THE IMPORTANCE OF PRENATAL DIET

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SEVERAL years ago Dr. Rose Nelson and I encountered a strange phenomenon. On a diet, the composition of which I will show you presently, anatomically normal female rats produced offspring one-third of which showed congenital malformations. The males, also anatomically normal, were fed an adequate stock diet.

The deficient diet, which we called Diet 1, consisted of yellow corn meal, wheat gluten, calcium carbonate, sodium chloride, the diet which you know as the Steenbock and Black diet. If you try to raise and breed rats on this diet you will encounter difficulties. The diet is deficient in many respects and so we added for certain reasons, viosterol to prevent rickets (Table 1).

On this diet we found that of 761 animals, 517 appeared anatomically normal and 244 (an average of 32 per cent) abnormal. Figure 1 shows some of these abnormalities. On the left is a normal newborn rat and on the right are three abnormals. These abnormals can usually be recognized by external inspection. You will notice short mandible, protruding tongue, shortness of the upper extremity, syndactylism of different degrees; then shortness of the lower extremity, and shortness of the lower extremity combined with clubfoot. The skeletal abnormalities can be seen much better if one clears the specimens with the Schultze-Dawson method.

In Figure 2 on the left, the skeleton of a normal newborn rat is seen, and on the right three abnormals. They show different degrees of abnormality. There is a pattern in this variety, in that certain bones are less affected than others. You will notice that the skull and the vertebral column are spared. The humerus and the femur are also usually spared, but the lower arm and the leg are affected.

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The defects of the ribs can also be seen. Some of these defects can be seen in detail in the three abnormals.

Note the normal chest and the regularity of the ribs in the normal newborn rat. In the first abnormal you may see just a slight approaching of two ribs. It is usually the middle ribs that are affected.

Table 1. Diet 1.

	Per Cent
Yellow Corn Meal	76
Wheat Gluten	20
Calcium Carbonate	3
Sodium Chloride	1
Vitamin D: 6 I.U. Daily as Viosterol	

The skeleton of the second abnormal newborn rat shows a further degree of fusion of ribs and in the third abnormal you see a complete fusion into a cartilaginous plate. By the way, the expression "fusion" is incorrect. One should say "nonseparation." We have studied such bones histologically and found that the ribs do not differentiate normally from the mesenchymal plate.

In Figure 3, there is a normal radius and a normal ulna, and in Figure 4A are slightly defective ones. You see that these two bones are shorter than normal. Figures 4B and 4C show further degrees of shortening, and Figure 4D, complete absence of the radius and ulna; a marked micromelia can be seen. If we speak of absence of a structure we mean the absence of bone. Cartilaginous structures may be present but they are defective too.

Figure 3 shows a normal tibia and fibula, and in Figure 4A a shortened tibia and a somewhat shortened fibula are shown. The tibia is more often abnormal than the fibula. Further degrees are shown in Figures 4B, C, and D.

Figure 5 shows various degrees of syndactylism. Figure 5A represents a normal hand of a newborn rat. In Figure 5B there is incomplete syndactylism of fingers III and IV and a slight separation is still visible. In Figure 5C there is complete syndactylism and in Figure 5D a marked form of syndactylism and brachydactylism.

I may add here that we have no control over the degree of these abnormalities. We may get normal and abnormal animals in one litter, and we may observe marked degrees of abnormality and milder abnormalities in the same litter.

Table 2. Frequency of osseous defects in 100 cleared abnormal specimens.

Tibia	93	Ulna	50
Mandible	80	Humerus	34
Ribs	75	Hindfoot	31
Fibula	63	Maxilla	8
Radius	58	Scapula	6
Hand	54	Clavicle	6
Sternum	52	Femur	1

Figure 6 shows on the left a normal palate of a newborn rat, and on the right a cleft palate. Interestingly enough, I think cleft palate was never described in the rat although it is described in many other animals. We never encountered a harelip in our specimens.

Figure 7 shows a mild degree of cleft palate, the so-called posterior palate cleft. Figure 8 represents a section through a head with a cleft palate in which you see a communication between the nasal cavity, the nasopharyngeal duct, and the mouth.

Table 2 lists the frequency of the various defects in 100 cleared abnormal specimens. The tibia is most often affected, shortness of the mandible follows, the ribs, fibula, radius, hand, sternum, ulna, palate, humerus, foot come next, and finally there are rarely affected bones like the scapula, clavicle, and femur.

We did not take it for granted that we were dealing with a nutritional phenomenon. We know that the appearance of normal and abnormal animals in one and the same litter could be interpreted as a result of a genetic combination. It was thought possible that we were dealing with a genetic abnormality which just happened to show up in rats fed a deficient diet. Yet the following experiments are hardly compatible with any other explanation than the assumption of a maternal nutritional deficiency which manifests itself in abnormalities of the offspring.

On an adequate stock diet, parent rats of the same strain, the Sprague-Dawley strain, never produced abnormal young of the pat-

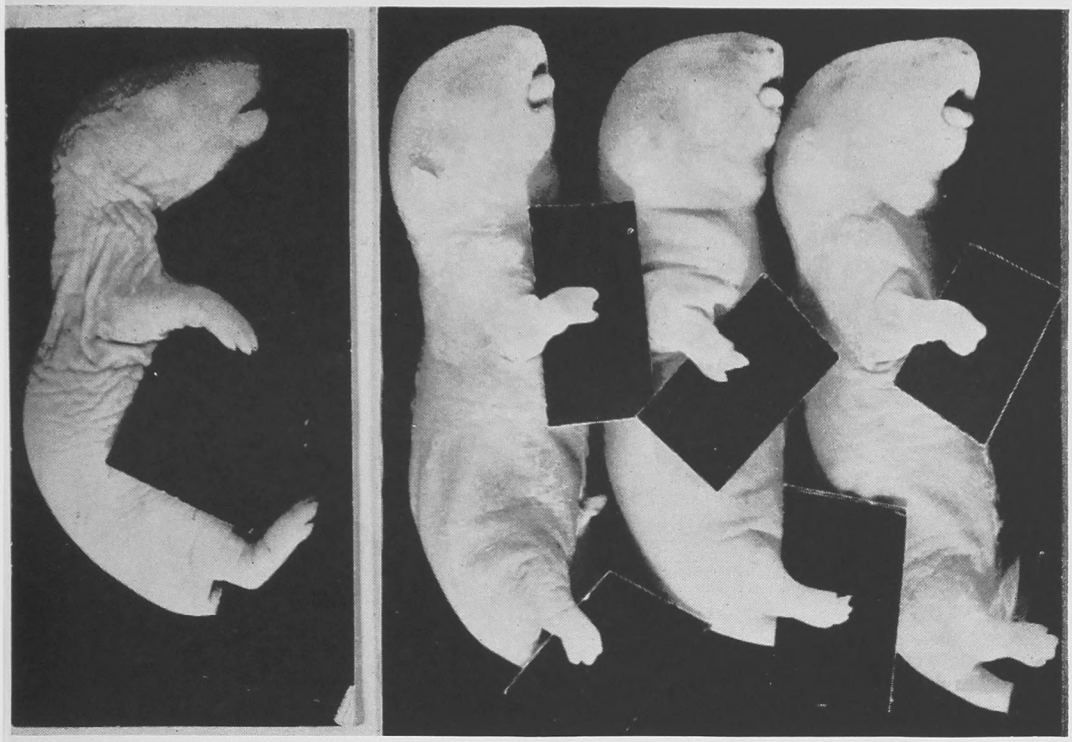


Fig. 1

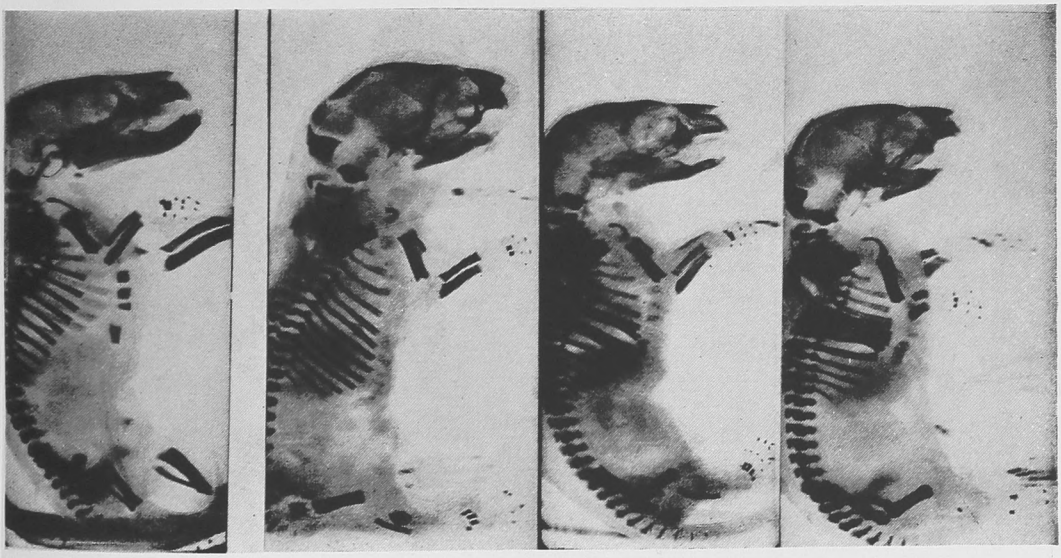


Fig. 2

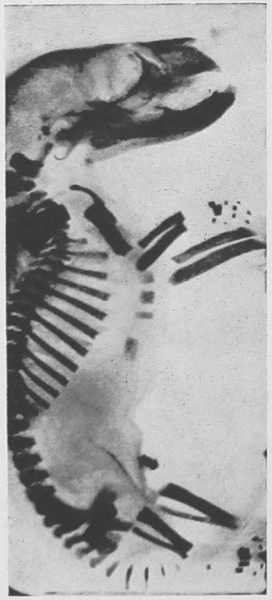
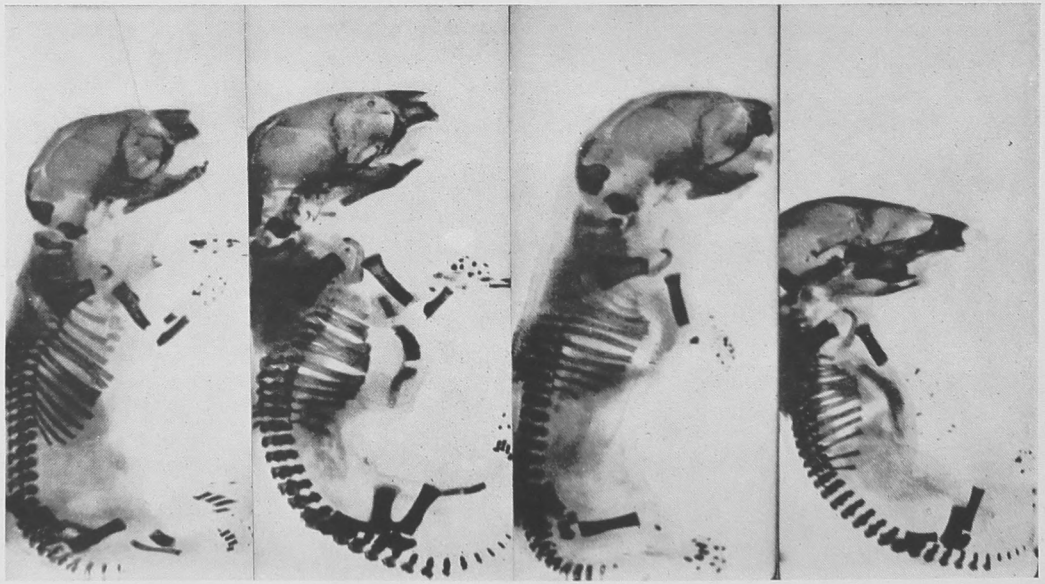


Fig. 3



A B C D

Fig. 4

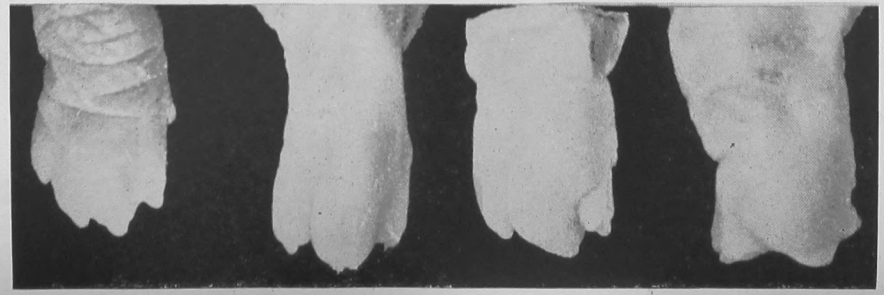


Fig. 5

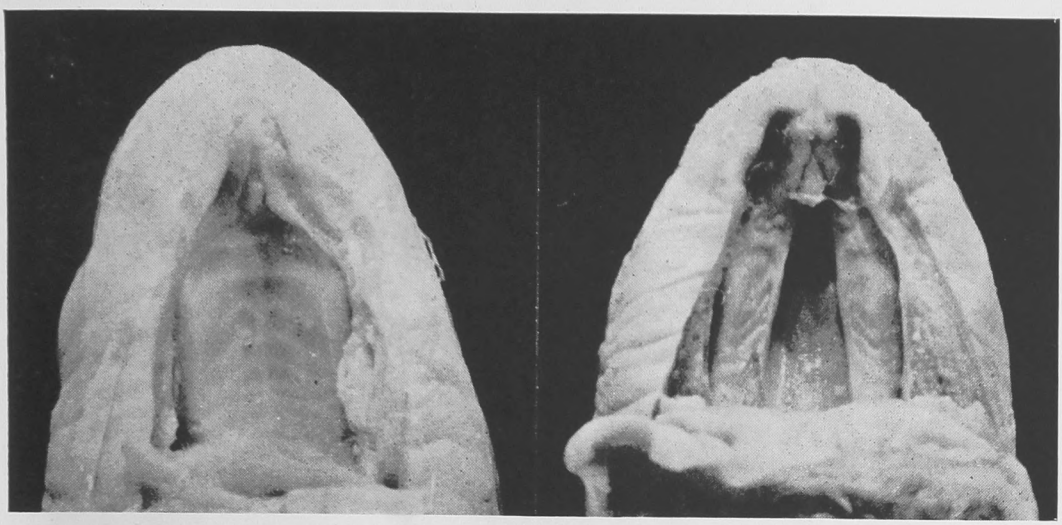


Fig. 6

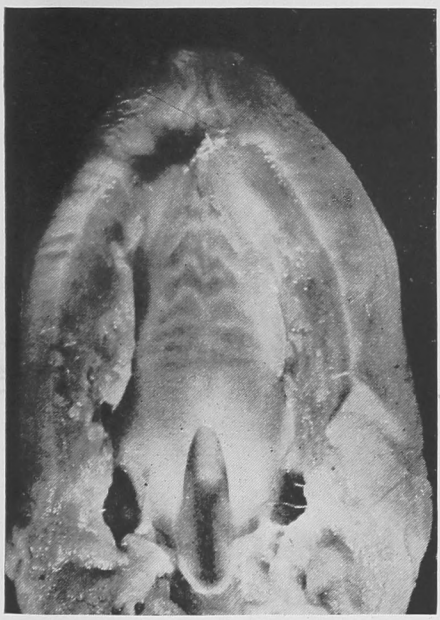


Fig. 7

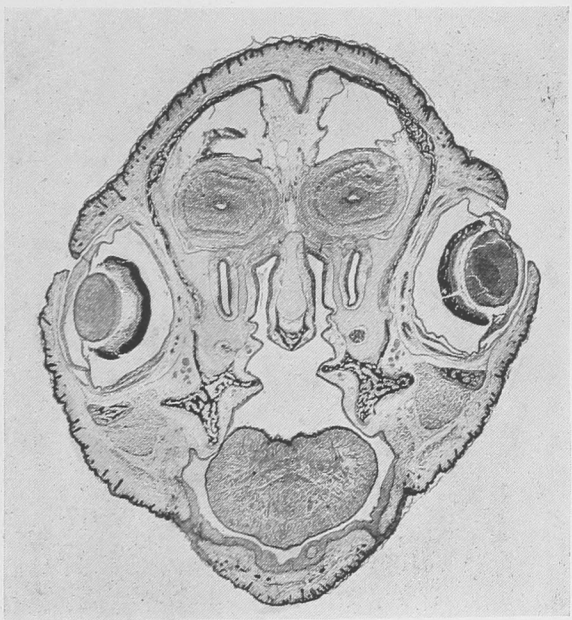


Fig. 8

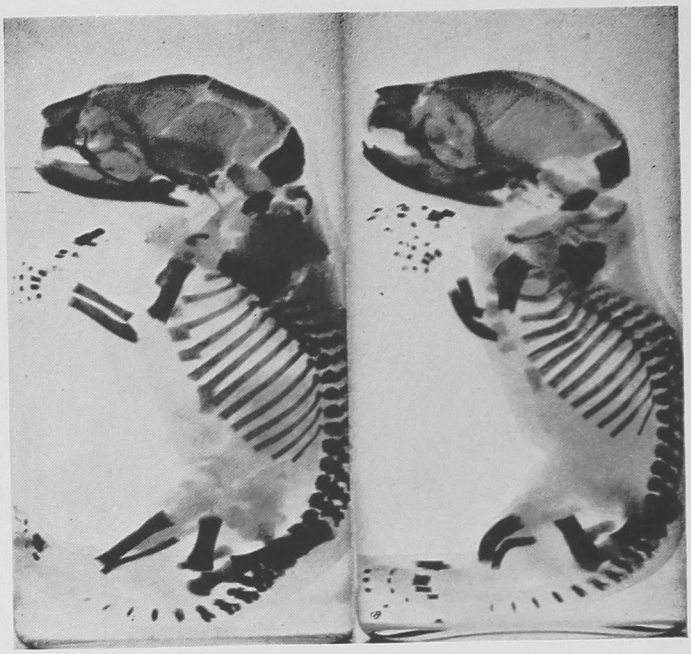


Fig. 9

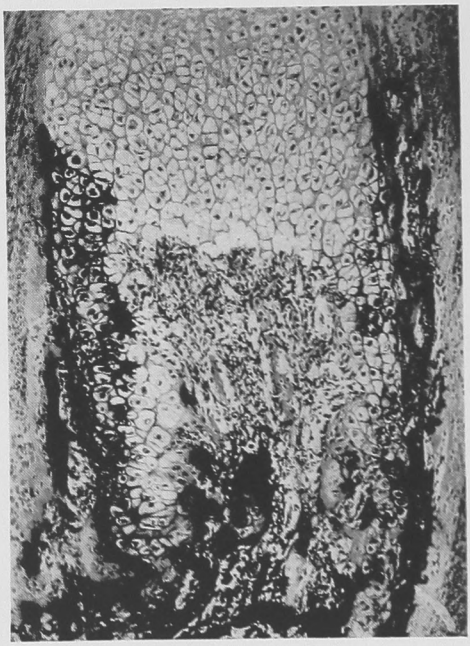


Fig. 10

tern of Diet 1. In 833 control rats we had 832 normals and only one abnormal. The one abnormal rat had a normal tibia and fibula and a short humerus and a short tail, a pattern which does not correspond to the pattern of Diet 1.

Very soon we found that the addition of 2 per cent of dried pig liver to Diet 1 prevented the abnormalities. We obtained on this diet 332 young; they were all normal.

The breeding results on alternating diets were as follows: Rat 45-B was raised on Diet 1 and bred and we obtained four abnormal rats. She was bred again on Diet 1, with the same result. Then liver was given and eight normals were obtained. If one adds liver to the diet of such rats, one obtains always normals. However, if one gives Diet 1 to a rat that had Diet 1 plus liver before, the first subsequent litter may be normal and it may be necessary to breed the rat again on the deficient diet before an abnormal litter is obtained. That can be explained easily by a storing of the factor in question, while the rat received liver. But this rat behaved very nicely. When she was put back on Diet 1 she produced one abnormal out of seven young; put back on liver, ten normals; and back on Diet 1, eight normals, and two abnormals; Diet 1, seven abnormals; changed back to Diet 1 and liver, three normals; after that she, unfortunately, died. This whole offspring was strictly legitimate and that is important, because the same male was used for all these matings.

I think no geneticist will be able to find a form of genetic transmission in which two normal rats can produce offspring in that way.

These experiments convinced us that we were dealing with a nutritional phenomenon. Diet 1 is obviously deficient in a factor which is present in large amounts in liver. The search for this factor was complicated by the fact that only one-third of the offspring of mothers on Diet 1 could be expected to be abnormal and that the first litters were less often abnormal than second and later litters. Therefore, a large number of normal offspring was necessary to prove that the supplement to Diet 1 was preventive, while it required only a

few abnormal young to eliminate a substance from the search.

Liver-ash added to Diet 1 did not prevent abnormality. There were twenty-one abnormals out of 163. The percentage given here should not induce us to draw conclusions because when we got a few abnormal litters frequently we stopped the experiment, since we knew that the substance tested was not preventive. Thus, liver-ash did not prevent; casein added to Diet 1 did not prevent; cod-liver oil did not prevent; wheat germ oil did not prevent. We tested a few other substances but it is not important to name them all.

We know that liver is a good source of the vitamin B complex, so we tried, together with Miss Elizabeth Schraffenberger, to ascertain the preventive power of some crystalline vitamins of the B complex.

A mixture of riboflavin, thiamine, niacin, pyridoxine, and pantothenate proved preventive. There were 371 young born of mothers on this supplemented diet, and they were all normal. It remained to be shown which of these five substances or which combination would prove preventive.

Thiamine, niacin, pyridoxine, and pantothenate together were not preventive. Then a pyridoxine-pantothenate mixture did not prevent. Thiamin alone and niacin alone did not prevent.

Riboflavin, on the other hand, prevented. We knew, therefore, that riboflavin was preventive, but we still were not quite sure if the rest of Diet 1 did not somehow contribute to the phenomenon. So we did the crucial experiment. We used a purified diet in which most of the food elements were of known chemical composition.

On a maternal diet of sucrose, casein, vegetable oil, salt mixture, vitamins A, D, E, K, thiamine, niacin, pyridoxine, pantothenate, and choline, the abnormalities were obtained when riboflavin was omitted. With riboflavin the abnormalities were prevented.

The abnormal offspring obtained from females on the riboflavin-free purified diet showed a pattern entirely identical with that of Diet 1, that is, shortness of the mandible, shortness of the arm, fusion of ribs, shortness of tibia, and cleft palate.

The abnormalities of the pattern of Diet 1 can thus be prevented by riboflavin. We have spent a great deal of time and effort to explain the pathogenesis of these skeletal defects. Time does not permit me to report these experiments today, but it may be mentioned that we assume at the present time that the rapid development from mesenchyme of the membranous skeleton, the forerunner of the cartilaginous and osseous skeleton, is inhibited by this nutritional deficiency.

Table 3. Diet R.

	Per Cent
Yellow Corn Meal	76
Wheat Gluten	18
Calcium Carbonate	3
Sodium Chloride	1
Dried Pig Liver	2

It is of interest that a very slight or quantitatively slight change of the maternal Diet 1 may induce congenital defects of an entirely different type.

Table 3 shows again the Steenbock and Black diet, but this time not supplemented by vitamin D but by dried pig liver. We had to give that supplement because on the Steenbock and Black diet the rats did not breed. When pig liver was added, they bred much better. We had even third and fourth litters on such a diet.

This diet is rachitogenic. The addition of dried pig liver does not change the rachitogenic qualities of the diet. If you put rats after weaning on this diet, they develop severe rickets.

We called this diet (the Steenbock and Black diet supplemented by dried pig liver) Diet R. On this Diet R, we had 136 normals and 112 abnormals, or 45 per cent, abnormalities. One cannot recognize these abnormalities on external inspection. The rats are usually born dead or they are in poor shape, but they appear anatomically normal. However, if you clear them with the Schultze-Dawson method, you can see the skeletal abnormalities.

In Figure 9 on the left, a normal rat can be seen and on the right you may see the bending of the tibia, of the fibula, of the ulna, and

of the radius. You can see a peculiar abnormality of the ribs; these ribs become suddenly broad and there is an angle in their bodies. The middle ribs chiefly are affected. There is never any overlapping of the two patterns of Diet 1 and of Diet R.

We thought we were dealing with congenital rickets. There is bending of the shaft and a definite cupping which might indicate that we are dealing with congenital rickets. But the situation is more complicated than we thought. You see in the abnormal tibia a mass of cartilage. The zone of cartilage is much deeper in the abnormal than in the normal bone, and there is a shortness of the osseous part.

The higher magnification in Figure 10 shows an interesting picture. There is marked cupping and a mass of cartilage, also blood vessels, but the histologic characteristic of rickets, the osteoid is missing; or the osteoid that is present is not abundant. We are confronted with an interesting fact: the vitamin D deficiency manifests itself in the fetus in a different way than in the infantile rat.

That a vitamin D deficiency is responsible for this condition can be deduced from the fact that only normal young were obtained when Diet R was supplemented by viosterol.

This brief outline of our experiments indicates that congenital malformations may be caused by maternal nutritional deficiency. The science of nutrition should be concerned, therefore, not only with the maintenance of the human machine but should also pay attention to its sound construction.

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