CLXIX. STUDIES ON THE MINERAL METABOLISM DURING PREGNANCY AND LACTATION. II.

BY KIRSTEN UTHEIM TOVERUD AND GUTTORM TOVERUD.

*From the Physiological Department of the University and the
Pedodontic Department of the Dental School of Norway.*

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FROM metabolism studies performed by us earlier [1931] it was evident that pregnant women on an unregulated diet often showed negative Ca and P balances during the last part of pregnancy. The diet examined contained 0.5–1.0 g. Ca and about 1.0 g. P daily and had a low content of vitamins A and D. This negative balance could be made positive or less negative by increasing the calcium content of the diet to 1.6–2.0 g. daily. In the majority of experiments cod-liver oil or a vitamin D preparation (vigantol) did not affect the negative balance when the Ca and P intake was low. If the Ca and P intake was fairly high, vitamin addition might in some instances change the balance to a positive one.

These studies led us to perform some metabolism experiments on dogs during gestation in order to follow the effect of such a diet low in Ca, P and fat-soluble vitamins on the mother and the young. The result was that the mother animal on a diet containing about 10 mg. Ca and 16–18 mg. P per kg. of body weight lost about 10 % of her original Ca content and developed a marked osteomalacia during gestation and lactation. During lactation manifest symptoms of tetany developed. The young got marked rickets with inability to walk.

As the Ca and P deficiency according to our experiments in women seemed to be the decisive factor in the negative salt balance, a parallel experiment on dogs was performed simultaneously in order to follow in detail the effect of a pure salt deficiency on mother and young.

The result was development of osteoporosis in all the young with poor formation of osteoid tissue in one of the puppies. As was the case with the first experimental litter, these puppies were also in a poor condition, they were unable to walk and could hardly move on account of multiple fractures of the hind legs.

In both these experimental litters the chemical changes taking place in the bony system of the new-born puppies were about the same, *i.e.* a distinct lowering was found in the Ca and P contents of the ash of the bones.

We wanted now to study more thoroughly this congenital osteoporosis found in all puppies by repeating the last experiment of a pure salt deficiency during gestation, and then following the development of these puppies under various nutritional conditions in post-natal life.

As in previous experiments a one year-old bitch was given a ration low in Ca and P, consisting of wheat, rye flour and cocoa fat cooked to a gruel, to this were added dried skimmed milk, raw horse meat, marmite, NaCl, Fe, lemon or orange juice and cod-liver oil.

The daily ration consisted of:

Meat	...	...	50 g.
Milk powder	...	...	10 g.
Cocoa fat	...	...	10-40 g.
Marmite	...	...	5 g.
NaCl	...	...	3 g.
Fe	...	...	0.1 g.
Lemon juice	...	...	5 cc.
Cod-liver oil	...	...	10 cc.

To this were added wheat and rye flour from 90 to 360 g. daily (according to the appetite of the dog). The Ca and P content of this diet calculated per kg. of body weight was somewhat higher in this experiment than in our earlier experiments as the dog had a lower weight. The Ca and P content ranged from 14 to 17 mg. Ca and from 37 to 40 mg. P per kg. of body weight. The Ca/P ratio was 0.4-0.5. The dog was kept in a large room with no free access to direct sunlight. A 3 days metabolism experiment was performed for Ca, P and Mg every week throughout the whole gestation period.

From Table I it is evident that with the exception of the two first metabolism periods all the remaining 7 balances were negative as far as Ca is concerned. The same may be said about phosphorus. Only during the 2nd and 8th weeks of gestation did the animal show a positive P balance. In all other experiments the P followed the Ca. The Mg balance was positive throughout the whole gestation period.

As far as the blood analyses are concerned serum-Ca (see Table I A) showed a slight lowering during the 4th, 5th, 6th and 7th weeks of gestation compared with the value of 12 mg. in the very beginning of gestation. These figures are however within the normal range. As to the P content of the serum there is a definite and constant lowering from 5 mg. in the 1st week to the lowest value of 2.9 mg. in the 9th week of gestation.

After the normal gestation period of 9 weeks the bitch gave birth to two normal-looking puppies. As the litter was so small, we were not able to perform any analyses of the new-born puppies, as were done in previous experiments. Both puppies were allowed to stay with the mother and suckled for 5 weeks. They were then given the same ration as the mother received during gestation and lactation, save that one of the puppies, Mosse, got a standardised and sufficient dose of cod-liver oil from the day of weaning.

Table I. *Metabolism studies of the dog Tulla.*

A. Gestation:		Daily output						Per 100 cc. serum	
Week of gestation	Minerals studied	Intake g.	Urine g.	Faeces g.	Milk g.	Total g.	Balance g.	Ca mg.	P mg.
1	Ca	0.1863	0.0287	0.1576	—	0.1863	0.0000	12.0	5.0
	P	0.4720	0.4200	0.1483	—	0.5683	-0.0963		
	Mg	0.1449	—	—	—	—	—		
2	Ca	0.2059	0.0267	0.1400	—	0.1667	+0.0392	11.7	4.9
	P	0.5846	0.3683	0.1415	—	0.5098	+0.0748		
	Mg	0.1923	0.0409	0.1014	—	0.1423	+0.0500		
3	Ca	0.1969	0.0184	0.3110	—	0.3294	-0.1325	11.4	4.9
	P	0.5333	0.4244	0.2416	—	0.6660	-0.1327		
	Mg	0.1706	0.0380	0.0760	—	0.1140	+0.0566		
4	Ca	0.1897	0.0190	0.2234	—	0.2424	-0.0527	10.8	4.2
	P	0.4924	0.4010	0.1941	—	0.5951	-0.1027		
	Mg	0.1534	0.0419	0.0879	—	0.1298	+0.0236		
5	Ca	0.1871	0.0127	0.2202	—	0.2329	-0.0458	10.9	3.4
	P	0.4772	0.4497	0.1863	—	0.6360	-0.1588		
	Mg	0.1470	0.0293	0.0622	—	0.0915	+0.0555		
6	Ca	0.1988	0.0130	0.2601	—	0.2731	-0.0743	10.8	3.2
	P	0.5443	0.4047	0.1984	—	0.6031	-0.0588		
	Mg	0.1752	0.0333	0.0914	—	0.1247	+0.0505		
7	Ca	0.2063	0.0137	0.2698	—	0.2835	-0.0772	10.8	3.4
	P	0.5873	0.4256	0.2108	—	0.6364	-0.0491		
	Mg	0.1932	0.0412	0.0957	—	0.1369	+0.0563		
8	Ca	0.2097	0.0276	0.2276	—	0.2552	-0.0455	11.2	2.9
	P	0.6066	0.4152	0.1772	—	0.5924	+0.0142		
	Mg	0.2013	0.0450	0.1301	—	0.1751	+0.0262		
9	Ca	0.2022	0.0272	0.1938	—	0.2210	-0.0188	11.1	2.9
	P	0.5637	0.4427	0.1985	—	0.6412	-0.0775		
	Mg	0.1833	0.0479	0.0980	—	0.1459	+0.0374		
B. Lactation:									
3	Ca	0.1933	0.0222	0.1159	0.3760	0.5141	-0.3208	10.4	3.8
	P	0.5126	0.2331	0.2222	0.2891	0.7444	-0.2318		
	Mg	0.1619	0.0345	0.1055	0.0196	0.1596	+0.0023		
3½	Ca	0.2153	0.0205	0.0659	0.5320	0.6184	-0.4031	10.6	3.9
	P	0.6389	0.1550	0.2365	0.3804	0.7719	-0.1330		
	Mg	0.2148	0.0258	0.0954	0.0206	0.1418	+0.0730		
4½	Ca	0.2270	0.0401	0.1022	0.6636	0.8059	-0.5789	10.4	3.4
	P	0.7056	0.0556	0.3460	0.5796	0.9817	-0.2761		
	Mg	0.2428	0.0476	0.1480	0.0308	0.2264	+0.0164		
After weaning.									
1	Ca	0.2270	0.0186	0.0895	—	0.1081	+0.1189	10.9	4.0
	P	0.7056	0.1746	0.3336	—	0.5082	+0.1974		
	Mg	0.2428	0.0263	0.1372	—	0.1635	+0.0793		

Three weeks after delivery a metabolism experiment was again performed on the bitch showing an increased negative Ca balance. This negative Ca balance was about 3 times as great as the highest negative balance during gestation (3rd week). Two other metabolism experiments performed during lactation, 3½ and 4½ weeks after delivery, showed even higher negative Ca balances with a daily loss of about 0.5 g. or more of Ca (Table I B). The serum-Ca showed values below that in the beginning of gestation. The same is also true of phosphorus.

The dog looked perfectly normal throughout the whole gestation and lactation period.

Analyses of the milk from the bitch gave the lowest value of Ca 18 days after delivery. All milk analyses gave rather low figures compared with milk analyses from previously examined dogs. 30 days after delivery the analyses showed an increase in the calcium figure up to 0.237 % (see Table II).

Table II. *Analyses of milk from Tulla.*

Days after delivery	Per 100 g. milk		
	Ca g.	P g.	Mg g.
13	0.213	0.246	0.009
18	0.173	0.133	0.009
25	0.207	0.148	0.008
30	0.237	0.207	0.011

When we consider the status of this dog (see Table III) we find that the calcium loss is decidedly greater in the lactation than in the gestation period. This is evidently of common occurrence in animals. Goss and Schmidt [1930] thus found a negative Ca balance in all lactating rats. The losses for full-sized litters were as great as 20 % of the entire body content of these elements in the mother.

Table III. *Status of the dog Tulla at the end of gestation and at the end of lactation.*

	Ca g.	P g.	Mg g.
Total intake during gestation	12.4803	34.0298	10.9284
Loss through urine and faeces	15.3335	38.1381	8.4175
Loss through two fetuses	4.7072	2.9048	0.1592
Total loss during gestation	20.0407	41.0429	8.5767
Balance at the end of gestation	-7.5604	-7.0131	+2.3517
Intake through food during lactation	7.4175	20.4818	6.9299
Intake through faeces from the puppies during lactation	1.9850	1.3650	0.4200
Total intake during lactation	9.4025	21.8468	7.3499
Loss through urine and faeces	4.8110	15.5449	5.5764
Loss through secreted milk	17.3236	13.9683	0.8328
Total loss during lactation	22.1346	29.5132	6.4092
Balance at the end of lactation not counting gestation	-12.7321	-7.6664	+0.9407
Balance at the end of lactation counting gestation ...	-20.2925	-14.6795	+3.2924

For the gestation and lactation period together a loss of about 20 g. Ca, 15 g. P and a Mg retention of about 3 g. were found in this dog. On account of the very small litter we had to use the average figures from our other experiments to estimate the Ca content of the new-born puppies. Furthermore, we had to calculate approximately the mineral intake which the mother may have received through eating the faeces of the puppies. The other figures, however, are arrived at by exact analyses of urine, faeces and milk. The great Ca loss during lactation is mostly due to the high output through the milk,

while the loss through urine and faeces is about half what it had been in earlier experiments. The final result of the gestation and lactation periods is a highly negative Ca and P balance, not as high however as in our first experimental dog [1931]. The calcium loss may be estimated at about 7 % of the original Ca content of the dog.

As stated before, the bitch gave birth to two well-developed and normal-looking female puppies with birth weights of 392 g. and 383 g. respectively. These puppies were allowed to feed at the breast for 5 weeks and were afterwards given the same diet as their mother, save that one puppy was deprived of the cod-liver oil.

The puppies developed quite normally during the lactation period and showed a good weight curve. When 50 days old however, the puppy Åse (with no cod-liver oil added to the diet after weaning) showed symptoms of what usually is called rickets. The clinical evidence of rickets appeared in this instance even 5 days earlier than in a puppy, Eva, described in our earlier experiments, where salt and fat-soluble vitamin deficiencies were present during foetal life and lactation as well as after weaning. This is evident from Fig. 1. Here we also see that the weight is somewhat higher than that of Eva.

Fig. 1. Weight curves of the 3 experimental puppies.

Mosse: deficiency: foetal life: Ca and P—lactating period: Ca and P—after weaning: Ca and P.
Åse: deficiency: foetal life: Ca and P—lactating period: Ca and P—after weaning: Ca, P, vit. A + D.
Eva: deficiency: foetal life: Ca, P, vit. A + D—lactating period: Ca, P, vit. A + D—after weaning: Ca, P, vit. A + D.

↑ indicates distinct lowering of serum-P.

| indicates clinical symptoms of a pathological condition of the bones.

Thus we cannot say that the cod-liver oil given to the bitch in the present experiment, at least when there is a deficiency of Ca and P in the diet throughout gestation and lactation, has protected her puppy Åse in any considerable way against the development of the rachitic process. The rachitic symptoms: bowing of the legs, enlargement of the costochondral junctions, development of the plantigrade condition so typical in these puppies, increased constantly so that the dog had a typical rachitic appearance at about 3 months of age, when the photograph reproduced in Plate V, fig. 1, was taken.

The Ca and P analyses of the blood showed definite decreases until they reached their lowest values of 6.4 mg. Ca and 4.6 mg. P when 105 days old (see Table IV). The usual roentgenological findings typical for rickets may be seen from Fig. 2, an X-ray picture taken when the puppy was 3½ months old.

The roentgenological changes were noticed after the blood changes and the clinical symptoms considered typical for rickets had appeared.

Metabolism studies performed every week (from the 9th week) in 3-day periods showed a positive balance for all experiments with the exception of the 7th experiment as far as P is concerned (see Table IV). The daily re-

Table IV. *Metabolism experiments of the puppy Åse.*

Ca and P deficiency during foetal life and lactating period.

Ca, P and vitamin A and D deficiencies after weaning.

Age in weeks	Minerals studied	Intake g.	Daily output			Balance g.	Per 100 cc. serum	
			Urine g.	Faeces g.	Total g.		Ca mg.	P mg.
9	Ca	0.0882	0.0018	0.0188	0.0206	+0.0676	11.0	7.4
	P	0.2355	0.1364	0.0593	0.1957	+0.0398		
	Mg	0.0603	0.0238	0.0354	0.0592	+0.0011		
10	Ca	0.0928	0.0300	0.0311	0.0611	+0.0317	12.1	8.0
	P	0.2619	0.1418	0.0712	0.2130	+0.0489		
	Mg	0.0714	0.0243	0.0333	0.0576	+0.0138		
11	Ca	0.0932	0.0080	0.0417	0.0497	+0.0435	9.2	8.0
	P	0.2641	0.1546	0.0695	0.2241	+0.0400		
	Mg	0.0724	0.0263	0.0367	0.0630	+0.0094		
12	Ca	0.0878	0.0086	0.0185	0.0271	+0.0607	8.5	6.4
	P	0.2333	0.1505	0.0384	0.1889	+0.0444		
	Mg	0.0594	0.0153	0.0199	0.0352	+0.0242		
13	Ca	0.0926	0.0106	0.0197	0.0303	+0.0623	8.2	5.6
	P	0.2608	0.1901	0.0354	0.2255	+0.0353		
	Mg	0.0710	0.0260	0.0166	0.0426	+0.0284		
14	Ca	0.0909	0.0077	0.0606	0.0683	+0.0226	8.2	5.2
	P	0.2508	0.1458	0.0995	0.2453	+0.0055		
	Mg	0.0668	0.0158	0.0263	0.0421	+0.0247		
15	Ca	0.0876	0.0119	0.0566	0.0685	+0.0191	6.4	4.6
	P	0.2322	0.2017	0.0661	0.2678	-0.0356		
	Mg	0.0590	0.0239	0.0156	0.0395	+0.0195		

tention, however, for Ca as well as for P, was very low, and insufficient for a growing animal. The weight increased until the last week when it dropped about 500 g. The general condition of the dog was fairly good, it was running about, had a good appetite and wanted to play.

The puppy was killed when 112 days old, as we had done with our previously examined puppies. The radius, a rib and the lower jaw were taken out for chemical and histological examination. As far as the chemical analysis of the radius is concerned a distinct lowering was found in nearly all figures for Ca, P and Mg, see Table V. For comparison with our earlier experiments, we have placed the figures for both these last two puppies in Table V together with the figures for bone analyses of the other experimental puppies all killed at the age of 112 days, and all with different dietary deficiencies throughout the foetal life, lactation period and after weaning.

It is evident from Table V that this last experimental puppy Åse in its bone composition was in a worse condition than Siss, a puppy with no dietary deficiency during foetal life and lactation period. The mineral deficiency

Table V. *Analyses of radius from 112 days-old puppies.*

Name of puppy	Dietary deficiency of			Fresh wt. g.	Dry wt. g.	% ash of fresh wt.	% Ca of fresh wt.	% P of fresh wt.	% Mg of fresh wt.
	Mother during gestation	Mother during lactation	Puppy after lactation period						
Lisken (normal)	None	None	None	9.733	4.709	22.5	8.9	4.0	0.15
Siss	None	None	Ca, P and vit. A + D	14.150	5.445	15.6	6.2	2.5	0.08
% difference from normal						-30.7	-30.2	-37.5	-46.70
Grete	None	Ca, P and vit. A + D	"	14.010	3.960	8.9	3.2	1.39	0.04
% difference from normal						-61.2	-64.1	-65.30	-73.30
Eva	Ca, P and vit. A + D	"	"	8.780	2.077	5.6	1.9	0.95	0.03
% difference from normal						-75.1	-78.7	-73.30	-80.00
Bobb	Ca and P	Ca and P	Ca and P	9.940	4.320	9.1	3.8	1.6	0.06
% difference from normal						-60.0	-57.6	-60.0	-60.00
Tell	"	"	"	13.200	4.695	12.1	4.8	2.0	0.06
% difference from normal						-46.2	-46.1	-50.0	-60.00
Mosse	"	"	"	6.830	2.783	13.5	4.9	2.5	0.08
% difference from normal						-40.0	-45.0	-37.5	-46.70
Åse	"	"	Ca, P and vit. A + D	8.610	2.984	9.3	3.1	1.6	0.07
% difference from normal						-58.7	-65.2	-60.0	-53.30

throughout foetal life and lactation period has thus had a definite influence on the bone composition of this puppy, Åse. The diets of these two puppies, Åse and Siss, after weaning were the same. It is the diet during foetal life and during lactation which is the sole cause of the difference between these two puppies.

The other puppy in this experiment, Mosse, was, as mentioned earlier, given exactly the same diet after weaning as the mother animal. This experiment may thus be considered as a repetition of the earlier described experiments with puppies Bobb and Tell, see Table V. This puppy also developed clinical symptoms of what is usually called rickets which progressed, and when 81 days old it developed a fracture of the tibia of the right hind leg and therefore had great difficulty in walking.

The general condition of this puppy was definitely worse than that of its sister. Plate V, fig. 1, demonstrates fairly well the bone condition of the two puppies. The bowing and the position of the legs are about the same in both animals. The enlargement of the epiphyses of the fore legs and the plantigrade condition is, however, more pronounced in Åse (with no cod-liver oil added to the diet). When 102 days old Mosse developed a complete fracture of the femur of the right hind leg with a great dislocation, which is evident from Plate V, fig. 3. From that day the puppy could not move at all and the general condition of the animal was extremely poor. It would hardly take any food for the last days before it was killed, when 112 days old.

Metabolism experiments performed in 3-day periods every week from the 9th week of age showed a positive balance of Ca and P throughout the whole

period, with the exception of a slightly negative P balance in the last experiment. The average daily retention was usually not higher than for the sister animal (see Table VI).

Table VI. *Metabolism experiments with the puppy Mosse.*

Ca and P deficiency during foetal life, lactation period and after weaning.

Age in weeks	Minerals studied	Intake g.	Daily output			Balance g.	Per 100 cc. serum	
			Urine g.	Faeces g.	Total g.		Ca mg.	P mg.
9	Ca	0.0905	0.0046	0.0216	0.0262	+0.0643	10.8	7.9
	P	0.2487	0.1304	0.0596	0.1900	+0.0587		
	Mg	0.0659	0.0196	0.0343	0.0539	+0.0120		
10	Ca	0.0951	0.0045	0.0230	0.0275	+0.0676	11.6	8.6
	P	0.2751	0.1426	0.0593	0.2019	+0.0732		
	Mg	0.0770	0.0229	0.0314	0.0543	+0.0227		
11	Ca	0.0946	0.0068	0.0480	0.0548	+0.0398	12.1	8.4
	P	0.2722	0.1277	0.1122	0.2399	+0.0423		
	Mg	0.0757	0.0263	0.0523	0.0786	-0.0029		
12	Ca	0.0943	0.0037	0.0537	0.0574	+0.0369	10.8	8.5
	P	0.2704	0.1228	0.1370	0.2598	+0.0106		
	Mg	0.0750	0.0135	0.0748	0.0883	-0.0133		
13	Ca	0.0950	0.0044	0.0371	0.0415	+0.0535	11.4	6.8
	P	0.2742	0.1540	0.1015	0.2555	+0.0187		
	Mg	0.0766	0.0172	0.0328	0.0500	+0.0266		
14	Ca	0.0916	0.0032	0.0307	0.0339	+0.0577	10.0	7.2
	P	0.2552	0.1495	0.1038	0.2533	+0.0019		
	Mg	0.0686	0.0138	0.0315	0.0453	+0.0233		
15	Ca	0.0834	0.0025	0.0119	0.0144	+0.0690	10.6	6.0
	P	0.2077	0.1746	0.0656	0.2402	-0.0325		
	Mg	0.0487	0.0131	0.0222	0.0353	+0.0134		

Blood analyses performed every week from the day of weaning did not show any lowering of the Ca. The P, however, dropped to 6 mg. per 100 cc. serum just before death.

In Plate V, fig. 3, is noticed the very poor calcification of the shaft of the femur, where the fracture occurred. No epiphyseal changes however can be noticed here.

When killed at 112 days of age, the radius was taken for chemical examination and the result of this chemical analysis may be seen from Table V. The figures indicating the bone composition are rather close to the figures of the puppy Tell, which was given the same pure salt-deficient diet during foetal life, lactation and after weaning.

The other radius, a rib and the jaw were taken for histological examination. This shows for the puppy Mosse:

(a) *Radius*. The bone trabeculae in the epiphysis are few and thin compared with the normal puppy Liskén [1931]. The osteoid zones are somewhat enlarged, but a little less than in the corresponding puppies Bobb and Tell [1931].

(b) *Costa*. The osteoid zones are about 3-4 times as broad as in the control puppy, Liskén, but a little narrower than in Tell.

(c) *Mandibula*. On the basis and in the alveolar septa the osteoid zones are about 4-5 times enlarged compared with the control puppy, Liskén. This is the same as in Tell. But in contra-

diction to this last-mentioned puppy, Mosse does not show any enlarged dentinoid zone or any other great disturbance in the calcification of the teeth.

The histological examination of the puppy Åse shows:

(a) *Radius*. The proliferating zone of the cartilage is 5-6 times as broad as in Mosse and very irregular. The bone trabeculae in the epiphysis are larger in number and rather thick. The osteoid zones in the epiphysis as well as in the diaphysis are greatly enlarged. Some of the trabeculae have not been calcified at all. The normal fatty tissue in the bone marrow has changed to a fibrous tissue.

(b) *Costa*. The costochondral junction is twice as broad as in Mosse. The osteoid zones are greater here than in the radius and many broad trabeculae have not been calcified at all.

(c) *Mandibula*. The osteoid zones in this bone are the broadest ones.

We have noticed as a common occurrence in our experimental work that the jaws among the different bones of the organism contain the broadest osteoid zones during an osteopathic process. This observation is of great clinical interest.

The calcification process of the teeth is greatly affected. The dentinoid zones are much enlarged and the calcified dentine very irregular.

These histological investigations show as far as the puppy Åse is concerned, that we have here a histological picture of what is called rickets. The other puppy, Mosse, which received a sufficient amount of cod-liver oil after weaning, also shows definite enlarged osteoid zones—especially in the ribs and jaws—considered as a typical sign of rickets. Enlargement of the proliferating cartilage zone, however, was not present.

DISCUSSION.

This experiment was performed in order to see what effect a pure congenital osteoporosis may have on the post-natal development of the bony system under various dietetic conditions after weaning.

We have presented two parallel experiments on puppies with Ca and P deficiencies during foetal life and during the lactation period. From our earlier experiments we know that puppies born under such conditions suffer from a general congenital osteoporosis, which is probably aggravated during lactation by the rather low Ca content of the mother's breast milk. When then one puppy was deprived from the day of weaning of the addition of cod-liver oil to the diet, while the other still received this addition, the further development of these two puppies, as far as the clinical appearance is concerned, was fairly similar. Both of them became bow-legged plantigrades and developed some enlargement of the epiphyses and the costochondral junctions, this last condition being however most marked in the puppy with no cod-liver oil. On the other hand the general condition of the latter puppy was decidedly better than that of the other which developed fractures.

While the chemical analyses show a lowering of all figures for Ca, P and Mg as a common finding for both puppies, the histological pictures are somewhat different. There is no doubt that the puppy without any cod-liver oil in the diet is suffering from rickets. The other puppy has no epiphyseal changes but has enlarged osteoid zones, a histological finding which is usually considered diagnostic of a rachitic process. Otherwise the condition of the bone corresponds rather with osteoporosis. It is at least evident from this experiment that cod-liver oil, even present in sufficient amount throughout foetal

life and lactation period, cannot protect the bones from undergoing an extreme osteopathic process resulting in multiple fractures when the mineral intake is low.

This finding agrees well with the statements of many previous investigators that the D-vitamin has less influence upon the balance of Ca than upon the intermediary distribution of Ca to the epiphyses of the long bones. Thus Brown and Shohl [1930] came to the following conclusion: "Vitamin D controls calcification of the skeleton by dissolution and deposition of the bone salts. The ash content of the bones is a result of these two activities. The amount of Ca and P in the diet determines the retention of these elements. Vitamin D controls their intermediary metabolism."

In this connection Hart *et al.* [1930] found that the Ca balance was not improved in milking cows by giving large doses of vitamin D in irradiated yeast. They were sure that vitamin D was absorbed into the blood as evidenced by the enrichment of the milk in this factor. According to their opinion this points to the fact, that there may be other factors influencing the Ca balance than the Ca level and the vitamin D intake.

Marek and Wellmann [1931] have furthermore recently shown by numerous animal experiments that the development of a true rachitic process is not strictly confined to the lack of D-vitamin in the diet. They have shown that the amount and the relationship of Ca and P in the diet together with the amount of organic matter, particularly proteins, irrespective of the lack of D-vitamin are, at least in animals, of great importance for the development of a rachitic process.

Another point of interest in this dog experiment is the influence of congenital osteoporosis on the time of development of a rachitic process in a growing organism in post-natal life. It is evident from Fig. 1 that the clinical symptoms of rickets appeared at about the same time as for the animal which experienced salt and vitamin deficiencies during foetal life as well as during lactation and after weaning. It is true that the clinical symptoms were more pronounced in the last-mentioned animal, but the time of development was about the same for both of them. In this connection we have to remember that the clinical findings of osteoporosis and of rickets in growing organisms are almost impossible to differentiate. In many instances we may therefore diagnose rickets when osteoporosis is the main pathological process. The two conditions may also be so combined in one individual case, that a true clinical differentiation is impossible. The difference may be evident only on histological examination.

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DESCRIPTION OF FIGURES IN PLATE V

- Fig. 1. Photographs of the puppies Mosse and Åse at 3 months of age. To the left Mosse *with* cod-liver oil. To the right Åse *without* cod-liver oil.
- Fig. 2. Radiograph of the distal end of tibia and fibula (puppy Åse) showing rachitic changes.
- Fig. 3. Radiograph of femur of the right hind leg (puppy Mosse) showing a complete fracture.

Fig. 1.

Fig. 2.

Fig. 3.