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.

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

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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).

vitamin.[3] Because of the importance of this substance in the chemistry of food and nutrition, all factors affecting the behavior of vitamin A in the body and the response of the body to it, or to the lack of it, should be studied as thoroughly as possible.

The experiments described in the present paper deal (1) with the age at which experimental animals (albino rats), under known conditions of feeding, attain their maximum store of vitamin A as indicated by the length of time they are able to survive when transferred to a diet devoid of this vitamin but adequate in all other respects, and (2) with the influence of previous feeding upon the relative store of vitamin A in the body at different ages as indicated by the period of survival when placed on vitamin-A-free food.

It was found that these questions could be more successfully studied in cases of rats put on the experimental diet during the winter months. The data are therefore drawn chiefly from experiments begun between the months of October and April, inclusive.

## General Method of Present Experiments

In all of the experiments described in this paper, the method has been to transfer healthy rats at a definite age from the diet on which they had been raised to a diet free from vitamin A but adequate in all other respects, and determine their survival period, when kept on this diet in individual round cages 23 cm. in diameter and 23 cm. high with raised, wire-screen bottoms to prevent the animal having access to its excreta. The vitamin-A-free diet here used (Diet 701) was the same as that used (under the designation 97–34) by Sherman and Kramer.[2]

## Storage of Vitamin A in Relation to Age

While it has been recognized that adults may survive deprivation of vitamin A considerably longer than young of the same species, and recently has been generally accepted that this is largely due to bodily stores of the vitamin, there has not hitherto been satisfactory quantitative information as to the relation of age to this storage of the vitamin in the body. In the present series of experiments, male white rats which had previously received Diet B (a mixture of one-third whole milk powder and two-thirds ground whole wheat, with sodium chloride added in the proportion of 2% of the weight of the wheat) were transferred to the same vitamin-A-free diet at different initial ages and found to show average survival periods as follows: four-weeks-old rats, 63 days; two months, 111 days; three months, 122 days; four months, 148 days; six months, 171 days; nine months, 140 days.

Thus, on an accurately known diet, which furnishes vitamin A in fairly liberal but not strikingly large proportion, the bodily store as indicated

[3] Sherman, MacLeod and Kramer, *Proc. Soc. Exptl. Biol. Med.*, **18**, 41 (1920).

by survival period on vitamin-A-free diet, was found to increase steadily up to six months or about the age at which full adult size has been attained. The trend of the weight curves during the survival periods are shown in Fig. 1.

These experiments also gave opportunity for observations upon the incidence of the different symptoms which have been considered more or less characteristic of the vitamin-A deficiency.

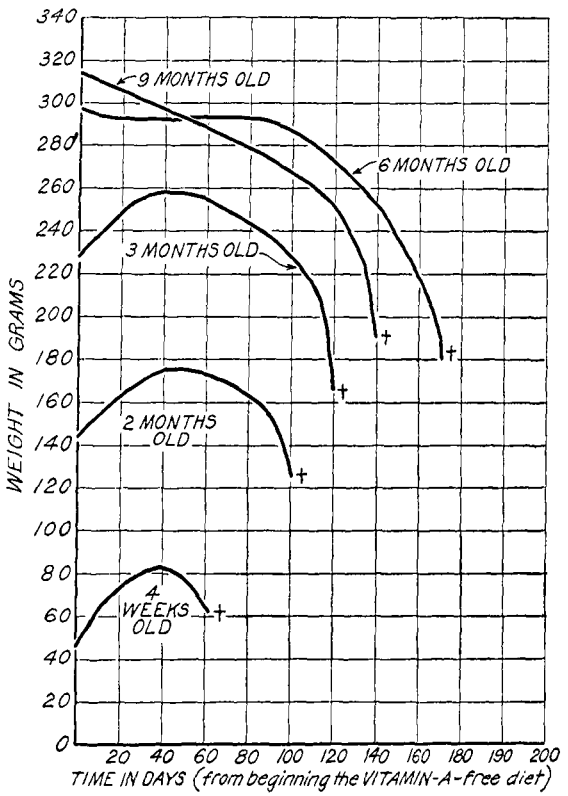


Fig. 1.—Typical or average weight curves of male rats taken from the same normal diet (Diet B) and placed on the same vitamin-A-free diet at different ages.

Three-fourths of the rats here placed upon vitamin-A-free food at the age of about one month developed the characteristic ophthalmia before death; but it developed in only about one-fourth of the rats that were from two to nine months old when subjected to the same dietary deficiency. Thus it is plain that, with all other conditions uniform, the older animal is distinctly less likely to develop the ophthalmia even though he dies from the vitamin-A deficiency.

The older rats, however, proved more susceptible than the younger ones to lung infection, the frequency of which, in our experience as in that of Steenbock and other investigators, is increased by diet deficient in vitamin A. In this series, the lungs of the rats that had been transferred to the vitamin-A-free diet at from four weeks to four months of age appeared normal at autopsy in nearly all cases, while nearly half the rats that were six or nine months old when subjected to the same dietary deficiency developed lung trouble.

Two-thirds of all the rats included in this series, and dying from vitamin-A deficiency, showed pus in one or more of theg lands near the base of the tongue. The younger rats showed this sign rather oftener, and the older animals distinctly oftener, than the ophthalmia. This sign has also been looked for in other rats than those of the series above mentioned and in a total of 190 rats examined after having died of vitamin-A deficiency, 142 have shown such abscesses near the base of the tongue. Pus in the middle ear seems to result with about equal frequency, having been found in 104 of 134 rats dying of vitamin-A deficiency; while sinusitis was found in 41 cases out of 79 examined. Thus, the sinusitis showed an incidence of about 50%, whereas the tongue and ear infections were found in about 75% of the animals subjected to the same dietary deficiency.

## Influence of Diet upon Body Store of Vitamin A

Parallel with the experiments described in the preceding section upon male rats taken from Diet B, a number of similar experiments have been made with females taken from the same diet and with both males and females from Diet A which contains less vitamin A than does Diet B.[4] The bodily store of vitamin A as shown by the length of time that an animal can survive on a diet devoid of this vitamin was found to be very materially influenced by a moderate difference in the vitamin-A content of the food which the animal had received. For each sex and for each of the three ages included in this comparison (namely, one, two and three months) Diet B had evidently resulted in a larger bodily store of vitamin A than had Diet A.

The data also show that for both sexes the difference in body store due to the animals' food increased with the age of the animal up to three months, which was as far as we had opportunity to carry this comparison.

## Influence of Family and of Sex

The value of litter controls has been emphasized in the vitamin work of the last few years and we believe rightly so; but this value must not be overestimated. In our experiments it has been found that family is undoubtedly an influence in some cases and, therefore, that litter controls

[4] For comparison of Diets A and B, see Sherman and Campbell, *J. Biol. Chem.*, 60, 5 (1924).

are unquestionably of greater value than controls drawn merely from stock; but that even the regular use of litter controls does not preclude the necessity of frequent repetitions of experiments if results of the highest quantitative accuracy are desired.

Since the main purpose of the present investigation was to study the relation of age to the bodily store of vitamin A and it was possible that the sexes might differ in this respect, our chief series of experiments have dealt exclusively with male animals in order that age should be the only variable factor. Somewhat incidentally, however, we have made certain observations upon females which seem worthy of brief mention here.

From the results as a whole it would appear that there is a difference in survival period in favor of the females; this difference is negligible at four weeks of age but becomes more distinct with age and development, at least up to early adult life, which is as far as we have had opportunity to extend the comparison. It is interesting that pregnancy and suckling of young have not, in the few cases yet studied, seemed to deplete the female organism of vitamin A to the extent of noticeably shortening her survival period. The possibility is therefore suggested that the development and exercise of the reproductive functions in the female may enhance the ability to conserve vitamin A, or in some way to use it more economically in case of need.

## Summary

Among animals coming from the same previous diet, age was found to have an important influence upon the length of the survival period upon food devoid of vitamin A. The maximum survival period and, therefore, presumably the maximum body store of vitamin A (or at least the maximum store in relation to daily need) was found at six months of age, or about at the beginning of full adult life.

A moderate difference in the vitamin-A content of the food was found to cause a marked difference in the amount of this vitamin stored in the body as indicated by the survival period on vitamin-A-free food.

The incidence of the characteristic symptoms as influenced by the age at which the experimental animal is subjected to the vitamin deficiency is discussed briefly.

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