EXPERIMENTAL RICKETS IN RATS.

V. THE EFFECT OF VARYING THE ORGANIC CONSTITUENTS OF A RICKETS-PRODUCING DIET.*

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WITH THE COLLABORATION OF S. M. SEIDLIN, J. V. LICHTENSTEIN, AND M. STANLEY-BROWN.

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PLATES 25 TO 29.

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In a preceding article (1) it was shown that rachitic lesions may be regularly induced in young growing rats by a deficiency of PO₄ in the diet, provided the Ca ion is present in sufficient quantity; and that a deficiency of both Ca and PO₄, or of Ca alone leads to bone changes which differ in certain respects from those of typical rickets.

The present study is concerned with the bone changes which result from modifying the organic constituents of the rickets-producing diet. We have sought, in particular, an answer to the following questions.

1. Is phosphorus, ingested in organic combination, of equal value, as compared with inorganic phosphorus, for the purposes of bone formation?

2. Do the water-soluble and fat-soluble vitamines exert any protective or curative effect, apart from the phosphorus content of the substances in which they occur? It has already been shown that the rachitic lesions which follow a diet lacking in phosphates may be prevented by merely remedying this deficiency (2), and that rats maintained throughout their growth period upon a diet deficient in fat-soluble vitamine, but adequate as regards salt content, fail to become rachitic (3). It was still conceivable that the addition of the fat-soluble factor to the rachitic diet might exercise some protec-

* A preliminary report of this work was presented before the Society of Experimental Biology and Medicine (1).
tive influence. A possible influence of the water-soluble factor also remained to be investigated.

3. Is it possible to make the rickets-producing Diet 84 more nearly adequate for proper nutrition and growth, without impairing its rickets-producing effect? Rachitic infants are often well nourished, whereas Diet 84, as has been pointed out, is not adequate for the normal growth and nutrition of rats. It was an obvious problem to attempt to bridge this discrepancy in amplying Diet 84 by the addition of the lacking food constituents.

The experimental procedure has been fully detailed in the preceding paper, and need not be again described.

**Effect of the Addition of Casein to Diet 84.**

In order to study the effect of phosphorus in the form of phosphoprotein, 10 per cent of casein was substituted for the same percentage of flour in Diet 84. The casein used was the ordinary commercial product which had been extracted with cold alcohol by slow percolation until the washings were colorless, and then extracted for 48 hours with two changes of ether. Analysis of this casein showed a P content of 0.840 per cent. The diet contained approximately the same amount of phosphorus (162 mg. per 100 gm.) as Diet 85.

The results of this experiment, as shown in Table I, require further comment. Rat 95, which showed moderate rickets on x-ray examination, was killed on the 22nd day (Fig. 1). Gross examination showed a slight beading and haziness of the chondrocostal junctions. Microscopically, there were found rachitic lesions of moderate intensity (Fig. 2).

**Rat 95.—Rib:** The zone of proliferative cartilage is very broad, averaging twenty-five to thirty cells in depth. From this, single or double rows of cartilage cells extend into the metaphysis, and between these the matrix is calcified. No demonstrable calcium, however, is present in the proximal or basal portion of the zone of preparatory calcification. There is a very dense spongiosa composed for the most part of uncalcified osteoid tissue. A few of the trabeculae show an inner ossified tissue enveloping the calcified cartilaginous matrix. The osteoid tissue is more than normally abundant along the cortex, especially about the perforating vessels. The marrow space of the shaft is broad, the marrow cellular. The lesion may be interpreted as early healing of a moderate rickets.
The other two rats of this series (Nos. 104 and 105) showed in radiographs taken on the 22nd day (Fig. 3) slight but definite changes which were interpreted as rachitic. Radiographs taken on the 36th day, however, showed apparently complete healing (Fig. 4). No rachitic deformities were found at autopsy, nor did microscopic study of the ribs show evidence of the previous lesion (Fig. 5).

These experiments strongly suggested that the casein phosphorus conferred a less complete protection than would be given by an equivalent amount of phosphorus in the form of basic potassium phosphate. The following experiments confirm this. Three rats (Nos. 292 to 294) were placed on Diet 84 with 5 per cent purified casein substituted for an equal percentage of flour. After 28 days all three rats showed, by radiograph, marked rickets; and one rat sacrificed at this time showed severe deformities and characteristic microscopic lesions without healing. Three other rats (Nos. 295 to 297) were given 15 per cent of casein, equivalent to a total P content of 200 mg. per 100 gm. of diet—an amount considerably in excess of that required to afford complete protection when administered as inorganic phosphate. Of these, all showed by x-ray examination on the 28th day moderate rickets. In one rat (No. 296), however, healing was apparently in progress as judged by the presence of a definite transverse shadow in the head of the tibia. Another rat (No. 297) killed on this day and examined microscopically proved to have moderate rachitic lesions with partial healing. The third rat of this group (No. 295) showed no healing radiographically on the 37th day. It was found dead in the cage and too decomposed for histological study.

The only conclusion that can be drawn from this series is that the protection afforded by casein during the period of most active growth is not wholly equivalent to that given by the same amount of phosphorus in the form of basic potassium phosphate. We have as yet no explanation for the discrepancies shown by individual rats. The difference cannot be correlated merely with the differences in the rate of growth.

Effect of the Addition of Lecithin to Diet 84.

Lecithin was added to the diet with the view of ascertaining whether phosphorus in the form of a phospholipin exerts an equivalent pro-
### Table 1

**Effect of the Addition of Casein to Diet 84.**

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<td>31</td>
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Controls from the same litters.

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<th>No.</th>
<th>Diet</th>
<th>Length of time on diet</th>
<th>Weight</th>
<th>Radiograph</th>
<th>Pathological examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>days</td>
<td>gm.</td>
<td>gm.</td>
<td>27th</td>
</tr>
<tr>
<td>92</td>
<td>Flour ...........................95.0 per cent.</td>
<td>44</td>
<td>32</td>
<td>32</td>
<td>27th</td>
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<tr>
<td>94†</td>
<td>Calcium lactate...... 2.9 &quot; &quot;</td>
<td>45</td>
<td>29</td>
<td>44</td>
<td>27th</td>
</tr>
<tr>
<td>102†</td>
<td>Sodium chloride..... 2.0 &quot; &quot;</td>
<td>35</td>
<td>43</td>
<td>53</td>
<td>25th</td>
</tr>
<tr>
<td>103†</td>
<td>Ferric citrate....... 0.1 &quot; &quot;</td>
<td>44</td>
<td>28</td>
<td>43</td>
<td>25th</td>
</tr>
</tbody>
</table>

* In all the tables the asterisk indicates that the finding was confirmed by microscopic examination.
† Rickets healed by cod liver oil, 5 drops every day, given for 5, 15, and 10 days respectively.

**TABLE II.**

Effect of the Addition of Lecithin to Diet 84.

<table>
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<td>days</td>
<td>gm.</td>
<td>gm.</td>
<td>27th</td>
</tr>
<tr>
<td>145</td>
<td>25</td>
<td>No. 84 + lecithin.</td>
<td>30</td>
<td>34</td>
<td>43</td>
<td>41</td>
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<tr>
<td>146</td>
<td>25</td>
<td>Flour ...........................93.0 per cent.</td>
<td>30</td>
<td>33</td>
<td>49</td>
<td>49</td>
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<tr>
<td>147</td>
<td>25</td>
<td>Calcium lactate...... 2.0 &quot; &quot;</td>
<td>30</td>
<td>36</td>
<td>51</td>
<td>51</td>
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</tbody>
</table>

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tection to that given by inorganic phosphate. Three rats (Table II, Nos. 145 to 147) were given a diet similar to Diet 84, except that 2 per cent of the flour was replaced by an equal weight of lecithin (commercial product). The lecithin was dissolved in ether and mixed with the flour in this way, thus ensuring an even distribution of the lecithin throughout the mixture. The ether was then allowed to evaporate at room temperature, and the salt mixture added. The 2 per cent of lecithin was calculated to contain about 79 mg. of phosphorus, making a diet which was comparable in its phosphorus content to Diet 85. The total phosphorus of the diet was found by analysis in duplicate to be 152.4 and 153.2 mg. per 100 gm. of diet.

After 30 days on this diet the rats showed no evidence of rickets either by x-ray examination or microscopically (Figs. 6 and 7). The conclusion is evident, therefore, that phosphorus in the form of lecithin afforded protection equal to that given by inorganic phosphates.

Effect of the Addition of Yeast to Diet 84.

Yeast extract was added to the diet with the double purpose of ascertaining the influence of the water-soluble vitamine and of another phosphorus-containing substance. The preparation used was Harris' yeast vitamine (Osborne and Wakeman (4)) which we found to contain 42 mg. of phosphorus per gm.

In a preliminary experiment about 100 mg. of the extract were measured roughly and sprinkled over each feeding. The rats always ate the yeast avidly as soon as it was placed in the cage. The three rats used for this test (Table III, Nos. 96, 108, and 109) showed no rickets on x-ray examination or section, after 37, 35, and 37 days respectively on the diet.

At the time of the experiment, it was not realized that the phosphorus content of this yeast preparation was as great as it was subsequently shown to be. Therefore, it was necessary to reduce the amount of the yeast extract so as to bring the phosphorus content to, and below the level of protection, and yet supply an adequate amount of water-soluble factor. Data for determining this were available from the work of Osborne and Wakeman (4), who showed that 20 mg. of such a yeast preparation carried ample vitamine to ensure proper growth when the other food constituents were present in
<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Litter No.</th>
<th>Diet</th>
<th>Length of time on diet</th>
<th>Weight</th>
<th>Radiograph</th>
<th>Pathological examination</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>days</td>
<td>gm.</td>
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<td></td>
<td></td>
<td>96</td>
<td>15</td>
<td>No. 84 + yeast. (100(2) mg. of Harris' yeast vitamin given daily to rats on basal Diet 84.)</td>
<td>37</td>
<td>37</td>
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<td></td>
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<td>108</td>
<td>17</td>
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<td>109</td>
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<td>166</td>
<td>28</td>
<td>No. 84 + 0.5 per cent yeast extract containing 21 mg. of phosphorus per 100 gm. of diet.</td>
<td>26</td>
<td>36</td>
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<td></td>
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<td>171</td>
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<td>165</td>
<td>28</td>
<td>No. 84 + 1.25 per cent yeast extract containing 52 mg. of phosphorus per 100 gm. of diet.</td>
<td>26</td>
<td>33</td>
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<td>167</td>
<td>28</td>
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* Denotes severe cases.
adequate amount. There has since accumulated additional evidence that the water-soluble B content of our diets is sufficient to permit of normal growth during the experimental period when the other deficiencies are rectified. Rats on Diets D and E, in which no additional water-soluble vitamine is added, have shown growth closely approximating the normal, at the same time that they were developing rickets (Text-fig. 1). These diets will be discussed under the section on amplified diets.

Two groups were given diets in which 0.5 per cent (Rats 166 and 171) and 1.25 per cent (Rats 165 and 167) of yeast extract replaced equal weights of the flour in Diet 84. In these experiments the extract was mixed evenly through the diet, and not added separately as before. 0.5 per cent of the yeast vitamine was calculated to give 25 mg. of extract per day (the rats each ate about 5 gm. of the total diet per day) and added 21 mg. of phosphorus per 100 gm. of diet. 1.25 per cent gave 62 mg. of extract per day, and 52 mg. of phosphorus per 100 gm. of diet. On these diets all the rats developed marked rickets, as shown by radiograph and section (Figs. 8 and 9).

This would indicate that the water-soluble vitamine may be present in the diet in large amount without any protective effect. A larger indefinite amount of this preparation protected, but here the phosphorus added was sufficient to account for the protection.

Effect of the Addition of Egg Albumin to Diet 84.

The phosphoprotein casein having been shown to afford at least a partial protection, probably dependent upon its phosphorus content, we attempted to improve the nutrition by the addition of a phosphorus-free protein. The phosphorus content of the egg albumin used was negligible, 10 gm. samples not yielding enough ammonium phosphomolybdate precipitate to titrate. 10 per cent of egg albumin (commercial product) was substituted in Diet 84 for an equivalent amount of flour. This addition alone did not strikingly improve the general nutrition. There was also no protective effect. Rat 159 (Table IV) kept on this diet for 26 days showed rickets by radiograph (Fig. 10) and at autopsy (Fig. 11). Rats 160 and 161, also on this diet for 26 days, showed even more severe rickets radiographically and were subsequently used for other work.
### TABLE IV.

**Effect of the Addition of Egg Albumin to Diet 84.**

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<td>gm.</td>
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<tr>
<td>159</td>
<td>26</td>
<td>No 84 + egg albumin. Flour: 85.0 per cent. Egg albumin: 10.0 &quot; &quot; Calcium lactate: 2.9 &quot; &quot; Sodium chloride: 2.0 &quot; &quot; Ferric citrate: 0.1 &quot; &quot;</td>
<td>26</td>
<td>33 43 43</td>
<td>24th</td>
<td>Moderate.</td>
</tr>
<tr>
<td>160</td>
<td>26</td>
<td>No 84 + egg albumin. Flour: 85.0 per cent. Egg albumin: 10.0 &quot; &quot; Calcium lactate: 2.9 &quot; &quot; Sodium chloride: 2.0 &quot; &quot; Ferric citrate: 0.1 &quot; &quot;</td>
<td>26</td>
<td>44 50 50</td>
<td>24th</td>
<td>Marked.</td>
</tr>
<tr>
<td>161</td>
<td>26</td>
<td>No 84 + egg albumin. Flour: 85.0 per cent. Egg albumin: 10.0 &quot; &quot; Calcium lactate: 2.9 &quot; &quot; Sodium chloride: 2.0 &quot; &quot; Ferric citrate: 0.1 &quot; &quot;</td>
<td>24</td>
<td>40 61 61</td>
<td>24th</td>
<td>&quot; &quot;</td>
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</table>
Effect of the Addition of Butter and of Butter Fat to Diet 84.

The addition of butter was of particular interest in view of the much discussed rôle of the fat-soluble vitamine in the prevention of rickets.

The conception of rickets as a vitamine deficiency disease was first propounded by Funk (5). The views and experiments of Mellanby (6, 7) in support of this theory have been so widely quoted that no further reference need be made to them.

Subsequent work in general has been unfavorable to Mellanby's conclusion. Harden and Zilva (8) in monkeys, Hess and Unger (9) in infants, Paton and Watson (10) in dogs, Hess, McCann, and Pappenheimer (11) in rats, Mackay (12) and Tozer (13) in kittens, Tozer (14) in guinea pigs, Zilva, Golding, Drummond, and Coward (15) in pigs have all failed to produce typical rachitic lesions on diets deficient in fat-soluble vitamine.

Shipley, Park, McCollum, and Simmonds (16) cite experiments in which a diet adequate in phosphorus, but deficient in fat-soluble A failed to produce rickets. In subsequent papers (17, 18) these authors state that the addition of 10 per cent of butter fat to a rickets-producing diet is ineffective in preventing the development of rachitic lesions, although 3 per cent of the butter-fat carried sufficient fat-soluble vitamine for the promotion of growth and fertility.

The latest publication on this phase of the subject is that of Korenchevsky (19), who concludes that ordinarily a deficiency in fat-soluble A produces in young rats merely an osteoporosis with deficient osteogenesis, but in some cases, namely, when the parents during conception, pregnancy, and lactation have been fed on a fat-soluble-deficient diet, changes more analogous to rickets are produced in the offspring. He concludes, therefore, that vitamine A has a relation to the metabolism of calcium and to the development of rickets.

In our first experiment four rats (Nos. 73, 74, 79, and 80, Table V) were given Diet 84 modified by the substitution of 5 per cent of pasteurized butter for flour. This gave about 0.20 to 0.25 gm. of butter per rat per day. This amount of butter of the same brand completely protected all control animals against keratomalacia and evoked a characteristic rise in the weight curve with cure of keratomalacia in two rats which had been maintained for a long time on a fat-soluble-deficient diet (11). The rats on Diet 84 plus 5 per cent butter, after 32, 43, 45, and 45 days respectively, were killed and found to show marked rickets (Figs. 12 and 13).

Incidentally, it may be mentioned that eye lesions occurred with extreme frequency (over 90 per cent), irrespective of the presence
<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Litter No.</th>
<th>Diet.</th>
<th>Length of diet in days</th>
<th>Weight (gm.)</th>
<th>Radiograph.</th>
<th>Pathological examination.</th>
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<td>Rickets</td>
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<tr>
<td>73</td>
<td>12</td>
<td>No. 84 + butter. (0.2 gm. of butter pasteurized, given daily to rats on basal Diet 84.)</td>
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<td>24</td>
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<tr>
<td>74</td>
<td>12</td>
<td>No. 84 + butter.</td>
<td>45</td>
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<td>46</td>
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<tr>
<td>79</td>
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<td>No. 84 + butter.</td>
<td>32</td>
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<td>80</td>
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<td>No. 84 + butter.</td>
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<td>No. 84 + butter. (0.4 gm. of fresh butter given daily to rats on Diet 84.)</td>
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<tr>
<td>143</td>
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<td>No. 84 + butter.</td>
<td>28</td>
<td>35</td>
<td>41</td>
<td>41</td>
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<tr>
<td>144</td>
<td>25</td>
<td>No. 84 + butter.</td>
<td>30</td>
<td>40</td>
<td>42</td>
<td>39</td>
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Controls from the same litters.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Litter No.</th>
<th>Diet.</th>
<th>Length of diet in days</th>
<th>Weight (gm.)</th>
<th>Radiograph.</th>
<th>Pathological examination.</th>
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<tr>
<td>71</td>
<td>12</td>
<td>Flour. 95.0 per cent.</td>
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<td>28</td>
<td>42</td>
<td>33</td>
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<tr>
<td>72</td>
<td>12</td>
<td>Calcium lactate 2.9, Sodium chloride 2.0, Ferric citrate 0.1</td>
<td>34</td>
<td>26</td>
<td>37</td>
<td>37</td>
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</table>
or absence of rachitic lesions, in rats on Diets 84 and 85, and the modifications described.

In spite of certain work indicating that the fat-soluble vitamine is fairly resistant to heat (Osborne and Mendel (20)), it seemed advisable to make certain that we were giving an excess of this factor by repeating the experiments, using butter which had not been subjected to heat.

To a second series of rats (Nos. 142 to 144, Table V) 0.4 gm. per rat of fresh butter, made every few days in the laboratory from fresh cream, was given daily. This butter contained 20 per cent of whey, and on analysis 2.4 mg. of phosphorus per gm. The 0.9 mg. of phosphorus given daily was thus far below the amount necessary to protect, as shown in the previous experiments. After 28, 30, and 30 days on this diet (Fig. 14) these three rats were killed, and all showed rickets of moderate degree microscopically. While the lesions were not so extreme as those which had been observed in some rats on other diets for a corresponding period, they were quite definite, and there was no evidence of healing or protection.

Moreover, butter fat, though given in amounts carrying ample fat-soluble vitamine, seems to have no curative effect upon rickets already established. Osborne and Mendel (20) found that 6 per cent butter fat carried enough for adequate growth and the prevention of eye lesions. In our experiments 10 per cent of two butter fats, one made from fresh raw (farm) butter and one from the same brand of pasteurized butter as used before, was substituted for an equivalent amount of flour in an amplified diet described below. Rachitic rats Nos. 161 and 190 were given the raw butter fat, and rachitic rats Nos. 160, 167, 169, and 171 (Table VI) the pasteurized butter fat. After 8 to 10 days on this diet these rats still showed marked rickets with no evidence of healing in spite of excellent general health.

The foregoing experiments seem to us to give convincing evidence that the fat-soluble vitamine, as usually defined (see, for example, Steenbock, Sell, and Buell (21)), plays no part in the pathogenesis of rat rickets.
Effect of the Addition of Meat to Diet 84 (Meat and Flour Diet).

Three rats (Nos. 281 to 283) were fed for periods of 33, 39, and 33 days on Diet 84, supplemented by the addition of chopped and dried round steak ad libitum. The growth on this diet was normal, and examination of the bones radiographically and microscopically showed a normal structure.

This diet was adequate in both calcium and phosphorus.

Three rats (Nos. 284 to 286) were placed for a period of 35 days upon a diet of dried chopped steak and patent flour, without the addition of sodium chloride, calcium lactate, or ferric citrate. This gave a diet rich in phosphorus, but deficient in calcium. Growth on this diet was poor. The lesions resulting in the ribs resemble rather closely those described in the previous paper on Diet 85C—low in calcium, but containing an excess of phosphate—namely, slight irregularity and widening of the zone of preparatory calcification, defective calcium deposition in the cartilage, and slight excess of osteoid. Marked rachitic deformities were not produced (Fig. 15).

It is interesting to compare these findings with those of Watson (22) in rats maintained on an exclusive meat diet. Watson's rats showed deformities and infractions resembling those of rickets, but microscopically the epiphyseal changes were very slight.

Amplified Rachitic Diet.

In the first paper of this series (2) it was pointed out that Diet 84 is deficient in the character of the protein, in its fat-soluble vitamine content, and in various inorganic constituents. The following diets were devised to remedy these deficiencies.

In a preliminary series rats already rachitic, as shown by radiograph, were placed on Diets 84R, 84R1, and 84R2 (Table VI), in which Diet 84 is enriched by the substitution of a more complete protein (egg albumin), butter or butter fat, and a salt mixture complete in every respect except phosphorus (Osborne and Mendel (23)). No tendency to healing of the bone lesions was shown microscopically after 8 to 10 days on this diet. Although the general condition of the animals was greatly improved during this time, as shown by sleekness and amount of body fat, the weight was not greatly increased.
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<tr>
<td>160</td>
<td>26</td>
<td>No. 84 + egg albumin.</td>
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<td>Marked.</td>
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<tr>
<td>161</td>
<td>26</td>
<td>No. 84 + egg albumin.</td>
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<td>167</td>
<td>28</td>
<td>No. 84 + 1.25 per cent yeast extract.</td>
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<td>169</td>
<td>29</td>
<td>No. 84 with Osborne-Mendel salt mixture.</td>
<td>26</td>
<td>&quot;</td>
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<tr>
<td>171</td>
<td>29</td>
<td>No. 84 + 0.5 per cent yeast extract.</td>
<td>26</td>
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<td>190</td>
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<td>No. 84.</td>
<td>28</td>
<td>Moderate.</td>
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**Diet 84R.**

Flour ..................................................78.0 per cent.
Osborne-Mendel salt (phosphorus-free) ..................5.0 "
Calcium lactate ........................................2.0 "
Egg albumin .............................................10.0 "
Butter (pasteurized) ...................................5.0 "

**Diet 84R.**

Flour ..................................................73.0 per cent.
Osborne-Mendel salt mixt. (phosphorus-free) ..........5.0 "
Calcium lactate .........................................2.0 "
Egg albumin .............................................10.0 "
Butter fat (pasteurized) ................................10.0 "

**Diet 84R.**

Like Diet 84R. except that raw butter fat was used.
The diet which is being used at present (Diet D) gives a growth curve which, as has been stated, approximates the normal during about 1 month. The composition of the diet is as follows:

- Patent flour ........................................ 80.9 per cent.
- Egg albumin ........................................ 10.0 " "
- Butter fat ........................................ 5.0 " "
- Salt mixture ........................................ 4.1 " "

The salt mixture furnished the following constituents.¹

- KCl ................................ 0.85 gm. per 100 gm. of diet.
- Na₂CO₃ ........................................ 0.85 " " 100 " " " "
- MgCO₃ ........................................ 0.286 " " 100 " " " "
- Ca lactate ..................................... 2.000 " " 100 " " " "
- Ferric citrate .................................. 0.1 " " 100 " " " "
- KI .............................................. 0.0002 " " 100 " " " "
- MnSO₄ ........................................ 0.00078 " " 100 " " " "
- NaF .............................................. 0.0024 " " 100 " " " "
- KAl(SO₄)₂ ................................. 0.00024 " " 100 " " " "

Total ................................................. 4.08962 " " 100 " " " "

This salt mixture is based on analyses of the milk of small animals instead of cow's milk, as in the Osborne and Mendel mixture.

Rachitic lesions develop regularly on this diet (Fig. 16). A few of the rats which failed to grow because of infections have shown less deformity than more actively growing rats. This has been noted on other rickets-producing diets, and is in accordance with clinical experience in children. An obvious explanation may be found in the greater phosphorus needs of all tissues in the rapidly growing animal.

In Diet E, 72 mg. of phosphorus were added to the above diet. Owing to the substitution of 15 per cent of non-phosphorus-containing substance for flour, the total phosphorus content was reduced to 145 mg. per 100 gm. of diet. This is an amount which has been shown to be on the border-line of phosphorus requirement. These rats showed excellent growth (Text-fig. 1). Of thirteen rats on this diet,

¹ The reasons for using this salt mixture will be discussed in a forthcoming paper.
eight showed no rickets, four minimal rickets, and only one typical, but moderate, lesions. It is in accord with the explanation offered that the rachitic changes in some of these rats were more marked than on simpler diets with equivalent phosphorus content.

CONCLUSIONS.

1. Casein phosphorus does not completely prevent the development of rickets when substituted in Diet 84 in amount equivalent to a protective dose of basic potassium phosphate.

2. The protection given by lecithin is equivalent to its phosphorus content.

3. The protection given by yeast is at least proportional to its phosphorus content. An amount carrying sufficient vitamine B to promote growth, but insufficient to provide adequate phosphorus, does not prevent rickets.

4. Vitamine A, in the form of butter or butter fat to the amount of 10 per cent of the diet, neither prevents nor cures rickets.

5. The substitution of 10 per cent of egg albumin in Diet 84 improves the nutrition, but does not prevent rickets.

6. The addition of meat to Diet 84, thereby supplying an abundance of phosphorus, promotes normal growth and normal bone formation. A diet consisting solely of meat and flour is inadequate for proper growth, and leads to changes in the bones comparable with those observed on a diet low in calcium, but rich in phosphorus.

7. A diet has been found which contains the necessary food elements for approximately normal growth, and in which the only known deficiency is phosphorus. This leads regularly to the production of rickets.

BIBLIOGRAPHY.


EXPLANATION OF PLATES.

PLATE 25.

FIG. 1. Rat 95. 22 days on Diet 84 with the substitution of 10 per cent purified casein. Radiograph showing moderate rickets.

FIG. 2. Rat 95. 22 days on Diet 84 with the substitution of 10 per cent purified casein. The rib shows moderate rickets; early healing (?) (see text). 8 days decalcification in Müller's fluid. Hematoxylin-eosin stain.

FIG. 3. Rat 105. 22 days on Diet 84 with the substitution of 10 per cent purified casein. Radiograph showing slight rachitic changes in the upper epiphysis of the tibia.

PLATE 26.

FIG. 4. Rat 105. Radiograph showing healing of rachitic lesion after 36 days.

FIG. 5. Rat 105. 38 days on Diet 84 with the substitution of 10 per cent purified casein. The rib shows a zone of provisional calcification, three to four cells deep; matrix calcified. Primary spongiosa composed of five or six short stout trabecula completely ossified, without osteoid margin. Secondary spongiosa poorly developed. Cortex without visible osteoid border. Marrow partly
fatty. No rickets. Osteogenesis inactive. 10 days decalcification in Müller's fluid. Hematoxylin-eosin stain.

**Fig. 6.** Rat 145. Diet 84 with the substitution of 2 per cent lecithin. Radiograph on the 28th day. No rickets.

**Fig. 7.** Rat 145. 30 days on Diet 84 with the substitution of 2 per cent lecithin. The rib shows a zone of provisional calcification four to five cells deep. Calcium deposition in matrix normal. Primary spongiosa, extremely delicate, trabeculae missing in places; those present, regular in arrangement and well calcified. Cortex thinned in subchondral region, elsewhere well developed. No excess of osteoid. Slight osteoporosis. No rickets. 5 days decalcification in Müller's fluid. Hematoxylin-eosin stain.

**PLATE 27.**

**Fig. 8.** Rat 166. Diet 84 with the substitution of 0.5 per cent Harris' yeast extract. Radiograph taken on the 25th day. Marked rickets.

**Fig. 9.** Rat 166. 26 days on Diet 84 with the substitution of 0.5 per cent Harris yeast extract. The rib shows moderate rickets. Zone of preparatory calcification twenty to thirty cells deep; calcium deposition limited to distal half of cartilage matrix and its prolongations. Dense spongiosa with great excess of osteoid; osteoblasts conspicuous. Osteoid margin about cortex much increased. 3 days decalcification in Müller's fluid. Silver nitrate-Van Gieson stain.

**Fig. 10.** Rat 159. 24 days on Diet 84 with the substitution of 10 per cent egg albumin. Radiograph showing moderate rickets.

**Fig. 11.** Rat 159. 26 days on Diet 84 with the substitution of 10 per cent egg albumin. The rib shows typical marked rickets. 9 days decalcification in Müller's fluid. Hematoxylin-eosin stain.

**PLATE 28.**

**Fig. 12.** Rat 79. 32 days on Diet 84 plus 5 per cent pasteurized butter. The rib shows definite rachitic lesions. The zone of provisional calcification projects irregularly and is greatly widened. Calcium deposition defective. Spongiosa dense, composed almost wholly of osteoid with inclusions of cartilage cells. Cortex surrounded by wide osteoid border. Marrow spaces reduced. 2 days decalcification in Müller's fluid. Hematoxylin-eosin stain.

**Fig. 13.** Rat 80. 45 days on Diet 84 plus 5 per cent pasteurized butter. The rib shows advanced rachitic changes at the chondrocostal junction. 3 days decalcification in Müller's fluid. Hematoxylin-eosin stain.

**PLATE 29.**

**Fig. 14.** Rat 144. 30 days on Diet 84 plus 0.4 gm. daily of fresh, unpasteurized butter. The rib shows a zone of preparatory calcification twelve to sixteen cells deep, with several prolongations into the metaphysis. Matrix calcium-free,
except for the central core of spongiosa. Trabeculae of spongiosa broad and abundant, almost wholly osteoid, bordered by conspicuous osteoblasts; osteoid border of cortex increased along entire length of shaft. Moderate rickets. 3 days decalcification in Müller's fluid. Hematoxylin-eosin stain.

Fig. 15. Rat 285. 35 days on meat and flour diet. Zone of preparatory calcification very slightly increased. Calcium deposition limited to matrix between distal two or three cells. Osteoid increased about spongiosa and cortex.

Fig. 16. Rat 498. 26 days on Diet D. Radiograph showing rickets.
(Pappenheimer, McCann, and Zuckerm Experimental rickets. V.)
(Pappenheimer, McCann, and Zucker: Experimental rickets. V.)
Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

(Pappenheimer, McCann, and Zucker: Experimental rickets. V.)
(Pappenheimer, McCann, and Zuckert: Experimental rickets. V.)