mum allowance of food which will induce an average gain in weight of 3 g. per week during the test period is thus ascertained.

The unit recommended for numerical expression of results is that amount of vitamin A which when fed daily induces an average gain of 3 g. per week in a standard test animal under the conditions described.

The choice and control of the basal diet, of the test animals, and of the experimental procedure, and the interpretation of the findings, are discussed.

New York, N. Y.

[Contribution from the Department of Chemistry of Columbia University, No. 472]

QUANTITATIVE EXPERIMENTS UPON THE OCCURRENCE AND DISTRIBUTION OF VITAMIN A IN THE BODY, AND THE INFLUENCE OF THE FOOD

By H. C. Sherman and L. C. Boynton

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A conspicuous feature of the experiments by means of which the existence of fat-soluble vitamin was demonstrated by McCollum and Davis[1] and Osborne and Mendel[2] was the fact that feeding tests of relatively long duration were required before the results of a lack of this substance in the food were made manifest in the condition of the test animal. This led to the general belief[3,4] that the animal body might be able to carry a store of this vitamin, and soon afterward it was shown[5] that animals kept under like conditions upon the same diet deficient in vitamin A survive for very different lengths of time, according as their previous diet has been rich or poor in this substance.

That the liver is especially concerned in this storage function was early suggested by Osborne and Mendel's[6] discovery of the high vitamin-A potency of cod-liver oil, and by the finding of liberal proportions of this vitamin in pig and beef livers.[7] In a paper appearing since the completion of most of the experiments here described, Steenbock has shown[8] that rat liver may be relatively rich or relatively poor in vitamin A, according to the nutritional history of the animal.

[1] McCollum and Davis, *J. Biol. Chem.*, **15**, 167 (1913).

[2] Osborne and Mendel, *ibid.*, **15**, 311 (1913).

[3] Hopkins and others, *Nat. Health Insur. Med. Res. Com. (Great Britain), Special Rept.*, No. 38, **1919**.

[4] Osborne and Mendel, *J. Biol. Chem.*, **45**, 277 (1921).

[5] Sherman, MacLeod and Kramer, *Proc. Soc. Exptl. Biol. Med.*, **17**, 41 (1920). See also Sherman and Kramer, This Journal, **46**, 1055 (1924).

[6] Osborne and Mendel, *ibid.*, **17**, 401 (1914).

[7] Osborne and Mendel, *ibid.*, **32**, 309 (1917); **34**, 17 (1918). McCollum, Simmonds and Parsons, *ibid.*, **47**, 111 (1921). Zilva and Drummond, *Lancet*, **1922**, I, 1243.

[8] Steenbock, Sell and Nelson, *J. Biol. Chem.*, **56**, 327 (1923).

According to Drummond, Golding, Zilva and Coward,[9] the diet influences the vitamin-A content of the body fat generally; and several investigators have reported that the vitamin-A content of milk is influenced by that of the food.[10] Coward and Drummond[11] also point out that young fish store this vitamin in their tissues in relatively large amounts when given food containing ample quantities, but not otherwise.

Thus previous investigations have amply established the ability of the animal body to store vitamin A, and have indicated to some extent the influence of food supply upon body storage and the relative prominence of the liver in connection with this storage function. It is the purpose of the present paper to present in brief summary the data of experiments, carried out in as strictly quantitative a manner as possible, to determine the relative amounts of vitamin A in several of the principal tissues of adult rats of known dietary histories. These experiments were planned as a study, first of the distribution of vitamin A in the body, and second, of the influence of the food of the animal upon the vitamin-A content of its tissues. The influence of the food is here shown both by differences in survival upon vitamin-A-free diet of animals whose previous dietary history had been different, and by the method of dissecting animals of different dietary histories and feeding their tissues as sole sources of vitamin A to test animals.

General Plan of Experiments

In the first series of these experiments, young rats of the same stock and of the same age, 28–29 days, but from families on different diets, were put upon the same basal diet (Diet 701), which has been shown by Sherman and Kramer[12] to be adequate in all other respects while free from vitamin A, and which has also been shown to yield the same results as the basal diet (Diet 380) used in the experiments of Sherman and Munsell.[13] These experiments, which were carried on simultaneously with those described in the preceding papers of this series,[12,13] served to show the influence of the dietary history of the young rat and its mother upon its body store of vitamin A at this early age, and to indicate the relative advantages of diets of differing vitamin-A content as food for the families from which test animals for experiments upon vitamin A are to be drawn. Diet B was thus selected as the standard for this work. In the second series of experiments, the distribution of vitamin A in the body was studied by feeding

[9] Drummond, Golding, Zilva and Coward, *Biochem. J.*, **14**, 742 (1920).

[10] McCollum, Simmonds and Pitz, *J. Biol. Chem.*, **27**, 33 (1916). Drummond, *Lancet*, **1918**, II, 482. Drummond, Coward and Watson, *Biochem. J.*, **15**, 540 (1921). Hughes, Fitch and Cave, *J. Biol. Chem.*, **46**, 1 (1921). Kennedy and Dutcher, *ibid.*, **50**, 339 (1922). Korenchevsky, *Med. Res. Council (Great Britain) Spec. Rept.*, No. 71, **1922**. Hume, *ibid.*, No. 77, **1923**.

[11] Coward and Drummond, *Biochem. J.*, **16**, 640 (1922).

[12] Sherman and Kramer, This Journal, **46**, 1055 (1924).

[13] Sherman and Munsell, *ibid.*, **47**, 1639 (1925).

tissues of healthy adult rats, which had been kept on Diet B, to test animals as sole source of vitamin A, according to the method described in the preceding paper. Similar experiments were next made with the tissues of rats which had been fed a different diet in order to determine the influence of the food upon the vitamin-A content of the different tissues; and finally, the fact that the differences thus found are unquestionably due to the diet was verified by a fourth series of experiments in which rats were fed with and without cod-liver oil, and the vitamin-A-contents of their tissues compared.

Influence of Previous Feeding as Shown by Survival of Young Rats on Vitamin-A-free Diet

Young rats of the same general stock were taken at 28 days of age from families on each of three diets: Diet A, 1/6 whole milk powder, 5/6 ground whole wheat; Diet B, 1/3 whole milk powder, 2/3 ground whole wheat; Diet D, 2/3 whole milk powder, 1/3 ground whole wheat. In each case the diet (A, B or D) contained also sodium chloride in the proportion of 2% of the weight of the wheat.

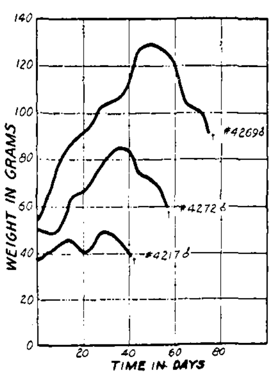

Fig. 1.—The influence of previous feeding upon the body store of vitamin A, as shown in survival periods upon vitamin-A-free diet. Rat No. 4217 had received Diet A (low in vitamin A); No. 4272 had received Diet B (which contains vitamin A in fairly liberal amounts); No. 4269 had received Diet D (which is still richer in vitamin A). All were placed at the same age (28 days) upon the same vitamin-A-free basal diet. Growth and duration of life upon this diet differed greatly, evidently because of the different amounts of vitamin A stored in their bodies at the beginning of the experiment.

These young rats were placed upon the basal vitamin-A-free diet which in this case was Diet 701, consisting of 10% of meat residue, thoroughly extracted with hot strong alcohol, 82% of patent flour, 4% of Osborne and Mendel salt mixture, and 4% of dried brewery yeast. Parallel tests have shown that this diet is interchangeable with that described in the preceding paper.

Typical results illustrating the wide differences in growth and survival when young rats from families on the three diets, A, B and D, were placed at the same age (28 days) upon the same vitamin-A-free diet are shown in Fig. 1.

As may be seen from Fig. 1, the young rats from Diet A soon cease growing when placed on vitamin-A-free diet. They are, however, hardly sufficiently vigorous or uniform to make satisfactory test animals; and those from Diet D are not satisfactory for this purpose because of the extended time required to deplete their initial bodily store of vitamin A. Hence, young rats from Diet B were preferred as test animals, and adult animals fed the same diet were used for dissection in the main series of determinations of the quantitative distribution of vitamin A in the body tissues.

Distribution of Vitamin A in Bodies of Adult Rats as Shown by Feeding their Tissues as Sole Source of this Vitamin to Test Animals

The rats whose tissues were used as sources of vitamin A, in this series of experiments to study its distribution in the body, were from families kept on Diet B and were practically all males in early adult life. All were healthy animals from families which, as described elsewhere,[14] have thrived on this diet for several generations.

Each animal, as required, was chloroformed, dissected and the tissues to be used for feeding were promptly transferred to glass containers, tightly closed and kept in a refrigerator. In dissecting out liver, kidney, etc., the blood vessels were cut close to the organ and the excess of blood was removed by gentle pressure and absorption on unglazed paper. Care was taken to allow as nearly as possible the same amount of drainage and absorption in all cases, and to handle the moist tissues, both at dissection and subsequently, in such manner as to insure the maintenance of their normal moisture content.

In general the muscle, liver, lung and kidney of the dissected rat were fed to test animals of the same litter in order that the comparisons of vitamin-A content of these tissues might be made as strictly quantitative as possible.

The feeding tests with graded portions of the tissues were carried out as described in the preceding paper.[13] Fig. 2 summarizes the results obtained in experiments with each of the four tissues chiefly studied: muscle, kidney, lung and liver. Since space is not available for the presentation of the numerical data of the experiments, the following brief notes will make clearer the results of the studies upon the distribution of vitamin A in the different tissues of the body.

Muscle.—Fresh skeletal muscle from which the visible fat had been removed was fed in daily amounts of 1, 2, 3 and 4 g. Four g. daily was found necessary to insure enough vitamin A to maintain the rat during the experimental period and permit some growth. The average growth curve of seven rats receiving this amount of muscle will be seen in Fig. 2 to be somewhat below those afforded by 0.1 g., respectively, of kidney and lung. Feeding 6 g. of muscle per day was attempted, but a considerable amount remained uneaten.

Kidney.—The fat was removed and the kidneys were cut into cross-section portions for feeding. Tests were made by feeding 0.5, 0.25, 0.10 and 0.075 g. daily. The average growth of ten rats fed 0.10 g. per day as the source of vitamin A, in comparison with comparable amounts of muscle, lung and liver, is given in Fig. 2.

[14] Sherman and Campbell, *J. Biol. Chem.*, **60**, 5 (1924).

Lung.—As will be seen from Fig. 2, 0.1 g. of lung tissue daily provided sufficient vitamin A to permit the standard increase in weight over a period of eight weeks. The average of nine tests gave results slightly above those obtained by feeding 0.1 g. of kidney, and of a somewhat greater difference over those obtained with 4 g. of muscle.

Liver.—In order to obtain growth curves corresponding to those with 4.0 g. of muscle and 0.1 g. of kidney or of lung, quantities of liver from 0.01 g. to 0.02 g. per day were found to be required. Vitamin A available from only 0.01 g. of liver daily, evidently was not entirely sufficient as four of eight rats fed this small amount died before the expiration of the experi-

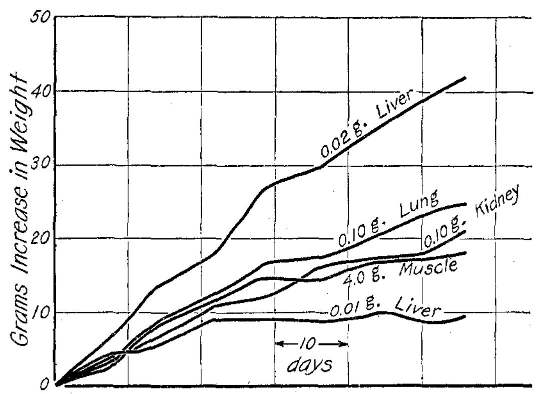

Fig. 2.—Average growth curves of young rats which were fed tissues of adult rats from Diet B as the sole source of vitamin A. The initial point of the curves represents the weight at the time of beginning the feeding of the tissues, after the animals had ceased to grow on a vitamin-A-free basal diet. Of eight rats receiving 0.01 g. of liver, four died during the experimental period, and the later part of this curve is based on the weekly weighings of the survivors.

mental period. The average weight curve of the survivors, as well as the average resulting of feeding twice this quantity, is shown in Fig. 2.

Other Tissues.—A number of other tissues were fed, for which it is not possible to present strictly quantitative data, either due to lack of sufficient fresh material to maintain growth over the experimental period or failure of the animals to eat sufficient of the tissue, as was the case with bone.

One test made with rats' blood points to the presence in it of significant quantities of vitamin A. The rat was fed an amount averaging 0.65 g. of fresh blood per day, and made a growth comparable to the rats of the same litter on 4.0 g. of muscle or on 0.1 g. of kidney.

The fact that the blood contained (per unit of weight) much more vitamin A than muscle and much less than kidney or lung makes it plain that the differences between these tissues cannot be ascribed to a difference in their retention of blood; if the blood were completely removed from them the difference between the low vitamin-A content of muscle and the relatively high content in liver and lung would become even more pronounced.

Bones (the long bone sand ribs) were scraped free from adhering tissue, ground in a mortar to a paste and fed, but were not always readily eaten by the experimental animals. In general, the amounts of bone which the animals would eat gave evidence of furnishing only barely measurable amounts of the vitamin. Weight for weight, bone and muscle appear, so far as can be judged from these few experiments with bone, to contain about the same small amount of vitamin A.

Brain, spleen, pancreas, or heart muscle fed at a level of 0.25 g. per day failed to maintain test animals over the experimental period. One-half g. of brain fed daily prolonged the survival period ten days beyond that of the negative control of the same litter. One experiment was made for the determination of vitamin A in the testes; all visible fat was removed and the gland fed whole to the rat. Weight was maintained over a period of six weeks before death occurred on an amount averaging 0.5 g. per day, indicating the presence of vitamin A in testes, but in concentrations much lower than in kidney or in lung tissue.

Summary of Distribution.—From the above results obtained with tissues of adult Diet B rats, the kidney appears to be at least 40 times as rich in vitamin A as the muscle, the lung more than 40 times, and the liver, 200 to 400 times as rich in this vitamin as the muscle. The relative importance of these tissues as storage organs may be further illustrated by the statement that of the total amount of vitamin A in the body of a typical (Diet B) adult rat, if adipose tissue and skin be ignored, about nine-tenths was found in the liver and the remaining tenth about equally divided between the muscles as a whole, the blood, the kidneys and the lungs.

Influence of the Food upon Vitamin-A Content of Individual Tissues

While the above-described studies of the distribution of vitamin A in the body were in progress, an extended comparison of the nutritive condition of rats kept on Diets A and B, respectively, was also being made,[14] and advantage was taken of the material thus afforded to study the influence of this difference in food upon the vitamin-A content of the body tissues. As explained above, both Diets A and B are mixtures of whole milk (dried) and ground whole wheat, Diet B containing the higher proportion of milk and, therefore, of vitamin A.

For the sake of economy of space, the numerical data of this series of experiments are omitted. The findings may be summarized in general terms as follows.

Liver.—Although both Diet A and Diet B are adequate, the latter, with its higher proportion of vitamin A, increased the concentration of this vitamin in the liver at least four- to ten-fold. In view of the fact that the storage outside of the liver appears relatively small, this must mean that the total store of vitamin A carried in the body is several times larger on Diet B than on Diet A.

Muscle.—In ten cases in which muscle was compared, there was evidence of only slightly more vitamin A in the muscle of the rats from Diet B than of those from Diet A, the difference being insignificant as compared with that found in the liver. Whereas the feeding of the diet richer in vitamin A increased the concentration of this vitamin in the liver at least 300%, it increased that of the muscles not over 30%.

Kidney.—No difference in the concentrations of vitamin A in the kidneys of the rats fed the two diets could be detected in three comparisons which were made with this tissue. This does not preclude the possibility of a small difference existing, such as that found in the muscle.

Lung.—In each of two comparisons, better growth was obtained with the lung of those rats receiving the larger proportion of milk (and, therefore, of vitamin A) in their diets. Apparently the lung tissue of rats on Diet B contained about twice as much vitamin A as did that of rats on Diet A.

Experiments with Ten-weeks-old Rats that Had Received Cod-liver Oil in Addition to the Basal Diet were made in order to verify the conclusion that the differences found in the preceding series were due to the differing vitamin-A contents of the foods that the animals had received. The rats of the new series had been fed for six weeks: (*a*) vitamin-A-free basal diet alone, (*b*) the basal diet supplemented with 1%, or (*c*) with 2% of cod-liver oil. A fresh supply of ten-weeks-old-rat tissue was maintained by placing rats from the same litter on each of the three diets each week. During the sixth week afterward they were killed and their tissues fed to test animals.

Amounts fed were the same as in the experiments summarized in Fig. 2, namely, 4.0 g. of muscle, 0.1 g. of kidney, 0.1 g. of lung and 0.01 g. of liver per day. In no case did there appear any appreciable difference between the test animals that were fed the tissues of the rats from the diet devoid of vitamin A, and their negative controls; but larger amounts were plainly present in the tissues of the animals that had received the same diet plus 1% or 2% of cod-liver oil.

The growth made on 4.0 g. per day of muscle was about the same, whether the muscle came from a rat on Diet B or from one that had received cod-liver oil. The vitamin-A content of the muscle appears to remain fairly constant at a low value, even when the food furnishes liberal amounts.

Both 0.1 g. of lung and 0.1 g. of kidney of rats receiving cod-liver oil resulted in a rate of growth at least twice that which resulted from the same amount of these tissues from Diet B rats.

Such experiments comparing tissues of rats receiving cod-liver oil additions to the basal diet, with those of rats denied such supplements, confirm the conclusion reached in the comparison of rats from Diets A and B that the differences in amounts of vitamin A in their tissues, particularly of this vitamin stored in the liver and present in the lung, are directly attributable to the different amounts of vitamin A received in their food.

Summary and Conclusions

The distribution of vitamin A in the body of the rat was studied by feeding the tissues of adult animals, as the sole source of this vitamin, to young rats which had ceased to grow on a diet otherwise adequate.

An average of all directly comparable results on adult rats reared on a diet of one-third whole milk powder and two-thirds whole wheat, showed the kidney to be at least 40 times as rich as the muscle; the lung more than 40 times, and the liver from 200 to 400 times as rich in vitamin A per gram as muscle.

The vitamin-A content of the food influences that of the body.

That the difference in the amount of vitamin A found in the liver and in the lung tissue was directly attributable to the different amounts of this vitamin in the food was further shown by a comparison of the tissues of ten-weeks-old rats that had received additions of cod-liver oil to the basal diet with tissues of rats of the same age and diet without cod-liver oil.

New York, N. Y.

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THE BODILY STORE OF VITAMIN A AS INFLUENCED BY AGE AND OTHER CONDITIONS

By H. C. Sherman and L. B. Storms

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Both of the papers in which the discovery of the substance now called vitamin A was recorded[1] contained suggestions that the body may be able to carry a store of this substance, and later experiments have shown definitely that this vitamin may be stored in important amounts both before and after the end of infancy.[2] There is no good evidence of its synthesis in the animal body of either sex at any age, and experience has suggested that it is essential at all ages and that the apparently diminished dependence of the adult as compared with the young may be largely due to the older animal having had opportunity to acquire a larger body store of this

[1] McCollum and Davis, *J. Biol. Chem.*, **15**, 167 (1913). Osborne and Mendel, *ibid.*, **15**, 311 (1913).

[2] Sherman and Kramer, This Journal, **46**, 1055 (1924).