CLXXIV. FURTHER EXPERIMENTS ON THE INFLUENCE OF THE PARENTS' DIET UPON THE YOUNG.

II. THE INFLUENCE UPON THE YOUNG OF AN EXCESSIVE AMOUNT OF FAT-SOLUBLE FACTOR AND CALCIUM IN THE MOTHER'S DIET DURING PREGNANCY.

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In our previous experiments [Korenchevsky, 1921, 1922, 1923; Korenchevsky and Carr, 1923, 1, 2], we have found that the mother's diet has a definite influence on the young. The results obtained may be summarised as follows.

The diet of the mother before conception and during pregnancy influenced the litter *at birth* in some respects: the mothers kept on normal diet, containing an excess of fat-soluble factor and calcium, bore litters larger in number and total weight than those born by mothers fed on usual or fat-soluble deficient diets. The larger total weight of the litters was due to the greater number of offspring in each litter and not to the increased weight of the individuals. The largest number of young were born dead in those litters whose mothers were fed on deficient diets. At the day of birth the H_2O, Ca, P and N content in the young was nearly the same, irrespective of the mother's previous diet. The normal chemical composition shown to exist at birth in foetuses born of mothers kept on a diet deficient in fat-soluble factors is in accord with the fact that the maternal organism will as far as possible yield all the necessary substances to her offspring by the sacrifice of her own tissues. Therefore, during lactation, a rapid exhaustion of fat-soluble factor from the mother's body was observed, when the mother during this period was on a diet deficient in this respect. However, the storage of these substances, though sufficient at birth, certainly did not suffice in the post-natal life of the offspring because they continued to be kept on a fat-soluble factor deficient diet.

A maternal sacrifice of a similar kind caused by a general fasting of the mother during pregnancy has been clearly shown by Rudolsky [1893]. He performed his experiments on a bitch and six rabbits. Nitrogenous and gaseous metabolism were studied in these animals during the whole of the experiment. He reduced their diets to four-fifths in the rabbits and to two-thirds in the

bitch of the normal minimal ration, required for the maintenance of the animals in metabolic equilibrium. Such a reduction of the ration was accompanied by a loss of protein and fat from the animals' tissues. In spite of this, these females, after being mated with normally fed males and kept during pregnancy on the same food régime as before, gave birth to young, the chemical composition of whose bodies was normal at birth. However, when the ration of food given to the mothers was reduced further, poor abnormal young were born. In this case the mothers were so emaciated that they often died, showing a great loss of weight and profound degeneration of their tissues, after giving birth to their young. If the mothers survived, they returned to their previous weight very slowly, if at all, in spite of ample food.

On the basis of the results of our experiments at *the day of weaning and later*, the following conclusions were drawn.

When the mother is fed during *pregnancy* or *lactation* on a diet deficient in fat-soluble factor or calcium there is a marked increase in the disorders of general nutrition and rachitic changes in the skeleton of the offspring kept after weaning on the same rickets-producing diet. Feeding the mother during *lactation* on a diet rich in fat-soluble factor and calcium hinders for a considerable time the disorganisation of general nutrition and the development of obvious rickets in offspring kept on a diet deficient in fat-soluble factor. The results obtained are due to the influence of the mother's diet during pregnancy and lactation upon her nutrition and therefore through the placenta and afterwards through her milk upon the nourishment of the offspring.

In the present investigation we studied the influence upon the young (1) of fat-soluble factor in the mother's diet during pregnancy, and (2) of an excessive amount of calcium in the mother's diet during pregnancy.

(1) *The influence of fat-soluble factor in the mother's diet during pregnancy upon the growth, general nourishment and skeleton of the young rats.*

In our previous paper [1923, 2] we really described only one experiment of this kind (litter 654, pp. 391 and 393), because in the second experiment only one young rat was investigated chemically and histologically from the whole litter (litter 650, p. 391). Owing to the importance of the results obtained, five new experiments were made in the present investigation.

Five pairs of adult rats were mated for this experiment. The mothers before and during pregnancy and the fathers before mating were given $N\,2$[1] diet which contained ample cod-liver oil and butter as the source of fat-soluble factor. The mothers during lactation and the young after weaning were given $-A$ diet, which was deficient in fat-soluble factor.

After birth four litters (Nos. 1–4) were each reduced to five young and one, litter No. 5, to seven young. The results obtained are shown in Tables I and III.

[1] For the composition of this and other diets mentioned in this paper see our previous paper in this journal, p. 1309.

If in these tables we compare the difference in the young born of mothers kept during pregnancy on *N* 2 diet with those born of mothers kept during pregnancy on *NB* 3, *NB* or − *A* diet, we see a definite improvement in the weight and in the degree of calcification of the skeleton of the young of the first group (mothers on *N* 2), which could only be explained by the good diet of the mother during pregnancy. These results are clear both at the day of weaning and when the young were 65 days old.

In these experiments the histological examination did not show the difference between the two groups so clearly as did the chemical analyses, because osteoporosis, or osteoporosis with slight rickets, prevailed. Histological examination was therefore not made in all litters. Slight histological changes from the normal were due to the normal and comparatively good feeding of the mother rats during pregnancy (*NB* diet). In spite of that, when cod-liver oil was added to the maternal diet during pregnancy, the chemical analyses showed quite definite improvement in the calcification of the skeleton of the young. These results prove once more the importance of chemical analyses and the unreliability of only making histological examinations in the investigation of the skeleton.

The general nourishment of the young rats (their size, state of the fur, development and condition of muscle, amount of fat in the body, presence or absence of xerophthalmia, diarrhoea, etc.) was also better in the group in which the mothers were better fed during pregnancy.

(2) *The influence of an excessive amount of calcium in the mothers' diet during pregnancy upon the growth, general nourishment and skeleton of their young.*

For this experiment two pairs of adult rats were mated. The mothers and fathers before mating and the mothers during pregnancy were fed on diet *NB* 3. This diet was similar to the normal *NB* diet (containing butter only as the fat), but the amount of calcium in it was trebled by the addition of $CaCO_3$, so that the fresh diet contained 0·77 % Ca and the air dry diet 1·18 % Ca. The mothers during lactation and the young after weaning were fed on − *A* diet, deficient in fat-soluble factor. The number of young in each litter was reduced after birth to five, ten young rats being used for the experiment.

Comparing the figures in Table II with those in Table III, it is clear that there is no difference at all between the weight or calcification of the skeleton of the *NB* 3 and the *NB* groups of rats. Their degree of nourishment, as well as the histological picture of the skeleton, also showed no essential difference.

So it can be concluded that enriching the normal *NB* diet of the mother during pregnancy with an excess of calcium does not improve the condition of the young nor, in particular, their rachitic skeleton. The marked improvement in the general and in the rachitic changes of the skeleton of the offspring kept on − *A* diet and born from mothers fed on *N* 2 diet during pregnancy, should therefore be ascribed to the beneficial influence of an excess of fat-soluble factor in the mothers' diet during pregnancy.

Table I. *Average chemical composition of the skeleton of young rats kept on a diet deficient in fat-soluble factor (− A), whose mothers before and during pregnancy and whose fathers always were fed on N 2 diet rich in fat-soluble factor and calcium. The diet of the mothers during lactation was deficient in fat-soluble factor (− A).*

	Killed when 24 days old						Killed when 65 days old					
	No. of rats taken for	Av. weight of rats		In bones			No. of rats taken for	Av. weight of rats		In bones		
Litter no.	investigation	♂ g.	♀ g.	H_2O %	Fresh Ca %	Dry Ca %	investigation	♂ g.	♀ g.	H_2O %	Fresh Ca %	Dry Ca %
1	2	35	32	59·6	6·1	15·2	3	68	74	46·7	7·9	15·0
2	2	40	34	57·3	6·3	14·7	3	88	88	45·2	8·7	15·8
3	2	42	40	58·5	6·3	15·1	3	120	112	41·9	11·0	18·8
4	—	—	—	—	—	—	5	85	76	46·5	9·2	17·2
5	—	—	—	—	—	—	7	82	67	45·7	9·4	17·2
Average	—	39	35	58·5	6·2	15·0	—	89	83	45·2	9·2	16·8

Table II. *Average chemical composition of the skeleton of young rats kept on a diet deficient in fat-soluble factor. Diet of the fathers (NB 3) adequate in fat-soluble factor and rich in calcium. Diet of the mothers before and during pregnancy the same as that of the fathers (NB 3); during lactation, deficient in fat-soluble factor (− A diet).*

	Killed when 24 days old						Killed when 65 days old					
	No. of rats taken for	Av. weight of rats		In bones			No. of rats taken for	Av. weight of rats		In bones		
Litter no.	investigation	♂ g.	♀ g.	H_2O %	Fresh Ca %	Dry Ca %	investigation	♂ g.	♀ g.	H_2O %	Fresh Ca %	Dry Ca %
6	2	34	34	64·1	4·8	13·4	3	64	52	51·9	6·8	14·2
7	2	34	33	60·3	4·8	11·9	3	42	71	52·3	6·2	13·2
Average	—	34	34	62·2	4·8	12·7	—	53	62	52·1	6·5	13·7

Table III. *The influence of different diets of mothers during pregnancy upon the young. The mothers' diet during lactation as well as that of the young after weaning was deficient in fat-soluble factor (− A)*[1].

		Weight of young		In bones		
Mothers' diet during pregnancy	Number of rats used for average	♂ g.	♀ g.	H_2O %	Fresh Ca %	Dry Ca %
		At the day of weaning (24 days old)				
N 2	10	38	34	58·4	6·2	15·0
NB 3	4	34	34	62·2	4·8	12·7
NB	63	31	30	61·4	5·0	12·7
−A	5	19	19	68·3	2·6	8·2
		When 65 days old				
N 2	25	90	85	44·5	9·3	16·6
NB 3	6	53	62	52·1	6·5	13·7
NB	62	62	53	50·9	6·8	13·8
Normal rats about 65 days old	19	140	108	43·8	11·5	20·5

[1] For this table data from previous papers have been used as well as from the present experiments.

General Conclusions.

1. When the mother's "normal" diet during pregnancy is enriched with an excess of the fat-soluble factor, there is a marked decrease in the disorders of general nutrition and in the rachitic changes in the skeleton produced in the young by a diet deficient in fat-soluble factor, even when the mother is also kept on the same insufficient diet during lactation.

2. This effect is not produced by giving an excess of calcium during pregnancy, provided that the mother's "normal" diet already contains an adequate amount of calcium.

3. Therefore, in order to decrease the above-mentioned disorders in the young, an excessive amount of fat-soluble factor and not of calcium is of great importance in the diet of the mother during pregnancy. At the same time, the importance of an adequate amount of calcium in the mother's diet is emphasised.

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Hess and Weinstock in their recent paper [1924] came to the conclusion that the improvement of the maternal diet during pregnancy and lactation mitigated rickets to a certain degree but they were unable to obtain such striking results as we did. This probably can be explained by the fact that we used a diet deficient in fat-soluble factor only, whereas the diet used by them was also deficient in phosphorus. Therefore the improvement to be expected by these workers would certainly be less pronounced owing to the greater deficiency of the diet used.

Unfortunately Hess and Weinstock did not make any chemical analyses of the skeletons of the experimental animals and we have been able to show several times that in experiments of this kind objective chemical data are often even more important than histological, the latter being sometimes too subjective.

Recent experiments by Byfield and Daniels, M. Jones, and Goldblatt have corroborated the results obtained in our investigations. In favour of our conclusions are also results obtained by Dutcher and Kennedy, Drummond, Coward and Watson, Reigher, and Luce, who showed, that the content of fat-soluble factor in the milk and its antirachitic value depend on the diet of the cow.

Hess and Weinstock's observations on 28 women, who each took 16 ounces of cod-liver oil during pregnancy, showed somewhat similar results to those, which we obtained in our experiments on rats. The babies of 15 of these

women developed rickets, but this diagnosis could only be verified by X-ray examination in eight cases. In our experiments we also were unable to obtain protection against rickets in young rats by giving cod-liver oil to the mother during pregnancy, but were only able to decrease the severity of the rachitic disorder.

REFERENCES.

Hess and Weinstock (1924). *J. Amer. Med. Assoc.* **83**, 1558.
Korenchevsky (1921). *Brit. Med. J.* ii, 547.
—— (1922, 1). *J. Path. Bact.* **25**, 366.
—— (1922, 2). *Med. Res. Council, Spec. Rep. Series*, No. 71.
—— (1923). *Brit. Med. J.* i, 802.
Korenchevsky and Carr (1923, 1). *Biochem. J.* **17**, 187.
—— —— (1923, 2). *J. Path. Bact.* **26**, 389.
Rudolsky (1893). *Dissertation* (Petrograd (Russian)).