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VITAMIN INVESTIGATIONS OF THE AVOCADO

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Address delivered at the Annual Meeting at Whittier

Mr. President and Members of the Avocado Association:

The establishment of the vitamin content of avocados is very important at this time in regard to the future sales' value of the Avocado because if it can be determined that it ranks high in these important principles, it will materially assist in further popularity and sales of avocados. Mr. Hodgkin of the Calavo Growers has been particularly interested in determining the vitamin content and at his request and through the cooperation of the Association the Chemistry Department of the University of Southern California has attempted to determine the vitamin content of avocados. I am going to report on a part of that work this morning but before I report on the results obtained, I want to discuss briefly with you something of the nature of vitamins and something of their history and occurrence.

Until recent years the value of a food was thought to be based on its composition as regards the food principles—carbohydrate, fat, and protein —and the mineral salts it contains. Today, however, it is recognized that there is yet another factor quite as important as this, which had previously been unrecognized. This factor we now term its "Vitamin" content.

A vitamin is a substance as yet not isolated in such a form by which its composition can be determined but which is recognized by its specific effect in aiding normal growth and development, and in curing or preventing certain diseases.

It was first thought that this was a substance necessary to life belonging to the family of chemical substances termed *amines.* Hence the name "vit-amine" or life-necessary amine. However, though it is generally recognized that nitrogen is present in its composition, no definite structure has been assigned to it. In order to keep the name so generally recognized, and yet not to assume its composition, it has been universally agreed to drop the final "e" of the term "amine" and call it not VITAMINE but VITAMIN.

At present there is accepted the occurrence of five such vitamins. Others are being added to the list, and it is thought that the effects now being ascribed to a single vitamin in one or two of those now recognized may be due to two or more distinct vitamins.

There are fully accepted at present Vitamins A, B, C, D, and E. These with their properties are listed on the chart, and we will take up the discussion of each one in order.

Vitamins

A. Anti-Xeropthalmic.

B. Anti-Neuritic and Growth Promoting.

C. Anti-Scurvy.

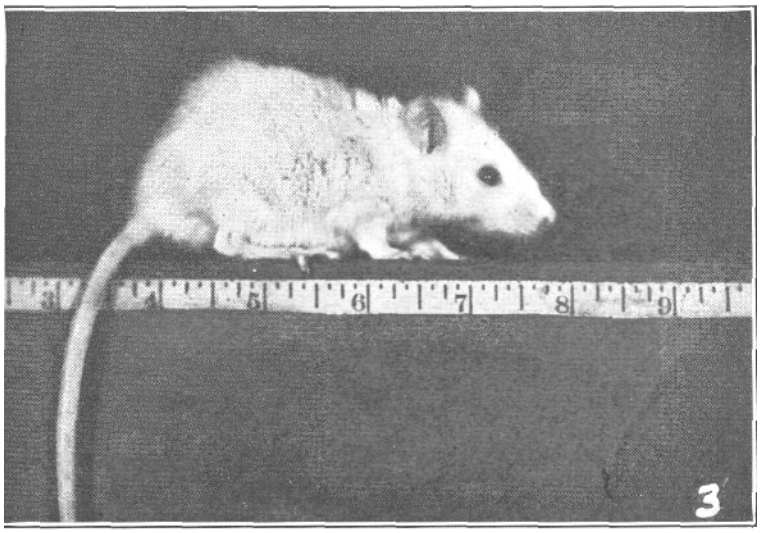
D. Anti-Rickets.

E. Anti Sterility.

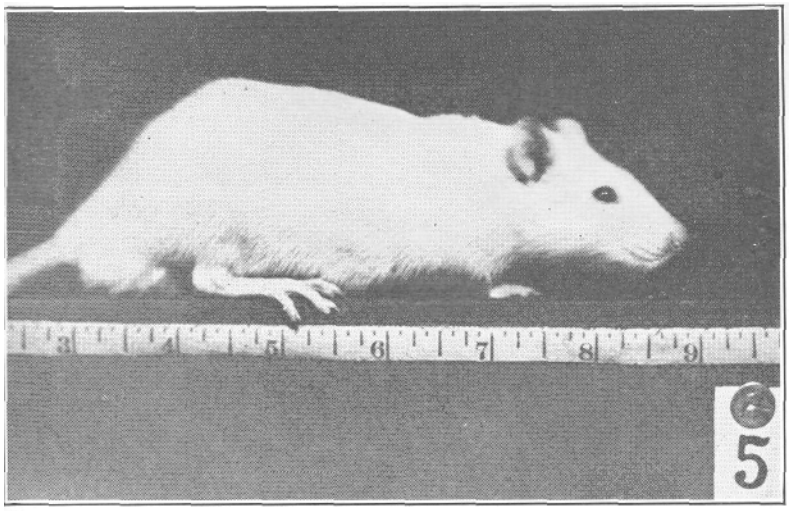
VITAMIN A: Anti-Xeropthalmic:

Xeropthalmia is a disease causing blindness. It is an inflammation of the eye membrane by which the eye-ball becomes protruded. In severe cases infection sets in and blindness results. It is caused by a deficiency of Vitamin A. This deficiency in experimental animals is also marked by the formation of pus sacks under the tongue and below the jaws, and also in the lungs and sometimes in other vital organs. Mori in Japan in 1904 at the time of a great food shortage noted 10,000 cases of Xeropthalmia. This condition was remedied by the feeding of chicken livers. In 1913 McCollum and Davis and Osborne and Mendel in America proved the existence of a fat-soluble vitamin which would prevent or cure this condition.

Since Vitamin A is soluble in fats, it occurs in the fat of certain foods. It occurs in butter, egg yolk, and cod-liver oil, but not in lard or most common vegetable oils Vegetables have the power of synthesizing vitamins. Animals, obtaining vitamines from vegetable sources, may store these in their body, thus Vitamin A, being fat soluble, is found in such occurrence as in butter and cod-liver oil. Vitamins seem to be a direct modification of the sun's energy. The effect of Vitamin D in preventing rickets may be duplicated by the irradiation of the body with sunlight or ultra-violet light from a mercury vapor lamp. Those vegetable oils in which the oil is manufactured or stored in the dark, protected from the chemical rays of the sun by a hull or husk, need not be expected to be a source of Vitamin A, as cotton-seed oil, corn oil, cocoanut oil, peanut oil, etc. The skin of the Avocado on the other hand is a live organ with the power of photo-synthesis and is thin enough to admit penetration of the chemical rays of the sun. Avocado oil might, therefore, be expected to contain a storage of Vitamin A.



Vitamin B free diet. Picture taken six weeks after diet started. Losing weight rapidly at the time picture was taken and would soon have died had not Avocado been added to its diet.



Avocado pulp 3.5 grams per day. Picture taken after 8 weeks on diet. Notice difference in size and condition of this rat and the one on the Vitamin B free diet above.

VITAMIN B: Called the Anti-Neuritis and Growth-Promoting Vitamin:

Beriberi, a nerve disease, was very prevalent in Japan so that about one-fourth of the men of the Japanese Navy were incapacitated for work and many deaths occurred on every voyage. In 1885 Tanaka varied the diet of the Navy by adding barley in place of a portion of the rice. As a result, Beriberi almost completely disappeared. In the Russo-Japanese War in1905, the diets of the Japanese Navy and Army were as follows:

NAVY	ARMY
1 lb. meat per day	50 oz. meat per day.
10 oz. barley per day	30 oz. rice per day.
20 oz. rice per day.	

No cases of Beriberi arose in the Navy while in the Army, there were over 200,000.

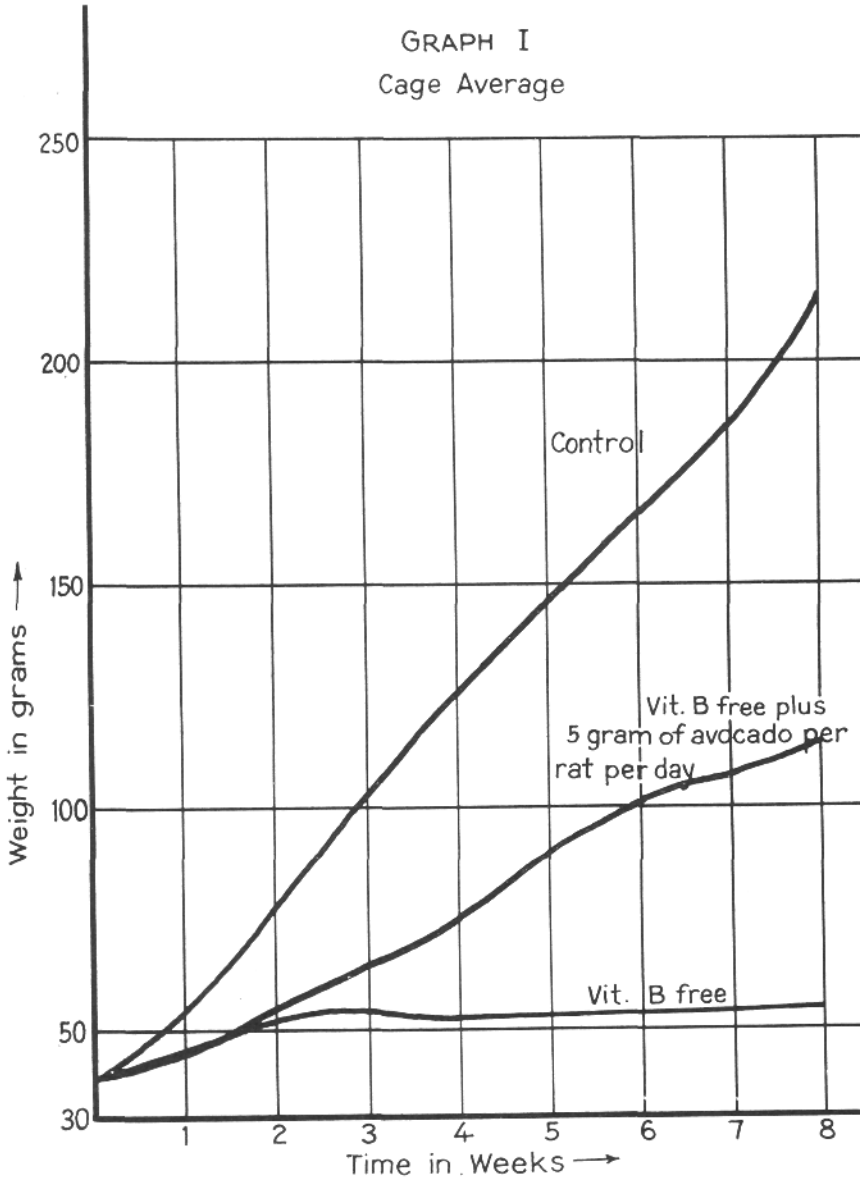
In 1897 Eijkman, a Dutch physician in Java, noticed that pigeons fed on polished rice left from the tables of prisoners developed a nervous condition called Polyneuritis similar to Beriberi in man. He found he could cure this condition by adding rice polishings or whole rice to their diet. He obtained permission to experiment on the diet of the prisoners in 100 jails in Java in which there were nearly a quarter of a million prisoners.

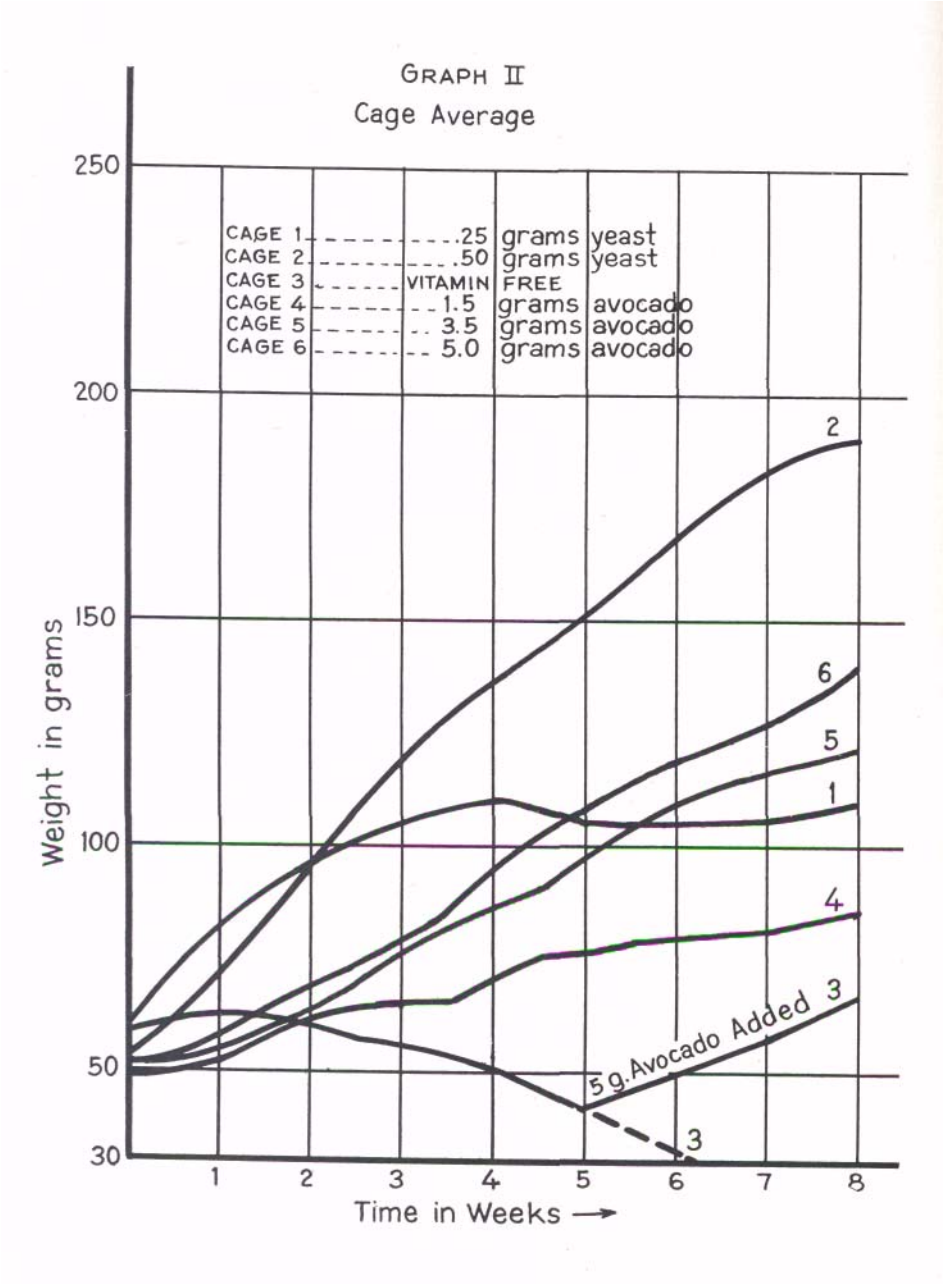
With polished rice, cases of Beriberi were 1 to 39. With mixed rice, cases of Beriberi were 1 to 400. With whole rice, cases of Beriberi were 1 to 10,000.

In 1911, Osborne and Mendel found a growth-promoting substance in yeast which seemed to have the same effect as rice-polishings. This is soluble in water and has received the name of the "Water-soluble Vitamin" or "Vitamin B." Vegetables and fruits are the chief source of Vitamin B. Its presence is tested by its ability to inhibit the nerve-disease condition and its ability to stimulate growth. The presence of Vitamin B in avocados comprises a major portion of the report to be made today. VITAMIN C: Called the Anti-Scorbutic or Anti-Scurvy Vitamin.

Scurvy, a disease to which sailors in the past were particularly susceptible on account of the lack of fresh vegetables, was found to be prevented by the addition to the diet of lemon juice. This has been proved to be caused not by the chemical composition of the lemon but by a third vitamin, named Vitamin C. This vitamin occurs in vegetables and fruit primarily. We have not as yet taken up the determination of Vitamin C in avocados as the work in our laboratory thus far has been with albino rats which are not susceptible to Scurvy. Guinea pigs must be used as the experimental animals. VITAMIN D: Called the Anti-Richitic or Anti-Rickets Vitamin.

Rickets is a disease caused by lack of proper calcium and phosphorus content of the blood causing improper calcification of the bones. This relationship seems to be controlled by a vitamin found in certain foods. Cod-liver oil, milk, vegetables, and fruits are anti-rachitic in property. Rickets may also be prevented by exposing the body to the direct rays of the sun, or of a mercury vapor light, or by *irridiating certain oils used as foods.* Avocado oil should be anti-rachitic since the fresh fruit receives irridiation from the sun, and as it grows only in a tropical or semi-tropical climate under the highest effect of sunlight. Our laboratory is starting investigation of the avocado in this respect.


GRAPH I
Cage Average
250
200
150
100
50
30
Weight in grams
Control
Vit. B free plus
5 gram of avocado per
rat per day
Vit. B free
1
2
3
4
5
6
7
8
Time in Weeks



VITAMIN E: Called the Anti-Sterility or Reproductive Vitamin.

Vitamin E is known as the Anti-Sterility Vitamin. Through work with experimental animals, it has been found that lack of this principle causes failure in reproduction. Sterility in the case of females on a Vitamin E-free diet results from the failure of the placenta to function properly in nourishing the embryo which results in the death of the latter and resorption before the time of birth. This may be prevented by the feeding of substances containing Vitamin E during this period. This Vitamin is also fat-soluble and oil obtained from the wheat germ is taken as a standard of comparison.

Sterility in the male on a Vitamin E-free diet is due to degeneracy of the reproductive

glands. Though complete sterility, once reached, is difficult to be overcome, the feeding of Vitamin E protects against the arising of this condition.

The chemical laboratory of the University of Southern California in co-operation with the Calavo Growers of California has undertaken the determination of the vitamins in avocados. The Fuerte Calavo has been used in the investigation and the fruit, which through exterior blemish was unsalable as first grade fruit, met the standard requirements in every other way.

The investigation of Vitamin B has been completed and will be reported in full here. Published report will occur in the near future in The Journal of Industrial and Engineering Chemistry, and in the reports of the Association.

A second series on Vitamin A is just being completed. This will be reported before a regional meeting of the American Chemical Society meeting with the American Association for the Advancement of Science, at Pomona early in June.

Work on Vitamin E has been in progress for about eight months. Work on the determination of this vitamin is very slow as all animals must have their fertility proved through the yielding of litters of young. A long time must then elapse for the depletion of the vitamin stored in the bodies of the animals. Their sterility must then be established through failure in a series of matings. After the re-addition of Vitamin E to the diet their fertility must again be tested through a series of matings. By the close of the summer we hope to have results in the cure of the female type of sterility through Vitamin E of avocados. Report on the cure of sterility in males cannot be reported until a considerable longer period.

I have prepared this chart to show some of the nature of the work we have been doing and explain the method of testing for the vitamins. I thought at first we would have a lantern to show these to you through pictures but I have brought these diagrams along instead.

Basal Diet

Meat Residue	20%
Starch	52%
Lard	15%
Butter Fat	9%
Salt Mixture	4%

1. *Normal Control:* Basal Diet +1 g. Yeast per rat per day.

2. *Vitamin B Free:* Basal diet alone.

3. *Avocado:* Basal diet +5 g. Avocado per rat per day.

This will show the results of our test on Vitamin B or the vitamin that has to deal with the Anti-neuritic property and which stimulates growth. Yeast, as we stated, is taken as the standard of comparison as to the Vitamin B content. So in order to make a comparison

with this standard, there is a diet prepared which contains all necessary principles for nourishment with the exception of Vitamin B. This diet is called "The Basal Diet". As shown here, we have a meat residue—20%-which is obtained by extracting finely ground steak with water a number of times to extract the Vitamin B since this is water-soluble, then drying and adding to the diet. Starch, 52%, Lard 15%, Butter-Fat 9% to furnish the necessary Vitamin A in order that the animal may be healthy in that respect, Salt Mixture 4% which furnishes the necessary salts for the existence of the animal. This contains the carbohydrates, fat, and protein in the necessary proportions, mineral salts and Vitamin A are taken care of and the other fat soluble vitamins but no food containing Vitamin B.

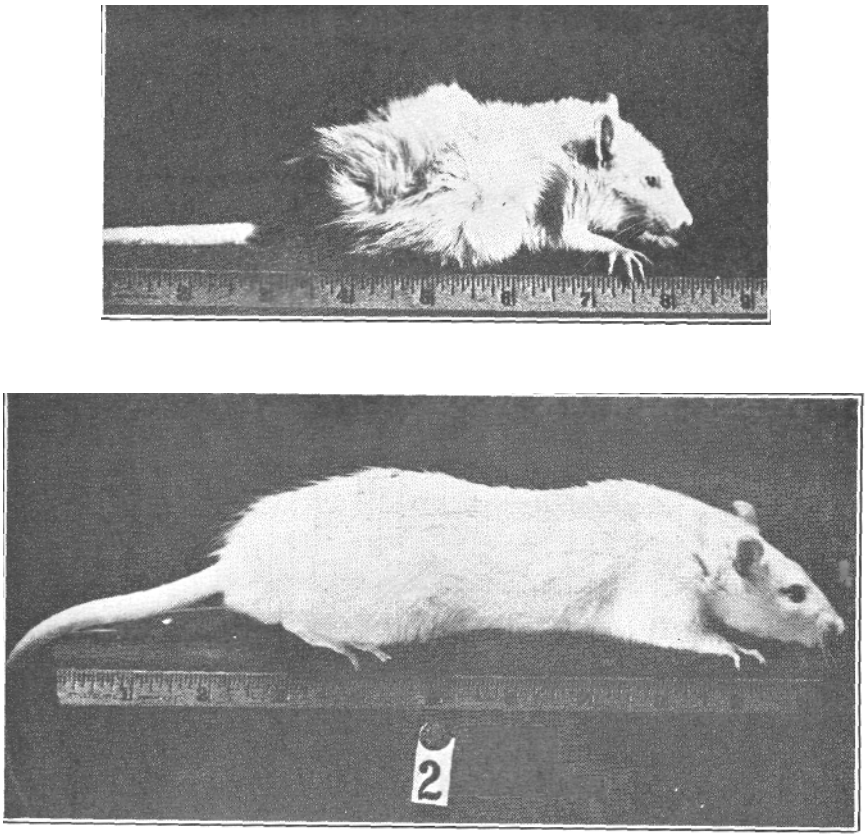
This Basal Diet is fed to various groups of animals. In this experiment there were three groups with about six or seven animals in each group. The first group is the normal or control group. They are fed this Basal Diet plus one gram of yeast per rat per day to give Vitamin B for comparison. The second is fed this basal diet alone and is called the "Vitamin-free Diet." The third group are fed this Basal Diet plus five grams of avocado per rat per day. The only difference in their diet is that this second group has no Vitamin B; this first group has Vitamin B in yeast; and this last group has five grams of avocado each and no Vitamin B unless there is vitamin B in avocado. The rate of growth of this last group per day indicates whether or not the avocado does contain this vitamin.

These results are plotted in the form of curves with which system many of you are familiar as most business propositions even are expressed in terms of graphic representation. We plot vertically on the curve the weight in grams of the animals. To the right the time in weeks. I could not well show here the growth of every animal. We have on our records the growth curves of every animal in the three groups and as there are seven in a groups that would be twenty-one animals. These curves shown on the chart in graph 1 are *average curves* obtained by adding the weight of all the animals and taking the average. This is really a better method for it does away with individual variation.

These three curves show the results of feeding on the diets I showed you before.

This top curve is the curve showing the growth of the rats that were fed the Basal Diet plus one gram of yeast per rat per day. The weight plotted upward, the weeks you see to the right. The minimums they started at were about 35 grams. At the end of the first week, it was nearly sixty grams, at the end of the second week about eighty grams, and carrying out the curve you see the growth through the whole period. There you have normal, good growth for rats fed on one gram of yeast per day.

Now this lowest growth curve shows those fed without any Vitamin B. They existed but didn't grow. They grew for a short time for Vitamin B is stored for a brief period in the body. These rats did not die. In the later series rats died on a Vitamin B-free diet. In this case probably the trouble was that we used the type of cages in which there was no screen at the bottom and the animal having access to its feces, ate them and by using again the Vitamin B previously stored in the body, they can maintain themselves for short period of time on the same vitamin used over and over again.



The upper picture shows a rat which has been on a Vitamin A free diet for six weeks during which time the Vitamin A stored in its body has become depleted. The lower picture is the same rat eight weeks later after there had been included in its diet 7½ grams of Calavo per day.

Note the condition of the fur and of the eyes in the upper rat. These conditions have entirely disappeared in the rat when fed on Calavos.

This middle curve shows the rats fed on five grams of avocado. There was good growth although not quite as great as in the curve for the ones having one gram of yeast. This is clearly indicative of the fact the Avocado does contain Vitamin B.

Series II.

The next series of experiments was to determine more nearly the quantitative relationship by varying quantities of yeast and avocado and compare them. This is the diet in the second series.

Group One: Low in yeast—the same basal diet as before plus 0.25 of a gram of yeast per rat per day.

Group Two: Yeast medium—the same basal diet plus 0.5 gram yeast per rat per day.

You see we have cut down the yeast to a minimum because the one gram is probably more than is necessary to enable growth to take place; So the yeast, in order to compare with the avocado, is cut down.

Group Three: The vitamin-free diet.

Group Four: The low-in-avocado diet—basal diet plus 1.5 grams per rat per day.

Group Five: The medium-avocado diet—basal diet plus 3-5 grams per rat per day.

Group Six: The Avocado-high diet—basal diet plus 5 grams avocado per rat per day.

The results are shown in the accompanying curves in graph II. Here we have more nearly what we wanted—quantitative comparison.

The highest curve is the growth curve for rats fed on 0.5 gram of yeast per rat per day which is practically adequate for normal, growth.

Curve 1 is the curve for those fed on the 0.25 gram yeast. Evidently not i enough for normal growth though there is normal growth for about two to three weeks but a bare existence after that.

These other curves show the growth of those fed on avocados.

With the five grams of avocado we get this curve—curve 6—not quite equal to that of the rats fed on 0.5 gram of yeast but considerably better than those fed on 0.25 grams of yeast. Growth has been continuously progressing and the animals are shown to be still growing.

The same is true on the 3.5 grams per rat per day. Growth continues and the animals are still growing though not as rapidly as with the five grams of avocado per day.

Those with the 1.5 grams of avocado did not get as good a growth as the rats fed on the 0.25 grams of yeast yet there is continuous slow growth.

Those on the Vitamin B-free diet are represented by this lower curve, curve 3. They had neither yeast nor avocado. There were three in each of these groups. Two died in this group before this point was reached and the other would have been dead by the sixth week but at the end of the fifth week, we took the one remaining rat and put it on five grams of avocado a day. Its mates—two of them—had died and it was on the point of death. In one or two days an improvement was noticed. It immediately began to pick up growth and had come up above its original growth at this point the sixth week and the curve is rapidly rising and would have continued to rise had the experiment been continued longer than eight weeks.

Vitamin B Results

3 g. Avocado=0.25 g. yeast.

12 g. Avocado=1 g. yeast; 12 oz. Av.=1 oz. yeast.

Av. Wt. 1 Fuerte Av.=12 oz.+(U.S. Bui. Ag. 1073).

1 Fuerte Avocado=1 oz. dry yeast.

(1 oz. dry yeast=3 oz. compressed yeast).

(3 oz. compressed yeast=6 cakes compressed yeast.)

1 Fuerte Avocado=6 cakes Compressed Yeast.

Here the results are tabulated as to a comparison of Vitamin B in avocado as to yeast taken as the standard. Compared with compressed cake of yeast, the kind that is popular in cafeterias and is advertised in the magazines, according to our curves five grams of avocado gives better results than 0.25 grams of yeast; and even 3-5 grams of avocado is more than 0.25 while 1.5 grams of avocado is not quite so much. So we might very justly conclude that 3 grams of avocado would have an equal or greater effect than 0.25 grams of yeast. So we conclude that "3 grams avocado equals 0.25 grams of yeast."

Arranging that in terms of value: 12 grams of avocado equals 1 gram of yeast. The measure "gram" we usually use in chemical laboratories. But the same ratio would obtain in any other measure. 12 oz. avocado is equal to 1 oz. of yeast.

The average weight of the edible portion of one Fuerte Avocado according to U. S. Bulletin No. 1073 is 12 oz. Therefore, one Fuerte avocado would equal in Vitamin B content 1 oz. of yeast. In the experiment we used Fleischman's dry yeast, its water content is considerably less than the moist compressed yeast. On the basis of solid matter 1 oz. dry yeast equals 3 oz. compressed yeast. By actual weight 3 oz. compressed yeast equals 6 cakes compressed yeast. Therefore, we can say that one Fuerte Avocado which is equal to one ounce of dry yeast has the Vitamin B content of six cakes of compressed yeast.

I have here the pictures of some of these animals. Here is the first series I referred to—experiments on the Vitamin B. Here are also pictures on the Vitamin A experiments which we have just taken which will show you comparisons although the experiment is not completed. See the appearance of this rat with no Vitamin A and with eye disease, compared to this one taken after Avocado had been added to the diet. The other chart is the second or quantitative experiment. Here is a picture of the one taken six weeks after the beginning of the experiment and which was just about to die for lack of the vitamin, after which avocado was added and this picture shows the improvement from feeding the Avocado.

Our conclusion from the work done thus far is that avocados contain Vitamin A, the quantity of which will be reported later. As to Vitamin B, it has been established that the vitamin content of one Avocado of Calavo grade is as much as that of six cakes of compressed yeast. It is also of course much better to taste and has other dietary values which we are not taking into consideration in this discussion.