains in body weight.
C-173	319	239	Received 5 cc. cod liver oil daily. Low P—low Ca rickets.
C-175	318	38	Received 5 cc. cod liver oil and 32 gm. $MgCO_3$ daily. Low Ca rickets.
C-188	520	140	Received 15 lb. corn silage daily. Low Ca—low P rickets.
C-224	163	53	Received 20 lb. whole milk daily. Healing last 23 days from low Ca rickets.
C-232	161	61	Received 20 lb. skimmilk daily. Low Ca—low P rickets.
C-237	151	61	Received 24 lb. whole milk daily. Low Ca rickets. Poor gains in body weight.
C-238	161	71	Received 20 lb. whole milk daily. Low Ca rickets.

Table 2. Growth data and blood plasma analyses.

Calf No.	Age	Weight		Height		Blood plasma		
						Ca	Inorg. P	Mg
	(days)	(lb.)	(per cent normal)	(cm.)	(per cent normal)	(mg. per 100 cc.)		
C-151(1)	10	85	85	...	...	...	...	...
	20	95	86	...	...	12.4	6.69	2.37
	30	105	87	76.8	101	12.3	6.10	2.30
	40	114	86	...	...	...	...	...
	50	122	84	...	...	...	...	...
	60	122	78	81.2	100	13.5	6.91	2.09
	70	126	74	...	...	13.6	7.53	2.11
	80	143	77	...	...	...	...	...
	90	163	82	85.2	99	13.6	8.06	2.34
	100	177	82	...	...	14.1	7.40	2.78
	110	193	83	...	...	15.4	8.23	2.75
	120	214	86	92.5	102	...	...	...
	130	231	87	...	...	...	...	...
	140	246	87	...	...	14.1	7.53	3.47
	150	253	84	97.8	102	12.8	8.39	2.33
	160	255	80	...	...	...	...	...
	170	253	76	...	...	13.0	6.01	2.45
	180	257	74	99.0	99	12.6	8.12	2.40
	190	276	76	...	...	12.8	7.96	2.57
	194(2)	288	78	...	...	13.8	7.44	2.58
C-176(3)	10	98	98	...	...	...	...	...
	20	108	98	...	...	13.8	8.87	2.40
	30	110	91	77.7	101	15.0	8.83	2.47
	40	121	91	...	...	...	...	...
	50	131	90	...	...	13.9	7.62	2.36
	60	142	90	81.7	100	13.3	6.79	2.07
	70	158	92	...	...	...	...	...
	80	176	95	...	...	13.9	7.14	1.98
	90	177	89	87.2	101	...	...	...
	100	202	93	...	...	12.9	6.72	2.37
	110	215	92	...	...	13.8	8.62	1.63
	120	233	94	91.3	99	...	...	...
	130	237	89	...	...	...	8.33	2.91
	140	230	81	...	...	9.6	5.17	1.56
	150	228	75	94.3	96	10.3	5.48	2.17
	160	250	79	...	...	...	...	...
	170	262	79	...	...	11.4	6.69	2.74
	180	277	79	95.5	94	10.6	7.58	2.80
	190	296	82	...	...	...	...	...
	200	311	83	...	...	11.2	7.71	2.74
	210	328	84	99.8	95	11.8	6.22	2.62
	220	345	86	...	...	11.5	7.35	3.31
	230	367	89	...	...	...	...	...
	240	373	88	101.2	94	10.8	6.28	2.60
	250	399	91	...	...	9.9	6.38	3.06
	260	416	92	...	...	...	...	...
	270	433	93	...	...	10.9	6.87	2.44
	280	440	92	107.8	98	10.7	6.69	3.68
	290	434	89	...	...	10.5	6.22	2.67
	300	453	90	...	...	...	...	...
	310	459	90	110.2	99	11.2	6.79	3.52
	317(2)	470	91	...	...	10.8	6.41	3.18

(1) Born March 4, 1932—male.
(2) Slaughtered.
(3) Born May 29, 1932—male.

Table 3. Growth data and blood plasma analyses.

Calf No.	Age	Weight		Height		Blood plasma		
						Ca	Inorg. P	Mg
	(days)	(lb.)	(per cent normal)	(cm.)	(per cent normal)	(mg. per 100 cc.)		
C-229(1)	10	80	80	....	....	....	....	....
	20	92	83	71.2	95	12.9	8.74	2.25
	30	100	83	....	....	13.8	8.56	2.21
	40	110	83	75.0	96	13.4	7.91	3.23
	50	129	89	....	....	12.9	8.39	2.55
	60	148	94	....	....	12.2	8.12	2.49
	70	165	96	81.8	98	12.3	7.58	2.72
	80	179	96	....	....	12.0	7.09	2.59
	90	197	99	....	....	12.3	6.41	1.90
	100	214	99	....	....	13.2	7.77	2.23
	110	233	100	86.7	96	12.1	7.27	2.32
	120	246	99	....	....	11.8	8.01	2.26
	130	256	96	....	....	12.1	9.29	2.41
	140	268	94	88.0	93	8.7	7.58	2.62
	150	284	94	....	....	12.1	9.24	2.55
	160	304	96	....	....	11.7	9.69	2.25
	163(2)	312	97	....	....	11.8	9.19	2.95
C-231(3)	10	97	97	74.5	101	12.6	6.19	2.33
	20	106	96	....	....	....	....	....
	30	116	96	78.2	102	11.7	6.98	2.83
	40	126	95	....	....	12.6	8.01	3.17
	50	144	99	....	....	12.3	8.22	2.52
	60	163	104	84.8	103	11.5	7.86	3.17
	70	181	106	....	....	11.6	8.25	2.77
	80	192	103	....	....	9.7	8.33	2.49
	90	202	101	....	....	12.1	7.86	2.07
	100	210	97	88.8	100	11.8	7.74	2.45
	110	228	98	....	....	10.7	7.02	2.38
	120	239	96	....	....	12.3	8.39	2.90
	130	236	89	94.2	101	11.4	8.74	2.43
	140	237	83	....	....	12.7	7.94	2.58
	150	249	82	....	....	11.5	8.68	2.32
	160	264	83	95.6	98	11.7	9.92	2.91
	161(2)	265	83	....	....	10.9	8.06	3.18
C-233(4)	10	104	104	73.5	100	13.4	7.53	2.69
	20	114	103	....	....	11.8	8.33	2.75
	30	123	102	77.7	101	....	....	....
	40	136	102	....	....	13.4	8.28	2.25
	50	146	101	....	....	12.7	8.56	2.61
	60	162	103	81.0	99	12.7	7.78	2.63
	70	174	102	....	....	11.9	8.01	2.67
	80	192	103	....	....	11.7	7.40	2.41
	90	206	103	....	....	12.1	7.77	2.27
	100	224	104	86.2	97	12.0	7.23	2.45
	110	245	105	....	....	12.3	7.03	2.48
	120	252	101	....	....	12.7	8.50	2.91
	130	278	104	92.8	99	12.9	6.31	2.63
	140	309	109	....	....	12.4	8.01	2.75
	150	337	112	....	....	11.6	8.23	2.17
	160	354	111	97.3	99	11.8	8.53	2.32
	161(5)	....	....	....	....	....	....	....

(1) Born October 16, 1933—male.
(2) Slaughtered.
(3) Born October 25, 1933—male.
(4) Born October 28, 1933—male.
(5) Slaughtered. Pitting of articulating surfaces of femur and humerus.

Table 4. Growth data and blood plasma analyses.

Calf No.	Age	Weight		Height		Blood plasma		
						Ca	Inorg. P	Mg
	(days)	(lb.)	(per cent normal)	(cm.)	(per cent normal)	(mg. per 100 cc.)		
148[1]	10	91	91	....	....	....	....	....
	20	98	89	....	....	12.5	5.61	2.40
	30	110	91	77.5	101	11.9	4.46	2.23
	40	118	89	....	....	11.3	5.84	....
	50	129	89	....	....	11.4	6.22	2.63
	60	144	92	83.5	102	10.1	5.43	2.15
	70	154	90	....	....	....	....	....
	80	166	89	....	....	10.0	5.04	2.28
	90	171	86	87.0	100	11.7	5.32	2.67
	100	171	79	....	....	11.8	4.09	3.31
	110	169	73	....	....	....	....	....
	120	168	67	87.7	96	12.2	3.95	3.02
	130	165	62	....	....	11.8	2.76	2.85
	140	165	58	....	....	....	....	....
	150	165	55	85.8	89	12.0	3.12	2.66
	160[2]	167	53	....	....	10.4	2.58	1.73
	170	172	52	....	....	9.7	3.93	2.80
	180	170	49	84.5	84	....	....	....
	190	171	47	....	....	9.9	2.96	2.23
	200[3]	175	47	....	....	9.6	3.23	2.15
	210	180	46	Down	....	....	....	....
	220[4]	190	47	....	....	12.3	5.00	2.12
	230	187	45	....	....	8.5	4.05	1.22
	232[5]	....	....	....	....	7.3	4.13	0.99
155[6]	10	88	88	....	....	13.7	6.83	2.29
	20	102	92	77.5	103	13.5	8.23	2.69
	30	115	95	....	....	12.7	7.27	2.80
	40	123	92	....	....	....	....	....
	50	134	92	83.7	105	10.9	6.28	2.31
	60	143	91	....	....	....	....	....
	70	149	87	....	....	9.3	7.53	2.13
	80	157	85	87.8	103	9.9	7.62	2.24
	90	166	83	....	....	9.3	8.33	2.69
	100	171	79	....	....	....	....	....
	110	177	76	90.0	100	9.8	7.53	2.05
	120	200	80	....	....	9.7	6.54	2.47
	130[7]	204	77	....	....	....	....	....
	140	199	70	91.8	97	9.3	7.31	2.19
	150	203	67	....	....	8.7	6.32	1.88
	160	206	65	....	....	....	....	....
	170	213	64	93.7	94	8.1	5.81	2.07
	180	217	62	....	....	8.4	5.27	1.77
	190	221	61	....	....	8.9	8.50	1.67
	195[8]	223	60	....	....	7.9	7.91	1.66

(1) Born March 1, 1932—male.
(2) Age 156 days. Legs bowed. Joints swollen.
(3) Age 197 days. Unable to walk to scales.
(4) Age 216 days. Unable to stand long enough to drink milk.
(5) Convulsion. Slaughtered.
(6) Born March 10, 1932—male.
(7) Age 134 days. Light convulsion.
(8) Slaughtered.

Table 5. Growth data and blood plasma analyses.

Calf No.	Age (days)	Weight (lb.)	Weight (per cent normal)	Height (cm.)	Height (per cent normal)	Blood plasma Ca (mg. per 100 cc.)	Blood plasma Inorg. P (mg. per 100 cc.)	Blood plasma Mg (mg. per 100 cc.)
C-170(1)	10	97	97	....	....	11.6	6.69	2.12
	20	96	87	....	....	....	....	....
	30	103	85	76.2	100	....	5.81	1.99
	40	111	83	....	....	12.6	7.02	2.14
	50	118	81	....	....	13.6	5.74	2.62
	60	128	82	80.2	98	....	....	....
	70	140	82	....	....	13.5	7.76	2.74
	80	146	79	....	....	14.1	6.65	2.88
	90	153	77	84.0	97	....	....	....
	100	161	74	....	....	13.2	6.38	2.45
	110	178	77	....	....	11.9	7.35	2.51
	120	189	76	88.2	96	....	....	....
	130	194	73	....	....	12.2	5.95	2.69
	140	198	70	....	....	10.6	4.90	1.90
	150	208	68	90.7	94	....	5.17	2.63
	160	220	69	....	....	....	....	....
	170	230	69	....	....	9.6	5.10	2.08
	180	243	70	....	....	8.7	5.58	2.13
	190	269	74	92.7	91	....	....	....
	200	286	76	....	....	8.7	6.54	2.57
	210	301	77	....	....	10.5	6.69	2.93
	220	326	81	98.2	94	9.5	7.53	2.75
	230	345	84	....	....	....	....	....
	240	355	84	....	....	8.8	6.13	2.60
	250	377	86	102.7	95	9.3	5.32	2.69
	260	396	88	....	....	....	....	....
	270	411	88	....	....	8.1	6.41	2.62
	280	429	90	103.7	95	7.3	5.71	2.58
	290	448	92	....	....	8.3	5.12	2.33
	300(2)	462	92	....	....	....	....	....
	310	463	91	107.7	96	8.0	5.34	2.78
	320(3)	461	89	....	....	9.4	4.73	2.59
	326(4)	473	89	....	....	....	....	....
C-188(5)	360	500	90	109.8	96	10.8	8.12	3.10
	370(6)	525	93	....	....	9.5	8.62	3.55
	380	524	92	112.8	98	8.3	9.19	2.56
	390(7)	523	91	....	....	10.3	5.93	2.26
	400	549	94	....	....	8.6	6.62	2.90
	410	520	88	114.8	98	8.2	8.28	2.61
	420(8)	538	90	....	....	8.3	6.95	2.58
	430	540	90	....	....	7.4	7.25	2.60
	440(9)	532	88	....	....	10.3	5.08	2.25
	450	520	85	114.2	96	7.8	4.63	2.83
	460	529	85	....	....	8.2	5.17	2.67
	470	521	82	....	....	7.9	5.63	2.45
	480	509	79	113.8	95	8.2	4.37	2.83
	490(10)	496	76	....	....	8.5	4.19	2.59
	500	466	71	....	....	8.6	3.29	2.49
	510	436	66	....	....	8.0	2.96	2.49
	520(11)	406	61	....	....	8.1	3.50	2.37

(1) Born May 1, 1932—male.
(2) Getting stiff in legs. Back arched.
(3) Very stiff in legs.
(4) Slaughtered.
(5) Born November 14, 1932—male.
(6) Joints enlarged. Rear legs stiff.
(7) Knees bowed.
(8) Stands cross legged in rear. Drags rear feet.
(9) Very stiff. Lies down most of time.
(10) Age 487 days. In coma.
(11) Died.

Table 6. Growth data and blood plasma analyses.

Calf No.	Age	Weight		Height		Blood plasma		
						Ca	Inorg. P	Mg
	(days)	(lb.)	(per cent normal)	(cm.)	(per cent normal)	(mg. per 100 cc.)		
C-171(1)	10	84	84	....	....	12.0	6.16	2.24
	20	87	79	....	....	....	....	....
	30	97	80	78.0	103	12.9	5.93	1.91
	40	110	83	....	....	12.2	6.95	1.94
	50	123	85	....	....	11.9	6.91	2.40
	60	133	85	80.3	99	....	....	....
	70	143	83	....	....	12.5	6.25	2.63
	80	151	81	....	....	13.4	6.83	3.10
	90	155	78	83.8	97	....	....	....
	100	158	73	....	....	13.1	6.22	2.38
	110	161	69	....	....	12.5	7.76	2.33
	120	168	67	85.2	95	12.5	6.25	2.58
	130	175	66	....	....	....	....	....
	140	181	64	....	....	11.2	5.00	2.13
	150	179	59	87.8	92	....	4.84	2.29
	160	178	56	....	....	....	....	....
	170	181	54	....	....	9.0	4.22	1.96
	180	179	51	....	....	9.0	3.50	1.61
	190	186	51	88.3	87	8.0	2.81	2.29
	200	198	53	....	....	....	....	....
	210	210	54	....	....	8.6	4.47	2.36
	220	215	54	89.2	85	9.1	7.02	2.72
	230	219	53	....	....	....	....	....
	240	225	53	....	....	9.9	4.63	2.53
	250	230	52	90.5	84	8.0	6.25	2.51
	260	241	53	....	....	7.5	5.68	2.60
	270(2)	257	55	....	....	....	....	....
	280	264	55	91.7	84	6.4	6.48	2.82
	290	269	55	....	....	7.3	4.58	2.17
	300	280	56	....	....	....	....	....
	310(3)	284	56	95.0	85	8.6	4.08	2.80
	320	283	54	....	....	8.6	3.81	3.02
	327(4)	283	54	....	....	....	....	....
C-224(5)	10	93	93	....	....	....	....	....
	20	106	96	76.8	102	12.5	8.45	2.27
	30	117	97	....	....	13.0	7.91	2.75
	40	127	95	....	....	12.5	6.98	2.15
	50	143	99	79.0	98	12.1	6.98	2.65
	60	162	103	....	....	12.0	6.41	2.39
	70	177	103	....	....	12.1	6.32	2.96
	80	192	103	83 5	98	11.4	6.10	2.62
	90	213	107	....	....	11.1	7.00	2.37
	100	229	106	....	....	10.0	7.06	2.56
	110	244	105	94.5	105	7.6	7.79	2.16
	120	264	106	....	....	8.0	6.76	2.15
	130	283	106	....	....	8.5	7.18	2.24
	140	298	105	96.5	102	11.1	10.24	2.37
	150	312	103	....	....	7.4	8.23	2.51
	160	329	104	....	....	9.8	7.95	2.34
	163(6)	342	107	....	....	9.6	7.02	2.97

(1) Born May 3, 1932—female.
(2) Age 275 days. Light convulsions.
(3) Age 315 days. Legs stiff. Difficult to rise to feet.
(4) Bloat probable cause of death.
(5) Born October 14, 1933—male.
(6) Slaughtered.

Table 7. Growth data and blood plasma analyses.

Calf No.	Age	Weight		Height		Blood plasma		
						Ca	Inorg. P	Mg
	(days)	(lb.)	(per cent normal)	(cm.)	(per cent normal)	(mg. per 100 cc.)		
C-173(1)	10	92	92	...	...	14.3	6.87	2.32
	20	102	92	74.8	100	14.3	6.69	2.19
	30	108	89	...	...	...	...	...
	40	109	82	...	...	13.9	5.10	3.43
	50	115	79	78.0	97	13.8	4.55	2.09
	60	117	75	...	...	14.8	7.81	2.53
	70	117	68	...	...	...	...	...
	80	123	66	81.0	95	12.8	4.46	3.02
	90	122	61	...	...	12.5	4.36	1.64
	100	129	60	...	...	...	...	...
	110	140	60	81.8	91	12.0	5.87	1.54
	120	153	61	...	...	12.9	5.56	2.34
	130	161	60	...	...	12.8	4.43	2.05
	140	176	62	84.5	89	...	5.90	2.86
	150	187	62	...	...	...	...	...
	160	200	63	...	...	10.3	6.04	1.76
	170	221	66	...	...	...	...	...
	180	239	68	89.3	89	9.9	6.32	1.78
	190	250	69	...	...	9.3	5.84	2.28
	200	262	70	...	...	10.2	5.53	1.80
	210	265	68	93.3	90	...	...	...
	220(2)	290	76	...	...	11.3	6.07	2.72
	230	316	77	...	...	11.2	5.63	2.74
	240	324	76	98.5	92	...	...	...
	250	336	77	...	...	10.8	6.01	2.88
	260(3)	342	76	...	...	10.4	4.60	2.10
	270	346	74	101.5	93	10.1	5.81	2.45
	280	355	74	...	...	...	...	...
	290	347	71	...	...	9.7	5.33	2.33
	300(4)	351	70	104.2	94	10.9	4.28	3.47
	310	342	67	...	...	9.0	5.17	2.43
	319(5)	323	62	...	...	9.0	3.90	2.93
C-232(6)	10	100	100	76.3	104	13.3	8.01	2.58
	20	109	98	...	...	12.8	8.74	2.90
	30	120	99	79.0	103	13.1	8.56	2.60
	40	134	101	...	...	12.9	8.39	2.46
	50	148	102	...	...	11.8	8.87	2.63
	60	158	101	85.7	105	12.0	7.94	3.25
	70	162	95	...	...	9.9	7.23	2.16
	80	174	94	...	...	10.0	7.49	2.07
	90	183	92	...	...	9.4	7.19	1.88
	100	201	93	91.3	103	7.7	6.91	2.97
	110	229	98	...	...	7.7	6.18	2.71
	120	231	93	...	...	8.0	6.65	3.16
	130	245	92	94.0	101	8.5	5.99	2.77
	140(7)	259	91	...	...	8.0	5.63	2.57
	150	276	91	...	...	8.0	5.79	2.25
	160	275	87	96.3	98	7.9	4.96	2.54
	161(8)	...	...	...	...	6.0	4.98	2.53

(1) Born May 8, 1932—male.
(2) Stiff in front legs.
(3) Knees bowed. Legs very stiff.
(4) Stiff in legs. Barely able to rise to feet.
(5) Slaughtered.
(6) Born October 28, 1933—male.
(7) Legs bowed.
(8) Slaughtered. Slight pitting of articular surface on ball of femur.

Table 8. Growth data and blood plasma analyses.

Calf No.	Age	Weight		Height		Blood plasma		
						Ca	Inorg. P	Mg
	(days)	(lb.)	(per cent normal)	(cm.)	(per cent normal)	(mg. per 100 cc.)		
C-175(1)	10	100	100	....	....	....	....	....
	20	110	99	....	....	14.0	7.49	2.74
	30	114	94	77.5	101	....	....	....
	40	132	99	....	....	14.0	7.23	2.38
	50	147	101	....	....	15.0	7.49	3.71
	60	159	101	84.3	103	....	....	....
	70	176	103	....	....	14.4	6.79	1.94
	80	193	104	....	....	13.3	8.01	2.16
	90	209	105	88.8	102	12.6	7.58	2.33
	100	240	111	....	....	12.7	5.95	2.36
	110	244	105	....	....	13.0	7.62	2.22
	120	266	107	93.2	101	....	....	....
	130	271	102	....	....	....	6.91	2.72
	140	292	103	....	....	11.4	7.62	1.98
	150	310	103	....	....	....	....	....
	160	329	104	97.5	100	11.3	7.27	1.61
	170	342	103	....	....	11.5	7.62	2.69
	180	352	101	....	....	12.2	7.35	2.59
	190	380	105	102.2	100	....	....	....
	200	409	109	....	....	12.1	8.06	3.10
	210	422	108	....	....	12.7	7.06	3.02
	220	439	109	105.5	100	....	....	....
	230	461	112	....	....	11.5	7.81	2.88
	240	468	110	....	....	9.8	8.74	2.75
	250	479	109	109.5	102	11.0	7.81	2.60
	260	505	112	....	....	....	....	....
	270	516	111	....	....	10.2	8.74	2.55
	280	535	112	114.5	104	9.6	8.28	3.31
	290	537	110	....	....	....	....	....
	300	553	108	....	....	9.5	9.13	2.91
	310	555	109	....	....	8.8	9.77	3.24
	318(2)	565	109	....	....	9.4	8.50	3.31
C-237(3)	10	102	102	....	....	....	....	....
	20	122	110	76.7	102	12.2	8.56	2.25
	30	134	111	....	....	12.4	6.38	2.80
	40	142	107	....	....	13.0	6.83	2.72
	50	155	107	81.3	101	13.5	6.58	2.33
	60	165	105	....	....	11.5	6.69	2.20
	70	181	106	....	....	11.7	6.21	2.07
	80(4)	186	100	....	....	10.1	7.62	1.70
	90	190	95	89.8	103	6.4	7.66	1.99
	100	197	91	....	....	8.5	6.76	1.75
	110	202	87	....	....	7.2	7.67	1.65
	120	214	86	90.3	98	8.8	8.06	2.45
	130	235	88	....	....	8.8	8.68	2.27
	140(5)	244	86	....	....	6.7	8.06	1.83
	150(6)	238	79	94.7	98	6.5	7.45	1.84
	151(7)	....	....	....	....	....	....	....

(1) Born May 28, 1932—male.
(2) Slaughtered.
(3) Born November 10, 1933—male.
(4) Convulsion at 82 days of age and very irritable at 83 days of age.
(5) Convulsions at 139, 140 and 143 days of age.
(6) Convulsion. Unable to stand on rear legs.
(7) Slaughtered. Fractured vertebra.

Table 9. Growth data and blood plasma analyses.

Calf No.	Age	Weight		Height		Blood plasma		
						Ca	Inorg. P	Mg
	(days)	(lb.)	(per cent normal)	(cm.)	(per cent normal)	(mg. per 100 cc.)		
C-238(1)	10	113	113	....	....	....	....	....
	20	109	98	78.0	104	....	....	....
	30	122	101	....	....	13.3	8.47	2.61
	40	130	98	....	....	12.6	7.62	2.47
	50	145	100	84.0	105	12.5	6.92	2.45
	60	160	102	....	....	12.0	6.13	2.37
	70	184	107	....	....	12.7	6.89	2.22
	80	205	110	....	....	11.6	7.49	3.11
	90	229	115	93.2	107	9.3	6.90	2.17
	100	253	117	....	....	9.5	7.02	2.12
	110	269	116	....	....	8.5	6.79	2.20
	120	290	116	96.7	105	8.7	7.16	2.16
	130	314	118	....	....	8.6	6.35	2.24
	140	336	118	....	....	8.2	5.64	2.23
	150	353	117	103.3	107	8.1	6.22	2.47
	160	370	116	....	....	8.0	5.92	2.47
	161(2)	....	....	....	....	....	....	....

(1) Born November 10, 1933—male.
(2) Slaughtered.

Fig. 1. Calf C-148. Severe rickets. Shows emaciation, humping of back, swelling of joints, knuckling of pasterns, and bowing of legs. This calf was unable to walk and was only able to stand long enough to be photographed.

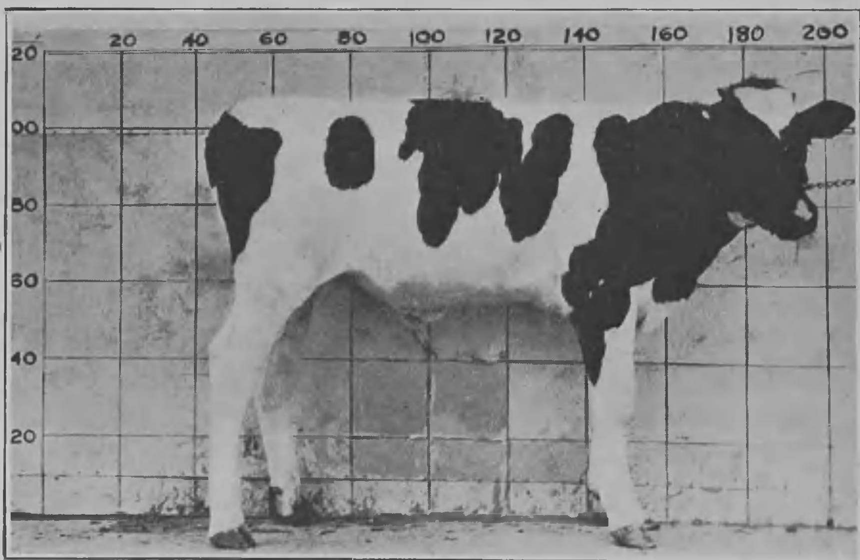

Fig. 2. Calf C-238. This calf received a basal ricketogenic ration supplemented with natural whole milk. Shows absence of definite clinical symptoms of rickets. Note, however, the severe ricketic changes in the rib as shown in Figs. 12, 20, and 26. Note also the blood analyses in Table 9. This case is a good illustration of the extensive ricketic alterations which may be produced in the rib before the leg bones show clinical evidence of the disease.

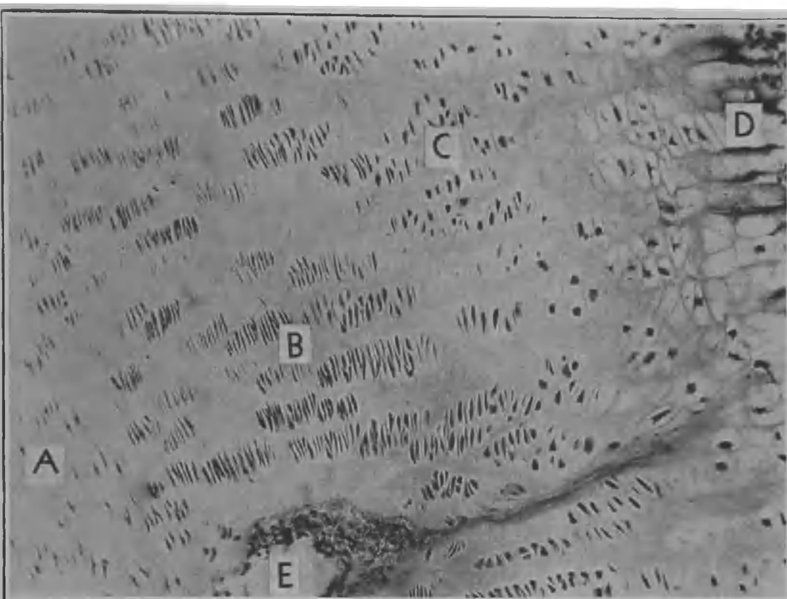

Fig. 3. Calf C-231. Normal. Shows: resting cartilage, A; cell proliferation, B; cell growth, C; mature cartilage cells with calcified matrix between the rows of cells, D; vascular bundle, E. (This and all succeeding photomicrographs illustrate mid-frontal sections through the left eighth costochondral junction. The diaphysis is to the reader's right, except in Fig. 14 where it is to the bottom of the page). 120X.

Fig. 4. Area A in Fig. 3. Shows: mature cartilage cell, A; isogenous pair of immature cartilage cells, B; cartilage matrix, C. 460X.

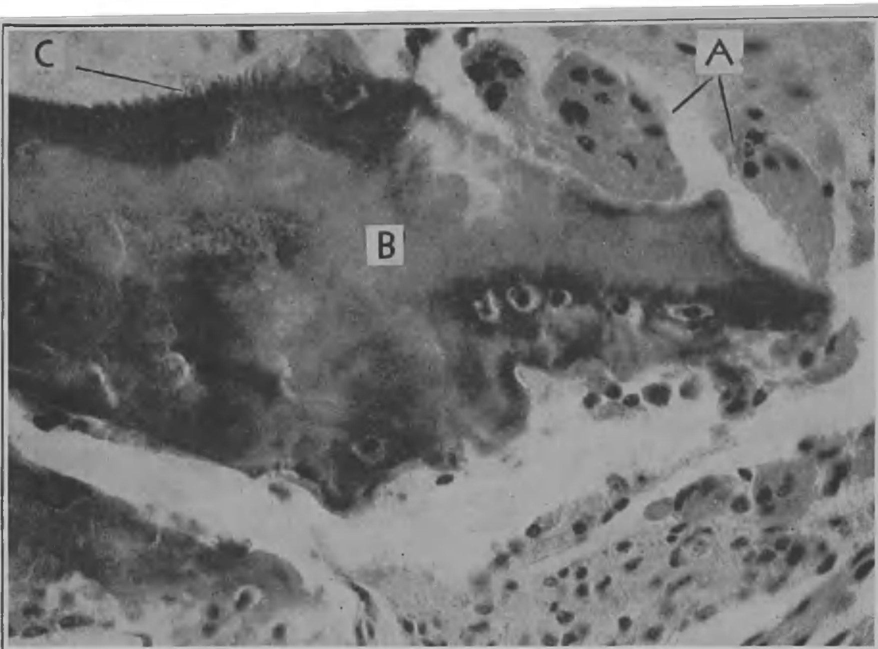

Fig. 5. Calf C-233. Normal. Shows: osteoclasts, A; core of calcified cartilage matrix, B; deposition of granules of inorganic salts in striations, C. 460X.

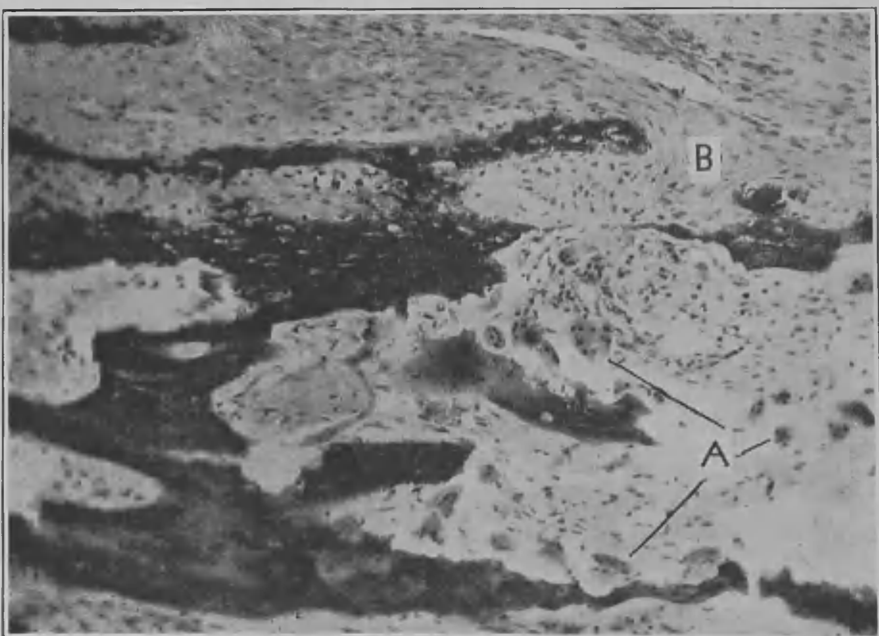

Fig. 6. From normal specimen. Shows: osteoclasts, A; periosteum, B. Note large numbers of osteoclasts here in the region where the trabeculae meet the periosteum. 120X.

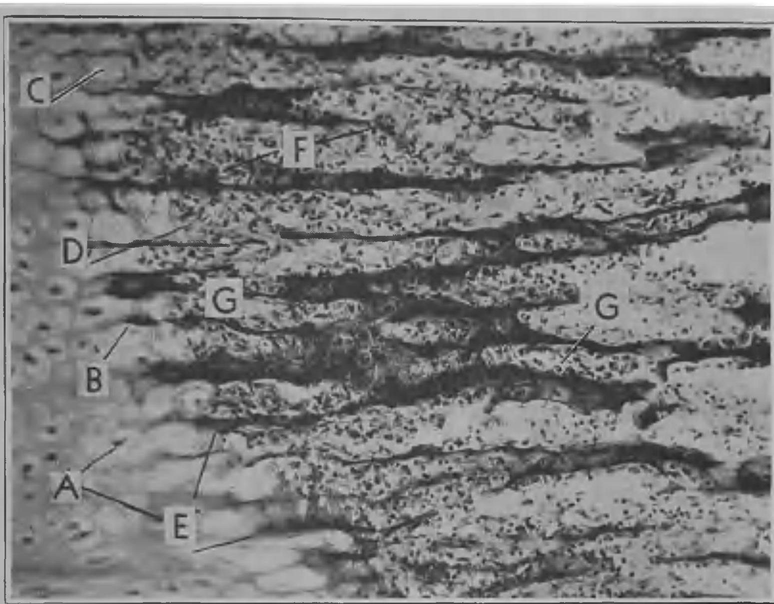

Fig. 7. Calf C-231. Normal advancement of embryonic marrow. Shows: mature cartilage cells, A; calcified matrix between mature and degenerating cartilage cells, B; coagulated blood elements in small sac-like areas in zone of cartilage removal, C; tongue of embryonic marrow, D; trabeculae of calcified tissue, E; osteoclasts, F; osteoblasts, G. 120X.

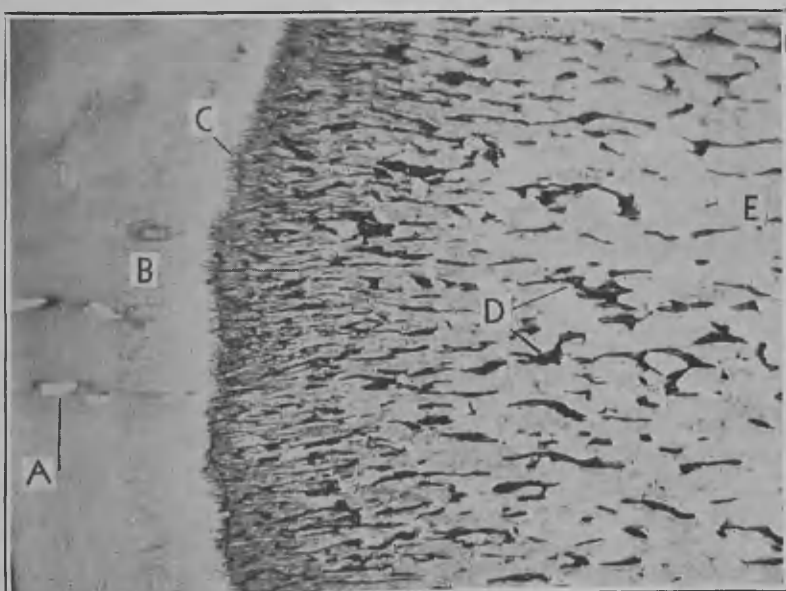

Fig. 8. Calf C-231. Normal. Shows: vascular bundles in resting cartilage, A; area of proliferation and cell rows, B; calcification at junction of cartilage with the diaphysis, C; trabeculae of calcified substance, D; marrow, E. Note the regular and even manner in which the diaphysis joins with the cartilagenous end of the bone. 30X.

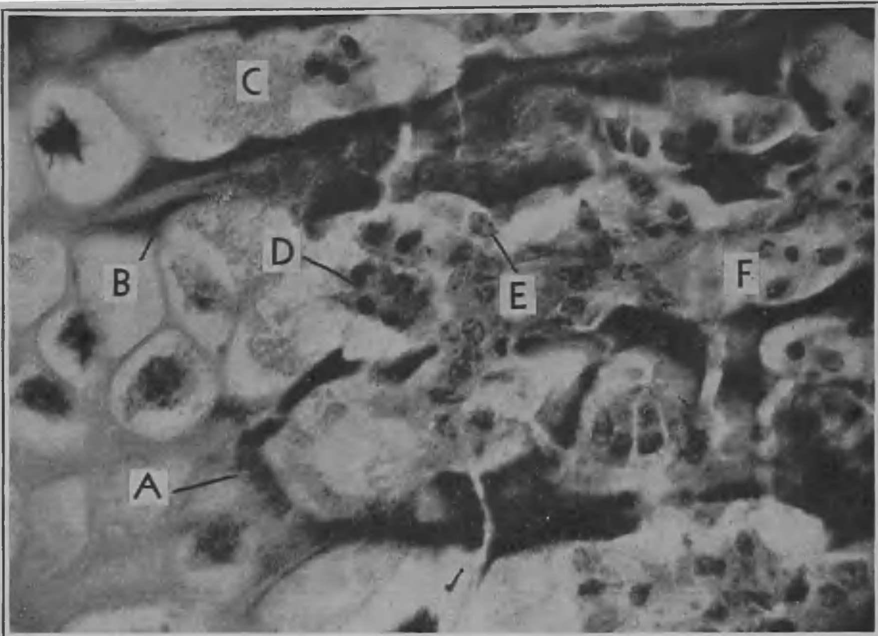

Fig. 9. Calf C-175. Mild rickets. Shows: retarded and irregular provisional calcification of cartilage matrix, A; heavier deposits of granules of inorganic salts in periphery of longitudinal bar of cartilage matrix, B; coagulated blood elements in sac-like structure, C; osteoclast, D; osteoblast, E; embryonic marrow, F. 460X.

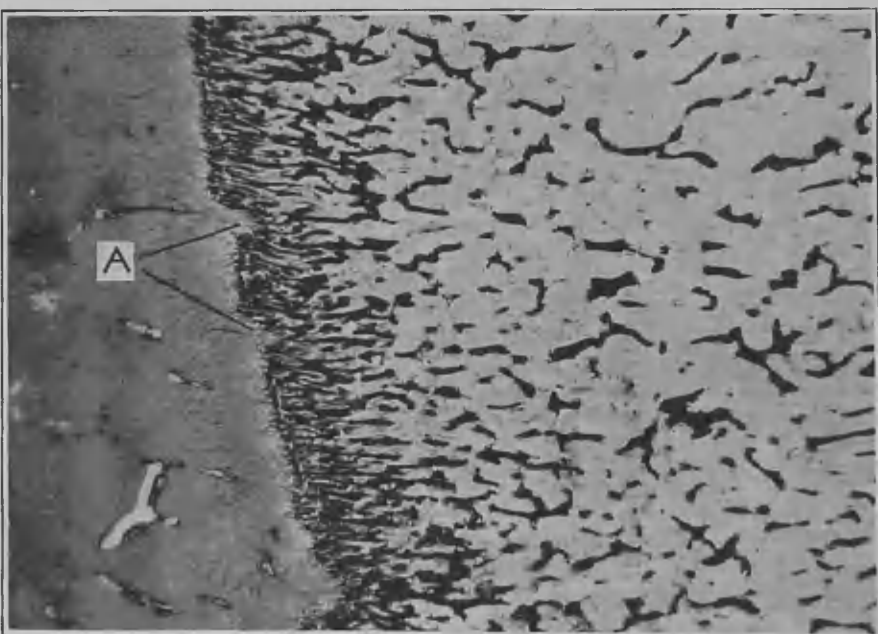

Fig. 10. Calf C-170. Mild rickets. Shows: extension of tongues of cartilage vertebrally into the diaphysis, A. 30X.

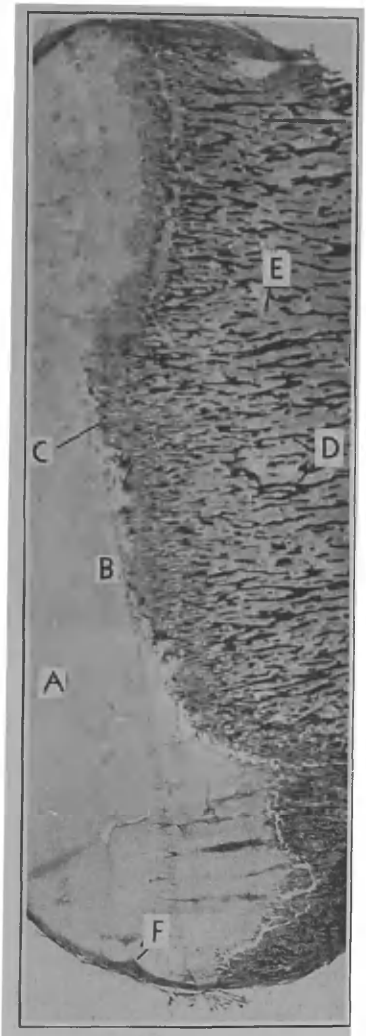

Fig. 11.

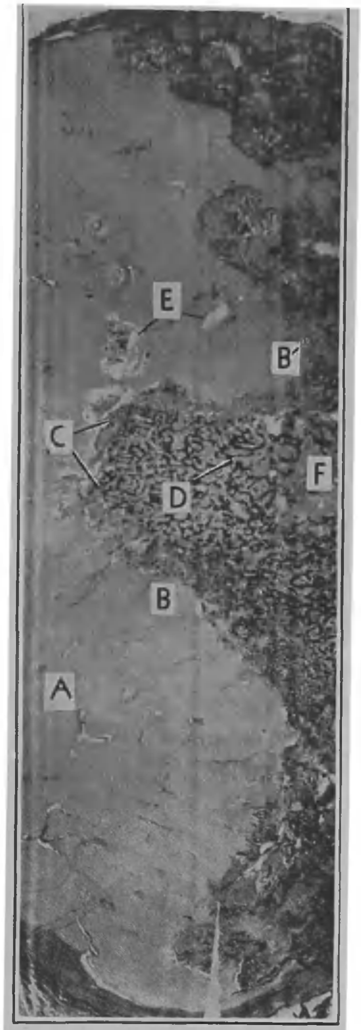

Fig. 12.

Fig. 11. Calf C-233. Normal. Shows: resting cartilage, A; area of proliferation and cartilage cell rows, B; junction of the diaphysis with the cartilagenous end of the rib—the slight separation noted here is an artifact, C; bars of bone trabeculae—note their dark stain, how they extend in a direction parallel to the long axis of the bone, and how they decrease in number and become heavier further in the diaphysis, D; marrow, E; thickening of perichondrium, F. 5X.

Fig. 12. Calf C-238. Severe rickets. Shows: resting cartilage, A; area of proliferation and cell rows, B; increased depth of zone of mature cartilage cells, B[1]; area of cartilage removal—note irregularity in this area, C; short, stubby trabeculae, D; sac-like structures isolated in irregularly-thinned cartilage, E; embryonic marrow and osteoid tissue, F. Note irregular advancement of the marrow and its extension ventrally in the axial region of the bone. 5X.

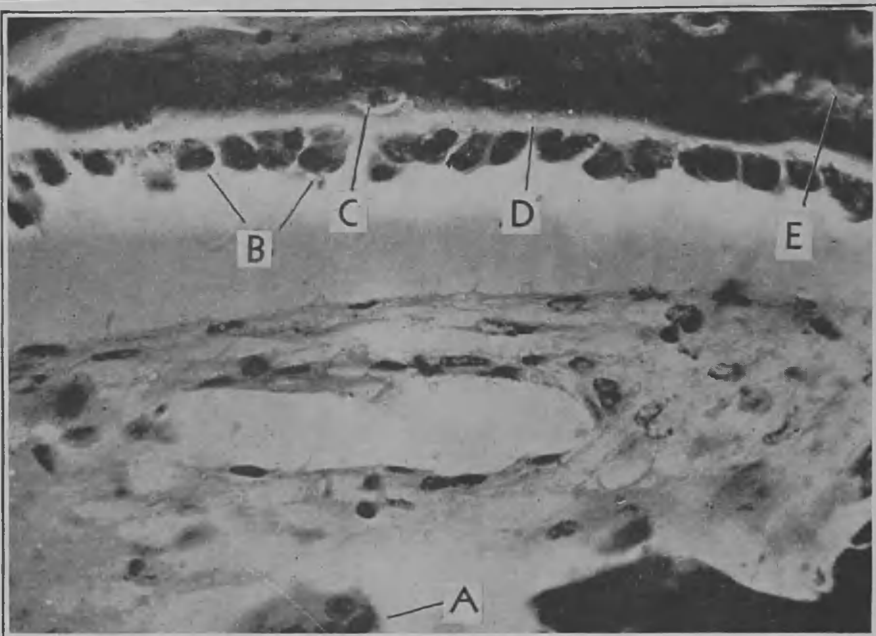

Fig. 13. Calf C-233. Normal. Shows: osteoclast, A; osteoblast bordering a trabecula of bone—note eccentrically placed nuclei, B; osteoblast surrounded by osteoid tissue and also partly surrounded by dark granules of inorganic salts—such cells are gradually changing into osteocytes, C; osteoid tissue, D; core of calcified cartilage matrix as yet unremoved by chondrolysis, E. 460X.

Fig. 14. Calf C-151. Normal. Shows: groups of osteoblasts as they occur on the borders of the larger trabeculae in the ventral end of the diaphysis, A. 460X.

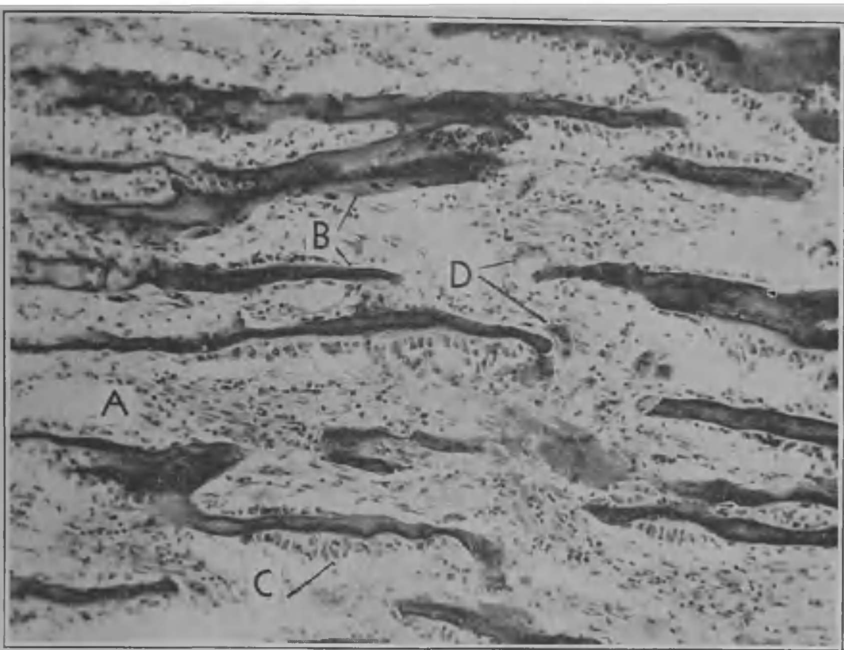

Fig. 15. Calf C-233. Normal. Shows: embryonic marrow, A; trabeculae of bone, B; osteoblasts bordering trabeculae, C; osteoclasts on ends of trabeculae where they are associated with terminal erosion of the calcified trabeculae, D. 120X.

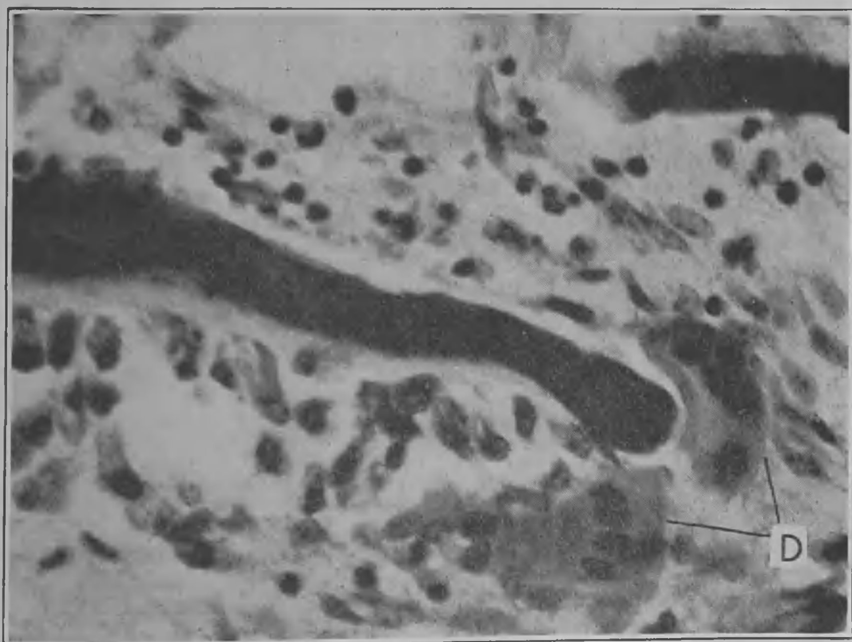

Fig. 16. Area D in Fig. 15. Shows: terminal erosion by osteoclasts, D. 575X.

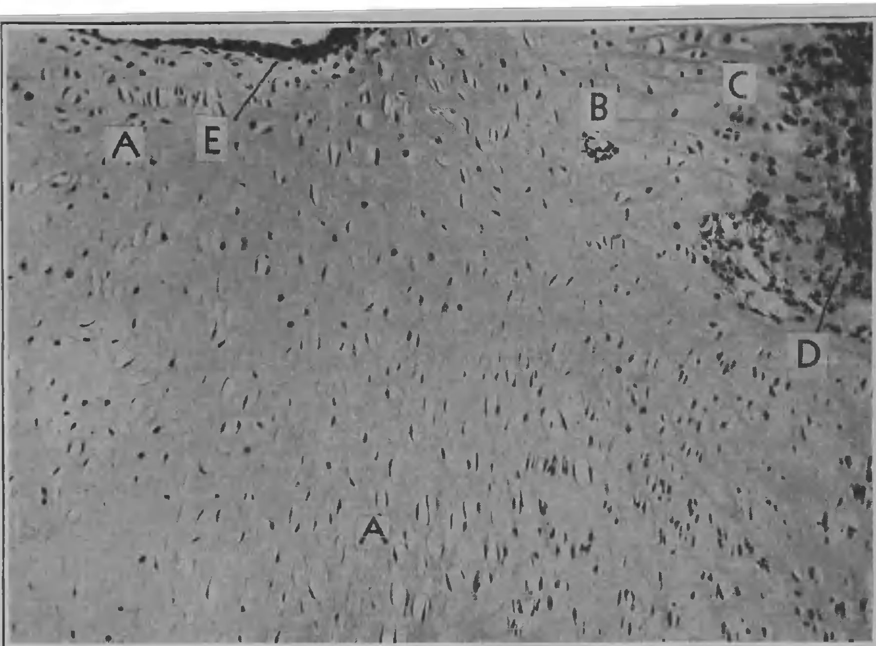

Fig. 17. Calf C-155. Severe rickets. Shows: resting cartilage, A; cell proliferation, B; cell growth, C; embryonic marrow, D; wall of blood vessel, E. 120X.

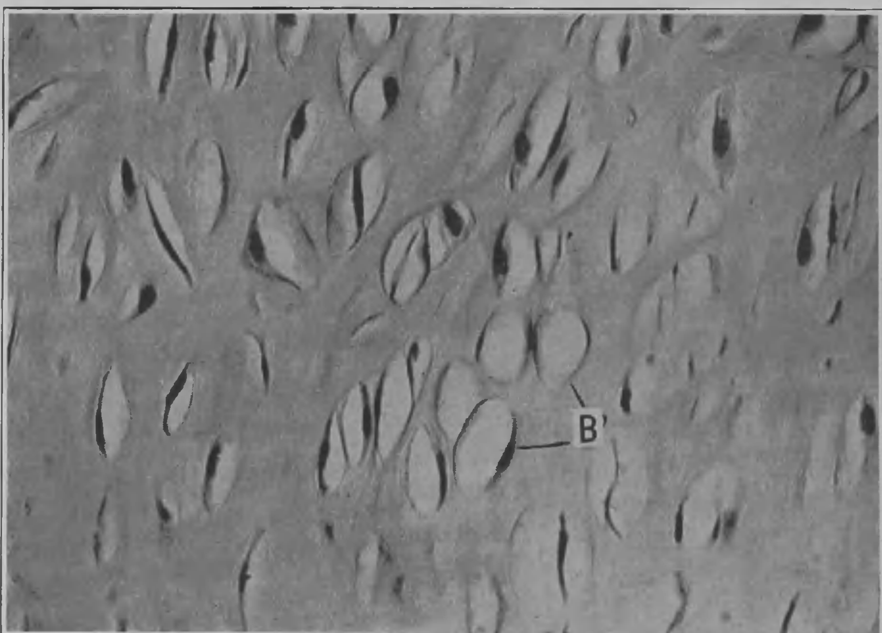

Fig. 18. Area A in Fig. 17. Shows: increased number of isogenous pairs of cartilage cells in shrunken condition, B. Note decrease in amount of matrix and comparative absence of mature cells. 460X.

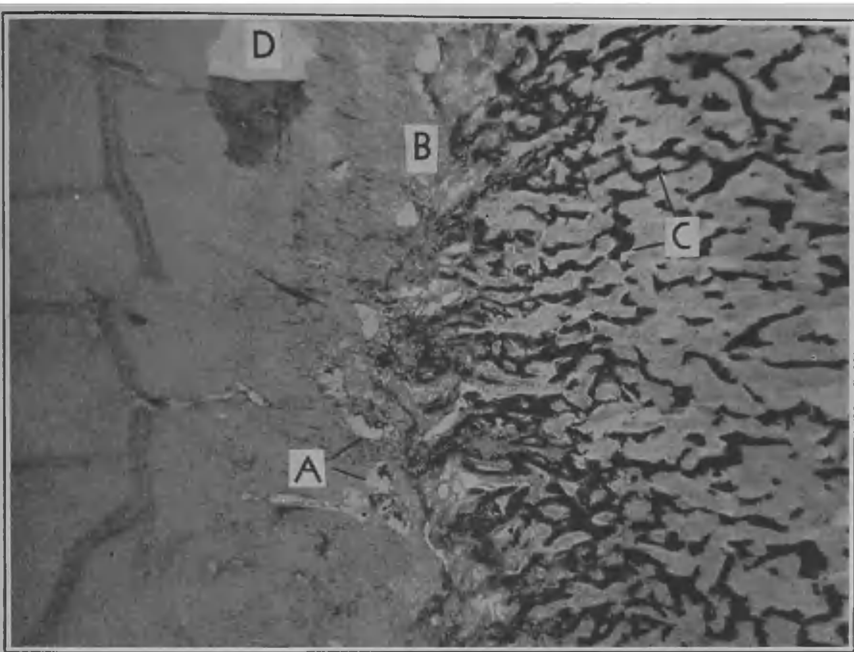

Fig. 19. Calf C-237. Advanced rickets. Shows: large number of sac-like structures, A; lengthened rows of mature cartilage cells, B; irregularly shaped trabeculae of calcified tissue, C; artifact, D. 30X.

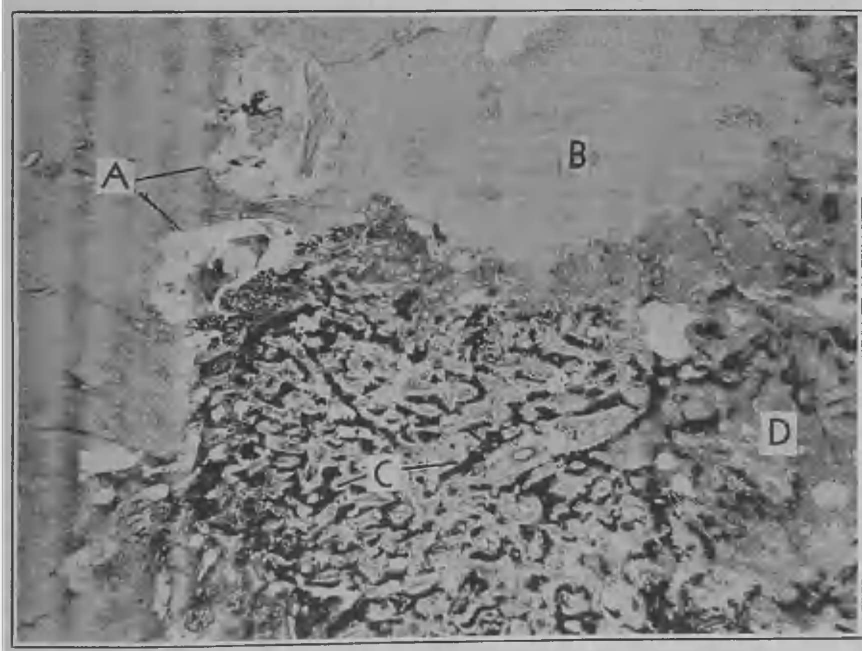

Fig. 20. Calf C-238. Severe rickets. Shows: sac-like structures, A; enormously increased depth of zone of mature cartilage cells, B; irregularly shaped trabeculae of bone, C; embryonic marrow and osteoid tissue, D. Note irregular advancement, ventrally, of the embryonic marrow in the axial region of the bone. Compare with Fig. 19, Calf C-237, which had rickets of similar severity, as indicated by the blood picture, for about the same length of time but made subnormal gains in body weight and developed much less severe bone lesions. 30X.

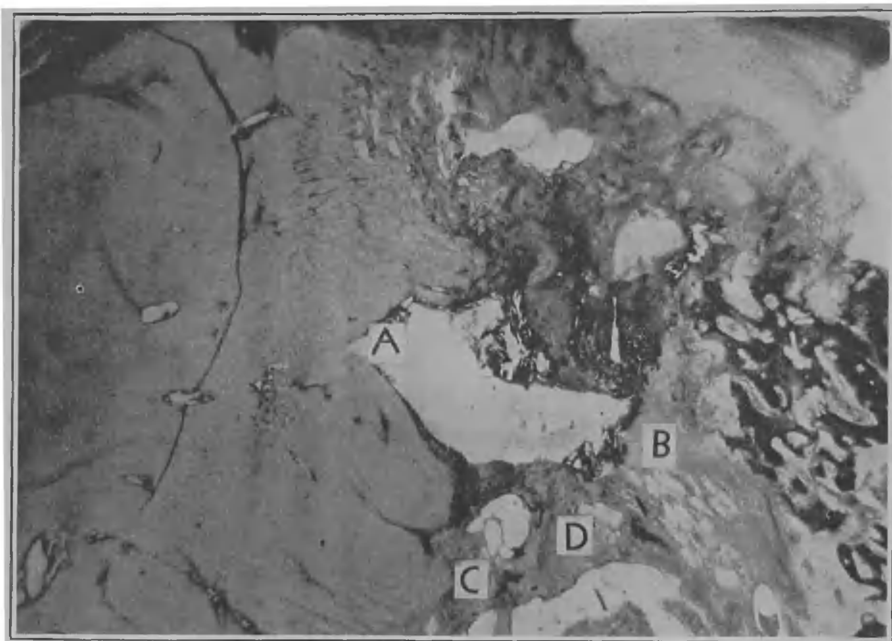

Fig. 21. Calf C-171. Severe rickets. Shows: splitting of cartilage, A; osteoid and fibrous connective tissues, B; crushing of cartilage cells, C (see Fig. 22 for higher magnification); patches of osteoid tissue about individual cells, D (see Fig. 24 for higher magnification). 30X.

Fig. 22. Area C in Fig. 21. Shows: crushing of cartilage cells, A; rejuvenated cartilage cells, B. 460X.

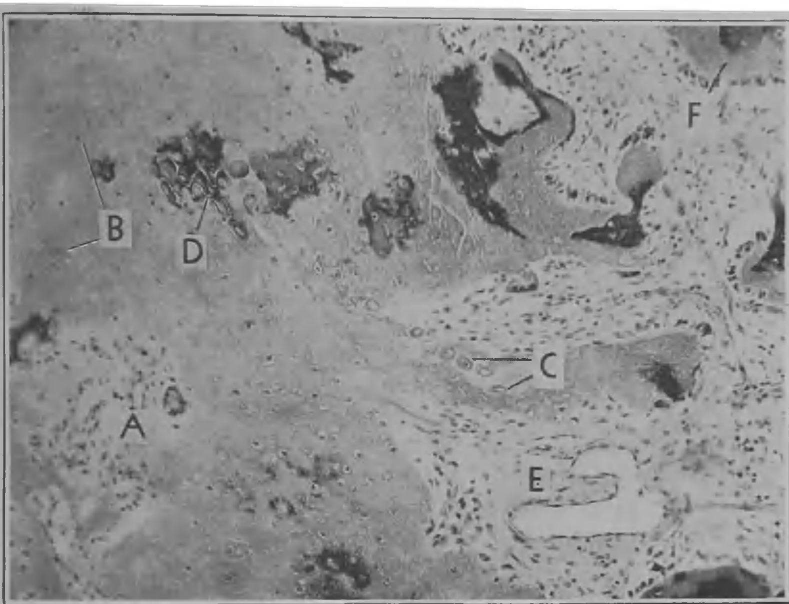

Fig. 23. Calf. C-171. Severe rickets. Shows: embryonic marrow, A; osteoid and fibrous connective tissue, B (refer to B in Fig. 21); patches of osteoid tissue surrounding individual cells, C; early ossification in patches of osteoid tissue similar to those at C, D, (note characteristic halo effect described in text); reorganization of the loose connective tissue between trabeculae giving rise to embryonic bone marrow, E; trabecula with core of bone and margin of osteoid tissue bordered by osteoblasts, F. 120X.

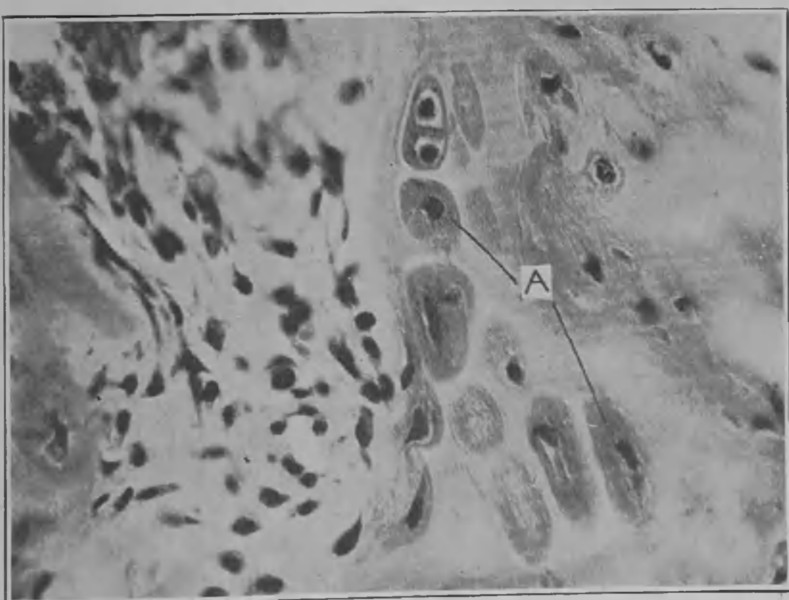

Fig. 24. Area D in Fig. 21. Shows: individual cells surrounded by circular zones of osteoid tissue which stain deeply acidophilic, A. 460X.

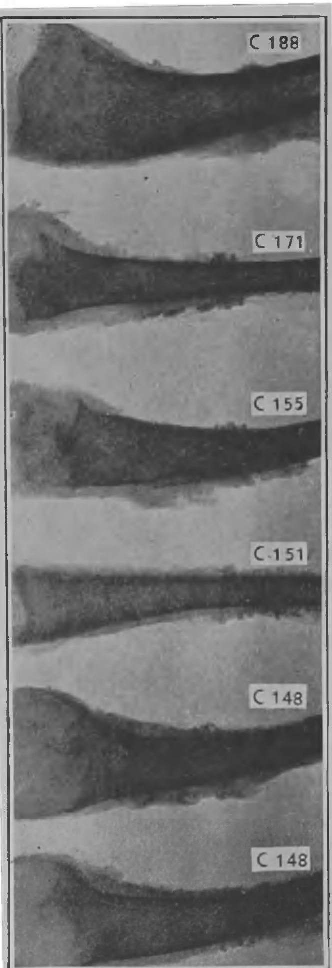

Fig. 25.

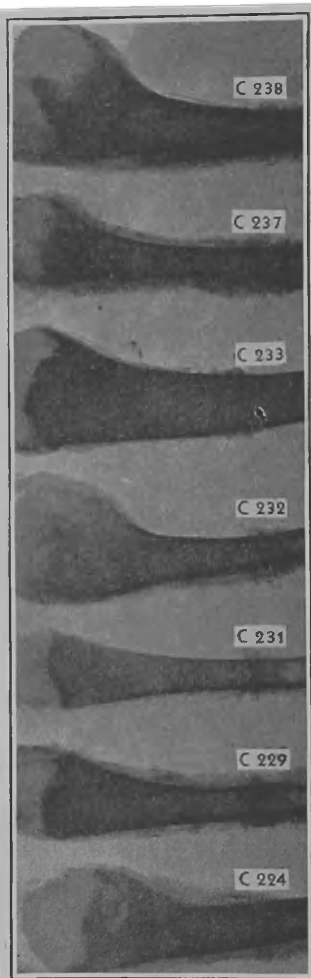

Fig. 26.

Fig. 25. Roentgenograms of mid-frontal sections 4 mm. in thickness taken from the left eighth costochondral junction of each calf as labeled. The varying shades of black show the distribution of calcified tissues. C-151 shows a normal condition. C-148, C-155, and C-171 show ricketic changes as evidenced by deficient calcification at the ends of the bones. The roentgenogram of C-188 shows slight evidence of at least two arrested-growth lines in the ventral end of the diaphysis. Note that beading of the ricketic ribs is more pronounced on the medial side. Also, note increased curvature of ricketic ribs in a medial direction.

Fig. 26. Roentgenograms of mid-frontal sections 4 mm. in thickness taken from the left eighth costochondral junction of each calf as labeled. C-224 shows evidence of epiphyseal type of healing rickets (refer to Table 6). C-229, C-231, and C-233 are from normal calves. C-232, C-237, and C-238 are from ricketic calves. Note that the ricketic alterations in C-237 are relatively mild when compared with C-238, because of poor growth (refer to Tables 8 and 9, and Figs. 19 and 20).

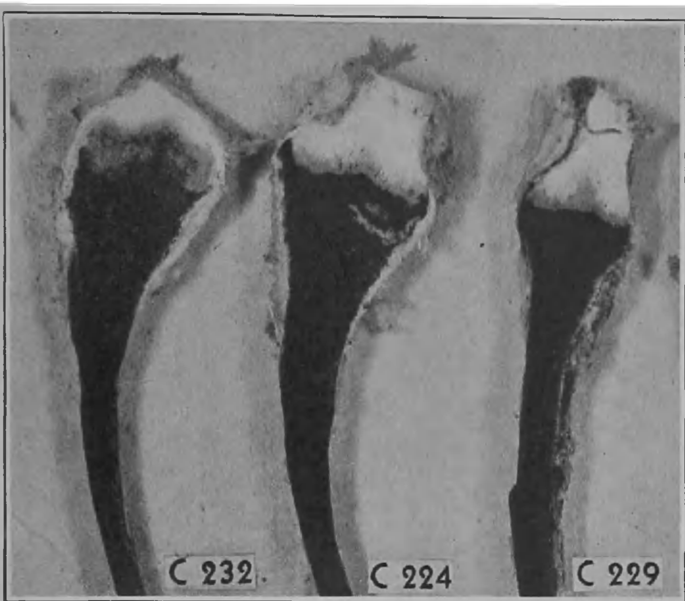

Fig. 27. Mid-frontal sections 4 mm. in thickness taken from the left eighth costochondral junction of each calf as labeled, and stained in silver nitrate solution. Shows typical appearance of normal and of ricketic ribs when treated in this manner. Compare with Fig. 26. Note that while the general appearances are the same, there is greater detail in the roentgenograms than in specimens stained in silver nitrate solution.

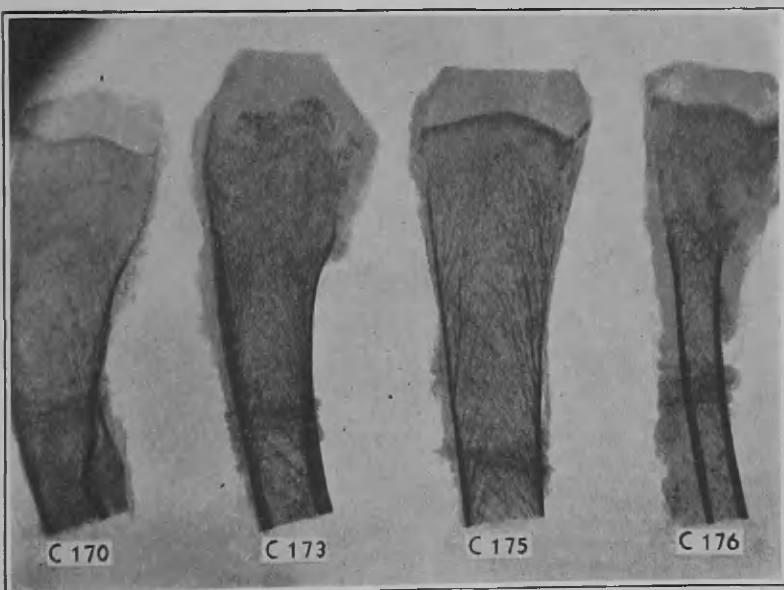

Fig. 28. Roentgenograms of mid-frontal sections 4 mm. in thickness taken from the left eighth costochondral junction of each calf as labeled. C-176 shows some rarefaction; this calf was free from rickets. C-170, C-173, and C-175 are specimens from ricketic ribs. In case C-175, definite evidence of rickets was indicated only in the blood analyses and in histological studies of the bone.

Fig. 29. Calf C-232. Severe rickets. Shows changes similar to those in Fig. 20. Compare with Fig. 10, Calf C-170, showing comparatively slight alterations. Fig. 29 is from a younger calf which had rickets for a much shorter time than did C-170. This illustrates the more extensive alterations which are produced in rickets in younger calves. 30X.

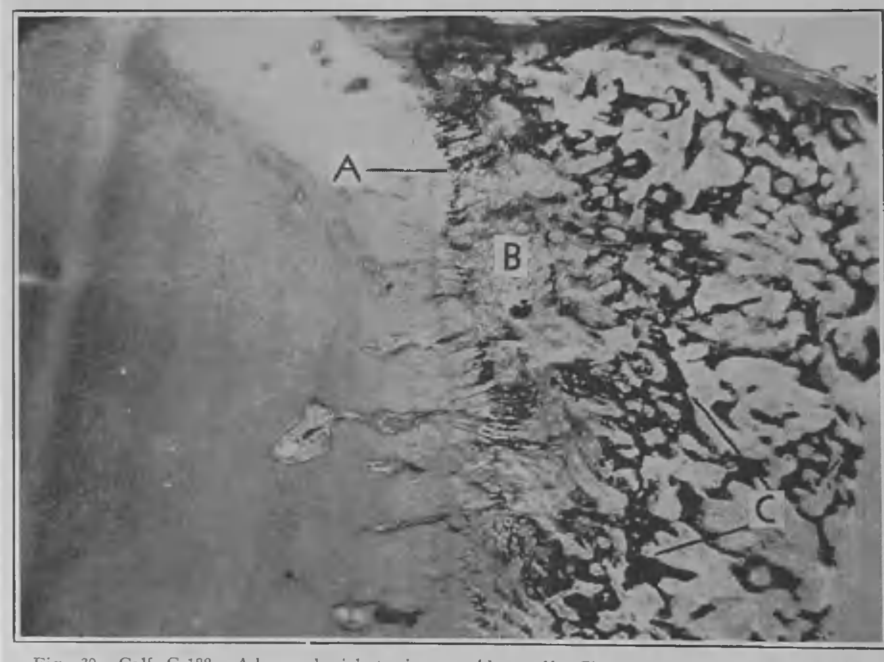

Fig. 30. Calf C-188. Advanced rickets in an older calf. Shows: calcification of cartilage matrix, A; embryonic marrow and connective tissue, B; more than usual number of transverse bone trabeculae, which account for the arrested-growth lines indicated in Fig. 25, C. 30X.

Fig. 31.

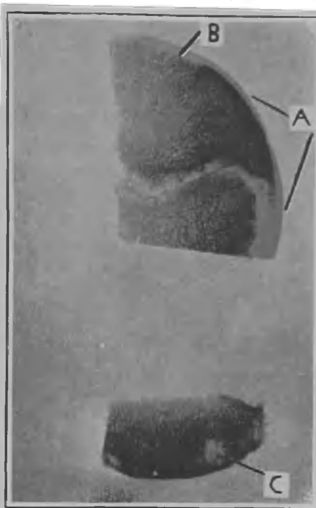

Fig. 32.

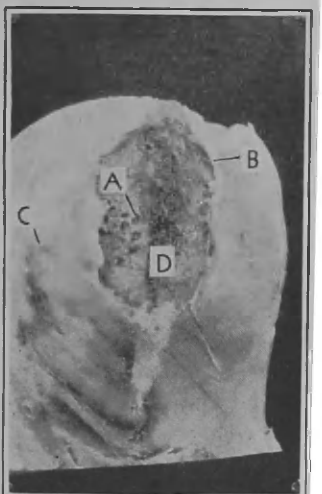

Fig. 33.

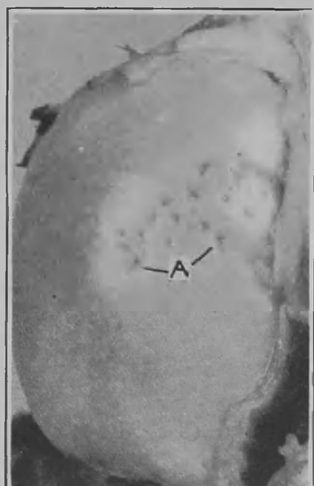

Fig. 34.

Fig. 31. Calf C-148. Ribs from the right side. Show enlargements of the ventral-epiphyseal ends of the ribs. This condition is commonly referred to as beading, and is best seen at A.

Fig. 32. Calf C-188. Roentgenograms of thin specimens of bone taken through the proximal end of the femur. There are no abnormalities at A, but note the moderate rarefaction at B and the loss of cartilage and rarefaction at C. See Fig. 33.

Fig. 33. Calf C-188. Proximal end of femur of ricketic calf. Shows: pitting of bone, A; destruction of cartilage, B; thinning of cartilage, C. (Note that this specimen has been reconstructed through the center at D where a section of bone was sawed out for histological study). Extreme pitting and erosion of the articular surfaces, such as illustrated here, occurred but once in calves used in this study.

Fig. 34. Humerus of a ricketic calf. Shows: pitting of articular surface A. This was a common condition in ricketic calves.