

VITAMINS C, D, AND E

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Introductory

A vitamin is a substance occurring in certain foods which is necessary to promote normal growth and development, and to protect against certain diseases, called dietary deficiency diseases.

There are five fully recognized vitamins. Recent researches tend to show that certain of these are complex, or the effects which we ascribe to them may be due to more than one separate vitamin.

These five vitamins are:

A. Anti-Xerophthalmic—promotes normal development and protects against xerophthalmia, an eye distemper.

B. Anti-Neuritic—promotes growth and protects against beriberi, a nerve disease.

C. Anti-Scorbutic—protects against scurvy.

D. Anti-Rachitic—protects against rickets.

E. Anti-Sterility—necessary for reproduction.

A, D, and E are the fat soluble vitamins.

B and C are the water soluble vitamins .

The results of our researches on Vitamins A and B in avocados in the chemical laboratory of the University of Southern California have been reported at previous meetings of this association. The avocado was found to contain both A and B in very high quantity compared to other fruit. The report, today, will be on Vitamins C, D and E.

VITAMIN C—ANTI-SCORBUTIC VITAMIN

Rats are not susceptible to scurvy, so for tests for Vitamin C, guinea pigs are used. The indications of scurvy are loss of weight, evident pain, and early death. Autopsy indications are hemorrhages of the joints and the ribs section, fragility of bones and loosening of teeth. The experimental period is ninety days.

Twenty-five guinea pigs, weighing 250 to 350 grams, were placed individually in cages and were fed ad libitum a basal diet as follows:

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| 1. Mixture of equal parts commercial rolled oats and wheat bran | 59 per cent |
| 2. Skimmed milk powder, heated on open trays at 110 C. (until anti-scorbutic vitamin is destroyed) | 30 per cent |
| 3. Butter fat, freshly prepared | 10 per cent |
| 4. Table salt | 1 per cent |
| 5. Water ad libitum. | |

During the preliminary period the animals received, in addition to this basal diet, a liberal allowance of fresh lettuce or cabbage. This preliminary period was continued until the animal weighed 300 grams or more for three consecutive weighings (weighings were made every five days).

The guinea pig is an animal very stubborn as regards its diet. It will not, of its own accord, take to new food. Experimentation is of no value unless it is assured that the animals eat the test food added to the diet. At first, practically all the animals refused the avocado, but, by a combination of forced feeding and coaxed feeding, developed in Dr. Sherman's laboratory at Columbia, the avocado was soon eaten with relish. In the case of the five gram portion, forced feeding was necessary to assure complete consumption of the portion.

The animals were distributed for testing in the following manner:

- (1) Four basal diet alone; negative controls.
- (2) Three basal diet plus 3 cc. orange juice; positive controls.
- (3) Four basal diet plus 0.5 gr. of avocado.
- (4) Four basal diet plus 1 gr. of avocado.
- (5) Five basal diet plus 2 grs. of avocado.
- (6) Five basal diet plus 5 grs. of avocado.

Summary of results:

1. With no antiscorbutic vitamin, there was good initial growth, followed, after about two weeks, by onset of scurvy symptoms, cessation of growth, and great loss of weight .before death from scurvy, which occurred at about twenty-eight days. Autopsy revealed in severe form all the typical signs of scurvy, notably hemorrhages, fragility of bones, and looseness of teeth.
2. The normal control group thrived throughout the entire experimental period with no indications of scurvy.
3. With 0.5 gr. of avocado per day the duration of life was prolonged and time of death was less uniform than on the completely scorbutic basal diet. The animals

became lame and stiff before death, and at death showed severe hemorrhages, fragile bones, and loose teeth.

4. With 1 gr. of avocado per day, the duration of life was prolonged still further, but the animals suffered badly from scurvy during their last days. They showed all signs of scurvy on autopsy.
5. With 2 grs. of avocado per day, life was prolonged somewhat further than in any of the previous cases, but the animals all died long before the end of the required experimental period of ninety days. Autopsy revealed all scurvy symptoms.
6. "With 5 grs. of avocado per day, forced feeding had to be resorted to. Animals seemed to lose a desire for any food. Autopsy revealed all scurvy symptoms. Death came earlier, due to their refusal to eat sufficient of the basal diet.

From these results, we would conclude that avocado contains a small amount of the Vitamin C, sufficient to defer severe symptoms of scurvy for a short period, but not sufficient to be considered as a protective against scurvy in the quantity that can be consumed.

VITAMIN D

The relation of Vitamin D, the antirachitic vitamin, to sunlight and to ultra-violet light is well established. Rickets may be prevented, and may even be cured, either by irradiating the body with sunlight, or with ultra-violet light, or by the addition to the diet of foods containing Vitamin D. A further relation between sunlight and Vitamin D is shown in the fact that certain foods containing fat-soluble sterols, cholesterol, phytosterol, or ergosterol, though in their natural condition not antirachitic in property, take on this property on being irradiated with the actinic rays of sunlight, or with ultra-violet light. The rays which possess this antirachitic property are those of wave length of about 300 millimicrons in length. These are the chemical rays, or those which take part in photosynthesis or manufacture of plant food by the chlorophyll in plant leaves.

Vitamin D does not occur in natural foods to an extent as do the previous vitamins. It occurs in green succulent vegetables, doubtless as a product of photosynthesis. Since Vitamin D is fat soluble, it occurs stored in certain fats, as egg yolk, and cod liver oil, and to some extent in butter fat; in which fats it has had origin either in green vegetation eaten by the animals producing this fat, or in the irradiation of the outer coating of their bodies, which contain cholesterol. Most fruits do not contain Vitamin D in measurable quantity. This is as might be expected, since this vitamin is fat soluble, and the ordinary fruits do not contain fat. The avocado is the one exceptional fruit, in that it is practically the only known fruit eaten as fresh fruit, which stores its food material as an oil. The California avocado has a high oil content. The minimum requirement for Calavo standard is twelve to fifteen per cent of oil. Since the avocado has a large spreading evergreen foliage, and grows in the sunlight of semi-tropical regions, it has ideal conditions for vitamin synthesis. The oil of the fruit furnishes a natural storage.

The test for Vitamin D in foods is the ability of the food to prevent, or to cure, experimentally produced rickets in test animals. White rats are used as the experimental animals.

The production of rickets through lack of Vitamin D appears to be through a development of a low phosphorus content of the blood, and thereby a lack of proper calcification of the bones. The ossification of a bone, as the tibia, takes place at the cartilage junction between the long bone shaft, or the diaphysis, and the enlarged end of the bone or the epiphysis (see diagram). A rachitic bone does not ossify at the junction, hence there is a bending or bow-legged condition.

The first and most specific test for rickets is the determination of this zone of non-calcification, and is accomplished by what is known as the "line test." ¹In this test the rear leg bone of an autopsied animal is dissected out. The femur is split lengthwise, placed in acetone for eight hours, then placed in a solution of silver nitrate, after which it is exposed to ultra violet light, which blackens the silver salts absorbed by the bone at the junction with the cartilage. This junction, when observed under a low power binocular microscope, shows a clearly defined white boundary line of cartilage if the bone is normal. If, on the other hand, there is no definite line of boundary, but the bone shows lesions, and is not ossified up to the proper margin, rickets is indicated.

A second rachitic condition is a malformation at the junction of the bone shaft of the ribs with the lower cartilaginous extension. This forms a knob, or bead-like formation. If a rachitic animal is autopsied and the ribs opened up and folded back, there appears a prominent chain of thin bead-like formations up the ribs on one side and down on the other, forming what is called a "rachitic rosary."

A third specific test for rickets is the finding of a low phosphorus content in the blood. The blood from a freshly killed animal is collected in a tube, and after standing twenty-four hours in the ice-box, the clear serum which has risen is tested for phosphorus by Tisdall's method.¹ This method depends on the treatment of the protein free serum with strychnine molybdate, which is separated by means of an electric centrifuge, a brilliant green color is developed by the reduction of the molybdate with potassium ferrocyanide. This is compared in a colorimeter with a standard color tube of known phosphorus value, from which the phosphorus content of the blood may be calculated. The phosphorous content of the blood of a normal rat is about ten to twelve mg. per 100 cc. of blood. In rachitic animals it may sink as low as two to four mg. per 100 cc. of blood.

The experimental procedure, with the results obtained, is shown in the accompanying tables.

DIETS

NORMAL DIET	RACHITIC DIET
2 parts ground whole wheat. 1 part evaporated whole milk. 2% wt. of wheat of sodium chloride. 1 leaf lettuce daily. Raw meat (ground round steak) twice a week.	(Steenbock & Black 2965) 76% yellow corn, ground. 20% wheat gluten. (Pure gluten meal. Battle Creek Co.) 3% calcium carbonate. 1% sodium chloride.

SERIES RUN

- I. Curative—Three weeks rachitic diet, followed by four weeks curative diet.
 II. Protective—Five weeks rachitic diet plus protective diet.
 Division 1. Using Fresh Avocado Pulp.
 Division 2. Using Avocado Oil. (a) Crude. (b) Refined.
 (Normal Controls, Rachitic Controls, Cod Liver Oil Controls used in each series.)

SERIES I—CURATIVE

Rachitic Diet, Three Weeks—Curative, Four Weeks

AUTOPSY RESULTS				
Group	Diet	Rosary	Line Test	Blood Phosphorus
1	Normal Diet	Normal	Normal	Normal
2	Rachitic Diet	Rickets	Rickets	Low
3	Rachitic Diet and 5 drops cod liver oil	Normal	Normal	Normal
4	Rachitic Diet and 1 gram avocado	Rickets	Rickets	Normal
5	Rachitic Diet and 2.5 grams avocado	Rickets	Rickets	Normal
6	Rachitic Diet and 5 grams avocado	Rickets	Rickets	Normal

SERIES II, DIVISION 1—PREVENTATIVE
Fresh Avocados Used
Total Time of Feeding—Five Weeks

AUTOPSY RESULTS				
Group	Diet	Rosary	Line Test	Blood Phosphorus
1	Normal Diet	Normal	Normal	Normal
2	Rachitic Diet	Rickets	Rickets	Low
3	Rachitic Diet and 5 drops cod liver oil	Normal	Normal	Normal
4	Rachitic Diet and 1 gram avocado	Rickets	Rickets	Medium
5	Rachitic Diet and 2.5 grams avocado	Rickets	Rickets	Normal
6	Rachitic Diet and 5 grams avocado	Mild Rickets	Mild Rickets	Normal

SERIES II, DIVISION 2—PREVENTATIVE
Avocado Oil Used
Total Time of Feeding—Five Weeks

AUTOPSY RESULTS				
Group	Diet	Rosary	Line Test	Blood Phosphorus
1	Normal Diet	Normal	Normal	Normal
2	Rachitic Diet	Rickets	Rickets	Low
3	Rachitic Diet and 5 drops cod liver oil	Normal	Normal	Normal
4	Rachitic Diet and 0.5 cc. crude avocado oil	Normal	Medium Rickets	Normal
5	Rachitic Diet and 1 cc. crude avocado oil	Normal	Mild Rickets	Normal
6	Rachitic Diet and 2.5 cc. crude avocado oil	Normal	Trace of Rickets	Normal
7	Rachitic Diet and 0.5 cc. refined avocado oil	Normal	Medium Rickets	Normal
8	Rachitic Diet and 1 cc. refined avocado oil	Normal	Mild Rickets	Normal
9	Rachitic Diet and 2.5 cc. refined avocado oil	Normal	Trace of Rickets	Normal

General Summary. These results prove the correctness of the assumption that the avocado, being an oil-bearing fruit, grown in semi-tropical regions, should contain the sunlight vitamin, Vitamin D, which is soluble in fats. It may be concluded that though avocados or avocado oil may not be compared as the equal of cod-liver oil in Vitamin D for the cure of rickets, the results obtained establish that it possesses this vitamin in liberal quantities, such that it may be considered a food source of Vitamin D, which would materially contribute to protection against rickets.

It is to be noted that this experimentation shows that this vitamin is held in the oil content of the avocado and that the oil may be extracted and refined without loss of the vitamin. It is to be presumed that the vitamin is in proportion to the oil content of the fruit, especially as the oil content is a measure of the maturity of the fruit.

VITAMIN E

Vitamin E, the latest child in the vitamin family, influences reproduction, and, as has been lately discovered lactation. It is, therefore, said to contain two components, the

anti-sterility vitamin and the lactation vitamin.

During the past years, it has been shown repeatedly that white rats, fed on synthetic diets, seldom bear young, in spite of an apparent normal growth and well being. Depending on the character and proportions of the constituents of the food, they sooner or later exhibit complete sterility; sterility simply meaning the fact that they cannot produce young. That sterility may be due to diet deficiency and can be cured or prevented by a change in the dietary regime, was first reported by Evans, of the University of California, in 1922. Vitamin E resembles A in some respects, and has been confused with it in the earlier studies. It is a fat soluble, though it occurs in plant tissues not considered oily. The chemical formula has not yet been found, but enough properties have been determined to give it a rather indefinite classification. It is soluble in the usual fat solvents, is stable to light, heat, air, and many ordinary chemical reactions. Prolonged irradiation with ultra violet rays produces oxidation, and it is destroyed as are also Vitamins A and D. Vitamin E is present in a great variety of animal tissues, though not in a concentrated form, the highest concentration being found in the musculature of the jaw. It is not stored in the tissues by feeding high percentages of the vitamin-containing foods.

Contrasted with the low concentration of Vitamin E in animal tissues is its general distribution and highly concentrated form in certain organs of the plants, especially green lettuce and seeds. Lettuce was first used as an anti-sterility agent. Two and one-half grams per day was used as the dosage in effecting cures. Wheat germ, or the extracted oil, has also been found to be a very potent source of the Vitamin E. Vitamin E has been proven present in lettuce, tomatoes, wheat germ, avocado, egg yolk, flax-seed, butter, crisco, and cocoanut oil. Note that animal fats do not contain this vitamin, with the exception of butter, which has very little.

Because of the high fat content of the avocado, and because Vitamin E is fat soluble, it was assumed that Vitamin E could be found present. To carry on the experiment, rats used in the test were given a synthetic diet containing all the food factors for growth and well being, including all the vitamins except Vitamin E. Litter mates of these rats were placed on a normal fertility diet and used as control animals. The depletion or sterility diet was that used by Evans in his original work. Casein, eighteen parts; starch, fifty-four; lard, fifteen; butter, nine, which was later replaced by Cod-liver oil, two; McCollins salt mixture, four. To this was added 0.5 per yeast per day to furnish Vitamin B. Control rats were given the usual diet plus lettuce every day and ground beef twice a week. The test animals were mated with the controls from time to time to test fertility. When complete sterility was obtained the test animals were fed, in addition to the original diet, varying amounts of avocado or the extracted oils. Five gms. avocado, or 7½ drops of the oil, were the minimum doses found effective in producing cures in the case of female rats.

In this research, it was definitely proven that female rats, proven sterile, were able to bear young after being fed avocado or avocado oil, thus definitely proving the avocado to contain Vitamin E. Since Vitamin E deficiency in the male causes degeneracy of the reproductive organs, it is more difficult to effect cures. Although in the experimentation, rejuvenation of the fully degenerate male was not accomplished, it was thought that prevention of sterility might be wholly possible. The proof of this needs further tests,

however, as the prevention of sterility was not covered in this experimentation.

It is interesting to note that in Central America, where the fruit is native, the story of its rejuvenating power for old men, and of its being fed to childless women to cure sterility, has come down even from the time of the Aztec Indians.