THE ABILITY OF THE DOG TO UTILIZE VITAMIN A
FROM VEGETABLE AND FROM ANIMAL SOURCES

by

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A Thesis
submitted to the faculty of the
Department of Nutrition
in partial fulfillment of
the requirements for the degree of

Master of Science
in the Graduate College
University of Arizona

1937

Approved: Margaret E. Smith
Adviser

Date  May 26, 1937
ACKNOWLEDGEMENTS

The writer desires to express her gratitude to Dr. Margaret Smith for her assistance and valuable criticism in selection and pursuance of the problem; to Dr. William Pistor for his assistance in the post-mortem examinations of the dogs; to Dr. R. B. Streets for the photographs; and to Dr. Louise Otis for her helpful suggestions.
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THE ABILITY OF THE DOG TO UTILIZE VITAMIN A
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Introduction

Since the discovery, in 1913, by McCollum and Davis (1), and by Osborne and Mendel (2) of a nutritional factor, later to be isolated and designated as vitamin A, a great many investigators have conducted experimental studies to test its physiological function, its requirement by different species of animals, its distribution in foodstuffs, and its chemical nature.

It had been suggested as early as 1881 by Voit (3) that a more logical method of studying the nutritional needs of animals would be to use purified foodstuffs instead of natural foods. Stepp (4) had found that lipoids or the materials which could be extracted with ether from a milk-bread diet were necessary for normal nutrition, and the addition of this extract to the extracted bread and milk led to normal growth. Osborne and Mendel (2) while studying the effect of single proteins in the diet of rats, discovered that mixtures of purified foodstuffs, such as they were using, maintained adult animals for some time, but did not support growth in young animals. At the same time McCollum and Davis (1) using rations of purified casein, carbohydrates and salt mixtures, and the same rations with a part of the carbohydrate replaced by lard, found that normal growth was produced for a period of seventy to one hundred twenty days, but beyond that time no gain in weight could be induced.
Changing the values of the different ingredients of the diet had no effect but growth was resumed promptly after the introduction of an ether extract of egg or of butter. They concluded that there were certain accessory materials in certain foodstuffs, either combined with the lipins or accompanying them, which were essential for normal growth. Osborne and Mendel (5) a little later reported the active factor to be contained in the clear fat fraction of butter, to be devoid of ash, and evidently not destroyed by heating with steam or by storage.

It has since been found that growing rats, when placed on a diet lacking in vitamin A, did not cease to grow immediately in most cases, but continued to grow slowly or sometimes nearly normally for a period of some weeks, at the end of which time there was a rather sudden cessation of growth followed by rapid loss of weight and general decline until death. The time elapsing between the beginning of the vitamin A deficient diet and the cessation of growth was found to be dependent upon the store of vitamin A in the body of the animal. Sherman and Storms (6) studying the factors influencing the store of vitamin A, transferred rats at various ages to a vitamin A deficient diet, and noted the survival periods at each age level, as an indication of the store of vitamin A. They found that longest survival, thus maximum storage, occurred when animals were taken from the stock diet at six months of age, or the beginning of adult life. They also found storage to be dependent upon the intake of vitamin A as did Sherman and Cammack (7) in a later study. Sherman and Boynton (8) studied the distribution of this store of vitamin A in the rat body and found the liver to contain two hundred to four hundred times as much vitamin A as did the muscle, in which the concentration was very slight.
The kidney, which was the next highest source of vitamin A, contained only forty times as much vitamin A as the muscle. Baumann, Riising and Steenbock (9) reported ninety-five per cent of the total storage of vitamin A in the rat to be concentrated in the liver. Newborn animals had very little vitamin A store, but this could be somewhat increased by feeding the mother a ration high in vitamin A during pregnancy and lactation. Administering definite amounts of vitamin A, they found absorption and storage to take place within six hours after ingestion.

At about the time growth ceased in the vitamin A deficient animal, there was noted also by Osborne and Mendel (2) a characteristic eye infection, beginning with a swelling of one or both eye lids, sensitivity to light as indicated by blinkiness, inflammation of, and a purulent discharge from the eyes, and finally ulceration of the cornea. Other workers (10), (6) corroborated these findings. The xerophthalmia, as it is now called, is more common in the young rat, and often does not appear at all in an adult rat fed on a vitamin A deficient diet.

A lack of vitamin A frequently manifests itself in the development of pus in one or more of the glands at the base of the tongue, in the middle ear, in the sinus cavities and in general along the entire respiratory tract (10), (6). Wolbach and Howe (11) reported abscess-like cavities in the pharynx, at the base of the tongue and in the sub-maxillary glands, occurring in vitamin A deficient rats. Their microscopic study of the lesions in vitamin A deficient rats showed a progressive epithelial keratinization along the upper respiratory tract, and also in the genito-urinary tract. Green and Mellanby (12) found that ninety per cent of vitamin A deficient rats showed tongue abscesses, nine per cent showed lung infections, forty-
four per cent kidney and bladder infections, while only two instances of such infections were found in several hundred rats on diets adequate in vitamin A. Because of the frequency of occurrence of epithelial lesions in the respiratory tract and elsewhere, vitamin A is regarded by some investigators as having anti-infective properties, at least insofar as maintaining the epithelial tissues of the membranes in good condition. Sherman and MacLeod (13) and Evans and Bishop (14) have shown that vitamin A is necessary for successful reproduction in rats. Van Leersum (15) has reported urinary and kidney calcium deposits to be present in eighty-eight per cent of rats on a vitamin A free diet. Cutaneous malnutrition, as evidenced by scabbiness of the tail or ears, sores on the nose and feet, thin and rough hair, are further symptoms noted in vitamin A deficient rats (16).

While most of the experimental work on the production of vitamin A deficiency has been done with rats, other animals have been found to be susceptible to a lack of vitamin A. Chickens, (17),(18), guinea pigs (19), rabbits (20), cattle (21), monkeys (22), cats (23), and dogs (24),(25) (26), (27), (28), (29), (30), have been shown to develop one or more of the deficiency symptoms characteristic of vitamin A deficiency.

There are on record a number of instances of the occurrence of avitaminosis A in humans (31), (32), (33). In the oriental countries, in particular, there appears to be a high incidence of xerophthalmia and "night blindness" which is generally conceded to be caused by a mild deficiency of vitamin A. Urinary calculi are prevalent in certain parts of India and are attributed in part to vitamin A deficiency (34).

During and following the World War, xerophthalmia among Danish children
was of frequent occurrence and responded to treatment with vitamin A (35). In the United States the occurrence of night blindness is much more frequent than is generally suspected (36) and is considered to be due to vitamin A deficiency.

That dogs require vitamin A for normal growth and develop deficiency symptoms when vitamin A is not present in their diet has been shown by several investigators.

The occurrence of vitamin A deficiency symptoms in puppies was reported by Steenbock, Nelson and Hart in 1921 (24). After five weeks on a Vitamin A deficient diet, they noted failure of appetite, unusual quietness, unsteadiness of gait, and in sixty-seven to ninety-four days development of xerophthalmia which recovered upon treatment with cod liver oil. Skeletal deformities were noted which suggests inadequate vitamin D as well as vitamin A.

Stimson and Hedley (25) also produced xerophthalmia in puppies, while other symptoms were irregular in appearance. In two litters of puppies, one in good condition when placed on the deficient diet, the other litter in poor condition, ocular symptoms occurred in eleven to thirty-two weeks and six to ten weeks respectively, suggesting storage as a factor in vitamin A depletion of the dog as well as of the rat.

Suzman, Miller and Ungley (26) were unable to produce xerophthalmia in adult dogs, but noted a dermatitis, weakness, loss of appetite, and anemia. Their results, however, are complicated by the fact that all dogs used in the experiment, whether receiving vitamin A or not, developed the same symptoms, indicating deficiency of one or more other factors.

Mellanby (27) considers a lack of vitamin A in early life to be re-
sponsible for poor formation of teeth and jaw bone in dogs and other ani-
mals. E. Mellanby (28) produced xerophthalmia in puppies, and found that
even after apparent outward recovery of the opthalmia following adminis-
tration of vitamin A, there were severe degenerative changes in the central
and peripheral nerves, of the type (Wallerian) which takes many months
to regenerate.

Ralli and co-workers (29) noted dermatitis, sores on the body and
loss of hair as the most constant finding in vitamin A deficient dogs.
Olcott (37) observed no symptoms of vitamin A deficiency except weakness
in three dogs on a vitamin A deficient diet, although all died after eleven
to fifteen weeks on the diet.

Storage of vitamin A in the livers of dogs was reported by Simonnet,
Busson and Asselin (30). They found no reserve in the livers of newborn
puppies, slightly detectable amounts in pups three days of age, and in
normal pups two months of age the concentration of vitamin A per gram of
liver was approximately one-half that of the mother.

Ralli (29) studying the metabolism of vitamin A in normal and depan-
creatized dogs, obtained widely variant results in determining the vitamin
A content of the livers, apparently dependent upon store previous to the
experimental period.

Because of its general use as a laboratory animal, the rat has been
widely used as a test animal for measuring vitamin A values of different
foodstuffs. With the adoption of a standard method of test, first introduc-
ed by Drummond (38) and further developed by Sherman and Munsell (10) and
modified by Sherman and Burtis (39) it has been found that rats within a
litter will respond fairly constantly to a given intake of vitamin A.
A basal diet which contains no appreciable amount of vitamin A is given
*ad libitum* to the animal, and when symptoms of depletion appear, the
supplement containing vitamin A is fed daily or weekly in graded amounts
and the weight gain at each level is recorded. The recommended procedure
aims for an average weekly weight gain of three grams per week during a
test period of eight weeks which weight gain has been adopted as that pro­
duced by a Sherman rat unit of vitamin A. This method has been used for
the determination of most of the available figures for vitamin A content
of various foodstuffs.

With the advance of knowledge concerning the chemical nature of vitamin
A, other methods for measuring its concentration in materials have come in­
to use. It was known by the first workers (1), (2), that vitamin A was
associated with fats. It was early found that when these fats were sapon­
ifed, vitamin A largely remained in the unsaponifiable matter. Drummond
(40) succeeded in obtaining a concentrate in this manner which was one
thousand times richer in vitamin A than the original sample. Distillation
of this concentrate, and examination of the fraction possessing the greater
part of the activity, showed at least three substances, at least one of
which was an unsaturated alcohol. Drummond and Watson (41) noted a purple
color reaction between liver oil and sulfuric acid and suggested that it
appeared to be parallel to the vitamin A value of the oil. Later, a similar
but more sensitive test was obtained with arsenic trichloride. Carr and
Price (42) in testing a number of reagents, found that antimony trichloride
was most suitable and convenient to be used for color tests of vitamin A
and proposed a method for such tests which with modifications is used now.
There has been considerable controversy as to the specificity of the color
tests. Spectroscopic examinations of the concentrate obtained by saponification have been found to exhibit a characteristic absorption band at 328 m\(\mu\), which corresponds with the color test, and the biological feeding tests (43).

The difficulty in using chemical methods for vitamin A assay lies largely in the possible error in extraction of the vitamin, and in different technique used by individual workers.

The earliest experiments with vitamin A showed that certain foods, as butter, egg, and cod liver oil, were rich in vitamin A, while lard and most of the vegetable oils contained very little. Since then a great many individual foods have been tested for vitamin A content. While vitamin A appeared at first to be associated or combined with fats it was soon found that other foods, not usually classed with fats, were also rich sources. Steenbock (44), (45), (46) first noted in 1919 that the vitamin A activity of plant foods appeared to be related to the yellow and green coloring matter and was apparently proportional to the degree of pigment-ation. Drummond and Coward (47) however, at about the same time, found no relationship between color in fats and their vitamin A content. Palmer and Kempster (48) also opposed the relationship of carotinoid pigments and vitamin A, obtaining good growth on rations containing only slight traces of carotene. Steenbock and associate workers (49), (50) however gave further evidence that richer vitamin A activity was found with higher carotene content. They suggested that during the metabolic processes the vitamin might be freed of pigment. Crist and Dye (51) have more recently shown a definite relationship between the degree of greenness in plants and their vitamin A activity. Xanthophyll (52) was found not to possess
vitamin A activity.

In 1928 Euler and associates (53) first reported promotion of growth in vitamin A deficient rats with carotene, and shortly afterwards Moore (54), (55) confirmed these results, and in attempting to explain controversial results previously obtained, suggested that carotene, while not identical with vitamin A, might be a vegetable precursor from which the vitamin is changed in the body. Weight was added to the theory by the fact that carotene, while intensely yellow, did not result in greatly increased pigmentation of the rat body. Also, the spectrographic examination of the absorption band formed by the reaction of antimony trichloride with the liver extract of the animal fed carotene was found to be characteristic of vitamin A rather than carotene. Olcott and McCann (56) succeeded in effecting this conversion in vitro by incubation of carotene with liver tissue, and suggested that the factor necessary for conversion was an enzyme, carotenase.

By 1931 the relationship between carotene and vitamin A was so strongly substantiated by experimental evidence that the London Conference of the League of Nations Health Organization selected carotene as a provisional international standard of reference for vitamin A, designating one microgram (.001 mg) of a mixed specimen of carotene as the international unit. Isolation of at least three different forms of carotene by Karrer and co-workers (57) led to the discovery, however, that pure \( \beta \) carotene was much more active biologically than either the \( \alpha \) or the \( \gamma \) forms of carotene. On the basis of these studies the International Conference on vitamin standardization in 1934 selected pure \( \beta \) carotene, in a solution of coconut oil, as the standard International Unit. The vitamin A
activity of the new standard was tested in comparison with the 1931 standard and 0.6 microgram of the pure β carotene was found equivalent in vitamin A potency to the old standard of one microgram of the mixed sample. This figure, 0.6 microgram of β carotene, is now accepted as the International Unit of vitamin A. The new United States Pharmacopoeia Unit of vitamin A, as referred to later in this paper, is of the same value as the International Unit, and is equivalent to 1.4 times the Sherman Rat Unit of vitamin A (58).

According to the formulae accepted for the isolated forms of carotene (57) and that proposed for vitamin A (59), the difference in biological activity of the different forms of carotene appears to be explained by the possible formation of two molecules of vitamin A from one molecule of β carotene, and only one molecule of vitamin A from each molecule of the other forms.
By the rat, \( \beta \) carotene appears to be equally as well utilized as vitamin A as shown by the work of Moore (60). Fowl have been reported by Capper and associates (61) and by Hart (17) to readily convert carotene into vitamin A. Cattle have been shown also to utilize carotene efficiently (62) and it would appear that all herbivorous animals must depend entirely upon the plant precursors for their supply of vitamin A. Rea and Drummond (23), however, were not able to convert carotene to vitamin A by incubation with the livers of cats, nor to observe a rise in vitamin A content of the liver of the cat following injections of colloidal suspensions of carotene. They suggested that possibly carnivorous animals could not convert a vegetable precursor to vitamin A. Although Frohring (63) and Ralli (29) both used carotene as a source of vitamin A in feeding dogs, no attempt was made to correlate the utilization of carotene with a comparable level of vitamin A. Ralli (29) reported the presence of the enzyme carotenase in the livers of some dogs
receiving carotene, while it could not be detected in others. Frohring (63) detected no vitamin A in the livers of puppies fed carotene for periods of three to twelve weeks, and only slight amounts of vitamin A in the liver of the control dog receiving a very high level of carotene (6500 units daily).

Simonnet and Busson (30) observed some storage of vitamin A in the livers of dogs previously fed carotene, but stated that there appeared to be a limit to the amount which could be stored. Mellanby (27) in the course of extensive studies on vitamins A and D, stated that dogs were not suitable for studying the calcifying effect of vegetables because of poor utilization of vegetable material, and it has been the belief of many practicing veterinarians that vegetable foods are not well utilized by dogs.

Thus, while there is ample evidence to show that dogs require vitamin A, and that they develop the characteristic symptoms of deficiency in its absence, the evidence is limited in regard to the dog's ability to utilize efficiently the vegetable precursor of vitamin A, and to store vitamin A in quantities sufficient to be quantitatively determined.

It is, therefore, the purpose of this paper to present evidence to show the comparative utilization by the dog of vitamin A and of its precursor carotene, fed in equivalent amounts, and to show the relative concentration of vitamin A in the liver of the dog upon administration of graded levels of vitamin A.
EXPERIMENTAL PROCEDURE

General Plan

Young puppies taken at five to seven weeks of age were used in the following experiments to compare the utilization and store of vitamin A obtained from animal and vegetable sources. The length of the experimental period varied from fourteen to twenty-two weeks, during which time the pups received a vitamin A deficient diet, and weekly supplements containing vitamin A provided in the form of cod liver oil, or as its vegetable precursor, carotene, in oil solution, or as it occurs in its natural form, in carrots.

Each animal was weighed at the beginning of the experimental period and each week thereafter. Notations were made at the time of the weekly weighing upon the condition of eyes, skin and hair, activity, and general appearance. At the end of the experimental period the dogs were killed by an injection of ether or chloroform into the heart, the body examined for lesions, and the liver dissected and prepared for assay of vitamin A content.

Description of Animals Used

When possible, mother and pups were brought into the laboratory before weaning, in an effort to limit the store of the pups by regulation of the mother's diet. As soon as the pups were able to eat well, they were weaned and given skimmed milk mixed with some of the vitamin A deficient diet in order to accustom them to the taste of the food, and so prevent refusal of food after the beginning of the feeding period. Four litters of pups were used, comprising a total of twenty-seven animals.

Litter I consisted of six puppies, five of which were used in this...
study, brought into the laboratory with the mother, a small mongrel terrier, at two days of age. The mother was given bread, skimmed milk and lean beef during the suckling period. The pups were weaned at between three and four weeks of age, and were given the basal ration and skimmed milk until they were started on the dietary regime at seven weeks of age.

Litter II consisted of four puppies, of the same age and type as Litter I. They were weaned and brought into the laboratory between three and four weeks of age, and thereafter followed the same regime as Litter I.

Litter III consisted of ten puppies from a mongrel collie-type mother, and were brought to the laboratory with the mother at two weeks of age, were weaned at four weeks, and were started on the dietary regime at five weeks of age.

Litter IV consisted of ten puppies, eight of which were used in this study. They were weaned at five weeks of age, brought to the laboratory and were started on the dietary regime at six weeks of age.

**Care and Housing**

At the beginning of the dietary regime, the pups were separated into wire metabolism cages, or wooden kennels with wooden or sand floors. Each pup was fed separately, the days' portion of food being divided and fed twice daily while the pups were small, and once daily towards the end of the feeding period. Tap water was given twice daily.

**Basal Ration**

The basal vitamin A deficient diet given was Morgan's (65) modification of the Karr-Cowgill diet, consisting of casein, 39.6%; sucrose, 42.7%; Osborne and Mendel salt mixture, 2.05%; agar 0.81%; brewers' yeast, 2.46%; and Crisco, 12.27%. Viosterol was given once weekly, except to those pups
receiving sufficient vitamin D in the cod liver oil supplement. This ration was adequate in all nutritional essentials except vitamin A. 47.5 grams of diet per kilogram of body weight were fed daily, adjustments for weight being made once weekly. The dry diet was mixed with warm water and fed in the form of a mash. In most cases it was eaten promptly, but in instances when a pup refused a portion of the daily allotment for several days, the amount of food given was reduced. Pups receiving carrots as the supplemental feeding were not given agar in the basal ration, in order not to increase the fiber content of their diet above that of the other dogs.

Supplemental Feeding

The basal ration was supplemented with vitamin A from cod liver oil, at daily levels of 20, 40, 100 and 200 U.S.P. units of vitamin A per 100 grams of body weight, or as the equivalent of 20 U.S.P. units of vitamin A in the form of a solution of carotene in oil, or in the natural form, carrots. The levels of administration of vitamin A were selected on the basis of Frohring's (63) determination of the quantitative vitamin A requirement of growing puppies. His results showed that the minimum effective daily curative dosage for vitamin A depleted puppies was 20 U.S.P. units of vitamin A per 100 grams of body weight. It was believed, then, that this level of vitamin A, when fed to young puppies as a preventive measure, would be ample for their daily requirements and might also allow storage which could be used as the basis of comparison of utilization of vitamin A from the various stores, while the higher levels of vitamin A administration might serve as a basis for measuring the ability of the dog for increased storage upon higher intake of vitamin A.
When the size of the litter permitted, one dog from each litter was placed upon each of the above levels, and one dog from each litter was kept as a negative control, receiving no vitamin A.

The cod liver oil used was obtained in one lot from Mead, Johnson & Company, and contained 1800 U.S.P. units of vitamin A per gram. Carotene in oil, equivalent in vitamin A value to 7500 U.S.P. units per gram was obtained from S. M. A. Corporation. The carrots used were of good color and grade, purchased on the open market. Their equivalent in vitamin A units was the average figure of 50 U.S.P. units per gram, taken from Rose's (65) table of average vitamin A content of foods. They were washed and brushed, or scraped, dried, weighed and cooked until tender. The weekly portion for each dog was divided and fed, mixed with the basal ration, three or four times weekly. Cod liver oil and carotene were measured and fed once weekly to each animal. Adjustments in the amount of supplement given were made, as with the basal ration, following each weekly weighing.

Method of Determination of the Vitamin A Content of the Liver

Antimony Trichloride Color Reaction:

The vitamin A content of the liver was determined essentially according to the method of Davies (66). The fresh liver, when dissected, was washed free of blood, and the gall bladder and large blood vessels were removed. It was weighed on a trip balance, ground and mixed thoroughly, and samples weighed for analysis. 5% to 10% aqueous KOH was used for preservation and digestion of the sample. In most cases 25 gram samples of liver were taken but if the expected yield of vitamin A was high, 10 gram samples were taken. These were transferred to Erlenmeyer flasks or wide mouthed bottles containing the KOH, (10 cc for each 5 gram sample of liver) and stored
in the refrigerator until analysis could be made. Davies (66) reported that the loss of vitamin A under these conditions was inappreciable in a period of time up to six weeks.

When ready for analysis, the liver samples were digested in the KOH solution for seven to eight hours. For digestion an electric oven was used, at a temperature of 95—99°C. It was found that this length of time was sufficient to dissolve the liver so that extraction could be made, while a much longer period of time resulted in partial or complete loss of the vitamin, as shown by lack of color development in the reaction with SbCl₃. At this point, the mixture was somewhat turbid, due to small particles of tissue. It was then transferred to a separatory funnel, shaken strongly with about one half the volume of 95% ethyl alcohol, and then with an equal volume of ether. A mixture of ethyl ether and petroleum ether was used in this extraction, at the suggestion of the S.M.A. Laboratories, to prevent the formation of troublesome emulsions. Emulsions did frequently form, however, in which case they were broken by the addition of a small amount of alcohol. The mixture was allowed to separate and the liver tissue-KOH layer was discarded. The ether fraction was washed free of alcohol by two or three washings with distilled water. After discarding the water, the ether was filtered by suction into a wide necked flask (Soxhlet flask) through a sintered glass funnel containing anhydrous sodium sulfate. The sodium sulfate was washed with ether, and the ether was evaporated off rapidly on a water bath. The residue, which had the appearance of a solid fat, was dried in a suction dessicator, and dissolved in chloroform. The chloroform used was dried over K₂CO₃ and distilled to remove all traces of moisture which might interfere with the
color determination. 0.5 cc samples of this solution were measured into the Lovibond Tintometer cell for that purpose and 2 cc of the antimony trichloride reagent added. The resultant color was matched quickly in the Tintometer and the readings recorded. Since the blue color which develops in the reaction between vitamin A and antimony chloride fades very quickly, care was taken to match the colors at the maximum intensity, 20 to 30 seconds after addition of the reagent. The color developed was expressed in terms of blue units only, any red, yellow, or neutral colors necessary for matching the solution with the standard glasses being disregarded in recording the results, as the blue color only is an indication of vitamin A value. The average of three or more separate samples checking within 0.5 of one blue unit was taken as the vitamin A content of the liver sample.

Bio Assay of the Vitamin A Content of the Liver:

Portions of the ground liver from some of the dogs of litter IV were used for bio assay. The method was essentially that of Sherman and Munsell (10) as modified by Sherman and Burtis (39). Albino rats, weaned at three weeks of age, were placed on a diet deficient in vitamin A. When depletion of bodily store of vitamin A occurred, as evidenced by cessation of growth and sensitivity of eyes, the animals were placed in separate, raised bottom cages and the basal ration, supplemented with weighed portions of liver, was given for an experimental period of six weeks. The livers of dogs having received 20 and 40 U.S.P. Units of vitamin A per 100 grams body weight, and those of dogs receiving the equivalent of 20 U.S.P. Units of vitamin A as carotene in oil, or carrots, were fed to rats so depleted, at a level of 0.1 gram liver daily. The liver of a dog
having received 200 U.S.P. Units of vitamin A per 100 grams body weight was fed at levels of .02 and .05 grams daily. Eight to ten rats were fed at each of the stated levels. The liver sample for the entire period was weighed at one time, stored in the refrigerator, and approximate weekly portions fed each week.

EXPERIMENTAL FINDINGS

Health Observations

In general, all dogs except the negative control animals were outwardly normal in appearance and action whatever the supplement given or the level of feeding. Some of the animals, particularly in litters I and II showed watery eyes during the first two or three weeks, but in all cases these had cleared up by the fifth week of the feeding period. There was no apparent superiority of general health in those dogs receiving high levels of vitamin A. The only consistent observation made on general appearance was that those dogs receiving a high unitage of vitamin A (100 and 200 U.S.P. Units per 100 grams body weight) had heavier, more lustrous coats than those animals on lower levels of vitamin A intake.

The appearance of the eyes of all dogs receiving vitamin A or its precursor was normal, and the dogs were active and alert throughout the experimental period. None of them at any time showed symptoms which could be attributed to vitamin A deficiency. (Plates IV and V are photographs of Dog B receiving 20 units vitamin A from cod liver oil and of Dog Z, receiving 100 units of vitamin A from cod liver oil. The general appearance and brightness of eyes of these dogs are typical of all dogs receiving vitamin A from any source.) Of the negative control animals, two developed un-
mistakable symptoms of vitamin A deficiency. Dog A, the control in litter I, exhibited watery eyes for the entire twenty-one weeks period, with, frequently, collections of purulent discharge in the corners of the eyes. This condition was most aggravated during the sixth to twelfth weeks of the experimental period. Subsequent to the twelfth week, the watery discharge abated somewhat but the eyes remained dull and squinty. After five weeks on the deficient diet, this animal was noted to hold his head constantly towards the left side, and to walk or run in circles when allowed out of the cage. After about eight weeks the appetite of this dog was poor and he often refused nearly all of his daily food allowance. He became very listless and was trembly and awkward in movement. He developed a rash or eczema on the legs and head, after eleven weeks on the deficient diet. However, this perhaps cannot be entirely attributed to vitamin A deficiency, since an invasion of fleas attacked all of the dogs at about this time, and many of them showed skin lesions from scratching. Most of the others responded promptly to treatment, while the skin roughness and irritation persisted in this animal until the end of the period. (Plate I is a photograph of this dog after twenty weeks on the basal ration.)

Dog V, the negative control of litter IV, grew normally for eight weeks, when several symptoms of vitamin A deficiency appeared rather suddenly. A watery discharge from the eyes had been noted for two weeks previous to this time, and when weighed on the eighth week after being started on the dietary regime, both eyes were glassy in appearance. Two days later the left eye was entirely opaque, and on the following day the right eye became opaque and an ulcer started to form on the left eye. At the same time the dog became very weak and unsteady, ran in circles in his cage,
refused most of his food, and was very apathetic. He became progressively weaker and more miserable. Within two weeks both eyes were badly ulcerated, discharging a moderate amount of pus. His coat was very rough and ragged in appearance, the hair coming out in large patches, especially on the legs and back. The skin on those patches was rough and scaly, and the nostrils were scabby. (Plates II and III show photographs of this dog after ten weeks on dietary regime.)

The two negative control animals of litter III, dogs K and N, showed no symptoms of vitamin A deficiency other than a slight discharge from the eyes, listlessness and poor appetite, none of which symptoms appeared until after ten or eleven weeks on the deficient diet. There were no gross pathological lesions found in any of the animals, when examined after death by Dr. William Pistor, Professor of Veterinary Medicine.

Growth Record

All of the dogs, except the negative controls, made rapid and consistent gains in weight. The growth record of all of the dogs used in this study are shown in Table I. The dogs in litters I and II gained consistently until about the twelfth week of the experimental period, after which time the weekly gains were considerably smaller and more or less erratic. Dog I, although receiving a high unitage of vitamin A, made very slow weight gains throughout the period and at the end of the dietary regime, being then seven months of age, weighed less than five kilograms. She was apparently normal in all other respects, being very active and possessing an exceptionally smooth and silky coat. Because of her small size she was believed desirable for breeding purposes and so was not sacrificed at the end of the dietary regime. The pups of litter III developed into very
large dogs. All of them, including the two negative controls, made striking gains and were still growing rapidly at the end of the fourteen weeks experimental regime. Litter IV, though smaller dogs than those in litter III, responded much the same to the dietary regime.

There was no consistent weight gain advantage in those animals receiving high levels of vitamin A, all of the gains being within normal range. Any differences which might be noticed were obviously due to individual variation rather than to the form or amount of supplemental feeding.

Vitamin A Content of the Liver

By the Antimony Trichloride Color Reaction:

The vitamin A content of the livers of dogs receiving 20, 40, 100 and 200 U.S.P. Units of vitamin A per 100 grams of body weight, or the equivalent of 20 units in the form of its precursor in carotene in oil or in carrots, is shown in table II. There was no detectable amount of vitamin A found in the livers of any of the negative control animals, as evidenced by a complete absence of blue color in the reaction of the antimony trichloride reagent with the extract from 25 gram samples of liver.

When vitamin A was administered in any form at a level of 20 U.S.P. Units per 100 grams of body weight, vitamin A was found to be present in the liver of the animal in amounts which could be determined in Lovibond blue units.

Again, no significant difference was found in the vitamin A content of the livers of dogs receiving 20 U.S.P. units of vitamin A in cod liver oil, and in those of dogs receiving an equivalent amount of its precursor as carotene in oil or as carrots. The vitamin A content of the livers of these dogs (with one exception, dog C) was within the narrow range of 1.4
and 6.8 blue units per gram of liver. The variations noted were not always in favor of any one supplement. Thus, it appears evident that vitamin A as cod liver oil or as its precursor, carotene, in oil or as carrots, are equally well utilized by the dog. When the amount of cod liver oil was increased to provide a higher level of vitamin A intake the liver content of vitamin A, as measured by Lovibond blue units, was proportionately increased.

The vitamin A content of the liver of dogs receiving 40 units of vitamin A per 100 grams of body weight was higher than that of litter mates receiving 20 units of vitamin A. The variation was wider, however, at this level than at the lower level of intake, ranging from 3.2 to 14.2 blue units per gram of liver. The total storage of animals fed at the level of 40 units was approximately 1.5 to 2 times that of litter mates fed at a level of 20 units per 100 grams body weight.

The vitamin A content of the liver of dogs receiving 100 and 200 U.S.P. units of vitamin A per 100 grams of body weight was in all cases much higher than that of dogs fed at the lower levels of 20 and 40 units. Roughly, dogs fed at a level of 100 units of vitamin A, five times the lowest level of 20 units, had a total vitamin A content of the liver 20 times as great as litter mates fed at the low level. Dogs receiving 200 units of vitamin A had a total vitamin A content 30 to 40 times that of litter mates fed at a level of 20 units per 100 grams body weight.

Thus, while a level of 20 units of vitamin A, either from animal or vegetable source, appears ample for the normal growth requirements for the young puppy, and permits a slight storage of vitamin A, an increased intake of vitamin A above this level results in proportionately greater
In general, although there were some individual variations, especially at the higher levels of intake, the greater the total amount of vitamin A administered, the greater the total vitamin A content of the liver in proportion to that intake.

Dogs in litters I and II showed somewhat higher liver content of vitamin A than did dogs of litters III and IV upon the same level of intake. The dogs of the first two litters remained upon the dietary regime for twenty-one and twenty-two weeks, while those of the latter two litters were killed at the end of a fourteen weeks experimental period. Thus the dogs on the longer experimental period, having passed the period of most rapid growth at twelve or thirteen weeks on the regime, required less vitamin A per unit of body weight, and therefore could store a larger proportion of the vitamin A ingested.

By Bio Assay:

Table III shows the growth record of rats whose sole source of vitamin A was the liver of dogs having received vitamin A in the form of cod liver oil, or as its precursor, carotene, in oil or in carrots. The increased average survival of rats fed 0.1 gram of liver from dogs having received 20 or 40 U.S.P. Units of vitamin A per 100 grams body weight, over the negative controls, indicates the presence of some vitamin A. However the level of liver fed was too low to prevent loss of weight, symptoms of vitamin A deficiency and death of the rat. The liver of a dog having been fed 200 U.S.P. Units of vitamin A per 100 grams body weight contained sufficient vitamin A to provide for an average weekly gain of seven grams in the rat, when fed at a level as low as .02 gram daily. This would cor-
roborate the findings by the antimony trichloride color reaction, that the dog is able to store vitamin A when fed at a level above his daily requirement.

SUMMARY AND CONCLUSIONS

Normal puppies, taken at weaning age, were fed a vitamin A deficient basal diet with supplemental feedings consisting of vitamin A fed as cod liver oil or as its precursor in the form of carotene in oil or in the natural state as cooked carrots for experimental periods of fourteen to twenty-two weeks. The ability of the dog to utilize and store vitamin A from these sources was studied by means of general health observations, growth records, and determination of the vitamin A content of the liver at the end of the experimental period. The liver content of vitamin A was determined by the antimony trichloride color reaction, the color being measured in the Lovibond Tintometer and results expressed in terms of Lovibond blue units. In a few selected cases bio assay of the liver tissue was made to confirm the findings by chemical assay.

The results obtained in the foregoing experiments showed that the normal growing puppy requires vitamin A and when given a diet deficient in that factor, exhausts any previous store of vitamin A and develops symptoms of deficiency. The most pronounced symptoms of vitamin A deficiency were loss of appetite, general apathy, loss of hair, and a watery discharge from the eyes, becoming progressively more serious, developing in one case into ulceration and complete loss of sight.

The normal growth requirements of the young puppy were amply provided for, and a slight storage was permitted, by the administration of 20 U.S.P.
units of vitamin A. When fed at a level of 20 units per 100 grams of body weight, vitamin A as cod liver oil or as its precursor carotene, in the form of carotene in oil or as the natural food carrots, appeared to be equally well utilized by the dog.

The ability of the dog to store vitamin A was made evident by the increased content of vitamin A found in livers of dogs fed levels of vitamin A intake above the requirement for growth and good nutritive condition. As the level of administration was increased, there was a proportionately higher concentration of vitamin A in the liver of the dog.

Although no weight gain advantage was afforded by levels of vitamin A intake above that required for normal growth, there were evidences of better nutritive condition in the superior lustre and heaviness of coat.

Following the period of most rapid growth, as the body weight tended to approach a more or less constant level, a given amount of vitamin A resulted in a higher concentration of vitamin A in the liver, than the same level given to an animal still growing rapidly. This finding appears to indicate that the vitamin A requirement for the puppy, per unit of body weight, is highest during the period of most rapid growth, that is, during the first five months of life.
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Dog A, Litter I. Negative control, after twenty weeks on a vitamin A deficient ration.
Dog V, Litter IV. Negative control, after ten weeks on a vitamin A deficient ration.
PLATE III.

Dog V, Litter IV, Negative control, after ten weeks on a vitamin A deficient ration.
Dog B, Litter I, after twenty weeks on vitamin A deficient ration, supplemented with cod liver oil at a level of 20 U.S.P. units of vitamin A daily per 100 grams of body weight.
PLATE V.

Dog Z, Litter IV, after ten weeks on vitamin A deficient ration supplemented with cod liver oil at a level of 100 U.S.P. units of vitamin A daily per 100 grams body weight.
## TABLE I.

THE COMPARATIVE WEIGHT GAIN OF DOGS RECEIVING VITAMIN A AS COD LIVER OIL OR ITS PRECURSOR, AS CAROTENE IN OIL OR CARROTS

<table>
<thead>
<tr>
<th>Litter I. Feeding Period 21 weeks</th>
<th>Dog and Sex</th>
<th>Daily Supplement per 100 g.body wt.</th>
<th>Weight at start of dietary regime</th>
<th>Final Wt.</th>
<th>Total Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U.S.P.Units of Vitamin A</td>
<td>Kg.</td>
<td>Kg.</td>
<td>Kg.</td>
</tr>
<tr>
<td>A ♀ None</td>
<td></td>
<td>1.77</td>
<td>5.89</td>
<td>4.12</td>
<td></td>
</tr>
<tr>
<td>B ♂ 20 units CLO</td>
<td></td>
<td>1.46</td>
<td>6.18</td>
<td>4.72</td>
<td></td>
</tr>
<tr>
<td>C ♂ 20 units carotene</td>
<td></td>
<td>1.43</td>
<td>7.09</td>
<td>5.66</td>
<td></td>
</tr>
<tr>
<td>D ♀ 20 units carrot</td>
<td></td>
<td>1.56</td>
<td>5.67</td>
<td>4.11</td>
<td></td>
</tr>
<tr>
<td>E ♀ 100 units CLO</td>
<td></td>
<td>1.65</td>
<td>7.43</td>
<td>5.78</td>
<td></td>
</tr>
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<table>
<thead>
<tr>
<th>Litter II. Feeding Period 22 weeks</th>
<th>Dog and Sex</th>
<th>Daily Supplement per 100 g.body wt.</th>
<th>Weight at start of dietary regime</th>
<th>Final Wt.</th>
<th>Total Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U.S.P.Units of Vitamin A</td>
<td>Kg.</td>
<td>Kg.</td>
<td>Kg.</td>
</tr>
<tr>
<td>F ♂ 20 units CLO</td>
<td></td>
<td>1.85</td>
<td>6.97</td>
<td>5.12</td>
<td></td>
</tr>
<tr>
<td>G ♀ 20 units carotene</td>
<td></td>
<td>1.85</td>
<td>6.35</td>
<td>4.50</td>
<td></td>
</tr>
<tr>
<td>H ♀ 20 units carrot</td>
<td></td>
<td>2.26</td>
<td>8.62</td>
<td>6.36</td>
<td></td>
</tr>
<tr>
<td>I ♀ 200 units CLO</td>
<td></td>
<td>1.475</td>
<td>4.31</td>
<td>2.835</td>
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<table>
<thead>
<tr>
<th>Litter III. Feeding Period 13-15 weeks</th>
<th>Dog and Sex</th>
<th>Daily Supplement per 100 g.body wt.</th>
<th>Weight at start of dietary regime</th>
<th>Final Wt.</th>
<th>Total Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U.S.P.Units of Vitamin A</td>
<td>Kg.</td>
<td>Kg.</td>
<td>Kg.</td>
</tr>
<tr>
<td>K ♀ None</td>
<td></td>
<td>1.81</td>
<td>9.64</td>
<td>7.60</td>
<td></td>
</tr>
<tr>
<td>N ♂ None</td>
<td></td>
<td>2.38</td>
<td>12.47</td>
<td>10.09</td>
<td></td>
</tr>
<tr>
<td>P ♂ 20 units CLO</td>
<td></td>
<td>2.55</td>
<td>12.19</td>
<td>9.64</td>
<td></td>
</tr>
<tr>
<td>Q ♀ 20 units carotene</td>
<td></td>
<td>2.10</td>
<td>11.57</td>
<td>9.47</td>
<td></td>
</tr>
<tr>
<td>R ♀ 20 units carrot</td>
<td></td>
<td>2.04</td>
<td>11.45</td>
<td>9.41</td>
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<tr>
<td>J ♂ 40 units CLO</td>
<td></td>
<td>2.50</td>
<td>16.16</td>
<td>13.66</td>
<td></td>
</tr>
<tr>
<td>O ♂ 40 units carotene</td>
<td></td>
<td>2.41</td>
<td>16.16</td>
<td>13.75</td>
<td></td>
</tr>
<tr>
<td>L ♂ 100 units CLO</td>
<td></td>
<td>2.72</td>
<td>19.96</td>
<td>17.24</td>
<td></td>
</tr>
<tr>
<td>S ♀ 100 units CLO</td>
<td></td>
<td>1.81</td>
<td>12.42</td>
<td>10.61</td>
<td></td>
</tr>
<tr>
<td>M ♂ 200 units CLO</td>
<td></td>
<td>2.50</td>
<td>14.23</td>
<td>11.73</td>
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<table>
<thead>
<tr>
<th>Litter IV. Feeding Period 14 weeks</th>
<th>Dog and Sex</th>
<th>Daily Supplement per 100 g.body wt.</th>
<th>Weight at start of dietary regime</th>
<th>Final Wt.</th>
<th>Total Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U.S.P.Units of Vitamin A</td>
<td>Kg.</td>
<td>Kg.</td>
<td>Kg.</td>
</tr>
<tr>
<td>V ♂ None</td>
<td></td>
<td>1.81</td>
<td>5.89</td>
<td>4.08</td>
<td></td>
</tr>
<tr>
<td>W ♂ 20 units CLO</td>
<td></td>
<td>1.93</td>
<td>10.43</td>
<td>8.50</td>
<td></td>
</tr>
<tr>
<td>X ♀ 20 units carotene</td>
<td></td>
<td>1.70</td>
<td>10.03</td>
<td>8.27</td>
<td></td>
</tr>
<tr>
<td>Y ♀ 20 units carrot</td>
<td></td>
<td>1.64</td>
<td>8.06</td>
<td>6.42</td>
<td></td>
</tr>
<tr>
<td>U ♂ 40 units CLO</td>
<td></td>
<td>1.81</td>
<td>11.90</td>
<td>10.09</td>
<td></td>
</tr>
<tr>
<td>Z ♂ 100 units CLO</td>
<td></td>
<td>1.25</td>
<td>9.07</td>
<td>7.82</td>
<td></td>
</tr>
<tr>
<td>T ♀ 200 units CLO</td>
<td></td>
<td>2.38</td>
<td>12.07</td>
<td>9.69</td>
<td></td>
</tr>
<tr>
<td>A1 ♀ 200 units CLO</td>
<td></td>
<td>1.53</td>
<td>8.22</td>
<td>6.69</td>
<td></td>
</tr>
</tbody>
</table>
TABLE II.

COMPARATIVE VITAMIN A CONTENT OF THE LIVERS OF DOGS RECEIVING VITAMIN A AS COD LIVER OIL, OR ITS PRECURSOR AS CAROTENE IN OIL OR IN CARROTS

<table>
<thead>
<tr>
<th>Litter I. Feeding period 21 weeks</th>
<th>Litter II. Feeding period 22 weeks</th>
<th>Litter III. Feeding period 13-15 weeks</th>
<th>Litter IV. Feeding period 14 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog and Sex</td>
<td>Daily Sup'1 per 100 gms.</td>
<td>Total Sup'1 U.S.P. Units</td>
<td>Total Body Weight U.S.P. Units</td>
</tr>
<tr>
<td></td>
<td>U.S.P. Weight of Liver kgs.</td>
<td>Weight of Liver gms.</td>
<td>Total Vit. A Content L.B.U.*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vit. A Content per gm. L.B.U.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vit. A Content per 100 gm. b.w. L.B.U.</td>
</tr>
<tr>
<td>A ϕ</td>
<td>None</td>
<td>5.89</td>
<td>none</td>
</tr>
<tr>
<td>B ϕ 20 CLO</td>
<td>130,312</td>
<td>6.18</td>
<td>287.0</td>
</tr>
<tr>
<td>C ϕ 20 carotene</td>
<td>142,086</td>
<td>7.09</td>
<td>380.0</td>
</tr>
<tr>
<td>E ϕ 20 carrot</td>
<td>120,890</td>
<td>5.67</td>
<td>250.0</td>
</tr>
<tr>
<td>D ϕ 100 CLO</td>
<td>747,670</td>
<td>7.43</td>
<td>278.0</td>
</tr>
<tr>
<td>F ϕ 20 CLO</td>
<td>143,199</td>
<td>6.97</td>
<td>290.5</td>
</tr>
<tr>
<td>G ϕ 20 carotene</td>
<td>134,190</td>
<td>6.35</td>
<td>255.5</td>
</tr>
<tr>
<td>H ϕ 20 CLO</td>
<td>167,055</td>
<td>8.62</td>
<td>361.0</td>
</tr>
<tr>
<td>I ϕ 200 CLO</td>
<td>947,970</td>
<td>4.31</td>
<td>not done</td>
</tr>
<tr>
<td>K ϕ</td>
<td>none</td>
<td>none</td>
<td>9.64</td>
</tr>
<tr>
<td>N ϕ</td>
<td>none</td>
<td>none</td>
<td>12.47</td>
</tr>
<tr>
<td>P ϕ 20 CLO</td>
<td>118,622</td>
<td>12.19</td>
<td>657.5</td>
</tr>
<tr>
<td>Q ϕ 20 carotene</td>
<td>110,642</td>
<td>11.57</td>
<td>623.0</td>
</tr>
<tr>
<td>R ϕ 20 carrot</td>
<td>118,034</td>
<td>11.45</td>
<td>488.0</td>
</tr>
<tr>
<td>S ϕ 40 CLO</td>
<td>306,040</td>
<td>16.16</td>
<td>684.0</td>
</tr>
<tr>
<td>T ϕ 40 CLO</td>
<td>273,336</td>
<td>16.16</td>
<td>670.0</td>
</tr>
<tr>
<td>L ϕ 100 CLO</td>
<td>941,570</td>
<td>19.96</td>
<td>796.0</td>
</tr>
<tr>
<td>M ϕ 100 CLO</td>
<td>569,520</td>
<td>12.42</td>
<td>556.5</td>
</tr>
<tr>
<td>N ϕ 200 CLO</td>
<td>1637,440</td>
<td>14.23</td>
<td>587.5</td>
</tr>
<tr>
<td>V ϕ</td>
<td>none</td>
<td>5.89</td>
<td>262.0</td>
</tr>
<tr>
<td>W ϕ 20 CLO</td>
<td>111,608</td>
<td>10.43</td>
<td>400.0</td>
</tr>
<tr>
<td>X ϕ 20 carotene</td>
<td>97,916</td>
<td>10.03</td>
<td>589.0</td>
</tr>
<tr>
<td>Y ϕ 20 carrot</td>
<td>82,460</td>
<td>8.06</td>
<td>355.0</td>
</tr>
<tr>
<td>U ϕ 40 CLO</td>
<td>198,044</td>
<td>11.90</td>
<td>550.0</td>
</tr>
<tr>
<td>Z ϕ 100 CLO</td>
<td>425,460</td>
<td>9.07</td>
<td>439.0</td>
</tr>
<tr>
<td>T ϕ 200 CLO</td>
<td>1,361,080</td>
<td>12.07</td>
<td>490.0</td>
</tr>
<tr>
<td>A ϕ 200 CLO</td>
<td>741,440</td>
<td>8.22</td>
<td>276.5</td>
</tr>
</tbody>
</table>

* L.B.U. is a Lovibond Blue Unit
TABLE III.

RESULTS OF THE BIO ASSAY OF LIVERS OF DOGS RECEIVING VITAMIN A AS COD LIVER OIL, OR ITS PRECURSOR AS CAROTENE IN OIL OR AS CARROTS

<table>
<thead>
<tr>
<th>No. of Rats at each level</th>
<th>Amt. of liver fed daily gms.</th>
<th>Level of sup’l. feeding in dog, per 100 gm. body wt.</th>
<th>Average Level of Survival of rat Days</th>
<th>Average Length of Survival of rat Days</th>
<th>Six weeks test period Av. wt. gain or loss gms.</th>
<th>No. rats surviving 6 wks.</th>
<th>No. rats showing deficieny signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.0</td>
<td>-</td>
<td>21</td>
<td>- 24</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.1</td>
<td>20 carrots</td>
<td>26</td>
<td>-22</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.1</td>
<td>20 carotene</td>
<td>31</td>
<td>-18</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td>20 CLO</td>
<td>40</td>
<td>-8</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td>40 CLO</td>
<td>31</td>
<td>-2</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.05</td>
<td>200 CLO</td>
<td>42 killed</td>
<td>53</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.02</td>
<td>200 CLO</td>
<td>42 killed</td>
<td>41</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>