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**Rickets in Rats. V.\* Comparison of Effects of Irradiated Ergosterol and Cod Liver Oil.**

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Hess, in collaboration with Windaus<sup>1</sup> and others, have achieved the cure of rickets by minute amounts of irradiated ergosterol. Most of the studies on the cure of rickets in experimental animals are based upon the X-ray pictures, histological preparations of the bones, and blood serum analyses for calcium and phosphorus. Quantitative data are, however, supplied only by metabolism studies<sup>2, 3, 4</sup> and the analyses of the ash of the dry fat-free bones.<sup>5, 6, 7</sup> By use of technique described elsewhere<sup>8, 9</sup> we desired to compare by the various analytical procedures the cure of standardized ricketic rats by cod liver oil and ergosterol.

Animals fed on Sherman Diet B, when 28 days old were placed on Steenbock and Black's high calcium-low phosphorus diet 2965 plus 10% lard. By analysis the diet contained 1.07% calcium and .178% phosphorus. The ratio of Ca/P was 6.0. They were kept on this regime for 21 days. Then the diet was altered by replacing 2% of the lard by cod liver oil, or irradiated ergosterol in olive oil.† Both were fed separately and not mixed with the diet. The amount of ergosterol represented .01 mg. per rat per day.

The animals were killed after 14 days on the curative diet. The autopsies showed intramuscular hemorrhages in the upper hind ex-

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<sup>1</sup> Hess, A. F., *J. Am. Med. Assn.*, 1927, lxxxix, 337.

<sup>2</sup> Hess, A. F., and Sherman, E., *J. Biol. Chem.*, 1927, lxxiii, 145.

<sup>3</sup> Boas, M. A., *Biochem. J.*, 1926, xx, 153.

<sup>4</sup> Schultzer, P., *Biochem. Z.*, 1927, clxxxviii, 427.

<sup>5</sup> Bethke, R. M., Steenbock, H., and Nelson, M. T., *J. Biol. Chem.*, 1923, lviii, 71.

<sup>6</sup> Dutcher, R. A., Creighton, M., and Rothrock, H. A., *J. Biol. Chem.*, 1925, lxvi, 401.

<sup>7</sup> Chick, H., Korenchevsky, V., and Roscoe, M. H., *Biochem. J.*, 1926, xx, 622.

<sup>8</sup> Karelitz, S., and Shohl, A. T., *J. Biol. Chem.*, 1927, lxxiii, 665.

<sup>9</sup> Shohl, A. T., and Bennett, H. B., *J. Biol. Chem.*, 1927, lxxiv, 247.

† The irradiated ergosterol was generously supplied us by Dr. Charles N. Frey of the Fleischmann Laboratories, New York City. This was irradiated in ether for 30 minutes at 28 inches with a mercury arc lamp. The ergosterol was taken up in olive oil.

tremities of 2 of the rats which received ergosterol. The rats were studied as to (1) blood serum analyses, (2) histological examination of the bones, (3) bone analyses, and (4) metabolism of calcium and phosphorus.

TABLE I.  
*The analyses of the blood serum for calcium and phosphorus.*

|                             | Calcium<br>mg. % | Phosphorus<br>mg. % |
|-----------------------------|------------------|---------------------|
| Cod liver oil .....         | 13.8             | 5.5                 |
| Irradiated Ergosterol ..... | 11.7             | 5.8                 |
| Rickets Control .....       | 9.9              | 3.7                 |

These rats were made ricketic on Steenbock's diet 2965 plus 10% lard for 21 days and then given additions of the curative agent for 14 days.

1. *The blood serum analyses*, as shown in Table I, indicate a marked rise in the serum phosphate above that of the rickets control with both cod liver oil and irradiated ergosterol. The calcium is also definitely raised. This degree of elevation of the serum phosphate is never present as a consequence of this diet unless rickets has been cured.

2. *The histological preparations* show marked unhealed rickets in the rickets control group. The bones of the group receiving cod liver oil and that with ergosterol show substantially the same condition. A narrow metaphysis is present; calcification in the provisional zone is complete and in a straight line; the trabeculae are straightening and osteoid tissue is being resorbed. All these are indications of moderately advanced healing.

TABLE II. *Analysis of femur.*  
Values in terms of one femur.

|                            | Rickets<br>Control | Cod Liver<br>Oil | Irradiated<br>Ergosterol |
|----------------------------|--------------------|------------------|--------------------------|
|                            | mg.                | mg.              | mg.                      |
| Wet weight .....           | 240.0              | 280.0            | 280.0                    |
| Dry weight .....           | 107.0              | 136.0            | 133.0                    |
| Fat free .....             | 90.0               | 100.5            | 110.0                    |
| Organic .....              | 61.7               | 56.4             | 60.5                     |
| Ash .....                  | 28.3               | 45.0             | 49.5                     |
| Ash in % of fat free ..... | 31.6               | 45.0             | 45.0                     |
| A/R .....                  | 0.46               | 0.80             | 0.82                     |

Analysis of the bones of the rats at the age of 63 days.

3. *The analysis of the bones*, as shown in Table II, indicate marked deficiency in calcification in the rickets control group. Those receiving cod liver oil and ergosterol have definite increase in their ash content. At this age the bones of normals contain about 60% ash, but the normal rate of increase of ash is exceeded by that of the animals which have been cured with cod liver oil or ergosterol.

TABLE III.  
*Metabolism of Calcium and Phosphorus.*

Figures in terms of 1 rat per week.

|                   | Food | Stool | Urine | Spill | Total | Bal. | Food | Stool | Urine | Spill | Total | Bal. | Ca/P<br>in Bal. | Stool | Weight<br>inc. |
|-------------------|------|-------|-------|-------|-------|------|------|-------|-------|-------|-------|------|-----------------|-------|----------------|
|                   | mg.  | mg.   | mg.   | mg.   | mg.   | mg.  | mg.  | mg.   | mg.   | mg.   | mg.   | mg.  |                 | gm.   | gm.            |
| <i>Irradiated</i> |      |       |       |       |       |      |      |       |       |       |       |      |                 |       |                |
| <i>Ergosterol</i> |      |       |       |       |       |      |      |       |       |       |       |      |                 |       |                |
| Period I          | 506  | 331   | 115   | -70   | 376   | 130  | 84   | 58.8  | 2.5   | -8    | 54    | 30   | 4.35            | 3.58  | 7.0            |
| Period II         | 506  | 360   | 110   | -60   | 410   | 96   | 84   | 63.   | 14.1  | -8    | 77    | 7    | 13.0            | 3.93  | 7.7            |
| <i>Cod L. O.</i>  |      |       |       |       |       |      |      |       |       |       |       |      |                 |       |                |
| Period I          | 548  | 254   | 99    | -10   | 343   | 205  | 90   | 51.4  | 2.1   | -1.3  | 52    | 38   | 5.4             | 3.64  | 4.1            |
| Period II         | 505  | 295   | 113   | -14   | 394   | 211  | 83   | 59.5  | 2.3   | -3    | 59    | 24   | 8.8             | 4.22  | 7.1            |

See footnote to Table I.

4. *The Metabolism of Calcium and Phosphorus.* The data given in Table III show that the food intakes, the weight of the feces and the growth of the rats are similar to those on the rickets control diet.<sup>9</sup> The analysis of the food revealed that the phosphorus con-

tent was extremely low compared to that used in other experiments. The phosphorus was .177% instead of .254%. The ratio of Ca/P, which in former experiments was 4.25, in this experiment was 6.0.

The metabolism data demonstrate that the cure of rickets is not necessarily associated with great increase in the phosphorus balance. On this severe diet, negative balances of phosphorus would probably result without the curative agent. In the rickets controls, the calcium is retained in excess of the phosphorus; in the cured animals even larger amounts of calcium were retained. This indicates that healing of the condition causing deficient bone deposition may be effected by much efficient utilization of the phosphorus as well as by increase in the retention.

*Summary:* Marked cure of rickets in rats is secured in 2 weeks, by cod liver oil and by .01 mg. daily of irradiated ergosterol. This is shown by histological preparations of the bones, the analyses of the blood serum for calcium and phosphorus and the ash analyses of the bone. The metabolism studies demonstrate that the cure is accomplished without great increase in the retention of calcium or phosphorus.

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#### Prediction of the Basal Metabolism of Infants from the Measured Insensible Perspiration.

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Measurement of the respiratory exchange of infants by present methods is time-consuming and technically difficult. The result is that only 3 or 4 pediatric clinics in America are making any attempt at present to study the subject. This paucity of data is regrettable since a knowledge of the energy metabolism furnishes the scientific basis for the feeding of infants in health and in nutritional disturbances, besides yielding valuable information in many other conditions. For these reasons, the attempt has been made to extend to infants the method proposed by Benedict and Root<sup>1</sup> for predicting the probable metabolism of adults from the insensible perspiration as measured with the aid of a delicate balance. The results thus far obtained with the method are presented in this preliminary report.

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<sup>1</sup> Benedict, F. G., and Root, H. F., *Arch. Int. Med.*, 1926, xxxviii, 1.