SUPPLEMENTARY PROTEIN VALUES IN FOODS.

I. THE NUTRITIVE PROPERTIES OF ANIMAL TISSUES.

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In 1915 McCollum and Davis¹ described a systematic procedure for evaluating each of the several essential factors in foodstuffs. This procedure involves the feeding of the food under consideration as the sole source of nutriment to one group of animals, and to another the same food supplemented with single and multiple additions of purified foodstuffs in every possible combination. Such additions include protein, inorganic salts, a source of fat-soluble A, water-soluble B, and as was later pointed out by Chick and her coworkers,² Cohen and Mendel,³ and others, water-soluble C. The latter factor is not essential in the diet of the rat. This procedure constitutes a biological method for the analysis of a foodstuff, and has been adopted by several students of nutrition. It has yielded results which have profoundly changed our basis of judgment as to the quality of a diet.

Studies from several laboratories have established the general landmarks which enable us to appreciate the lines of procedure which must be followed if satisfactory diets are to be made up by combining the various types of animal and vegetable foods. In order to make such combinations of foodstuffs it is necessary that we should understand in detail the special qualities of each of the important natural foods. Such an understanding can be secured only through carefully planned experiments on animals in which

each food is studied as the sole source of nutriment, then studied in combination with each of the other foods with which it may be used in practice. It is necessary to proceed from the simple to the complex mixtures in these studies. Ultimately it is hoped that diets can be planned which will promote the optimum of physiological well being, and therefore lead to the optimum in physical development, length of life, and the preservation of youthful characteristics.

In publishing this series of papers dealing with the studies of the dietary properties of several types of food mixtures, several new observations will be pointed out. The interpretation of the results is based upon more careful and thorough observations than have hitherto been described in any similar studies. They include not only the rate and extent of growth, the fertility, and success in rearing of young, but also the period of life up to and including the onset of old age with its characteristic changes.

Since animal tissues have a very prominent place in the diet of man in most parts of the world, it is of great moment to understand the value of these with respect to each of the essential dietary factors. Our knowledge of the nutritive qualities of animal tissues is still very incomplete. Watson and Hunter\(^4\) showed that rats fed exclusively on muscle meats suffered severe malnutrition. Liver has, however, found great favor as a food for young fish in hatcheries. It has been shown\(^5\) that lard does not contain appreciable amounts of fat-soluble A, whereas fats extracted from a glandular organ (pig kidney or cod testicle) are a good source of it.\(^6\) Liver and kidney have been shown\(^7\) to be a good source of both fat-soluble A and water-soluble B, whereas muscle tissue is very poor in both.

Heart, a variety of muscle, was found on the other hand to contain sufficient of both fat-soluble A and water-soluble B to support growth for a time at least in young rats.\(^7\)

\(^4\) Watson, C., and Hunter, A., *J. Physiol.*, 1906, xxxiv, 111.
McCollum pointed out that the mineral content of animal tissues such as muscle and glandular organs resembles that of seeds of plants sufficiently to indicate that it would not prove satisfactory as a source of inorganic elements in animal nutrition. No data exist showing the comparative values of the proteins of the several kinds of animal tissues. Osborne and Mendel have shown that growth takes place in young rats restricted to 18 per cent of protein derived solely from liver, kidney, muscle, or brain. This is a liberal amount, and is far above the plane of intake necessary for the support of normal growth in the rat when the quality of the protein is good.

There is also reason to inquire into the possibility of the presence of toxic substances in the glandular organs. The presence of such powerful physiological stimulants as thyroxin and adrenalin in the thyroid and suprarenal glands, respectively, makes them unfit for human or animal food. We have been informed that the Eskimos do not eat the liver of the reindeer. This fact may perhaps be accounted for by the absence of a gall bladder in this species, and consequent high content of bile, which would give it a bitter flavor. The Eskimos are said not to eat the liver of the polar bear, to avoid that of a certain species of seal, and to believe the liver of the dog poisonous. The liver is concerned with so many types of transformations of organic substances which have their origin in metabolism that it seemed possible that certain of these may be present in sufficient amounts to be detrimental to one who eats freely of it. Similar consideration might lead one to inquire whether the kidney of an animal may contain sufficient amounts of certain metabolic products as to render it undesirable as a food.

The glandular organs are rich in cell nuclei and consequently yield considerable amounts of purines when metabolized. These ultimately are converted in great measure into uric acid. There are conditions of perverted metabolism in man in which the excretion of uric acid and urates is interfered with and doubtless under such circumstances liver or kidney should not be eaten. There would seem little reason, however, why these organs should not be eaten by healthy persons as adjuvants to the diet. They possess

8 McCollum, E. V., The newer knowledge of nutrition, New York, 1918.
dietary properties as distinct from those of muscle tissue as the leaves of plants do in contrast to the seeds.

In regions such as Labrador and Newfoundland where the diet of the habitants consists essentially of wheat flour, molasses, fish, meats, tea, and raisins, beri-beri and scurvy are common. A condition popularly called night-blindness is also of frequent occurrence among these people. On such a diet one would suspect the danger of developing xerophthalmia and apparently this is the case. The successful treatment of night-blindness has recently been reported by the administration of cod liver oil. This oil is a good source of fat-soluble A and is very effective in the cure of xerophthalmia.

Fresh liver is rich in fat soluble-A, water-soluble B, and water-soluble C, the protective dietary factors for ophthalmia, beri-beri, and scurvy, respectively. It should be easily possible to eradicate these dietary diseases in such regions as Labrador and Newfound-land by the use of fish livers as food. It is strange indeed that the natives of these regions have failed to discover the value of a by-product of their fishing industry which would serve in a great measure to correct the conspicuous faults in their diet.

For the purpose of comparing the biological values of the proteins of kidney, liver, and muscle, we have followed the procedure described by McCollum, Simmonds, and Parsons of feeding the tissues singly as the sole sources of protein at planes so as to intro-
duce 9 per cent of protein into the food mixture. They were sup-
plemented with respect to all other factors so as to make a satis-
factory diet, with the possible exception of the protein moiety.
Lots 2475, 2476, and 2474, Chart 1, represent experiments of this type. Such experiments make it possible to compare the proteins of these animal tissues with those of a number of combinations of cereal and other seed proteins with milk which we have previ-
ously studied. It has been shown that normal growth is secured when the diet contains 9 per cent of protein of high biological value. If much less than this content of protein is furnished by the food mixture, even when the protein is excellent, the growth falls distinctly below the curve of normal expectation.

When proteins of several types such as those of cereals, legume seeds, and milk were compared in this manner it was found that the proteins of milk were superior to those of most seeds of plants yet examined. The seed which furnishes proteins nearest to milk in value for conversion into body proteins during growth is wheat, which therefore stands first among the cereals in value. From the records of Chart 1, which show the effects of feeding kidney, liver, and muscle proteins respectively at 9 per cent of the food mixture, it is apparent that the proteins of these substances are scarcely superior to those of certain cereals, especially wheat. We base this conclusion on the growth of young rats restricted to one of these sources of protein at the critical 9 per cent level. Normal growth is not secured with wheat proteins fed below this plane of intake.

This result is very surprising indeed. It seems best explainable on the assumption that the patterns on which the proteins of the muscle are constructed differ very decidedly from that of the liver or kidney, and presumably from the glandular organs as a class. The nutritive needs of the body involve the replacement of tissues of both organ and muscle types. Apparently glandular organs or muscle as the sole source of nitrogen in the food fails to serve for efficient transformation into new body proteins during the symmetrical growth of the body tissues. They are complete but their transformation into body proteins cannot be very effectively accomplished (Chart 1).

We have shown in a previous communication that the cereal grain proteins do not in general make good each other’s deficiencies or enhance to any great extent each other’s biological values when two of them are combined. Rye and flaxseed meal proteins form a notable exception. These, when combined in certain proportions, form a mixture which is distinctly superior to the proteins of either component alone. The supplementary value of one protein for another depends on the yields by each of those indispensable amino acids which are present in each of the sources in smallest amounts. We shall show in one of the following papers that kidney, liver, and muscle proteins have much greater values as supplements to the cereal proteins than the cereal or legume proteins have, with few exceptions, among themselves. The data in the succeeding paper illustrate the importance of animal tissues in the food supply
when the diet consists mainly of such vegetable products as do not yield a mixture of proteins having a high biological value. For the special purpose of enhancing the quality of the protein in the diet they have the highest value. It must be kept in mind, however, that their use does not correct the mineral deficiencies of a cereal, tuber, and legume seed diet, and that when muscle meats are used as food they have little effect in raising the content of fat-soluble A in the resulting mixtures. In no instance, therefore, will one of these types of animal tissue supplement a cereal, tuber, and fleshy root type of diet so as to make it highly satisfactory.

Our observations on the rats described in these experiments do not show any definite evidence of injury to the animals as the result of being fed excessively high protein diets. These diets were, however, essentially complete and fairly well proportioned as regards all factors other than protein. It is not justifiable to generalize from these results that such a high protein intake is safe for man. Our animals were not kept to determine the possible span of life or the time of appearance of the signs of senility, owing to the necessity of temporarily vacating the room in which the animals were kept. It is the custom for people in the United States to derive a high protein diet, when such is taken, in great measure from muscle meats, fish, poultry, eggs, and legume seeds. Menus containing such high protein foods will only in exceptional cases be completely supplemented by other constituents of the diet. The evil effects often attributed to excessive protein consumption may now with some confidence be attributed in many instances to faults in the composition of the diet in factors other than protein. Further studies are required to demonstrate the relative merits of diets of high and low protein contents when other factors are of comparable value in the two cases. We shall discuss this phase of nutrition on the basis of carefully planned experiments on animals in a later communication.

There are some very interesting and important instances of successful nutrition among people who have lived almost exclusively upon a diet very rich in protein and derived from foods of animal origin. We are indebted to Mr. Vilhjalmur Stefansson for the information that previous to about 1850 dental caries were very rare or absent from Iceland. During the last half of the 19th century cases have gradually become more and more common.
until today infected teeth are perhaps as common there as in most parts of the United States. No carious teeth were found among 96 skulls disinterred by Stefansson from a cemetery in Iceland dating from the 9th to the 13th centuries. These skulls are now in the Peabody museum at Harvard University and have been described by Hooton.\textsuperscript{11} The diet of the Icelanders previous to about 1850 consisted essentially of milk, mutton, fish, and fowl, but in some parts of the island they ate the eggs of wild birds. The only vegetable food eaten regularly was carrageen moss, but potatoes and turnips were eaten to some extent. The teeth of the natives and their general health were excellent as long as this diet was taken. The deterioration of the teeth apparently began about the time when cereals and sugar were regularly imported into Iceland as sources of food.

The teeth of the primitive Eskimo were excellent. The younger generation in northern Alaska, whose diet is derived in a large measure from cereal grain products, canned foods, and muscle meats, similar to what would be purchased in a grocery store in the United States, has poorly calcified teeth which are often carious.

We have collected numerous observations on the effect of dietary faults on the quality of the skeleton in the rat. These data make it clear that most profound differences in the extent of calcification and density of the deposited calcium phosphate can be effected by such faults as are found in the cereal, tuber, and muscle meat type of diet.

The diet of the primitive Eskimo was very rich in protein but it was at least fairly satisfactory with respect to other factors. It consisted of muscle tissue and fat as the principal components, but all blood was carefully saved and eaten, and the glandular structures were regarded as dainties of especial delicacy. In addition, they regularly ate bone marrow and chewed the softer parts of bones, such as ribs and the epiphyses of the long bones. Such a selection of tissues suffices for the satisfactory nutrition of the rat and produces the fine physical development seen in the Carnivora such as the lion, tiger, jaguar, etc. The deterioration of the teeth of the Eskimo which occurred simultaneously with the modification of the diet due to contact with the white man is in

\textsuperscript{11} Hooton, E. A., \textit{Am. J. Physical Anthropol.}, 1918, 1, 53.
harmony with what we have been led to expect as the result of experimental studies of the types of combinations of ordinary foods which enter into the diet of man and animals in different parts of the world. The cereal and muscle meat diet or its equivalent, the bread, meat, and potato type of diet, is in all probability the cause of the deterioration of the teeth of the present generation of "civilized" Eskimos as it is among the people of the United States and Europe.

CONCLUSIONS.

The kidney, liver, and muscle of the ox contain proteins which, when they serve as the sole source of nitrogen, and are fed singly as the sole source of protein, but completely supplemented with respect to all necessary factors other than protein, are shown to possess about the same biological value as those of the wheat kernel.

There is no distinct evidence of toxicity in either muscle, kidney, or liver tissue when fed at planes of intake sufficiently high to introduce from 35 to 70 per cent of protein into the diet.

The first limiting factor in the kidney, liver, and muscle tissue is a lack of calcium. It is also necessary to add sodium chloride in order to insure prolonged well being. Carnivorous man and animals secure their sodium chloride by eating blood, and calcium by eating bone. Liver and kidney contain an abundance of fat-soluble A and of water-soluble B, and when fresh and raw, of water-soluble C. Muscle tissue is very deficient in these factors but does not entirely lack any one of them. Kidney proteins appear to have higher biological value than those of the other animal tissues yet studied.

It has been our custom for years in preparing experimental diets to thoroughly grind the several components of the food and to make a uniform mixture from which the constituent parts cannot be picked out by the animals. In all cases iodine was given once a week in the form of potassium iodide-iodine in the drinking water, which was distilled. The liver, kidney, and steak, except when the contrary is stated, were steamed in a sterilizer until thoroughly cooked, subsequently dried, and ground. Practically all visible fat was removed from both organs and muscle. The curves presented in the charts are typical representatives of a group of four to six animals which composed each experimental group.
Chart 1.—The curves in this chart illustrate the growth of young rats fed diets containing 9 per cent of protein derived solely from beef kidney, liver, and muscle, respectively. The inorganic additions were of a character which completed, at least in a fairly satisfactory manner, the mineral content of these animal tissues. There can be no doubt that kidney proteins have a somewhat higher biological value than those of liver. Liver proteins, surprising as it may seem, have not been found in our experiments to be superior to those of muscle (steak).

In Lot 2475 there were two females. One of these died in parturition. The other at the age of 4 months had one litter of four young and successfully reared them. She died from unknown cause about 40 days after weaning her young. Two of her daughters grew up and produced one and three litters of young (a total of seventeen), respectively. Of these ten were successfully weaned.

Two granddaughters of the female described in the original experimental group were kept 6½ months on the family ration, and although they appeared to be in good condition, neither proved fertile. The rats fed the kidney ration did not exhibit early signs of aging. In this respect they were superior to the groups fed diets containing comparable amounts of liver and of muscle.

The records of these experimental groups fed 9 per cent of protein from kidney, liver, and muscle, respectively, all other factors being more or less satisfactorily adjusted, show them to be typical examples of nutritional instability. In Lot 2475 on the kidney diet, each succeeding generation was inferior to its parents. Lack of uniformity of vitality among individuals of the same group or family is observed with striking frequency in animals whose diets fall but little below the quality necessary to maintain the vigor of the species unimpaired throughout successive generations.

It might be suggested that the failure of the animals fed liver to develop more satisfactorily, was due to the presence of toxic substances in this organ, which performs the function of degrading numerous foreign and poisonous substances derived from metabolism and absorption from the alimentary tract and elsewhere. The records of Chart 2 show clearly that this is not the case.
CHART 1, LOT 2476

<table>
<thead>
<tr>
<th>Ration</th>
<th>Lot 2476</th>
<th>Lot 2476</th>
<th>Lot 2476</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney (beef)</td>
<td>12.7</td>
<td>Liver (beef)</td>
<td>12.3</td>
</tr>
<tr>
<td>Salts (10%)</td>
<td>3.7</td>
<td>Salts (10%)</td>
<td>3.7</td>
</tr>
<tr>
<td>Agar-agar</td>
<td>2.0</td>
<td>Agar-agar</td>
<td>2.0</td>
</tr>
<tr>
<td>Dextrin</td>
<td>70.5</td>
<td>Dextrin</td>
<td>79.0</td>
</tr>
<tr>
<td>Butter fat</td>
<td>3.0</td>
<td>Butter fat</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Part of the dextrin carried the alcoholic extract of 10 grams of wheat embryo.

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Graph showing the growth of young over time with different lines representing various dextrin and butter fat levels.
Neither of the two females fed the steak diet had any young, although they appeared to be well nourished and were kept under observation during more than 12 months.

Three females on the liver diet were somewhat undersized, aged early, and never had any young, although they were kept under observation for more than a year. In the case of both the liver and muscle diets this was apparently due to the proteins from these sources not being of sufficiently good quality to make 9 per cent of protein from these sources sufficient to promote well being at the optimum, since in these diets all other factors were corrected.

**Composition of Salt Mixture 185.**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.173</td>
</tr>
<tr>
<td>MgSO₄ (anhydrous)</td>
<td>0.266</td>
</tr>
<tr>
<td>NaH₂PO₄ + H₂O</td>
<td>0.347</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.954</td>
</tr>
<tr>
<td>Ca₃(PO₄)₂·H₂</td>
<td>0.540</td>
</tr>
<tr>
<td>Fe citrate</td>
<td>1.300</td>
</tr>
<tr>
<td>Ca lactate</td>
<td></td>
</tr>
</tbody>
</table>

*Chart 2.*—The rats in these experiments were fed food mixtures which were satisfactorily constituted except for possible shortage of water-soluble B and fat-soluble A, which in each case were derived entirely from 25 per cent of kidney, liver, or muscle, respectively. The growth curves and fertility of the animals on the kidney and liver diets show that these amounts were satisfactory as sources of fat-soluble A and water-soluble B. In these diets the protein of the animal tissues was supplemented with 9 per cent of casein. Chart 4 shows, however, that 20 per cent of either kidney or liver suffices as the sole source of protein for normal growth, reproduction, and rearing of young, for the fourth generation on the kidney diet appeared to have normal vitality. The rats of the fourth generation on the liver diet were inferior, although they were successfully weaned.

Two females on the kidney diet, Lot 2163, had collectively four litters (fourteen young), all of which were reared. Two of the daughters were maintained during 10 months on the diet on which their mothers had lived. One remained sterile, the other had a single litter (five young) at about 4 months of age and never became pregnant afterwards. The young were in good condition when weaned.
<table>
<thead>
<tr>
<th>Ration</th>
<th>Lot 2162</th>
<th>Lot 2163</th>
<th>Lot 2160</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (beef)</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Casein</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>KCl</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Dextrin</td>
<td>62.5</td>
<td>62.5</td>
<td>62.5</td>
</tr>
</tbody>
</table>

**Period 1**
- Cooked muscle

**Period 2**
- 5% beef fat replaced part of dextrin.

**Ration**
- Periods 1 and 2 like Lot 2160 but raw muscle replaced the cooked round steak.

Y = birth of young
There were three females raised on the liver diet (Lot 2162). They had collectively fifty-six young (nine litters), of which thirty-three were weaned. The young which died became lethargic just before weaning time and died in this condition. We have at present no explanation for this peculiar behavior.

Lots 2160 and 2161 were fed diets comparable to Lots 2162 and 2163, except that the factors, fat-soluble A and water-soluble B, were entirely derived from 25 per cent of muscle tissue (cooked and raw beefsteak, respectively). In both cases there was failure of growth after about 4 weeks. After a period of suspended growth during which one female in Lot 2161 had a litter of four young which were eaten by the mother shortly after birth, 5 per cent of butter fat was added to the diet of each group. In both cases there was a marked response to growth following this addition. This demonstrates that 25 per cent of round steak does not contain sufficient fat-soluble A to meet the needs of the young rat. Lot 2142, Chart 3, shows clearly that 20 per cent of steak does not furnish sufficient water-soluble B for the normal nutrition of young rats during growth.

The records in this chart show clearly the remarkable difference between the glandular organs as compared with muscle tissue in respect to their content of both the factors, fat-soluble A and water-soluble B. This supports the view which we have repeatedly stated, that the dietary properties of a substance can be fairly accurately predicted from a knowledge of their biological function.

Chart 3.—The three groups of animals whose curves are shown in this chart were fed diets comparable in all respects, except that the sole source of water-soluble B and protein was 20 per cent of the dry matter in the diet in the form of kidney, liver, and muscle, respectively. A comparison of these curves with those of Chart 1 shows that the protein furnished by this proportion of liver, kidney, and steak, respectively, suffices to support normal growth when other factors in the diet are properly adjusted.

The animals fed the kidney ration, Lot 2144, were markedly superior to the other two groups. Representatives of four successive generations were grown upon this diet as their sole source of water-soluble B, and with no evidence of deterioration. In the original group there were three females. These produced collectively ten litters (forty young) but only six individuals were
successfully weaned. The mothers destroyed their young soon after birth.

Two daughters of the females just described were kept on the family diet. They had together six litters (forty young) and weaned only four individuals altogether. Here again the mortality was the result of cannibalistic tendencies in the mothers. But one granddaughter of the original group was kept on the family diet. She grew normally and had six young (two litters) and successfully weaned all of them. One great granddaughter was brought up on the diet. She had three young (one litter) and weaned them successfully. With optimum nutrition these litters would have been two or three times as large as those produced.

It might be suggested that perhaps the content of water-soluble B in this diet was below the optimum, and that for this reason the nutrition of the nervous systems of the rats restricted to this diet was faulty, and that the tendency to destroy the young was an expression of an abnormal psychology analogous to the psychosis observed in beri-beri or pellagra. The fact that the third and fourth generation females in this family were less cannibalistic than their mothers and grandmothers militates somewhat against this view.

Lot 2143 derived all its antineuritic factor and protein from 20 per cent of liver in the diet. This was equivalent to 14.4 per cent of protein. The growth curves were normal. In the first group fed this diet there were two females. These had forty-six young (eight litters) and of these but twenty were weaned. Two second generation females had eighteen young (four litters) and weaned ten of them. Two third generation females had thirteen young (two litters) of which they weaned six. One fourth generation female was kept 5½ months, when she died, never having had any young. None of these animals in any generation was kept to an advanced age. The young of the rats fed the liver diet were somewhat undersized but appeared to be vigorous.

Lot 2142 was fed a diet comparable to the others described in this chart, but with all protein and water-soluble B derived from 20 per cent of muscle (round steak). These animals were markedly inferior both as regards growth and fertility to those fed kidney or liver at the same plane of intake. Two females were kept to the age of 15 months, an age which usually marks the end of
fertility in the rat. One had a single litter of eight young but they died when about 2 weeks old. The other remained sterile. The rats in this group were still in good nutritive condition after a year on the diet. This chart, like the other records in this paper, shows clearly the superiority of glandular organs over muscle tissue as sources of water-soluble B.

Chart 4.—These records show the behavior of young rats fed cooked dry beef kidney as the sole food, Lot 1253. Lot 1254 shows the growth curves of rats fed 97.5 per cent of kidney supplemented with sodium chloride and calcium carbonate. Lot 1255 was fed kidney, 94.5 per cent, supplemented with sodium chloride, calcium carbonate, and butter fat. Lot 1256 was fed a diet like that of Lot 1255 except that 14 per cent of the kidney was replaced by lactose. This last ration was designed to show whether lactose would tend to modify the bacterial flora of the alimentary tract. No beneficial effects of the modification were apparent.

The rats restricted to cooked dried kidney as their sole food grew in a fairly normal manner. Two females had collectively fourteen young (a litter each), five of which were weaned. One of these young developed abnormal ribs suggestive of rickets. None of these young grew up.

It is very remarkable that young rats confined to a diet of kidney could develop so successfully and reproduce and rear young. This tissue is very poor in calcium, and yields a great excess of acid on being metabolized. The protein content of this diet was not far from 71 per cent, yet because all essential food factors except calcium were so abundant, the animals were able to tolerate this deficiency and the abnormal protein content remarkably well.

Lot 1254 contained two females, one of which became pregnant and died in parturition. The other had a tumor which became so large that she was chloroformed.

There was but one female in Lot 1255. She had sixteen young (two litters) of which nine were weaned. Two second generation females had collectively seventeen young (a litter each) of which ten were weaned. Two third generation females had each a litter of young (seventeen) of which twelve individuals were weaned. The young appeared to be well developed, but were always greasy from their food. They drank much water. The cage had a strong odor. The protein content of the ration of Lot 1254 was 69 per
The results show that the rat is capable of growing and remaining in a state of health on a diet comparable to that of the strictly carnivorous animals. At least among the carnivorous animals of the Arctic regions the proportion of protein in the diet is not necessarily excessive at all times. Mr. Stefansson has informed us that it is not unusual for travelers to come upon a seal that has been killed and skinned, the subcutaneous layer of fat eaten, and the remainder of the carcass left practically untouched. The polar bear evidently prefers fat to protein as a source of energy.

Lot 1256 contained two females. They had collectively twenty-two young (two litters each) of which fifteen were weaned; two second generation females had together nineteen young, and weaned fourteen of them. One third generation female had one litter of six young and weaned them all.

Chart 5.—The diet of the four groups of rats whose curves are shown in this chart was in all instances comparable to those in Chart 4, the only difference being the substitution of liver for kidney in the diets of Lots 1277, 1278, 1279, and 1280.

Lot 1277, which was confined to liver as its sole food, grew very little and died from 4 to 6 months after being confined to the diet.

Lot 1278 was fed liver supplemented with sodium chloride and calcium carbonate. On this diet growth was approximately normal. The group contained three females. One of these had twelve young (two litters) and weaned eleven of them. The other two remained sterile. One second generation female remained sterile.

Lot 1279 was fed liver supplemented with sodium chloride, calcium carbonate, and butter fat. Lot 2162, Chart 2, shows that even 25 per cent of liver as the sole source of fat-soluble A furnishes a sufficient amount of this factor.

There were three females in this group. They had thirty-four young (six litters) and weaned thirty-one of them. One second generation female grew up on the diet, and had six young in one litter. She weaned four of these. One third generation female in this family had a litter of six and weaned them all. The reproduction records of this group were distinctly better than those of Lot 1278, whose diet was identical except for the 3 per cent of but-
ter fat. We cannot explain the reason for the superiority of Lot 1279.

Lot 1280 was like Lot 1279 except that 14 per cent of lactose replaced a like amount of liver. This did not exert any noticeably favorable effect on the well being of the animals. Two females each had a litter (collectively fourteen young) and weaned thirteen individuals. One second generation female had one litter of eight young and weaned seven of these. The young in Lots 1279 and 1280 appeared to be strong and in good condition but showed in all cases a peculiar condition which we have observed occasionally in abnormal animals; viz., a wet and stained area around the urethral orifice. This not infrequently occurs in poorly nourished animals.

**Chart 6.**—The animals whose records are shown in this chart were fed a diet made adequate in all respects as far as could be judged, and with the protein derived solely from 50 per cent of dry beef liver. The protein content of the diet was about 35 per cent.

Lot 1281 contained three females. These had collectively thirty-five young (five litters) and weaned twenty-two. Two second generation females had one litter each, collectively nineteen young, of which number they weaned seventeen. These young appeared to be very well nourished.

Lot 1282 contained two females. These collectively had five litters (thirty-four young). They weaned thirty-one of these young.

Two second generation females were restricted to this diet. One had a litter of six, which she successfully weaned. The other female remained sterile. The young were apparently well nourished but badly urine-stained. There was no evidence that the inclusion of lactose benefited the animals.

**Chart 7.**—The animals whose records are shown in this chart were fed either muscle tissue or blood, with and without certain purified food additions. Lot 1232 was restricted to a diet of cooked, dried, beef muscle. They were able to grow very slowly, but remained very much undersized, and died early. None of this group had any young. Two of the rats showed, toward the end of their lives, distinct signs of xerophthalmia, due to the lack of fat-soluble A in muscle tissue.
<table>
<thead>
<tr>
<th>Ration:</th>
<th>Muscle (beef)</th>
<th>Ox blood</th>
<th>Muscle</th>
<th>NaCl</th>
<th>CaCO₃</th>
<th>Lactose</th>
<th>Butter fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td>100.0</td>
<td>100.0</td>
<td>97.5</td>
<td>1.0</td>
<td>1.5</td>
<td>14.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Period 2</td>
<td>50.0</td>
<td>50.0</td>
<td>94.5</td>
<td>1.0</td>
<td>1.5</td>
<td>14.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

- Muscle: 91.0
- Ox blood: 80.5
- NaCl: 1.0
- CaCO₃: 1.5
- Lactose: 14.0
- Butter fat: 3.0

*Y = birth of young*
Lot 2027 was fed dried ox blood as their sole food in Period 1. They declined rapidly on this and after 2 weeks were changed to a diet of muscle meat 50 per cent and dried blood 50 per cent. On this diet they were able to grow at a rate approximately half the normal. A female in this group had a single litter of four young, which she destroyed within a few days. All these animals showed early signs of old age.

Lot 1233 was fed cooked dried muscle (beefsteak) supplemented with sodium chloride and calcium carbonate. They grew but little better on this diet than on muscle alone, but one female had a litter of three young, which she destroyed soon after birth. Two other females remained sterile on this diet. That fat-soluble A was the limiting factor in this diet is shown by the records of Lots 1234 and 1235.

Lot 1234 was fed muscle, sodium chloride, calcium carbonate, and butter fat. They not only grew in an approximately normal manner, but were fairly fertile and had moderate success in the rearing of their young. Two females had together twenty young (three litters) of which sixteen were weaned. One second generation female was kept on the diet. She had one litter of five young and weaned them all. The young were somewhat inferior. Further studies are necessary to determine what modifications of this diet are necessary to secure greater fertility and higher vitality in the young.

Lot 1235 was fed a diet of beefsteak supplemented with sodium chloride, calcium carbonate, butter fat, and 14 per cent of lactose. They were not superior in vigor, fertility, or success in rearing young to Lot 1234 which had more beefsteak in place of the lactose. Two females had thirty-two young collectively (five litters) of which twelve were weaned. Since these were all males no further reproduction records were secured.

Chart 8.—The animals whose records are shown in Chart 8 derived their protein and water-soluble B from 50 per cent of cooked, dried, muscle tissue (beefsteak). The protein content of the diet of Lot 1236 was about 35 per cent. The growth curves approximated the average and the animals appeared to be in a satisfactory state of nutrition.

There were two females in Lot 1236. Together they had thirty-four young (five litters) and weaned twenty-four of them. One
daughter of one of these mothers was kept on the diet. She had one litter of eight young and weaned them all. Two of her daughters grew up on the diet and each had a litter, together thirteen young, and weaned but three of them. The young in most cases appeared normal. They were not urine-stained as many were on the liver diets.

Lot 1237 differed from Lot 1236 in that 15 per cent of lactose replaced an equivalent amount of dextrin. There was no evidence that this carbohydrate exerted any beneficial effect over dextrin.

Two females in this group had together thirty young (six litters) and successfully weaned them all. Two of the second generation females had each a litter (collectively fifteen young), of which eleven young were weaned. One third generation female had a litter of seven young and weaned them all.

Chart 9.—These records show the histories of three groups of young rats fed diets in which all protein and antineuritic factors were derived from raw muscle tissue (round steak). Lot 2056 was restricted in the first period to raw round steak as the sole source of nutriment. They grew no better on this than on cooked steak (Chart 7, Lot 1232). In a second period sodium chloride and calcium carbonate were added and these caused a slight response with growth for a time. The diet was too poor in fat-soluble A to admit of much growth. This is seen in the record of Lot 2058.

Lot 2058 was fed a diet containing 50 per cent of raw, dried muscle (beefsteak) supplemented with sodium, potassium, calcium, chlorine, and fat-soluble A in butter fat. The diet contained 43.5 per cent of dextrin. On this ration growth was normal and the animals were apparently in good nutritive condition. Three females each had a litter of young (collectively twenty-two) of which twenty were reared. Two second generation females each had a litter (fourteen young) and reared them all.

There was a period between the ages of 18 and 28 days when the young of the second generation appeared lethargic. They recovered later and appeared to be as alert as rats on the cooked steak. This was probably due to the effects of eating so much raw meat while still in a very immature condition.

Lot 2057 had a diet like that of Lot 2058 except that 15 per cent of dextrin was replaced by a like amount of lactose. There was
<table>
<thead>
<tr>
<th>Period 1</th>
<th>Lot 2056</th>
<th>Lot 2058</th>
<th>Lot 2057</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw muscle</td>
<td>100.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CaCO3</td>
<td>1.5</td>
<td>1.5</td>
<td>25.0</td>
</tr>
<tr>
<td>XCL</td>
<td>1.0</td>
<td>1.0</td>
<td>CaCO3</td>
</tr>
<tr>
<td>Dextrin</td>
<td>42.5</td>
<td>1.0</td>
<td>XCL</td>
</tr>
</tbody>
</table>

replaced part of the steak.

<table>
<thead>
<tr>
<th>Period 2</th>
<th>Lot 2056</th>
<th>Lot 2058</th>
<th>Lot 2057</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butter fat</td>
<td>3.0</td>
<td>3.0</td>
<td>28.5</td>
</tr>
</tbody>
</table>

Y = birth of young
no noticeable benefit from this modification of the diet. There were two females in this group. They had collectively twenty-three young (four litters) of which twenty-two were weaned. No young have as yet been secured from any of the daughters. These records when compared with those of Chart 8 indicate that no noticeable difference exists in the nutritive value of raw and cooked steak. Although steak is very poor in water-soluble B, there was a sufficient amount of it in 50 per cent of cooked steak to permit young rats to grow up and rear young.
SUPPLEMENTARY PROTEIN VALUES IN FOODS: I. THE NUTRITIVE PROPERTIES OF ANIMAL TISSUES
E. V. McCollum, Nina Simmonds and H. T. Parsons

J. Biol. Chem. 1921, 47:111-137.

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