STUDIES ON EXPERIMENTAL RICKETS.

I. THE OCCURRENCE OF RICKETS IN YOUNG RABBITS BORN OF MOTHERS INFECTED WITH SCHISTOSOMUM JAPONICUM.

By R. KAWAMURA, M.D., and Y. KASAMA, M.D.

(From the Pathological Laboratory of Niigata Medical University, Niigata, Japan.)

Plates 43 and 44.

(Received for publication, June 4, 1925.)

In spite of the fact that rickets can be produced by a variety of experimental methods, and this disease can be cured or prevented, the real mechanism by which rickets is produced remains uncertain. Believing, therefore, that any new method or condition which can cause this disease may be a matter of great interest, we wish to record here our findings.

A parental influence on the predisposition of the young to this disease was suggested as early as the seventeenth century by Glisson (1668). Since then, numerous investigators have shown either by statistics or by experiments, that certain maternal nutritive factors such as calcium, phosphate, and the antirachitic vitamin can exert a considerable influence on the generation following after. Byfield and Daniels (1923) state that in a series of animal experiments, it has been possible to produce gross rickets by faulty diet into the second generation only.

During the course of our investigations on the study of certain parasitic diseases, we have accidentally encountered rickets in young rabbits born of mothers infected with the parasites. The preliminary report of this investigation was made before the Japanese Pathological Meeting in 1922.

EXPERIMENTS.

Female rabbits were infected with Schistosomum japonicum by rubbing on the well shaven and scarified skin of their abdomen the cercariae, obtained by emulsifying a fresh water snail, Katayama 793.
nosofora robsoni. Some of these infected females survived and became pregnant. During the whole course of the experiment, the experimental and normal control rabbits were fed with exactly the same diets, which, we had assured ourselves through other experiments in producing experimental rickets, did not give rise to this condition. To all appearances, both groups of the animals ate equally well, and no evidence of a deficiency was noted. Young rabbits born of the normal mothers showed a normal rate of growth, whereas those born of infected mothers developed signs of rickets between 25 and 35 days after birth; their growth also was considerably retarded, as shown in Table II, and the necropsy gave gross evidence of rickets. The joints of the extremities, especially of the wrist, ankle, and knee, were enlarged and thickened. The rosary at the rib joints was evident, and sometimes fractures of ribs were observed. A kyphoscoliotic disturbance of the vertebrae occurred in some cases (see Fig. 1).

<table>
<thead>
<tr>
<th>Infected mother No</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Total offspring born</td>
</tr>
<tr>
<td>Rachitic young</td>
</tr>
</tbody>
</table>

Microscopically, there were typical rachitic changes, namely proliferation of the cartilage zone, thickening of unossified metaphyses, fibromatosis, and hyperemia of the bone marrow in the epiphyses. The calcium of the bones was greatly reduced (see Fig. 1, 2, and 4).

The ratios of the rachitic young to the total offspring are shown in Table I.

Of all the young born of the infected mothers, we found 67.7 per cent rachitic. In these animals, the rachitic change reached its maximum in 50 to 60 days and in 90 to 100 days it had either declined or disappeared.

In order to see whether these rachitic changes were identical with those produced experimentally in rats and dogs by various investigators, we fed some of the affected young with cod liver oil. We selected two groups of the young which were afflicted in about the same degree with rickets, as shown in Figs. 6 and 7. The right fore
paws of both groups of animals were amputated, under ether anesthesia and aseptic conditions, when they were 54 days old, and to one group, 0.2 to 0.5 cc. cod liver oil was given daily thereafter, the other group being kept as a control. At the end of 27 days, both groups were killed, and the left hind paws were examined. As shown in the x-ray photographs (Figs. 8 and 9), there was no sign of rickets detectable in those young fed with the cod liver oil, while the controls were still markedly rachitic.

Thus it seemed evident that the rickets produced in the young born of mothers afflicted with Schistosomum japonicum is the same as that produced experimentally by the usual methods in other animals. Details of the experiment are given in Table II.

Since, as stated before, the experimental conditions were such that neither dietary nor environmental factors could be the direct cause of these changes in the young, it is safe to conclude that the congenital factor is responsible for this disease. The question, however, whether it is of hereditary nature or is due to a direct transmission of toxin from the parasites of the mother to the young is yet to be answered.

In order to see whether the parasites can cause rickets directly, we infected young healthy rabbits with them.
As shown in Table III, when young weighing less than 500 gm. were infected with *Schistosomum japonicum*, slight signs of rachitic change are noticeable within 13 days after inoculation. These changes reach their maximum within 30 to 60 days and gradually diminish but they are detectable even after 75 days. After 90 to 140 days, however, even a microscopic examination reveals no sign of rickets. It may be added here that this parasite causes rickets only in the young, never producing this disease in adults.

**TABLE III.**

<table>
<thead>
<tr>
<th>Method of experiment</th>
<th>Mother No.</th>
<th>No. of young taken</th>
<th>No. of rachitic animals</th>
<th>Time between autopsy and amputation (days)</th>
<th>Age at amputation (days)</th>
<th>After amputation</th>
<th>Body weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young rabbits infected with parasite</td>
<td>I</td>
<td>4</td>
<td>4</td>
<td>24</td>
<td></td>
<td></td>
<td>360</td>
</tr>
<tr>
<td>Control</td>
<td>IV</td>
<td>2</td>
<td>0</td>
<td>76</td>
<td></td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>Young rabbits infected with parasite</td>
<td>6</td>
<td>6</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td>320</td>
</tr>
<tr>
<td>Control</td>
<td>III</td>
<td>1</td>
<td>0</td>
<td>68</td>
<td></td>
<td></td>
<td>550</td>
</tr>
<tr>
<td>Late findings in young rabbits</td>
<td>II</td>
<td>6</td>
<td>0</td>
<td>30</td>
<td>32</td>
<td>97</td>
<td>170</td>
</tr>
<tr>
<td>Control</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>570</td>
</tr>
<tr>
<td>Adult rabbits infected with parasite</td>
<td>VI</td>
<td>2</td>
<td>0</td>
<td>82</td>
<td>74</td>
<td>144</td>
<td>550</td>
</tr>
<tr>
<td></td>
<td>VII</td>
<td>3</td>
<td>0</td>
<td>82–136</td>
<td></td>
<td></td>
<td>1520–1700</td>
</tr>
</tbody>
</table>

**TABLE IV.**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mother No.</th>
<th>No. of young</th>
<th>No. of rachitic young</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed with egg yolk</td>
<td>EI</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>VIII</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VIII</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Published December 1, 1925
In order to see whether the action of the parasite is specific or not, we have conducted a few preliminary experiments, the results of which are given in Table IV. As shown in the table, the young rabbits born of mothers fed with fowl egg yolk have rickets. From the mothers which were used in experiments in connection with the investigation of a tumor problem some of the young born became rachitic. These rachitic changes became milder as the young grew. If, however, the young rabbits are fed directly with the egg yolk, no rickets is produced. It is conceivable that the changes after feeding egg yolk may come about through the same mechanism as those induced by the parasites.

DISCUSSION AND CONCLUSION.

Our observations show that young rabbits born of mothers afflicted with Schistosomum japonicum develop typical rickets. Rickets can also be produced if we infect the young, healthy rabbits with the same parasite. It is natural to suppose that the rachitic changes are caused by the parasite itself. Since, however, a similar disease can be produced in the offspring, when the mother is fed on egg yolk, the causation is not limited to the action of this parasitic toxin alone.

The toxin of Schistosoma may disturb the calcium and phosphorus metabolism of bone in young animals, especially in the period of vigorous growth; that is, 20 to 40 days after birth of the rabbits. Or it may exhaust some element important in the calcium and phosphorus metabolism such as vitamin A or D. The fact that exhaustion of the antirachitic factor in the mother causes rickets in the young, as Grant (1924) showed, and that certain low grade infections can exhaust vitamin B as shown by Wedgewood (1924), is in line with this conception.

It may be added here that most investigations on rickets have been carried out on rats and dogs. We have found a simple and excellent way of producing rickets in rabbits by dietary deficiency. Concerning this method, we shall report elsewhere.

BIBLIOGRAPHY.

STUDIES ON EXPERIMENTAL RICKETS. I

Marfan, A. B., Semaine méd., 1906, xxvi, 481, cited above by Byfield and Daniels.

EXPLANATION OF PLATES.

PLATE 43.
Fig. 1. X-ray photograph of Rabbit 1, born of mother injected with Schistosomum japonicum.
Fig. 2. Photomicrograph of a section of radius of Rabbit 1 (rachitic).
Fig. 3. Section of radius of Rabbit 2, born of normal mother.

PLATE 44.
Fig. 4. Section of radius of Rabbit 4 (33 days old), born of injected mother.
Fig. 5. Section of radius of Rabbit 5 (33 days old), born of normal mother.
Fig. 6. Right hind paw of Rabbit 20, amputated at 54 days old.
Fig. 7. Right hind paw of Rabbit 19, amputated at 54 days old.
Fig. 8. Left hind paw of Rabbit 20, fed with cod liver oil for 27 days after the first amputation (cf. Fig. 6).
Fig. 9. Left hind paw of Rabbit 19, 27 days after first amputation, no cod liver given (cf. Fig. 7).
(Kawamura and Kasama: Experimental rickets, I.)