SUPPLEMENTARY PROTEIN VALUES IN FOODS.

V. SUPPLEMENTARY RELATIONS OF THE PROTEINS OF MILK FOR THOSE OF CEREALS AND OF MILK FOR THOSE OF LEGUME SEEDS.

By E. V. McCOLLUM, NINA SIMMONDS, AND H. T. PARSONS.

(From the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

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The nature of the dietary deficiencies of the cereal and legume seeds is now well understood. It has also been shown that milk and the leafy vegetables occupy a unique place among our ordinary foodstuffs in that they are the only foods regularly consumed in moderate quantities which are of a nature to correct the mineral deficiencies of cereals, legume seeds, tubers, and fleshy roots, or to adequately supplement them with respect to fat-soluble A. It is well known from laboratory experiments on animals as well as from agricultural experience, that milk proteins tend to enhance the value of vegetable proteins generally. Specific information as to the extent to which milk proteins supplement those of individual vegetable foods is still wanting. The studies reported in this paper form a contribution to this phase of our knowledge of practical dietetics.

In these experiments the proteins of the diets were derived from half skimmed milk powder (Merrell-Soule) and a single plant seed or tuber. In order to bring out more clearly the extent of the supplementary relations between the proteins employed we have in all cases limited the content of this factor to 9 per cent of the food mixture. We have already pointed out that in order to secure a normal growth curve in the rat on this low plane of protein

1 McCollum, E. V., The newer knowledge of nutrition, New York, 1918.
2 McCollum, E. V., J. Biol. Chem., 1914, xix, 323.
intake, the quality of protein for growth must be good.\textsuperscript{3} When the growth curves fall below normal the extent of retardation of development serves as a good index to the extent to which the quality of any mixture under investigation falls below that of the best combinations which we have been able to discover. When, in addition, we observe the animals throughout their reproductive period and secure records of fertility and infant mortality, and the time at which the first signs of old age appear, we have the most sensitive indexes to physiological well being which it seems probable will ever be observable. Proteins of good quality will induce normal growth when fed at the plane of intake of 9 per cent of the food mixture, and may induce a fair degree of fertility. In order to secure high fertility and low infant mortality the proteins must be of excellent quality.

In a former paper\textsuperscript{4} we have described comparable studies with diets in which the proteins were derived from combinations of either liver, kidney, or muscle with cereal or legume seed proteins. The plane of protein intake was uniform (9 per cent) in all cases. These records, together with those presented in the present paper, form, therefore, a contrast between the value of milk proteins on the one hand and animal tissue proteins on the other, as supplements for a number of vegetable foods with respect to the protein moiety.

The results bring out in a very striking way the unexpected superiority of animal tissue proteins for the special purpose of enhancing the value of various plant seed proteins. In our numerous studies of this phase of nutrition we have demonstrated that with diets of the type containing 9 per cent of protein, and with all other factors satisfactorily adjusted, the proteins of kidney produce the best results we have yet observed. The biological value of the proteins of animal tissues for growth or for maintenance of health differs in an easily demonstrable degree.

An inspection of the results of our experiments with diets of the type here employed warrants arranging the proteins of a number of animal and vegetable foods of great importance in a series show-

\textsuperscript{3} McCollum, E. V., Simmonds, N., and Parsons, H. T., \textit{J. Biol. Chem.}, 1919, xxxvii, 155.
\textsuperscript{4} McCollum, E. V., Simmonds, N., and Parsons, H. T., \textit{J. Biol. Chem.}, 1921, xlvii, 139.
ing their relative nutritive values. In the following scheme we have arranged the several foods in order of the biological value of their proteins. The best is placed at the left and the series is a descending one.

\[
\text{Beef kidney—Wheat—Liver—Barley—Maize—Oats—Navy beans—Pea}
\]

In our earlier studies of the cereal grains\(^5\) we became convinced that wheat proteins were of somewhat lower biological value than more recent experimental data would seem to indicate. This may perhaps be accounted for by differences in the proteins of various samples of wheat. It is well known that the dough-forming quality varies markedly in wheats. This property depends upon the peculiar nature of the proteins of this grain and may be due to lack of uniformity in the relative amounts of the individual proteins contained in the seed.

It should be kept in mind that such a differentiation in biological value of proteins from these foods will not be apparent unless the experimental procedure is appropriate to bring them out. The several foods included in the scheme must be fed, with other dietary factors satisfactorily constituted, at such a plane of intake as to furnish the critical level of 9 per cent of protein in the diet. This is the only method we have been able to devise to show these differences. The observations must include not only the period of growth but also the fertility, the success with young, and the period following the completion of growth to the point where senile characters are apparent.

We were surprised to find how consistently combinations of milk proteins and cereal or legume proteins fail to show as high biological values as can be demonstrated for kidney, liver, and muscle proteins combined with those of certain cereals.\(^4\) It should not be lost sight of that milk has an effective supplementary relation to cereals both with respect to the inorganic and fat-soluble A deficiencies of the latter, whereas muscle meats supplement them

only with respect to the protein factor, and glandular organs only with respect to protein and fat-soluble A. This fact is brought out in a striking way by the records in Chart 5. When pasteurized or boiled milk, or cooked glandular organs are contained in the diet they do not effectively supplement a cereal and legume seed mixture with respect to the antiscorbutic factor since this is a labile substance. This factor is essential in the nutrition of man, monkey, and guinea pig, but need not be furnished by the diet of the rat or prairie dog, since they are apparently able to synthesize it.6

SUMMARY.

We have described in this and preceding papers experiments which were so planned as to compare the relative merits of animal tissue proteins and of milk proteins for enhancing the value of the proteins of each of the following vegetable foods: barley, peas, soy beans, rye, maize, navy beans, wheat, potato, and rolled oats. These indicate clearly that animal tissues such as liver, kidney, or muscle, are superior to milk for the specific purpose of making good the deficiencies of the proteins of the seeds and tuber mentioned above.

Milk, however, is an effective supplement for these vegetable foods with respect to other factors as well as protein. This is especially true of calcium and fat-soluble A.

In making deductions from these results it should be kept in mind that muscle tissue supplements seeds, tubers, etc., only with respect to the protein factor, and that other deficiencies of even greater importance for the well being of the body are always met with in that group of vegetable foods which are functionally storage tissues of plants; viz., seeds, tubers, and roots.

Chart 1.—Lot 2391 was fed a diet containing 9 per cent of protein derived from barley (6 per cent) and milk (3 per cent). All other factors in the diet were made satisfactory by suitable additions of inorganic elements and fat-soluble A (in butter fat). Growth took place at a subnormal rate and the full adult size was never attained. The animals in this group looked rough coated and old at 15 months.

<table>
<thead>
<tr>
<th>Chart 1, Lot 2391</th>
<th>Lot 2390</th>
<th>Lot 2389</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Barley</strong></td>
<td>55.0</td>
<td></td>
</tr>
<tr>
<td><strong>Peas</strong></td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td><strong>Soybeans</strong></td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td><strong>Powdered milk</strong></td>
<td>9.3</td>
<td>9.3</td>
</tr>
<tr>
<td><strong>Powdered milk</strong></td>
<td>1.0</td>
<td>NaCl</td>
</tr>
<tr>
<td><strong>Dextrin</strong></td>
<td>1.5</td>
<td>CaCO₃</td>
</tr>
<tr>
<td><strong>Dextrin</strong></td>
<td>35.2</td>
<td>63.0</td>
</tr>
<tr>
<td><strong>Dextrin</strong></td>
<td>68.6</td>
<td></td>
</tr>
</tbody>
</table>

**Grades**

- Barley
- Peas
- Soybeans
- Powdered milk
- Dextrin

**Rations**

- Lot 2391
- Lot 2390
- Lot 2389

**Butterfat**

- Lot 2391: 3.0
- Lot 2390: 3.0
- Lot 2389: 3.0

*Y = birth of young*
Two females grew up on this diet. One remained sterile. The other had eleven young (three litters) but destroyed them soon after birth.

Lot 2390 derived its diet, containing 9 per cent of protein, from peas (6 per cent) and powdered milk (3 per cent). The protein of the diet was the limiting factor. The animals all grew poorly, but better than they could have grown on 9 per cent of pea protein alone. These rats were very apprehensive and could be weighed only with difficulty because of constant efforts to escape. They were so excited when handled that they would not sit still in a small covered box but would constantly spring up and strike the lid with their heads. The hair was very short and fine, and had a silky appearance, which we have never seen on rats fed highly satisfactory diets. The same type of coat has been frequently met with in rats restricted in a great measure to maize as a source of protein. Less frequently we have seen these "mole-skin" rats in groups which derived their protein from kafir corn.

Two females were restricted to this diet but never had any young.

Lot 2389 was fed soy beans and powdered milk as a source of protein. The total protein content of the diet was 9 per cent. The soy beans furnished two-thirds and the milk one-third of the total. While the growth was much better than we have ever seen on 9 per cent of soy bean protein alone, the rate of growth was distinctly below normal. The animals remained undersized, and their fertility was very low. There were three females in this group. One had a litter of seven. At 14 days they weighed 57 gm. At 21 days one had died. The remaining six weighed 62 gm. At 27 days but four survived and these weighed collectively but 45 gm. They were very puny and incapable of growing on the mother's milk. The other two females remained sterile.

Chart 2.—The rats of Lot 2386 were fed 9 per cent of protein derived from rye (6 per cent) and milk powder (3 per cent). Growth was slow but the animals reached nearly the adult size after some delay. These rats aged decidedly early. They looked old at 14 months. The second generation were all more undersized than the first. We have observed in many cases where the food mixture was faulty to a slight degree and a family was con-
CHART 2, LOT 2386

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>LOT 2385</th>
<th>LOT 2386</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye</td>
<td>50.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Powdered milk</td>
<td>9.3</td>
<td>9.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Dextrin</td>
<td>25.2</td>
<td>25.2</td>
</tr>
<tr>
<td>Butter fat</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Y = birth of young

2nd gen. 2nd gen. <4> Weeks
fined to the diet through several generations, each succeeding generation was smaller than the preceding one when growth was completed.

There were three females in the group. Two of these remained sterile. The other had twenty-eight young (three litters) and weaned eleven. The nursing period was, however, very long. A litter of eight was reduced by death to five by the 32nd day. These weighed 108 gm. At 39 days they weighed 121 gm., and were in a poorly developed condition. They were less than one-third the normal size for their age. One second generation female had a single litter but destroyed it soon after birth.

Lot 2385 was fed 9 per cent of protein derived from maize (6 per cent) and milk powder (3 per cent). All other factors in the diet were made satisfactory by suitable additions. Growth was slow but the animals reached nearly the full adult size. There were three females in the group but none ever had any young. The hair of this group was short and silky and suggestive of a mole skin.

*Chart 3.*—Lot 2388 derived the 9 per cent of protein in its diet from navy beans (6 per cent) and milk powder (3 per cent). The animals grew slowly and remained permanently undersized. They lived surprisingly long on this diet on which they grew so poorly. The history of the group on 9 per cent of protein from navy beans and milk is comparable to that of Lot 2390 (Chart 1), which was identical except that peas replaced the beans. The same diet with soy beans in place of peas or navy beans produced distinctly better growth (Lot 2389, Chart 1).

Lot 2384 derived the 9 per cent protein in its diet from wheat (6 per cent) and milk powder (3 per cent). The combination of wheat and milk proteins is better than a similar amount of protein from wheat alone. With the exception of the diet of oats and milk (Lot 2387, Chart 4), this food mixture was superior to any other combination of seed with milk proteins which we have studied. The animals appeared old after about 19 months on this diet.

There were two females in the group, one of which died after being 4½ months on the diet. The other had forty-one young (seven litters) and weaned nineteen. The nursing periods were long in all cases. The young were not destroyed in the ruthless
manner frequently observed, but died at intervals from undetermined causes.

A litter of five young weighed but 103 gm. at 23 days of age. At 58 days four weighed 141 gm. They appeared to be in good condition but were undersized. Two second generation females had but five young (one litter) and weaned one.

Chart 4.—Lot 2403 derived the 9 per cent of protein which its diet contained from potatoes (6 per cent) and milk powder (3 per cent). Growth was somewhat below normal and they remained undersized. These rats aged very early. They looked as old at 1 year as many better nourished animals do at 18 months.

Three females had twenty-seven young (five litters) and weaned only two. The nursing periods were long. A litter of five weighed 40 gm. at 15 days. At 26 days they were reduced by death to three, which weighed collectively 47 gm. At 60 days but two were left. These weighed together 70 gm. This is less than half what they should have weighed at this age.

The nitrogen of the potato is in great measure in the form of simple substances of a non-protein nature. These substances are evidently not of a character which supplements the proteins of milk to any marked extent. We have pointed out elsewhere that the nitrogen of the potato when fed as the sole source of this factor is not of so high a value as some have reported.\(^7\)

Lot 2387 was fed 9 per cent of protein, two-thirds of which was derived from rolled oats and one-third from milk powder. This combination of proteins seems to have a higher value than any other cereal and milk mixture we have investigated. But little inferior to this is the wheat and milk combination. We have in some earlier experiments seen better curves of growth on about this amount (8 per cent) of protein from a mixture of oats and milk. After 16 months on the diet their coats (Lot 2387) were somewhat rough, but the animals were still vigorous.

Three females had thirty-three young (five litters) of which six were weaned. Three other litters were destroyed before their numbers could be determined. The nursing periods were long. A litter of seven young weighed 66 gm. at 15 days of age. At 34

\(^7\) McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1918, xxxvi, 197.
days they weighed collectively 107 gm. At 60 days six of these weighed 202 gm., or less than half the normal for this age. Two second generation females grew up on the diet. One was sterile. The other had one litter of three after she had been 8 months on the diet. She weaned them all after a long nursing period.

Chart 5.—Lots 2148 and 2147 show the relative merits of milk and of muscle tissue as supplements to a mixture of foodstuffs consisting of articles which are functionally storage organs of plants. The mixture, aside from the milk or muscle meat, consisted of two fleshy roots, red beet and yellow turnip; a tuber, the potato; two legume seeds, pea and navy bean; and two degenerated cereal products, wheat flour and corn-meal (maize).

Notwithstanding the wide variety in such a list of foods, and an appropriate chemical composition as indicated by the ordinary food analysis, it does not promote growth in young animals nor support the vitality of adults as measured by fertility, success in rearing young, or in deferring the onset of old age.

Muscle meat (round steak) supplements a mixture of cereals, legume seeds, fleshy roots, and tubers only with respect to the protein factor. Milk on the other hand enhances not only the protein of these vegetable foods, but likewise makes good their mineral deficiencies and also the shortage of fat-soluble A which all such mixtures exhibit.

Lot 2147 in which the vegetable diet of storage organs is supplemented only with muscle meat, failed to grow normally. The curve shown is typical of a group of six animals restricted to this diet. They never had any young and aged very early. They looked extremely rough coated after 6 to 8 months on the diet. Lot 2148, on the other hand, whose diet was similar in all respects but contained 10 per cent of whole milk powder, grew normally and remained in much better condition to an age of about 18 months. These animals showed fair fertility and success in rearing their young. The milk supplemented not only the proteins of vegetable origin but accomplished what was of greater importance; viz., the correction of the inorganic deficiencies and made good in great measure the lack of fat-soluble A. The bearing of such observations as these on practical human dietetics will be easily appreciated.
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