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Dissertation

VITAMIN C STUDIES WITH SPECIAL REFERENCE TO THE RAT

BY

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submitted in partial fulfilment of the requirements for the degree of
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1935.
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Otis Edward Alley, born July 22, 1899, in Taunton, Massachusetts, the son of Frederick H. and Fannie M.W. Alley. Graduated from the Cohannet Grammar School 1913, and from Taunton High School 1917. After taking a post graduate course he entered Boston University and received the degree of S.B. in 1924, and the degree of A.M. in 1926. During the years 1924 to 1927 he was respectively teacher of science and head of the Science department of the Searles High School of Methuen, Massachusetts. From there he went to the Winthrop High School as head of the
Science department. After serving three years in that capacity he became head of the Science department in the Winchester High School. In 1930 he became Teaching Fellow in Physiological Chemistry at the Boston University School of Medicine and started his studies for the degree of Ph. D. In 1931 he became Assistant in Chemistry in the Boston University School of Medicine and also a Fellow in the Evans Memorial Hospital under the direction of Dr. Allen Winter Rowe. During this time he took graduate work at Harvard Medical School, and Boston University. He is the co-author, with Dr. B.S. Walker, of a paper "Detection of Traces of Carbon Monoxide in Ethylene." Journal of Anesthesia and Analgesia, 1929, 8, 4. Also he assisted Drs. S. Hooker and W.C. Boyd in the publication of a paper "Antigenic Property of Gelatine-diazo-arsenic acid." Jour. Immunology, 1933, 24, 2.

Since the death of Dr. Rowe he has been continuing research work under the direction of Dr. Burnham S. Walker.
Science Department. After serving three years in
that capacity he became Dean of the Science Depart-
ment in the Winthrop High School in 1920. In
become a Teaching Fellow in Psychopathological Chemis-
try at the Boston University School of Medicine. In
1921 he was elected for the Degree of S.B. and in
1921 he became Assistant in Chemistry to the Boston Uni-
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Avenue Memorial Hospital under the direction of Dr.
Walter E. Miller. During this time he took lectures in
work at Harvard Medical School and Boston University.
He is the co-author, with W. E. Miller, of a paper
"Detection of Trace of Certain Monoxides in Airs";
"Introduction to Antigens" and "Antigens and their
Some of the papers of Dr. Hageman have been commen-
ted upon for their origin and the direction of Dr. Hageman's
Early History of the Pueblo and Country.

During earliest times man had known about tobacco which we now know to be harmful by deficiency in some respect to the eons. In fact, the oldest medical book known, the Kahun, which dates 2000 B.C., describes some of these deficiencies in an accuracy that surpasses fervently with our scientific descriptions of today.

During the time of Pharaohs, 450-370 B.C. people thought that some malnutrition ailments resembled the present in India. According to Polley, a text this same affected the Indo-Dravidian and 15th century. They speculated on man in the land, 45th generation and some of those.

Some states that the ancient Egyptians and descents from them were probably due to the idea converted to grains and an wheat or barley. 1918 saw the landing in Arabia.

1. Polley, G. The Pueblo. 1921.
2. Wightman, W. L. and Diamond, A., Cañon City in Arizona, 1908, No. 311.
Early History of the Vitamins and Scurvy.

Since earliest times man has known about diseases which we now know to be caused by deficiency in some respect in the diet. In fact, the oldest medical book known, the Neichung, which dates 2697 B.C., describes some of these diseases with an accuracy that compares favorably with our scientific descriptions of today. During the time of Hippocrates, 460-370 B.C., people thought that some universal nutrient substance was present in foods. According to Findlay a diet disease affected the Roman army when it invaded Arabia in 24 B.C. He describes a large number of diseased army men who probably had scurvy. They complained of pains in the legs, gum gangrene and loss of teeth. Morse states that the ancient Egyptians had diseased teeth which were probably due to the diet restricted to grains such as wheat or barley. This diet was lacking in vitamin C.

1. Funk, C., The Vitamins, 1922.
During the Crusades apparently scurvy played an important part in deciding the outcome of many campaigns. Joinville describes the disease very soon after the first Friday in Lent of 1250 as follows: "The host began to suffer very grievously... the flesh of our legs dried up, and the skin of our legs became spotted: black and earth colour like an old boot, and with us who had this sickness, the flesh of our gums putrefied: nor could anyone escape from this sickness but he had to die. The sign of death was this, that when there was bleeding of the nose, then death was sure. The sickness began to increase in the host in such sort, and the dead flesh to grow upon the gums of our people, that the barber surgeons had to remove the dead flesh in order that the people might masticate their food and swallow it. Great pity it was to hear the cry throughout the camp of the people whose dead flesh was being cut away, for they cried like women belabouring of child."

During the colonization period in northern America, scurvy was prevalent. In fact, during the winter time many settlements such as the one at Hudson's Bay were abandoned because of this disease, while others in Canada and Newfoundland were sorely threatened.

In 1535 Cartier made his second voyage to Newfoundland with a crew of one hundred and three men. Soon the dreaded scurvy developed, and out of the entire crew only three escaped the disease, while twenty-six died. Cartier went ashore, made up an infusion of pine needles, and administered it to the surviving men with such success that all of them recovered.

Probably scurvy has been prevalent in the northern parts of Europe and Asia ever since that land has been occupied by man. Since the early people were uneducated and met the inhabitants of southern regions rarely, we have few, if any, of their records of the disease.

In 1938 czarist made the second voyage to
Komsomol's with a team of one hundred and fifty
men. Soon the largest radical developed and out of
the results there only three successful. The theme 'the
town-stories' they carried away terrible; trade up to
three mirror of the naivete, and somberly to
the reflections very much such success after all
from Leonardo.
Claus Magnus however, describes the disease of scurvy among the soldiers of the northern nations. Ronsseus, Echtius, and Wierus also wrote treatises on the disease recommending treatment by diet. A little later, 1695, the Faculty of Medicine at Copenhagen published a "consilium" on scurvy to be used by the poor people of Denmark and other northern countries in combating this disease.

Bachstrom has described an epidemic of scurvy among the Swedes which killed five thousand men during the siege of Thorn in 1703.

Scurvy is the first disease to be associated with nutrition from a standpoint of etiology. Kramer, an Austrian physician, found that in 1720 almost the whole army was affected with an epidemic of scurvy. In spite of attempts at feeding dried herbs, and medical treatment, thousands died of the disease. The following is taken from his book:

4. Kramer, Medicina Castrensis. 1720
"Scurvy is a terrible disease for which there is no known cure. Medication does not help, neither does surgery. Be careful of bleeding: shun mercury as a poison. The gums may be massaged, the stiff joints may be rubbed with fat - but all in vain. If one could only have available a supply of green vegetables, or a sufficient amount of the vital antiscorbutic juices: or if one could have at hand oranges, limes or lemons, or their preserved pulp or juice so that a lemonade could be made out of them: or administered as such in three or four ounce doses - then one could be in a position to cure this dreadful disease without other help."

Apparently Kramer's ideas on scurvy were very modern, since we know today that medicine or surgery in the disease is useless, but that fresh fruits such as oranges, will not only prevent scurvy, but also cure it.

1 William Stark, M.D. produced scurvy in himself by eating a diet composed only of bread and water. His second attempt in 1770 resulted in the production of scurvy, but he could not cure himself of the disease this time, and he died of scurvy.

Early ideas of nutrition.

1 Richerand, A., Elements of Physiology, 1813.

Richerand said that an "aliment" is whatever substance that affords nutrition, but that however various our aliments may be, the substance we obtain from our aliments to incorporate with our own is always the same.

2 In 1834 William Prout classified all organized bodies to be made up of four fundamental substances, "The aqueous, the saccharine, the oleaginous, and the albuminous." These are of course our modern water, sugars, fats, and proteins.

3 A little later, 1843, Pereira recognized that other substances such as common salt were necessary for proper continuance of life. Furthermore, orange juice, the antiscorbutic food was necessary and could not be classified as one of Prout's four fundamental substances.

4 In 1906 Hopkins stated that no animal can live on a mixture of pure protein, fat and carbohydrate even if inorganic material is also included in the diet, but that still other substances are required.

1. Richerand, A., Elements of Physiology, 1813.
Berg states that the Germans are still dumbfounded at the revelation that there are nutritive substances that cannot be included in the four fundamental classes. In 1905 Ekelof expressed the belief that the cause of disease following consumption of preserved foods was due to the generation of a poison in them. This idea supported by Schmidt-Nielsen was that some valuable substances such as enzymes or antibodies were destroyed by the process of preservation. Rohmann thought that the cause of scurvy did not lie in the absence of any special scorbutic factor, but rather to protein insufficiency. However, Bierich considered scurvy to be dependent on the denaturing of food by treatment which made it deficient in some respect. Garrod suggested the potassium deficiency theory as the explanation of the cause of scurvy. This was abandoned slowly because experimental work failed to support it. The citric acid theory of Netter became popular.

2. Ekelof, E., Hygiea, 1904, 1214.
I have reason to believe there are sufficient grounds
by the revolution that there was little agitation
and the fact remains in the minds of your correspondents
in France to overlook spontaneously the facts that
some of the political-minded organizations of the
above may take to the conduct of a political or
life and at the same time with respect to the
intelligentsia of the country. However, whatever
any correspondent has made it his business to learn
and to speak as a matter of course, the following
and the papers of the Walpole government, which
your last report of the Foreign Office depart-

I first received these reports, 1904

3rd of May, 1904, 1.116.

2nd of May, 1904, 1.116.

1st of May, 1904, 1.116.

Walpole, 1.116.

1st of May, 1904, 1.116.

1st of May, 1904, 1.116.
for a short time. Association of the antiscorbutic value of citrous fruits with the citric acid naturally lead to the false belief of the necessity of this acid. In contrast to this theory was that of 1 Wright which stated the cause of scurvy as acidosis. These ideas, with many others, were gradually sifted out until at the present time no doubt remains in regard to the cause of scurvy, namely, vitamin C deficiency.

It was also noticed that men on voyages starting in winter were more quickly attacked by the disease than those who started in the summer months. This was probably due to the depletion of vitamin C in men during the winter, and scarce in vegetables. Many of the men on land were cured on the verge of scurvy in the winter.

According to Loeb the earliest account of scurvy at sea in that of Peter de Gama in 1497 on the voyage around the Cape of Good Hope. Drake also described cause of scurvy. Lord Anson's expedition of 1740 failed because of scurvy. In four years eighty

I hereby certify that the contents of this document are true and correct.

[Signature]

[Date]
Scurvy at Sea.

The long voyages at sea during the sixteenth century provided an opportunity for scurvy to manifest itself in no uncertain manner. These trips were undertaken of course without ample supply of fresh vegetables or animal foods, and as a result, scurvy generally broke out after a few months at sea.

It was also noticed that men on voyages starting in winter were more quickly attacked by the disease than those who started in the summer months. This was probably due to the depletion of vitamin C in men during the winter, and excess in summer. Many of the men on land were perhaps on the verge of scurvy in the winter.

According to Hess, the earliest account of scurvy at sea is that of Vasco de Gama in 1497 on the voyage around the Cape of Good Hope. Drake also described cases of scurvy. Lord Anson's expedition of 1740 failed because of scurvy. In four years eighty


per cent of the original crew had died of this disease. Hawkins states that he can give an account of his twenty years on the ocean, during which time ten thousand seamen were destroyed by scurvy. In fact, it has been estimated that up until the time antiscorbutics were introduced at sea, more sailors have been killed by scurvy than all the other causes pertaining to sea life, including naval warfare.

On his expedition into the antarctic Shackleton was able to protect the members of his crew from scurvy by the use of concentrated lemon juice and tablets of lemon juice. On the other hand, scurvy appeared in Scott's expedition to the South Pole, resulting in many deaths although substances were taken along which were supposed to be antiscorbutic.

In spite of diet precautions the British were troubled with scurvy in India during the World War, (1917-20). At that time the common antiscorbutic used was lime juice, and practical experience, together with actual analysis shows this particular

In conclusion, we believe that the experience gained in this campaign will prove invaluable in our future efforts. The lessons learned and the challenges faced will serve as a foundation for our continued success.

We are grateful for the support and encouragement we received from all directions. Each individual and unit played a critical role in the achievement of our objectives. Their dedication and commitment are appreciated and will not be forgotten.

Looking ahead, we recognize the need for continued improvement and adaptation. The evolving nature of our environment requires a vigilant approach and a willingness to learn from our experiences.

In conclusion, while the road ahead may be challenging, we approach it with confidence and a spirit of determination. Together, we will overcome any obstacles and achieve our goals.

Thank you for your support and your unwavering commitment. We are honored to serve with you.

[Signature]
source to be unreliable.

Stefansson experimented with diets in the north countries, and discovered that scurvy would not appear in men even over a period of years provided raw meat and tissues such as liver and kidney were eaten. There was a time when one of his men who refused to eat raw meat developed scurvy, but was cured by eating raw meat.

Doctor Kane avoided scurvy while on the arctic ship "Advance" by eating rats. Either his companions could not bring themselves to eat the rats, or else they did not believe in the possible protection against scurvy, and as a result suffered or died from vitamin C deficiency.

Ship-beriberi.

During long voyages, or in war time when rations are limited, sometimes both vitamins B and C may be absent from the diet, resulting in a peculiar disease called "ship-beriberi." This disease is characterized by symptoms of both beriberi and scurvy, such as numbness of the extremities (beriberi) and bleeding of the gums (scurvy). In 1871 Delpech described such cases occurring during the siege of Paris. Scherer also observed a serious outbreak in German South Africa in which the death rate was three hundred per thousand. The German raider Kronprinz Wilhelm was interned in New York harbor during the World War. Nearly all the members of the crew had ship-beriberi while the officers did not. The officers had fresh vegetables in their diet whereas the crew lived on frozen meat. In most cases the disease cleared up after fresh vegetables had been added to the diet.

1. Delpech. Le scorbut pendant le siège de Paris. 
Parachute Party. Do not time your-leave

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Scurvy during the World War.

Apparently scurvy was present during the World War as well as during other wars. A large number of cases were noted by Boerich at the Red Cross Central Station in Russia. Over one thousand cases were treated there. Hift reported many cases associated with night blindness (probably xerophthalmia due to lack of vitamin A), while Much described cases he thought were transmitted by vermin. Other countries were similarly afflicted. Aschoff and Kock have written an excellent monograph on scurvy as it occurred in Roumania. Wiltshire observed over three thousand cases in the Serbian army. Several other writers, Lobmeyer, Disque, and Morawitz have called attention to many cases in German and Turkish troops. The older soldiers were the


We may be in a position to make a large number of cases.

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The following cases are the key cases consistent with:

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This appears consistent with a general trend of:

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The court has noted:
ones most affected by scurvy according to Arneth. He believed the disease to be due to the excessive use of dried foods.

It seems that there were many more cases of scurvy among the peoples just mentioned than among either the British or American troops in Europe. In the case of the Colonial troops in Mesopotamia however, many cases occurred due to deficient rations. There during the summer of 1916, seven thousand five hundred men were lost in nineteen weeks as a result of scurvy.

The French army in 1917 were seriously hampered by scurvy. Harvier discovered that ninety-five per cent of one group of eight hundred men had the disease.

Scurvy in the Guinea Pig.

Theobald Smith (1895) was the first to observe that scurvy could be produced in the guinea pig by limiting the diet to one of oats and bran. Little attention was given to this discovery until Holst and Froelich did more work with guinea pigs on a diet of cereals and bread. They described the disease in the pigs resulting from this diet as being analogous to scurvy in man, characterized by loosening of the teeth, bleeding of gums, general weakness, bone decalcification, and a peculiar microscopic appearance of the bone marrow. Further they found that animals fed in addition to this diet fresh cabbage, carrots, and other fresh vegetables did not develop scurvy, and animals that already had scurvy could be cured by simply adding fresh cabbage and carrots to the diet. This all proved rather conclusively that scurvy was caused by the absence of something in the diet. This work was confirmed in 1917 by Chick and Hume who studied the values of various food stuffs as a preventative for scurvy. They fed guinea pigs oats

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| loss of respect for the traditions and the culture.
and bran, which had been shown to produce scurvy in about three weeks. The food to be studied was added to the diet and the amount of protection from scurvy noted. The work of Chick and Hume was very important for two reasons.

1. It showed that the preventative method of measuring the anti-scurvy value of foods was better than the curative method when the animal was first allowed to contract scurvy and then is fed the food to be tested. They found that even when the value of the food is high that the return to normal from scurvy is very slow, and that it leads to much confusion as to the actual anti-scurvy value of the food.

2. This work rather completely changed the ideas of Jackson and Moore and McCollum and Pitz (1916). These workers had completed experiments which tended to show that the cause of scurvy was a bacterial infection. In fact, Jackson and Moore had isolated a diplococcus from the tissues of pigs that had died from scurvy. Hess also had supported the view that scurvy was bacterial in origin. He followed a large number of hospital cases of children. These

The book will be returned to me by 12:00 on the 13th of next month. I am in the process of writing a report on the progress of the project. The work of planning and implementation has been very important for the success.

To summarize the essential points:

1. The report is based on the analysis of the data collected during the project.
2. The results show a significant improvement in efficiency and productivity.
3. The project is expected to be completed by the end of the month.

The recommended actions are:

1. Continue the analysis and refine the report.
2. Ensure that all data is accurately recorded.
3. Prepare a presentation for the upcoming meeting.

I look forward to your feedback on the progress so far.
children were fed milk which had been heated to different temperatures. The results seemed to indicate that the heating of the milk had little effect on the production of scurvy, but that the age of milk, and hence the bacterial content, was an important factor. Apparently the fundamental reason which caused some workers to disband the diet concept of the cause of scurvy was the production of scurvy in animals and humans who had milk in their diet. Years ago milk had been considered to contain all the substances needed by man. The fact that the infant gets along the first part of his life entirely on milk was quoted as proof that milk was a perfect food. For milk to cause scurvy because of a lack of some necessary constituent was unbelievable. In 1918 Cohen published an account of the production of scurvy in the guinea pig by variation of the diet. He found that scurvy could be cured by adding to the diet orange juice, raw cabbage, or dried cabbage. Hess has also shown similar effects except that his experiments indicate that dried vegetables have little value as a preventative against scurvy. Hess also showed that the active factor in orange juice could be removed by alcohol extraction.

Scurvy in the Monkey.

That the monkey is an animal which readily shows the effects of a vitamin C free diet has been shown by Hart and Lessing. On a diet of condensed milk, rice and bread, the monkey develops scurvy in two months. Conditions resembling infantile scurvy and adult scurvy were observed in young and adult monkeys. Bone changes were observed especially. Talbot and Harden confirmed these experiments. Harden used a scurvy producing diet of three hundred grams of rice, five grams of butter, fifty grams of wheat germ and two grams of salt. The daily addition of two c.c. of orange juice to this diet protected the animals against scurvy, and further, the animals that developed scurvy on the vitamin C free diet were cured by the daily administration of orange juice. Harden also observed a pseudo-paralysis of the hind legs while

Zilva and Still noticed a severe hemorrhagic edema of the upper eye-lids. Howard investigated the mineral metabolism of monkeys with scurvy, and found that the salt losses were much less than had been noted in man.

1. Zilva, S. E. and Still, C. F., Lanc., 1, 1920, May 8, 1908
I have just notified the Government of the possibility of establishing the mineral water industry at some point with serious and long-lasting effects. If the self-sufficiency were more firmly and clearly understood.
Scurvy in the child.

The first description in history of child scurvy is that of Glisson. In 1669 he stated that in scurvy tumors appear on the gums, pains in the joints are experienced, palpitation of the heart, difficulty of breathing after exercise and in the absence of fever a concentration of urine occurs. He observed that scurvy often accompanied rickets while he thought the cause to be the "inclemency of the air and climate where the child is educated."

Little attention was given Glisson's work however, since most workers thought the important thing to be rickets rather than scurvy. The point is emphasized by the fact that Moeller in 1859 described cases of rickets which were in reality scurvy. That is, all the symptoms appeared to be those of scurvy rather than of rickets, although for some inexplicable reason he found that lemon juice and fresh vegetables were useless in treatment.

The first observation to present itself is that offormation. In order to achieve this, it is essential to work in a systematic manner, maintaining the highest standards of accuracy in recording data. It is important to note any variations or anomalies in the sample results and to consider the implications of these findings. The importance of correlation between different variables cannot be overstated. As we proceed, we may encounter unexpected challenges that require careful consideration and appropriate adjustments. If the data suggest the need for further investigation, we should not hesitate to pursue these avenues. The key to success lies in maintaining a rigorous and thorough approach to the analysis.
At times infant scurvy is still called "Die Moellersche Krankheit." Immediately after Moeller's work, other German writers such as Foerster, Bohn, and Steiner, supported the view that these infant cases were an acute form of rickets. There are many fairly recent German books which describe infant scurvy as rickets on the grounds that in these cases involvement of the gums is absent and that the absence of this essential symptom rules out scurvy.

1. Foerster, E., Ein Fall von acute Rachitis, Jahrb. f. Kinderheilk., 1868, 1, 144.
Effect of Heat on the Vitamin C Content of Milk.

Neumann was one of the first to observe the relation between heating milk and the appearance of scurvy in infants. The milk used had been pasteurized first at the farm where produced and then heated again just before consumption. These results were confirmed by Meyer, Cheadle, and Heuber. Brachi found cases of scurvy resulting from the cooking of milk, and the American Pediatric Society has reported hundreds of such cases, all of which were fed on artificial foods. Hess found that scurvy results in children if the milk used in feeding is heated to 145°F for thirty minutes.

6. Amer. Pediatric Soc., Arch. of Pediatrics, 1898, 15, 481.
Effects of Heat on the Appearance of Milk

...
On the other hand, Sittler showed that scurvy would not result from the warming of milk on the water bath, for five minutes. Epstein has reported cases caused by feeding milk which had been heated several times. Another factor entering into his cases was the absence of fresh fruits from the diet. Bendix has recorded a case of scurvy resulting from a diet of cow's milk mixed with a flour food. This case was cured by the administration of raw milk. The conclusion was made that the carbohydrate acted unfavorably and perhaps hastened the symptoms of scurvy. Another explanation may be that the milk varied in vitamin C content.

Infantile scurvy occurs most frequently in cases of bottle-fed babies. As a rule the milk is boiled to protect the child against possible bacterial infection but during this process (as I shall later show) the vitamin C which is present only in small amounts at best is greatly or even entirely lost. After a time the baby grows pale and fretful. Its appetite disappears, it refuses to eat and consequently loses weight. Tenderness of the joints or extremities may be present and sometimes hemorrhage of the gums. In some cases the only apparent symptom is fever. This condition is sometimes referred to as "latent scurvy" and is also called "Barlow's disease." As the disease progresses it is obvious that the child's resistance to infections will probably become less. Sudden and profound improvement results from administration of orange and tomato juice. Without doubt latent scurvy is fairly common and is not recognized at all unless it reaches a dangerous stage and a physician is called.

It is interesting to note that scurvy generally appears in the infant between the ages of six
to nine months. **Rohmer** has shown that vitamin C is excreted in the urine of an infant during the first six months of life but not after that period. This fact would seem to indicate that the infant is born with a supply of vitamin C which is gradually used up until at the end of six months it no longer has an excess of the vitamin, so that unless the supply is replenished from some outside source, scurvy is imminent.

to mine ore from the ground.
Symptoms of Scurvy.

The disease develops slowly. Commonly the gums become swollen and bleed easily. Mouth infections result and at times the teeth become so loose as to fall out. The skin becomes pale. Large blue-black spots may appear following injury or without apparent reason. Sometimes there are regions of very hard, stiff skin and subcutaneous tissue. The patient becomes weak, loses weight, develops anemia, swollen ankles, and hemorrhages of the mucous membranes.

As the disease progresses nervous symptoms appear including headache and delirium. Convulsions appear in the final stages. In the case of the very young infant, fretfulness, loss of weight and fever may be the only symptoms. Very little is said in the early literature about scurvy in infants. The reason for this is probably that the infant fed on a diet other than mother's milk died before it had a chance to develop scurvy. It was not until 1894 that Barlow described a combination of scurvy and rickets. This paper is one of the early descriptions of scurvy in infants.

Symptoms of Gonorrhea

The disease develops slowly. Commonly, the same
becomes watery and slightly-yellowish. Intense irritation
may at times be felt. Foul-smelling, grayish-white or-
the skin becomes hot. Take any antibiotic except
beer. Following infection with one apparent reason: Some-
time before the lesion of very pain, within skin and
appearance is serious. The bacterium becomes more.
Some weight, headache, nausea, vomiting in nausea, and
remittent.

As the disease progresses various symptoms begin
in the initial infection. In the case of the very young
intense irritation, loss of sleep, and fever may be
the only symptom. Very little is said in the study
intercourse without causing in infants. The reason for the
interruption of sexual contact was repair. The disease may
be properly treated for the infection by any other form
medication. If we cannot achieve these specific symptoms.

It is necessary to assess the symptoms and make an
one of the early symptoms of syphilis in infants.

J. Hatton, Jr., Thomas, John, 1839, M.D.
Pathology of Scurvy.

At autopsy the subcutaneous tissue is found to be infiltrated with a bloody fluid. There are hemorrhages in the periosteum including such bones as the jaw and tibia. Very rarely are there hemorrhages in the muscles themselves. "Beading" or "rosary" of the ribs is often observed which is something like that occurring in rickets. There are bone fractures, disappearance of the osteoblasts and in the case of infants there is degeneration of the bone marrow resulting in improper bone formation. Accumulation of fat is often found resulting in fatty degeneration of the heart muscles. There are also specific changes of tooth structure. These changes will be dealt with in some detail later in this paper.
Perception of society.

At any point the experimenter might be forced to
be initiated with a thought that there are
resemblances in the perception involving some
use of any titles. Very likely the same perception
in the specimen percept this "search of "laundry" in
the type to offer a view which in some of the
view of occurrence in circles. There are none common.
interference there is generalization of the normal late.
start in immediate and perception of each
case of the heart measure. There are also specific cases
of each experiment. These chances will be dealt with
in some detail later in this paper.
Early attempts at the isolation of vitamin C.

One of the first attempts to isolate vitamin C was made by Agopian in 1921. This was done by precipitating the substance from fruit juices with heavy metals. This substance contained nitrogen but in 1925 Bezsssonoff isolated a substance which contained no nitrogen. The non-nitrogen containing substance protected guinea pigs from scurvy when given in doses of 2 mg. daily. Bezsssonoff was not sure of the analysis of this compound but thought it to be an ortho diphenol derivative or a glucoside. In the light of later work it appears that he may have been working with a solution containing fairly pure vitamin C.

The rather important work of Szent-Gyorgyi should be mentioned. He examined various oxidizing systems of plants and animals and finally the peroxidase system with the idea of determining the function of the interrenal system. As a result of these investigations he isolated a strongly reducing substance from

of the first attempts to isolate vitamin C

One of the first attempts to isolate vitamin C was made by Holman in 1931. The same group of scientists at the University of Wisconsin conducted further research on this vitamin. The experiments continued until in 1933 the scientists succeeded in isolating a substance containing vitamin C. This work was of great consequence as far as the synthetic manufacture of vitamin C is concerned. Further work is necessary that can have been made with artificial substances containing ascorbic acid. 

The latest information made of synthetic substances

I am writing to say that I am very pleased to receive the information you have sent me. It is very important to me to learn all I can about the latest advances in this field. I would like to express my appreciation for the information you have provided. It is very valuable to me. 

If you have any further questions or need additional information, please do not hesitate to contact me. I would be more than happy to assist you in any way I can.

Thank you again for your help.

Sincerely,

[Signature]
the adrenal cortex which he called hexuronic acid because he thought it was similar to glycuronic acid. This same substance he also isolated from oranges and cabbages, in small amounts although he was unable to isolate it on a large scale. He concluded the function of hexuronic acid to be that of a catalytic hydrogen carrier between the peroxidase and other oxidizing, or reducing systems in plant and animal life. The formula of hexuronic acid was found to be $C_9H_{14}O_7$. Szent-Gyorgyi isolated hexuronic acid from orange juice by precipitation with lead acetate from a solution containing the juice with ammonia and sodium cyanide. The precipitate is decomposed with sulphuric acid, reprecipitated with hot lead acetate from alcohol solution. Sulphuric acid is again added and fluid reduced in vacuo. Methyl alcohol is used to dissolve the crude product and ether added to precipitate the vitamin. This last process is repeated several times.

Unfortunately Rygh was much opposed to the idea that hexuronic acid had any strong connection with vitamin C. He said that the active constituent of vitamin C was independent of any reducing substance.

The scientific notation is called exponents and powers.

Let's some examples be the following:

1. $10^3 = 1000$
2. $10^{-2} = 0.01$
3. $10^{1.5} = 31.62$

Scientific notation is used to express very large or very small numbers.

For example, the distance between the Earth and the Sun is approximately 93,000,000 miles, which can be written as $9.3 	imes 10^7$ miles.

The advantage of using scientific notation is that it makes it easier to read and compare numbers.

For example, the speed of light is approximately $3 	imes 10^8$ meters per second.

In summary, scientific notation is a useful tool for expressing very large or very small numbers.
and that the hexuronic acid must be present therefore only as an impurity. Apparently this discouraged the idea of hexuronic acid being identical with vitamin C, because it was not until Tillmans, Hirsch and Dick in 1932 again pointed out the possible identity of the two substances in question that Szent-Gyorgyi further examined hexuronic acid by feeding it to guinea pigs. He found that it protected the animals from scurvy and that it cured induced scurvy, even if vitamin C containing substances other than hexuronic acid were withheld from the diet. Derivatives of hexuronic acid were made, then reverted back to the original substance and again tested with guinea pigs. In each case the substance was found to be ascorbic acid, and these facts precluded any possibility of vitamin C being present with hexuronic acid as an impurity. As a result of this work, Szent-Gyorgyi changed the name from hexuronic acid to ascorbic acid, and this latter name is now universally used.

1. Tillmans, Hirsch, and Dick, Biochem. Jour., 1933, 27, 581...
any fact that the transportation does not plan to place the
function only as an exposure. A previous type of
exposure, therefore, appeared to be of interest

the use of paradoxes and path integrals with

a function of the two variables in a certain field of

which function is examined a function only by testing it

in some other way. The only thing that is known to

from the fact that it makes the function have a

of a dominating substance in place than presence

To be able to use the substance we know to an accuracy of

To say, any space tends to become an possibility of

attain a certain degree with paradoxes only as an for

bulldozer as a means of this work. Some other that may

be the most from paradoxes both to express or ever

the letter name to you immediately enough

,, 935
\textbf{L. T. I. Closing}, \\
\textit{Vol. 98, Issue 8, 1953}, \\
\textit{Page 5, 1953}
At about this time Rygh attempted to make a systematic study of orange juice concentrate in order to determine once and for all the nature of the active principle. He finally isolated a nitrogen containing substance which he called "nor-narcotine" and which he believed to be the real vitamin C. The formula of his vitamin is indicated below.

\[
\text{CH}_2\text{O}
\]

\[
\text{CH}_2\text{N}\text{CH}_3
\]

\[
\text{CH}_3\text{O}
\]

\[
\text{CH}_3\text{OH}
\]

\[
\text{OH}
\]

I. Rygh. Z. physiol. chem. 1932, 204, 105-11

3. Rygh, W. J., Z. physiol. chem., 1932, 204, 105-II
In the family of the skeletal structures of natural products, the molecules often contain a high degree of complexity, which is reflected in their chemical properties and functions.

The structure of the molecule shown is a typical example of a natural product with multiple functional groups and stereochemical features. The presence of these groups can lead to a variety of biological activities, making these molecules valuable for both research and development purposes.

[Chemical structure diagram]
During the past two years about twenty different investigators (some of whom are listed below) have attempted to repeat the work of RYGH. The bulk of this work has been on the "alleged" anti-scorbutic activity of methylornarcotine, narcotine, and its derivatives and mixtures of these substances. The conclusions that most of these workers reach is that vitamin C has no nitrogen, and that methylornarcotine has no anti-scorbutic powers. On the other hand, the substance discovered by Szent-Gyorgyi has been investigated thoroughly, and the majority of workers confirmed his results. Waugh and King in 1932 isolated and identified vitamin C, finding it to be identical with the hexuronic acid of Szent-Gyorgyi. Hirst and ZILVA reach the same conclusion. Almost immediately the world of science recognized the work of Szent-Gyorgyi

5. Smith and Zilva, Chem. and Ind., 1932, 51, 166.
Information (else of how the Initials ... love it in the work at the “allege” filled-up with the activity of materialization and the verification of mixture of these elementary. The combination and work of these mixture to the activity of seeing on-traction and ... materialization

The interesting of the content of the particulate how it been important of science reporting the make of anti-counterpart...
to be fundamental. To him is given the credit of discovering the long sought after vitamin so that now vitamin C is known universally as ascorbic acid.

The color which is characteristic of ascorbic acid.

Cyclization of vitamin C with \( \text{H}_2\text{O} \) at room temperature produces \( \text{H}_2\text{C}_6\text{O}_7 \), showing that there is a CO group adjacent to the COOH group. Oxidation with \( \text{NaIO}_4 \) or in glacial \( \text{AcOH} \) did not produce \( \text{C}_6\text{H}_8\text{O}_7 \) and hence there are not two free alcoholic groups at the ends of the chain.

Ascorbic acid has been prepared (Verhoffs) by action of 6 eq. of pyridine in which was dissolved 1 g. of ascorbic acid and 1/4 g. of \( \text{NH}_2\text{OH}. \) Vitamin C contains a primary group, is not so stable, and has the CO group as a ketone. According to Keutmann and others, ascorbic acid exists in two forms as follows:

\[ \text{CH}_2\text{CO}_2\text{-CH}\text{CHOH}-\text{CH}_2\text{CO}_2\text{H} \]

\[ \text{CH}_2\text{CO}_2\text{-CH\text{CHOH}-CH}_2\text{CO}_2\text{H} \]

To the Preface...
Structure of Ascorbic Acid.

Vitamin C reacted with FeCl₃ produces the typical blue color which is characteristic of enols.

Oxidation of vitamin C with H₂O₂ at room temperature produces H₂C₂O₄ showing that there is a CO group adjacent to the COOH group. Oxidation with Pb(OAc)₄ in glacial AcOH did not produce CH₂O and hence there are not two free alcohol groups at the ends of the chain.

Tritylascorbic acid has been prepared (Vargha) by action of 5 cc. of pyridine in which was dissolved 1 g. of ascorbic acid and 1/4 g. of Ph₂C Cl. Vitamin C contains a primary group, is not an aldehyde, and has the CO group as a ketone. According to Haworth and others, ascorbic acid exists in two forms as follows:

1. CH₂OH-CHOH O-CO
   \[ C-\text{CO-CHOH} \]

2. CH₂OH-CHOH O-CO
   \[ C-\text{COOH} \]

Structure of Acetone with D.C. Spectroscopic Data

The infrared absorption of acetone at 9.81 μm in the 3000 cm⁻¹ region was measured and compared with the spectra of other ketones. The spectra were recorded using a Perkin-Elmer 21 spectrophotometer. The following structure was observed:

\[
\begin{align*}
\text{CH}_3 - \text{CO} - \text{O} - \text{CH}_3 \\
\text{H} & \quad \text{H} & \quad \text{H} & \quad \text{H}
\end{align*}
\]
The second form is the enol form which can be oxidized to the following product.

\[
\begin{align*}
CH_2OH-CHOH - & C \\
& \text{(enol form)} \\
& \text{CO - CO}
\end{align*}
\]

The ease with which ascorbic acid is oxidized is one of the outstanding properties of this substance. In fact, according to Szent-Györgyi the reducing powers of ascorbic acid will be "sans seconde parmi les substances composées seulement de C, H, et O." It will reduce silver nitrate in acid neutral or alkaline solution in the dark. Furthermore, it reduces Fehlings solution, potassium permanganate, and 2-6 dichlorophenol-indophenol.

P. Karrer, E. Schopp, and F. Zehnder reacted Me MgI on the lactone formula of acetonatedimethyl-ascorbic acid which resulted in the introduction

The second form of the partial equation can be expressed as:

\[
\begin{align*}
\text{CH}_2\text{CHOH-CH}_2\text{OH} & \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

The case with which extractions could be obtained is one of the controversies discussed in the text. In light of the evidence presented by Dr. Smith, the suggestion that these phenomena are due to some lesser degree of surface tension (as compared to taurocholic acid) will be "naive". However, the significance of these phenomena will require further investigation in the future.

"Interference with Taurocholic's solubility" seems to require further discussion. In the presence of sodium and other hydrophilic substances, the matter of solubility and the character of the solution as a whole, may be an important feature in the investigation of such phenomena."


of two Me and 2 OH groups. This evidence is against the furane formula which would take on one Me and one OH group.
Synthesis of Ascorbic Acid.

Ascorbic acid has been synthesized recently by Haworth, Hirst and others. In general their method was to react xylosone with potassium cyanide in a water solution of calcium chloride. Nitrogen was passed through the solution resulting in the addition of HCN and the evolution of ammonia.

The calcium was precipitated with oxalate, the resulting syrup concentrated under CO₂ and extracted with ethyl alcohol.

The \( \beta \)-keto-nitrile above hydrolizes almost immediately to the \( \beta \)-keto-acid.

This $\beta$-keto acid is referred to as $\psi$-ascorbic acid since it is not true ascorbic acid. This $\psi$-ascorbic acid was treated with eight per cent hydrochloric acid twenty-six hours under carbon dioxide, acid nearly all removed, the syrup concentrated and extracted several times. After several concentrations, ether was added, producing turbidity. The final solution when concentrated gave a colorless crystalline mass of ascorbic acid.

This synthetic product has been tested biologically (guinea pig method) by Haworth, Hirst and Zilva and found to have the same degree of

\[ 
\begin{align*}
\text{H}_2\text{CO}_3 + \text{H}_2\text{O} & \rightarrow \text{H}_3\text{O}^+ + \text{CO}_3^{2-} \\
\text{CO}_3^{2-} + \text{H}_2\text{O} & \rightarrow \text{H}_2\text{CO}_3 + \text{OH}^- \\
\text{H}_2\text{O} & \rightarrow \text{H}^+ + \text{OH}^- \\
\end{align*}
\]
anti-scorbutic potency as the natural product. This seems to be very strong evidence in favor of the following:

(1) the synthesis of ascorbic acid

(2) the formula resulting from this synthesis as shown above.

Equation for the reaction of \( \psi \)-ascorbic acid to vitamin C (ascorbic acid).

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{CH(OH)} \cdot \text{CH} \cdot \text{OH} \\
\text{C} = \text{C} \quad \text{COOH} & \quad \text{OH} \quad \text{OH} \\
\text{CH}_2\text{OH} & \quad \text{CH(OH)} \cdot \text{CH} \quad \text{CO} \\
\text{C} = \text{C} & \quad \text{HO} \quad \text{OH}
\end{align*}
\]

Vitamin C (ascorbic acid).
ol-p"Jrpuril participation or the control identity of the enzyme to be very similar situation to that of the following

(1) the appearance of saccharides with
(2) the formation resulting from the substrate as shown above.

Diagram for the reaction of X: saccharide with

\[ \text{X} + \text{SACCHARIDE} \rightarrow \text{PRODUCT} \]
Synthesis of d- and l- ascorbic acid by T. Reichstein, and others.

d-Xylosone was prepared from d-Xylose. This in turn was treated with HCN and then hydrolyzed with HCl. The vitamin was precipitated as the lead salt. This compound was recrystallized until the melting point reached 185-9°C and \( [\alpha]_D^{27} = -80^\circ \). In a similar manner l-ascorbic acid was prepared from l-Xylose producing a substance with a melting point of 187-9°C.

A mixture of the d- and l- forms prevented scurvy but the d-form had only about one fourth the power.

I. Reichstein, T., Grussner, A., and Oppenauer, R.

Sympathetic, if I may I would like you to imagine

If you could imagine a world where all my predictions come true.

I-Xylene was prepared from 2-Xyloes.

This was in turn tested with HCN and then purified with the

Where else? The victim was described as the

Deaf ear. The something was testifying with.

the melting point ranging 158-160°C and a

in a similar manner I prepared a solid mass prepared from

I-Xylene prepared a substance with a melting point

of 187-188°C.

I mixtures of 4- and I-toluene by

Forming part the 4-toluene, only present one fourth

the power.

...
Isomers and homologues of ascorbic acid.

Several isomers and homologues of ascorbic acid have been prepared by Reichstein and studied by DeMole. The lactone formulas of d-Erythro-3-ketohexonic acid and l-Erythro-3-ketohexonic acid have been fairly well established by them.

\[
\text{d-Erythro-3-ketohexonic acid.}
\]

\[
\begin{align*}
\text{HO} & - \text{C} - \text{C} - \text{C} - \text{C} - \text{C} - \text{C} = 0 \\
\text{H} & \text{OH} \quad \text{O} \quad \text{OH}
\end{align*}
\]

\[
\text{l-Erythro-3-ketohexonic acid.}
\]

\[
\begin{align*}
\text{HO} & - \text{C} - \text{C} - \text{C} - \text{C} - \text{C} - \text{C} = 0 \\
\text{H} & \text{H} \quad \text{H} \quad \text{H}
\end{align*}
\]

d-Erythro-3-ketohexonic acid was found to have a slight ascorbic action but l-erythro-3-ketohexonic acid had none.

I. Reichstein and Grossner, 1934.

Several successful attempts by researchers at the Chemical Research Laboratories of the National Institute of Health have been reported for the synthesis of D-ribo-5-ketopentonic acid and L-ribo-5-ketopentonic acid.

D-ribo-5-ketopentonic acid

\[
\begin{align*}
  &\begin{array}{c}
  H \\
  O = C - O - C - O - C - O - H \\
  H \\
\end{array} \\
  &\begin{array}{c}
  \text{HO} \\
  \text{HO} \\
  \text{HO} \\
\end{array}
\end{align*}
\]

L-ribo-5-ketopentonic acid

\[
\begin{align*}
  &\begin{array}{c}
  H \\
  O = C - O - C - O - C - O - H \\
  H \\
\end{array} \\
  &\begin{array}{c}
  \text{HO} \\
  \text{HO} \\
  \text{HO} \\
\end{array}
\end{align*}
\]

The stereochemical assignments for D-ribo-5-ketopentonic acid have a slight stereochemical preference for 1-ribo-5-ketopentonic acid over 2-ribo-5-ketopentonic acid.
The following compounds had no ascorbic properties at least in amounts up to twenty mg. per day.

d-Lyxo-3-ketoheptonic acid.

\[
\begin{align*}
\text{H} & \quad \text{H} & \quad \text{OH} & \quad \text{OH} & \quad \text{O} & \quad \text{H} & \quad \text{O} \\
\text{HO} & \quad \text{C} & \quad \text{C} & \quad \text{C} & \quad \text{C} & \quad \text{C} & \quad \text{C} & \quad \text{OH} \\
\text{H} & \quad \text{OH} & \quad \text{H} & \quad \text{H} & & & & \quad \text{OH}
\end{align*}
\]

1-Xylo-3-ketoheptonic acid.

\[
\begin{align*}
\text{H} & \quad \text{OH} & \quad \text{H} & \quad \text{OH} & \quad \text{O} & \quad \text{H} & \quad \text{O} \\
\text{HO} & \quad \text{C} & \quad \text{C} & \quad \text{C} & \quad \text{C} & \quad \text{C} & \quad \text{C} & \quad \text{OH} \\
\text{H} & \quad \text{H} & \quad \text{OH} & \quad \text{H} & & & & \quad \text{OH}
\end{align*}
\]


The following compounds may on escopo but
perform at least in amounts up to twenty
per.

4-Furo-8-keopenitone scale.

\[ \begin{align*}
&\text{O} & & \text{H} & & \text{O} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{H} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{O} & & \text{H} & & \text{O} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{O} & & \text{H} & & \text{O} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{O} & & \text{H} & & \text{O} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{H} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
\end{align*} \]

5-Furo-9-keopenitone scale.

\[ \begin{align*}
&\text{O} & & \text{H} & & \text{O} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{H} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{O} & & \text{H} & & \text{O} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{O} & & \text{H} & & \text{O} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{O} & & \text{H} & & \text{O} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
&\text{H} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} & & \text{H} & & \text{O} \\
\end{align*} \]
Recent developments in the occurrence of vitamin C and its use in the treatment of scurvy.

In recent years vitamin C has been found in foods and organs little suspected as a source previously. Gough and Zilva, Bessey and King, and others listed below have investigated the distribution of vitamin C in various animals and man.

While it is fairly widespread, it occurs in high concentration in the adrenals and pituitary. Citrous fruits, of course, have long been recognized as a potent source of the vitamin and recent analysis confirms this. Other fruits and vegetables, however, have been found to contain vitamin C in fairly high concentration.

Recent developments in the treatment of allergy

In recent years allergy and the treatment of allergic reactions have become a more widespread concern. Several factors, including increased awareness and the availability of new treatments, have contributed to the rise in the popularity of allergy treatment methods. Among these methods, immunotherapy has proven effective in controlling allergic reactions in many cases. While it is widely accepted, its exact mechanism of action is still under investigation.
Peppers and iris leaves seem to represent perhaps, the most concentrated sources of the vitamin.

1-3

Other new sources are the black currant and raspberry, the grape, tea, tomatoes, and various fodder plants. The vitamin has been isolated by several investigators and synthesized by Ault and Reichstein.

The text on the image is not legible due to the quality of the scan or the nature of the handwriting. It appears to be a page with text, possibly a legal or official document, but the content cannot be accurately transcribed.
Gothlin and Falk have determined the vitamin C requirements of man by the use of a skin capillary resistance method. Their results indicate that 0.7-1.0 c.c. of orange juice daily per kilogram of body weight is sufficient to prevent the onset of scurvy.

Dann, working with guinea pigs found that the vitamin C requirement is directly proportional to the body weight and is one c.c. of lemon juice per 100 gm. weight.

Goettsch's and Eddy's results are partially confirmed by these figures but Gothlin found that the growing child required an amount of vitamin C greater in proportion to his body weight than did the adult.

I...

Coaching and staff have determined the athlete's

inability to perform at a high level due to a skill deficiency

in coordination and balance. The athletes involved must

be carefully monitored to prevent the onset of serious

injury. The coaching staff has been notified of the

situation and will be monitoring the athlete closely for

any signs of improvement or deterioration.

A complete medical report is to be submitted to

the coaching staff. Any necessary adjustments to the

athlete's training program will be made.

In light of this situation, the coaching staff has decided to

suspend the athlete's participation in all varsity activities until

further notice. Any questions or concerns should be directed

to the athletic director.
The use of substances containing vitamin C such as citrous fruits has been mentioned before in this paper. Pure vitamin C is now being used in hospitals in different parts of the world.

1. Brugsch reports the cure of a case of infantile scurvy by the administration of 0.03 grams of
2. ascorbic acid per day. Wright describes cases of
3. adult scurvy cured by 40 mg. per day. Bauke and
4. Schultzer have injected the vitamin intravenously
5. (40-50 mg.) resulting in rapid recoveries. In some cases the temperature dropped on the second day of
6. treatment. Kramar gave 20-25 mg. of ascorbic acid
daily to newborn infants and found development to be accelerated. Premature infants were able to
tolerate 10-15 mg. per day. A case of Moeller-
7. Barlow's disease treated by Neumann recovered completely in three weeks by the administration of

4. 10, 1934.
The new test equipment for some of the more important types has been manufactured.

In this report, the principle of operation is developed in detail, followed by a description of the experimental equipment.
0.02 g. per day. Mettier, Minot and Townsend have found that vitamin C has a specific effect in the bone marrow of a patient with scurvy. Also interesting results of improvement in tuberculosis cases by administration of vitamin C have been reported by Robertson. Hemorrhagic diatheses have been treated with ascorbic acid by Böger. It is believed that possibly the permeability of the capillary walls may be altered by the vitamin. The coagulation time of the blood is also decreased enormously and the plasma albumin increased.

It would seem from the number of cases already reported in so short a time that the treatment of scurvy and some other diseases with pure ascorbic acid is rapidly coming into use.

2. Robertson, E.C., Medicine, 1934, 13, 123-206.
In the experimental part the idea of the author
was to investigate five various tests and methods for
the analysis of vitamin C which are found in the literature
with the view of determining the one most effective.
Special attention was to be given pure chemical
tests as compared to the so-called "biological tests.”
From these tests was based on the investigations
on the rat. During the course of these investigations
it was also thought that improvement of known methods
might be discovered since the field is, from a stand-
point of pure chemistry, comparatively new. One of the
first substances to be investigated was the well known
methylamine base. Kolbe and Welti have studied the re-
solution of mephalene base by means containing vitamin
C. A little later Martini and Bonalietta developed
this test into a quantitative method for the determi-
nation of vitamin C in tissues and yeast juices. After
experimenting with various methods the author found
the following to be good:
Vol., 1880, 111. 1.
Vol., 1898, 9, 350-9
In the experimental part the idea of the author was to investigate first various tests and methods for the analysis of vitamin C which are found in the literature with the view of determining the ones most effective. Especial attention was to be given pure chemical tests as compared to the so called "biological assay." Then these tests were to be used in the investigations on the rat. During the course of these investigations it was also thought that improvement of known methods might be discovered since the field is, from a standpoint of pure chemistry, comparatively new. One of the first substances to be investigated was the well known methylene blue. Euler and Malin have studied the reduction of methylene blue by organs containing vitamin C. A little later Martini and Bonsignore developed this test into a quantitative method for the determination of vitamin C in tissues and fruit juices. After experimenting with various methods the author found the following to be good:

In the experimental part of the paper, we see to understand the nature and behavior of the molecules of the material to which are exposed to the influence. For the view of determining the above most interest.

The experimental situation was to be given prior occupation. "Physiological necessity" seems to be a concept to be made in the investigation. Then there were ways to be made in the investigation. We also thought that the idea to form a state of mind may be conservative since the idea to form a state of mind may be conservative. A point of the experimental was the new known.

"That we believe that we have arrived at the-"...
1. Method of Martini and Bonsignore.


The method of Martini and Bonsignore was used by the author in the analysis of fruits and tissues. The principle of the method as outlined by these investigators depends on the utilization of the reducing property of ascorbic acid. Apparently, one mole of methylene blue is reduced by one mole of ascorbic acid in the presence of sodium bicarbonate, sodium citrate, and sodium thiosulphate. Under these conditions methylene blue reduces with difficulty and this fact is an advantage in the analysis of vitamin C, because it eliminates the possibility of reduction of the dye by other substances. According to Martini and Bonsignore, reducing substances such as glutathione and cysteine have no effect on the dye under the conditions of the test. The author has found that glucose, sucrose, lactose, tannic acid, pyrogallol, and cysteine do not affect the dye under the conditions of the test enough to produce an appreciable error.

The author also modified the methylene blue method somewhat.

The method of reducing any probability was kept by the section in the method of trials and formulae. The principles of the method as outlined by these investigators are based on the differentiation of the learning process in an attempt to eliminate such factors of learning and memory. The sections under these conditions would extend the learning plane to a point whereby one minute of learning could be the measure of learning in which the difficulty may rise from the standpoint of the memory and memory processes.

The difficulty may arise from the standpoint of the memory and memory processes, extending and extending on the effect on the learning plane.

The conclusion of the book. The method has shown that the sections, therefore, produce, produce, produce, produce, by production and occurrence, to not affect the end the conclusion of the method, extending and extending on the effect on the learning plane.

The section on reducing the learning plane when somewhat.

F. H. Winsor. "Mental and Psychical, A. April 1917.
The method of Martini differs from that of the author in several respects. Martini standardizes the methylene blue by comparison of ascorbic acid or lemon juice with a 0.005 M iodine solution while the author standardizes the methylene blue directly against pure ascorbic acid. The end point is determined by Martini by matching the unknown with a control. This control is made by mixing 0.2 c.c. of methylene blue (1:10,000) with 8 c.c. of water. The author found that a sharper end point could be reached if 0.3 c.c. of methylene blue were used instead of 0.2 c.c. Also instead of using water the reagents citrate, bicarbonate, thiosulphate and trichloracetic acid were used and in this way similar conditions were produced in the control to those in the unknown. Difficulty was experienced in matching the final colors. This is affected by the diameter of the tubes used, the color of the unknown and the source of light. To avoid these difficulties the author used a larger amount of methylene blue and compared the unknown with the standard with the colorimeter.

It was found by experiment that tissue extracts must be analyzed immediately in order to obtain consistent values. The following table shows titration of
liver extract on successive days.

Effect of the time element on the methylene blue reaction.

<table>
<thead>
<tr>
<th>Days</th>
<th>c.c. of methylene blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The above effects may be caused by the action of bacteria on the substances dissolved in the extract. At any rate it is obvious that the longer the extract is allowed to stand, the higher its titration with methylene blue becomes.

Solutions required:

1. Citrate bicarbonate solution made up so as to contain fifteen per cent of sodium citrate and four per cent sodium bicarbonate.
2. Sodium thiosulfate solution of five per cent.
3. Methylene blue solution 1:10,000.

Title: Effect of the Gene Element on the Melting Point Reactant

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Melting Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
</tr>
</tbody>
</table>

From the above table, we can see that the reaction rate increases with the higher the reaction temperature.
Method of analysis of fruit juices for vitamin C.

The method used was a modified method of Martini and is outlined as follows:

One c.c. of lemon juice was diluted to twenty-five c.c. with eight per cent trichloracetic acid. To five c.c. of this solution was added two c.c. of citrate bicarbonate solution and one c.c. of thiosulphate solution. Then methylene blue solution was added in 0.3 c.c. amounts until the color nearly approached the color of the standard after exposure to direct sunlight. Then the two solutions were checked with the colorimeter.

Solutions required.

1. Citrate bicarbonate solution made up so as to contain fifteen per cent of sodium citrate and four per cent sodium bicarbonate.

2. Sodium thiosulphate solution of five per cent.

3. Methylene blue solution 1:10,000.

Mixture of solutions of first jargon for

Activity.

I

The mixture was a molten mixture of twenty-one
and two parts as follows:

One part of iron tris was added to twenty-one
parts with slight heat until the mixture was
five parts of the solution was added two parts of

sulfuric acid solution. Then, the solution was

mixed with the solution.

It is observed that the solutions were used as the

containing fifty per cent of rubber and forty

per cent of rubber

and one part of the

sulfuric acid solution is 1:10,000.

I. Mixture of any benzenes at 380° C., 400° C.

Then, 140° C.
4. The standard is made by adding to 5 c.c. of trichloracetic acid 2 c.c. of citrate bicarbonate solution followed by 1 c.c. of thiosulphate solution and then 0.3 c.c. of methylene blue.

Calculations:

Methylene blue is titrated against pure crystalline vitamin C solution of known per cent, and the amount of methylene blue equivalent to a standard amount of vitamin C determined. The graph below shows the results of titration of methylene blue against pure vitamin C.

**Titration of methylene blue against crystalline Vitamin C.**

![Graph showing titration of methylene blue against vitamin C.](image)
Titration of 

methylamine 

against 

phenolphthalein 

Alcohol (g)
Analysis of fruits for the presence of vitamin C by a modified methylene blue method.

At the present time lemon juice is the international standard for vitamin C assay and as such is accepted by the Permanent Commission for Biological Standardization. Key and Morgan have analyzed lemons for the quantitative determination of vitamin C. Their results show an ascorbic acid value of 1.35 mg. per c.c. of juice. They suggest that this rather high value might be due to differences in the juice from different lemons. In order to investigate the possibility of the variation in the standard the following analyses were carried out on lemons from different and the same sources. Each sample was made by extracting the juice of several lemons, mixing thoroughly and then analyzing. The following table shows the results of these analyses.

The following table shows the receipt of these

<table>
<thead>
<tr>
<th>Year</th>
<th>Money</th>
</tr>
</thead>
<tbody>
<tr>
<td>1928</td>
<td>$1050</td>
</tr>
<tr>
<td>1929</td>
<td>$1350</td>
</tr>
</tbody>
</table>
Lemons.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Vitamin C content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>.50 mg/cc</td>
</tr>
<tr>
<td>2.</td>
<td>.45 &quot;</td>
</tr>
<tr>
<td>3.</td>
<td>.75 &quot;</td>
</tr>
<tr>
<td>4.</td>
<td>.60 &quot;</td>
</tr>
<tr>
<td>5.</td>
<td>.90 &quot;</td>
</tr>
<tr>
<td>6.</td>
<td>.80 &quot;</td>
</tr>
<tr>
<td>7.</td>
<td>.20 &quot;</td>
</tr>
<tr>
<td>8.</td>
<td>.05 &quot;</td>
</tr>
<tr>
<td>9.</td>
<td>.10 &quot;</td>
</tr>
<tr>
<td>10.</td>
<td>.55 &quot;</td>
</tr>
</tbody>
</table>

Samples number 8 and 9 seemed to be from lemons that had been stored for some time. They were rather soft and appeared to be over ripe. This may account for the low vitamin C content. On the other hand, no value was obtained which reached the value obtained by Key and Morgan. In fact most values of vitamin C in lemon juice in the literature do not seem to be nearly as great as those of Key and Morgan.

<table>
<thead>
<tr>
<th>Sample</th>
<th>10</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td></td>
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<tr>
<td>3</td>
<td></td>
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<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>.5</td>
<td></td>
</tr>
<tr>
<td>.3</td>
<td></td>
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<td>.2</td>
<td></td>
</tr>
<tr>
<td>.1</td>
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There are several possible explanations. The time of year, the source, the length of time of storage, and the variety of lemons, all may be important conditions which affect the amount of the vitamin present. The method of analysis also probably has an important bearing on the results. These same tests carried out by the biological method do not show small individual variations which the chemical methods do show. Key and Morgan did not use any chemical methods. Further, an error in the chemical method due to the presence of other reducing substances than vitamin C cannot have entered into these analyses, because that would result in higher, rather than lower values.

However, the conclusion to the work is the same as that drawn by Key and Morgan, namely that the vitamin C content of lemon juice may vary, Since there is considerable variation in the vitamin C content it seems an obvious objection to the continued use of lemon juice as an international standard. Birch, Harris and Ray as well as Key and Morgan believe that ascorbic acid is a better standard for vitamin C than lemon juice. The results of the author also

favor the adoption of pure ascorbic acid as the standard.

Cartier's experience in 1886 at Quebec with scurvy and pine needles seemed to infer the presence of vitamin C in the pine needles, although not much is known of other factors in the food which one might have contained vitamin C. In order to settle the point of vitamin C content, two kinds of needles, samples of northern white pine and hard pine, were tested. An extract was made by titrating with trichloroacetic acid and then titrating with methylene blue for vitamin C.

<table>
<thead>
<tr>
<th>White pine Extract</th>
<th>Methylene blue average</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 cc.</td>
<td>0.6 cc.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hard pine Extract</th>
<th>Methylene blue average</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 cc.</td>
<td>0.6 cc.</td>
</tr>
</tbody>
</table>

I. Cartier

Vitamin C in pine needles.

Cartier's experience in 1535 at Quebec with scurvy and pine needles seemed to infer the presence of vitamin C in the pine needles, although not much is known of other factors in the food which also might have contained vitamin C. In order to settle the point of vitamin C content, two kinds of needles, samples of northern white pine and hard pine, were tested. An extract was made by triturating with trichloracetic acid and then titrating with methylene blue for vitamin C.

White pine Extract Methylene blue average
5 c.c. 0.6 c.c.

Hard pine Extract Methylene blue average
5 c.c. 0.4 c.c.

I. Cartier

Attachment 0 to the report

I

Refer to expirations in I & II of report with

some one of these seems to have the

presence of Acetamin 0 in the urine, because

not much is known of this factor in this test. If

some have complained of Acetamin 0 in order to

the point of Acetamin 0 containing two kinds of urine,

some of our tests were made on this line and only one

tested by extract as were of fluctuation with this

contraceptive 099 and hear it stated with methylene

White line extract

Methylene blue extract

Red line extract

.0.0.0

.0.0.0

.0.0.0
From this experiment it appears that the cures of scurvy may have been due at least in part to the administration of the pine needle infusion. At any rate Cartier attributed the cure to the infusion and the above experiment shows the presence of vitamin C in an extract of the needles.

<table>
<thead>
<tr>
<th>Germinated seedlings</th>
<th>Ascorbic mg. per g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 hours</td>
<td>.00</td>
</tr>
<tr>
<td>24 hours</td>
<td>.10</td>
</tr>
<tr>
<td>48 hours</td>
<td>.15</td>
</tr>
<tr>
<td>72 hours</td>
<td>.40</td>
</tr>
<tr>
<td>110 hours</td>
<td>.55</td>
</tr>
<tr>
<td>154 hours</td>
<td>.55</td>
</tr>
<tr>
<td>10 days</td>
<td>.50</td>
</tr>
</tbody>
</table>

Sterile precautions were used in so far as was possible in order to prevent the destruction of the vitamin C content by bacteria and molds. Agar containing salts was used as a medium for growth. The results indicate that preservation is associated with increase of vitamin C content, the amount increasing
Now this experiment is supposed to be done
as soon as may have passed rae of least to best to
the determination of the time needed for each
of the above cases or appropriate the same to the
information by the space experiment above the press
since all arrive 0 in an extract of the merger.
Change of Vitamin C Content of Seeds With Germination.

For many years it has been a common belief that sprouted seeds would cure scurvy. Among peoples such as the Chinese who eat sprouted seeds scurvy seems scarce. The following experiments were conducted to test this belief by chemical and biological means. Been seeds were sprouted and tested at intervals for the vitamin C content.

<table>
<thead>
<tr>
<th>Germinated seedlings</th>
<th>Ascorbic mg. per g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hours</td>
<td>.00</td>
</tr>
<tr>
<td>24 hours</td>
<td>.10</td>
</tr>
<tr>
<td>48 hours</td>
<td>.20</td>
</tr>
<tr>
<td>72 hours</td>
<td>.40</td>
</tr>
<tr>
<td>110 hours</td>
<td>.55</td>
</tr>
<tr>
<td>134 hours</td>
<td>.55</td>
</tr>
<tr>
<td>10 days</td>
<td>.50</td>
</tr>
</tbody>
</table>

Sterile precautions were used in so far as was possible in order to prevent the destruction of the vitamin C content by bacteria and molds. Agar containing salts was used as a medium for growth. The results indicate that germination is associated with increase of vitamin C content, the amount increasing
The text is not legible due to the quality of the image. It appears to be a page from a document containing a table and text, possibly discussing a topic related to weather or climate, with columns and rows that are not clearly visible.

---

**Table**: Weather Conditions

<table>
<thead>
<tr>
<th>OC</th>
<th>OH</th>
<th>AS</th>
<th>AS</th>
<th>IA</th>
<th>TD</th>
<th>TD</th>
<th>GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hole</td>
<td>Hole</td>
<td>Hole</td>
<td>Hole</td>
<td>Hole</td>
<td>Hole</td>
<td>Hole</td>
<td>Hole</td>
</tr>
</tbody>
</table>

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The text seems to mention the conditions of certain locations, possibly for weather or climate studies, but the exact details are not clear due to the quality of the image.
the first few days and then remaining constant. Dry seeds when tested for vitamin C showed none, or at best, only a slight trace. These results were checked by feeding varying amounts of sprouted seeds to guinea pigs on an otherwise vitamin C free diet. Protection was obtained with one gram of full sprouted seedlings per day. Harris and Ray have also noted an increase of vitamin C during germination in the case of peas and oats.

Other Tests for Vitamin C.

According to Salvatori the Bezssonoff test with molybdophosphotungstic acid is not only a test for vitamin C but also other reducing substances such as hydroquinone, metol, and others. Furthermore phosphomolybdic acid also reacts with vitamin C and other reducing substances in a manner similar to the Bezssonoff reagent. However Mouriquand has used the Bezssonoff reaction in analyzing vitamin C in the urine of guinea pigs. Hahn finds the Bezssonoff reaction gives no indication of the vitamin content of fruits, fresh and canned vegetables, and orange juices when compared to results of biological tests. It is worth while noting that this reagent is really the well known "Folin reagent" applied to the test for vitamin C. When boiled with HCL, ascorbic acid gives off CO₂ and loses water. The resultant substance produces a pink color when reacted with aniline indicating

3. Hahn, F.V., and Wieben, M., Z. Untersuch. Lebensm., 1932, 63, 481-95
1. Certificate of Absence

Accident to 200000°F and other 2000°F and over temperatures not only
with water and water vapor, and also with other liquid and
steam, may be hydrogenated catalysts, may provide
appropriate bypasses where temperatures above 2000°F are
involved or any other temperatures encountered in
ommnent allergic to the presence of the presence of
inhalation and may require treatment as in a
warfarin allergy 0 may occur later in time in the
in time of contact where the
Hepatitis virus is prevented by the presence of the presence of
in time of contact give no indication
of the allergic condition of plant. Keep away from
allergens, may cause intense skin reactions to
sensitization of plants. Keep at 100°F to 80°F within
noting that this one is highly important. The table of "2000°F
Molarity table." Applies to the special cases of the
new policy with the second and third lines at 2000°F
and those wanted. The temperature of the presence of
is blank except when necessary with simple interconnection

2. 1955, 60, 36, 76, 198, 341, 208, 62, 389, 42, 39, 58, 32, 260
the presence of furfural. Phloroglucinal and orcinol tests may be employed for the furfural reaction. Of course the production of furfural and its subsequent analysis from ascorbic acid would not be a specific test for vitamin C since pentoses, pentosans, hexoses and hexosans also give the same reaction but it may be possible that these interfering substances could be removed and the test used as a method of quantitative determination of vitamin C.

Szent-Gyorgyi found that a one per cent solution of ascorbic acid made slightly alkaline and ferrous sulphate added would produce a dark violet color on standing in the air. This color disappears upon the addition of sodium thiosulphate solution and reappears on shaking in air. The color is due to a primary Fe$^{++}$ vitamin complex.

The indophenols and their use in determining vitamin C.

The oxidation reduction systems of the indophenols have been introduced and studied at length by Gibbs¹ and Cohen². Tillmans³ has recommended the use of dichlorophenolindophenol for the determination of vitamin C. Birch, Harris and Ray⁴ have a modified method of Tillmans' reduction test for vitamin C which is outlined as follows:

A measured volume of 2-6-dichlorophenolindophenol (0.01M) is placed in a tube and an extract of the substance to be tested is added until the disappearance of the red color of the dye. The ordinary substance used to extract vitamin C from plant or animal tissues is trichloracetic acid. This treatment dissolves out the vitamin well and precipitates the proteins. Some work has been done which indicates possible error in this method such as the interference of other reducing substances such as glutathione and cystine. Moreover it is difficult to be sure that all the vitamin present

in the tissues is removed by the extraction. Guha\textsuperscript{1} and Ahmad\textsuperscript{2} have shown that trichloracetic acid reacts slowly with 2,6-dichlorophenolindophenol and that the lower the concentration of acid the longer it takes for the reduction to take place. Boiling the fruits in order to extract the vitamin had no effect on the vitamin content except in the case of cabbage. Guha also found that any treatment with hydrogen sulphide to reduce any of the oxidized form of the vitamin may produce unknown changes.

In the 1933-34 Harvey Lectures Clark\textsuperscript{3} has discussed in an admirable manner the oxidation reduction systems including various indophenols as well as methylene blue. The general formula indicating the structure of the indophenols is as follows:

\[ \text{Diagram of indophenol structure] } 

In the reaction of benzyl alcohol with 1,4-benzodioxane, the addition of the nucleophile occurs as an intramolecular reaction. The reaction mechanism involves the formation of a carbocation intermediate, followed by nucleophilic attack. The structure of the product is as follows:

![Molecular Structure](image)
One of the typical dyestuffs representative of this class is indophenol blue.

These substances are closely related to diphenyl amine as can be seen from the comparison to its formula.

The general method for making the indophenols is to oxidize a mixture of a p-diamine and a phenol or by the reaction of nitrosodimethylaniline with a phenol. These two reactions are represented below.

1. Paraphenylenediamine with phenol.

\[ \text{H}_2\text{NC}_6\text{H}_4\text{NH}_2 + \text{C}_6\text{H}_5\text{OH} + \text{O}_2 \rightarrow \text{HN=C}_6\text{H}_4=\text{N-C}_6\text{H}_4\text{OH} + \text{H}_2\text{O} \]

Indophenol

2. Nitrosodimethylaniline with naphthol
The image contains a chemical equation and a diagram. The equation is:

\[ \text{reaction} \]

The diagram appears to be a molecular structure, possibly related to the reaction shown in the equation.
Indophenol can be written as follows:

\[ \text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{N}^\equiv-\text{C}_6\text{H}_4\equiv \text{H}_2\text{N} \]

Svirbely\(^1\) has used dibromophenolphindophenol as an indicator in the determination of vitamin C. One of the common substances used by Bessey\(^2\) and others for this purpose is 2,6-dichlorophenolphindophenol. This dye is prepared as follows:

2.25 g. of phenol are added to 5 g. of powdered 2,6-dichloroquinonechloroimid with stirring. The solution is maintained at 5 degrees centigrade by surrounding the contents with ice outside the container. Then 18 c.c. of 3N sodium hydroxide are added, stirred 30 minutes, followed by the addition of 150 c.c. of 15% sodium chloride solution. This last addition of salt precipitates the dye which is filtered off on a Buchner funnel. The dye is then redissolved in two liters of warm water and salted out again by the slow addition of salt. The product is again filtered, dried and then extracted with ether to remove the last traces of impurities.

The synthesis of 2,5-dichloroaniline is achieved as follows:

$$\text{C}_6\text{H}_4\text{Cl}_2\text{NH}_2$$

The synthesis involves the reaction of aniline with 2,5-dichloroacetic acid.

1. Dissolve 2,5-dichloroacetic acid in water.
2. Slowly add aniline to the solution while stirring.
3. The reaction mixture is then heated to 100°C to ensure complete reaction.
4. The resulting product is then purified by crystallization.

The product is then further purified to ensure optimal quality.
The use of the indophenols in the determining of the amount of vitamin C present in various substances is dependant on its oxidizing power. 2,6-dichlorophenolindophenol is represented as an oxidant by the formula

$$2,6\text{-dichlorophenolindophenol}$$

The following formula is the same substance as a reductant:

Reduced 2,6-dichlorophenolindophenol

Ascorbic acid has two available hydrogens so that when it reacts with 2,6-dichlorophenolindophenol it gives up these two hydrogens and in that way reduces the dye, as shown by the equations below:
The use of the inductive effect in the polarization of the chemical bonds in an aromatic compound is important in understanding the behavior of the molecule. The following formula is an example of the inductive effect as it pertains to the formation of 2,4-dichlorophenol:

\[
\begin{align*}
&\text{CH}_2-\text{Cl} \\
&\text{CH}_2-\text{Cl}
\end{align*}
\]

The inductive effect in aromatic compounds is a key concept in organic chemistry, as it influences the reactivity and stability of the molecule. For instance, in the case of 2,4-dichlorophenol, the chlorine atoms can withdraw electrons from the aromatic ring, making it more electron-deficient and less reactive than a corresponding unsubstituted aromatic compound.
Ascorbic acid  

Sodium salt (blue)

\[
\begin{align*}
\text{Ascorbic acid} & \quad \text{Sodium salt (blue)} \\
\text{H} & \quad \text{Cl} \\
\text{H-C-OH} & \quad \text{N} \\
\text{H-C-OH} & \quad \text{ONa} \\
\text{H-C} & \quad \text{Cl} \\
\text{HO-C} & \quad \text{Cl} \\
\text{HO-C} & \quad \text{N} \\
\text{O=C} & \quad \text{ONa} \\
\text{O=C} & \quad \text{O=C} \\
\text{O=C} & \quad \text{O=C}
\end{align*}
\]

The use of sodium N-Naphthylleucochrome X. Methylleucochrome as a reagent for the quantitative determination of ascorbic acid.

After experimenting with various responses for estimating vitamin C in the author used the compound 2,6-dichlorobenzidine-N-methylleucochrome with good results. The method is briefly as follows:

The eye solution is dissolved in successive portions of warm water, filtered, and kept cool. Under these conditions the eye solution will keep well. In order to determine the concentration of the eye solution it is necessary to prepare a blank juice by adding sodium N-methylleucochrome solution containing 1.6 g. of Na per liter until a blue color is permanent with ascorbic acid. The end point may be approached and then kept titrated with sodium thiosulfate solution. The equivalent of the juice can be determined in terms of ascorbic acid.

From the fact that two moles of iodine react with one mole of the salt according to the following
The Use of Sodium 2, 6-Dichlorobenzene-oneindo -3- Methylphenol as a Reagent for the Quantitative Determination of Vitamin C.

After experimenting with various reagents for determining vitamin C the author used the compound sodium 2,6-dichlorobenzenoneindo -3- methylphenol with good results. The method is briefly as follows:

The dye can be dissolved in successive portions of warm water, filtered, stoppered in a glass bottle and kept cool. Under these conditions the dye keeps fairly well. In order to determine the concentration of the dye solution it is titrated against fresh lemon juice by adding measured amounts of the dye to the juice until a permanent pink results. Then the lemon juice is titrated with 0.01 N iodine solution containing 15 g. of KI per liter until a blue color is permanent with starch. The end point may be exceeded and then back titrated with sodium thiosulphate solution. The equivalent of the lemon juice can be determined in terms of ascorbic acid from the fact that two atoms of iodine react with one molecule of the acid according to the following
The Use of Sodium 3,5-Diphenylpyrazole

Conclusions and Recommendations

After examining with various techniques for the quantitative determination of vitamin C:

- Sodium 3,5-Diphenylpyrazole has been found to be a sensitive and specific reagent for the determination of vitamin C.

The method involves the formation of a colored complex with vitamin C in aqueous solution. This complex is then measured spectrophotometrically.

Under these conditions, the method shows a good linearity and reproducibility. It is, therefore, recommended for the quantitative determination of vitamin C in biological samples.
equation:

\[
\text{C}_6\text{H}_8\text{O}_6 + 2 \text{I} \rightarrow 2\text{H}_2\text{I} + \text{C}_6\text{H}_6\text{O}_6
\]

Hence one c.c. of 0.01 N iodine solution is equivalent to 0.89 mg. of ascorbic acid. Knowing the concentration of the lemon juice in ascorbic acid the value of the dye can be calculated easily.

In order to check the above results the author also determined the value of the dye by titrating it against pure crystalline ascorbic acid. The results checked well with the combination of dye, lemon juice and iodine solution as shown by the following tables.

Ascorbic acid in lemon juice determined as mg. per c.c.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Determined by dye</th>
<th>Determined by iodine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.62</td>
<td>0.63</td>
</tr>
<tr>
<td>2.</td>
<td>0.62</td>
<td>0.64</td>
</tr>
<tr>
<td>3.</td>
<td>0.61</td>
<td>0.63</td>
</tr>
<tr>
<td>4.</td>
<td>0.62</td>
<td>0.62</td>
</tr>
<tr>
<td>5.</td>
<td>0.61</td>
<td>0.60</td>
</tr>
<tr>
<td>6.</td>
<td>0.62</td>
<td>0.65</td>
</tr>
<tr>
<td>Sample</td>
<td>Percentage of Sample</td>
<td>Percentage of Total</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>25.0</td>
<td>25.0</td>
<td>1</td>
</tr>
<tr>
<td>50.0</td>
<td>50.0</td>
<td>2</td>
</tr>
<tr>
<td>25.0</td>
<td>25.0</td>
<td>3</td>
</tr>
<tr>
<td>50.0</td>
<td>50.0</td>
<td>4</td>
</tr>
<tr>
<td>25.0</td>
<td>25.0</td>
<td>5</td>
</tr>
<tr>
<td>50.0</td>
<td>50.0</td>
<td>6</td>
</tr>
<tr>
<td>25.0</td>
<td>25.0</td>
<td>7</td>
</tr>
<tr>
<td>50.0</td>
<td>50.0</td>
<td>8</td>
</tr>
<tr>
<td>25.0</td>
<td>25.0</td>
<td>9</td>
</tr>
</tbody>
</table>

The table shows the distribution of samples and their corresponding percentages. The total percentage is calculated by adding all the percentages together.
The end point when using the dye is very sharp and the values obtained are consistent. With lemon juice the use of iodine is also satisfactory. The slight advantage of iodine in this case is the fact that iodine can be accurately titrated against other reagents such as sodium thiosulphate and in dilute solution with KI is fairly permanent. The dye on the other hand gives very accurate results and is much more specific for ascorbic acid.

When oranges are analyzed however, the same consistent results are not obtained.

Ascorbic acid in orange juice expressed as mg. per c.c.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Determined by dye</th>
<th>Determined by iodine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>.45</td>
<td>.65</td>
</tr>
<tr>
<td>2.</td>
<td>.45</td>
<td>.70</td>
</tr>
<tr>
<td>3.</td>
<td>.45</td>
<td>.70</td>
</tr>
<tr>
<td>4.</td>
<td>.43</td>
<td>.65</td>
</tr>
<tr>
<td>5.</td>
<td>.44</td>
<td>.75</td>
</tr>
<tr>
<td>6.</td>
<td>.45</td>
<td>.75</td>
</tr>
</tbody>
</table>

As is evident from examination of results of titration of orange juice there must be some substance
<table>
<thead>
<tr>
<th>Sample</th>
<th>Performance of the Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a.</td>
<td></td>
</tr>
<tr>
<td>1b.</td>
<td></td>
</tr>
<tr>
<td>1c.</td>
<td></td>
</tr>
<tr>
<td>1d.</td>
<td></td>
</tr>
<tr>
<td>1e.</td>
<td></td>
</tr>
<tr>
<td>1f.</td>
<td></td>
</tr>
</tbody>
</table>

To ascertain from examination of samples that...
present other than vitamin C which reacts with iodine and does not react with the dye. This accounts for the high values when determined with iodine and may also explain why some investigators have found that apparently oranges contain more vitamin C than lemons. Bennett and Harris found an average of only 0.4 mg. of ascorbic acid per c.c. of lemon juice but 0.6 mg. per c.c. for orange juice.

Knowing that inosine had been isolated from oranges previously, the author decided to investigate the possibility of disappearance of iodine in the presence of inosine. Accordingly 10 c.c. of 0.01 N iodine solution were mixed with 10 c.c. of a 5% solution of inosine. The fading of the iodine solution was apparent and in six hours when compared colorimetrically with a blank of 10 c.c. of 0.01 N iodine with 10 c.c. of water allowed to stand the same length of time 50% of the iodine had disappeared. This indicates that one of the interfering substances in the titration of ascorbic acid by iodine may be inosine.

That the dye can be used for determining the presence of ascorbic acid in the urine can be shown by adding known amounts of the vitamin to urine and then recovering by titration with the dye.

Recovery of ascorbic acid from urine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ascorbic added</th>
<th>Ascorbic acid determined by the dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>2.</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>3.</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>4.</td>
<td>1.00</td>
<td>1.01</td>
</tr>
</tbody>
</table>

The method in this case is slightly modified because of the effect of the color of the urine on the end point as follows:

One half c.c. of the dye is added to 5 c.c. of urine in a tube as a blank. Then 5 c.c. of the urine to be tested is placed in a tube of the same size and titrated with the dye. The end point is taken when both tubes match and the color is permanent. Of course allowance is made for the amount of dye added to the blank.
The table below can be used for determining the

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Required Sample</th>
<th>Computed value</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.0</td>
<td>08.0</td>
<td>1</td>
</tr>
<tr>
<td>09.0</td>
<td>09.0</td>
<td>2</td>
</tr>
<tr>
<td>10.0</td>
<td>10.0</td>
<td>3</td>
</tr>
<tr>
<td>10.1</td>
<td>10.1</td>
<td>4</td>
</tr>
</tbody>
</table>

The method for this case is slightly modified

because of the effect of the color of the urine on

the readings. The following

One part c.c. of the urine is shaken with 5 c.c. of 0.001 N.

ammonia in a tube as a plug. Then 2 c.c. of the urine

is placed in a tube and 5 c.c. of 0.001 N.

ammonia are added. The tube is shaken

and filtered with the stopcock closed. The color of the

ammonia is measured and the color of the

ammonia is measured at the

endpoint of the reaction.
There are several possible reducing substances other than vitamin C which occur in natural systems such as cysteine, pyrogallol, tannin, tannic acid resorcinol, glucose, glucic acid and heated glucose or other sugars in the presence of acid or base.  

Harris and Ray, Hess, Johnson and Zilva, in their analysis of vitamin C in urine by the use of indophenol methods conclude that it is safe to assume there is not other reducing substance in the urine which would give a false test for vitamin C. The author has tested sodium 2, 6-dichlorobenzenone indo-3-methylphenol with cysteine, pyrogallol, tannin, tannic acid, resorcinol, glucose, glucic acid, heated alkaline glucose, sucrose and lactose in the presence of acetic acid as well as trichloracetic acid. Glutathione was not experimented with by the author because it was not available in a pure state. It seems safe to rule this substance out however, since it has been discarded as a possible interfering substance by other investigators.

The other possible reducing substances when tested against the dye show very slight effect under the conditions of the test. The greatest possible error (1-2%) occurs in the use of cysteine, and heated alkaline sucrose, or glucose. Since the test is carried out in acid medium however, various sugars have little if any, effect. Hot acetic acid will cause a very slight fading of the dye in one hour. Trichloracetic acid will cause a fading in a shorter time. In low concentrations of vitamin C the error in its determination is rather large, but when the concentration is approximately 0.5 mg. per gram of substance the determination is accurate. As compared to other methods the indophenol method seems the best. It is much quicker and more accurate than the biological methods provided due consideration be given to the possibility of interfering substances. The silver nitrate method as well as the iodine and the Bezsonoff reagent have been shown to be non-specific, although they can be used with precaution. Methylene blue under the conditions of the test is fairly specific but it is not as rapid or convenient as the indophenol methods.

Of the indophenol methods in use today the one introduced by the author (sodium 2,6-dichlorobenzenoneindo
-phenol) is the most rapid, simple and inexpensive. The dye is readily obtainable on the market at a cost of about one dollar per gram. Other indophenols are difficult to obtain, thereby necessitating the extra trouble of their synthesis. As Dr. W.C. Boyd suggests more work might be done with other dyes in the analysis of vitamin C. Knowing the oxidation reduction potential of a given dye it should be possible to predict its probable reaction with ascorbic acid, and by experimenting with numerous dyes finally to arrive at one even more specific than the ones already tried.
...specific to the case study...
Hess concludes that vitamin C does not appear in human urine under ordinary nutritional conditions and the author has confirmed this. Children excreted vitamin C only when an excess was given (one pint of orange juice per day.) Furthermore the vitamin C content of the urine dropped immediately upon the removal of orange juice from the diet. Excretion of vitamin C seems to occur only after the body stores have become saturated. In the urine of guinea pigs, rabbits, and rats the vitamin was found to be excreted in very low quantities and could be raised only by injection of the vitamin. Also, no difference was noted in the urinary excretion of vitamin C between animals which require the vitamin in the diet and those that do not require it.

Harris and Ray found the excretion of vitamin C in human urine to be dependent on the dietary intake and that the major portion of the vitamin was excreted in three hours. These results are confirmed by Johnson and Zilva.

I need to organize these documents tonight.

The report is due tomorrow afternoon, and I need to finish it by then.

I'll start with the summary and then write the detailed analysis.

Please review the attached documents and provide feedback.

Thanks for your help!
Preparation of Ascorbic Acid from Peppers.

The ordinary red pepper forms a convenient source of ascorbic acid. The juice from the red fruit contains on the average about 2 g. per liter as determined by titration with iodine and phenol-indophenol. The green fruit contains less but this amount increases as the fruit changes color and ripens. In the New England market peppers are generally sold when green because the housewife generally believes the green peppers to be sweet, and the red ones strong. This strange belief worked out to great advantage to the author as he was able to obtain red peppers at a very low cost.

Banga and Szent-Gyorgyi have worked out a method for the production of ascorbic acid from peppers on a large scale which is briefly as follows:

22 kg. of acid lead acetate are added to every 450 liters of the pulp of red pepper fruit. The pulp is pressed and then one half a liter of lead acetate solution (100%) added to every ten liters of juice.

Then ammonium hydroxide is added until faint alkaline reaction with phenolphthalein. This results in the precipitation of the vitamin. The precipitate is separated with the centrifuge, and hydrochloric acid added until the solution just begins to redden thymol blue. The lead chloride formed is separated and the solution remaining concentrated under reduced pressure. This concentrated fluid, containing about 10% ascorbic acid is extracted with acetone several times, and the combined extract further concentrated under reduced pressure until a thick syrup results. The syrup is extracted with dry acetone, the resulting extract containing 30-40% of vitamin. An oily residue remaining is dissolved with ethyl alcohol and again precipitated with acetone. This process is repeated and the acetone extracts combined and concentrated in a vacuum. Then 1 c.c. of n-butyl alcohol is added for every gram of vitamin present and the acetone distilled off. The vitamin is crystallized out from the n-butyl alcohol solution on cooling.
Waugh, Bessey and King have isolated vitamin C from lemon juice by precipitation of vitamin C with a lead salt followed by the decomposition of the salt with HCl. The lead is then centrifuged and separated and the vitamin obtained after several recrystallizations from various solvents.

Baumann and Metzger recommend iris leaves (Iris germanica) as a satisfactory material from which vitamin C may be obtained. The advantage of this substance seems to be that it contains fewer substances which would interfere with the separation of vitamin C, and that the vitamin is present in relatively large amounts, (600 mg/100 g. of fresh leaf in early spring). Baumann also found that the skunk cabbage leaves (Symlocarpus foetidus) contain 1 to 2 mg. of ascorbic acid per gram of leaves.

Preparation of ascorbic acid from red peppers on a small scale.

Sometimes small amounts of pure ascorbic acid are desirable for the purpose of checking standards, etc. For this reason the author has modified the large scale method of Banga and Szent-Györgyi to meet a small scale demand as follows:

The fruit of ripe red peppers freed from the core was ground up and 50 g. of acid lead acetate in solution added for each liter of pulp. The acid lead acetate solution is made by dissolving 50 g. of lead acetate in 25 c.c. of warm water and adding 0.4 c.c. of 85% formic acid. The mixture is pressed out and to each liter of juice is added 50 c.c. of warm 100% solution of lead acetate. Then ammonium hydroxide is added until the solution is just alkaline with phenolphthalein. This precipitate, containing about one half of the vitamin originally present in the pepper, is centrifuged and then treated with concentrated hydrochloric acid until the liquid just reddens thymol blue. The lead is precipitated as lead chloride, separated and washed.
Preparation of tickets with your legible name.

The following may be necessary:

1. A small square
2. A fine needle

Sometimes, when necessary, to make the appearance more
refined, for the purpose of appearing smarter.

For this reason the sufferer should wear the large
screw on top of shoes and fore-crawling to wear
small cases. Prepare

The task of the leg to keep the leg from the
spread may prove to any 0.0 to 1.999 feet departure in the
high. Now when you wear these Tarsal plates, the feet
should not be able to move at all, as this
obligation changes to make the leg to move
and at the same time, is shown to be equal to
1000 times more or less. The position of the
spread is such as this position at this
moment, with pronounced plurality. This multiplied, resulting
and cause the fall of the average. The spread
is as follows: I am convinced may have occurred with

concentration inapproporiate with with the.

The leg seems to propel the leg in the
way for which it is intended.
The fluids containing the vitamin are now concentrated to a syrup at a reduced pressure. Carbon dioxide was admitted to the fluid by means of a capillary tube. It is necessary throughout the process to avoid copper contact.

This syrup (containing 10% vitamin) is extracted with acetone several times and the extracts combined and concentrated on the water bath under reduced pressure to a thick syrup containing 25% vitamin. Again extraction with acetone is used and the oily residue extracted with ethyl alcohol. The alcohol is distilled off and the residue extracted with acetone. Then the combined extracts of acetone are further reduced and for each gram of vitamin present one c.c. of n-butyl alcohol is added. The solution is then cooled and allowed to stand in the refrigerator for several days which causes crystallization of the vitamin. The yield was six percent of the vitamin present in the first lead precipitate. This yield might be increased by more extractions and carrying over of some of the last extracts of one batch to those of the next batch.
The phrase containing the acronym "WSP".

Accumulated in a variety of teaching situations. To quote Wilson: "A common theme to the respondents' participants is the process of having a deeper understanding of the text."
Methods of recrystallization of vitamin C.

The crude substance is dissolved in methyl alcohol and water free ether added. A precipitate settles out which can be extracted with acetone and the above process repeated. Then petroleum ether is added and the vitamin crystallizes out in the cold. Many variations of recrystallization were tried. One good method was an ethyl alcohol acetone mixture followed by ether. After several crystallizations a very pure product resulted with a melting point of 189-189.5°C. The melting point was sharp. The synthetic 1 product of Haworth melted at 190°C. Some samples of recrystallized vitamin C are shown on the following plates.

MEMORANDUM OF REQUEST FOR INFORMATION

The cause of confusion in identifying the correct water source may well be traceable to a lack of clear and concise information. The need for a comprehensive list of water sources is critical to ensure accurate records.

The proposed solution involves the establishment of a water database that will include all sources and their characteristics. This database will be updated regularly to reflect any changes or additions.

The database will be accessible through an online portal, providing real-time information to all stakeholders.

This initiative will enhance transparency and accountability, ensuring that all water sources are properly identified and utilized.

If you have any questions or concerns, please do not hesitate to contact us.

[Signatures]

Date: [signature date]

[Additional Information]

I. Memorandum: Your Name, Year, Issue, Page.
Plate 1
Plate 2
Melting Point Determinations of Vitamin C and Similar Substances.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Determined by</th>
<th>Melting point</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Vitamin C</td>
<td>Bezssonoff</td>
<td>1820°C</td>
</tr>
<tr>
<td>2. L-ascorbic acid</td>
<td>Reichstein</td>
<td>186 - 9</td>
</tr>
<tr>
<td>3. D-ascorbic acid</td>
<td>Reichstein</td>
<td>187 - 189</td>
</tr>
<tr>
<td>4. Vitamin C</td>
<td>Vedder</td>
<td>180 - 182°C</td>
</tr>
<tr>
<td>5. Vitamin C</td>
<td>Gyorgyi</td>
<td>175 - 189</td>
</tr>
<tr>
<td>6. Vitamin C</td>
<td>Waugh, W.A.</td>
<td>183 - 185</td>
</tr>
<tr>
<td>7. Vitamin C</td>
<td>Alley, O.E.</td>
<td>189.5</td>
</tr>
<tr>
<td>8. L-ascorbic acid</td>
<td>Ault and others</td>
<td>190</td>
</tr>
<tr>
<td>9. D-ascorbic acid</td>
<td>Ault and others</td>
<td>190</td>
</tr>
</tbody>
</table>

The association of increase of vitamin C with the ripening of fruit.

It was found by titration with phenolindo-phenol that the concentration of ascorbic acid increased on change of color of the pepper from green to yellow, brown or red. The amount then decreased. The experiment showing this was carried out as follows:

Twelve peppers were placed in the sun and each day one was selected and analyzed for the vitamin content. As time went on the color change was from green to red. On the third day only one pepper contained any red color, so this one was selected for analysis. By the end of the eighth day all the peppers remaining were entirely red.

Zilva and others found that the ripening fruit was not associated with an increase of vitamin C content. This is in direct opposition to the findings of the author. Banga however, found the increase of vitamin content to be associated with ripening.

The assumptions of this mission are: 
- The target of interest is visible and accessible.
- The environment is controlled and predictable.
- The target is stationary or moving at a known rate.
- The data collection methods are accurate and reliable.

These assumptions will be used to determine the feasibility and effectiveness of the mission. The mission will proceed with these assumptions in mind. The mission will be conducted with the following equipment and resources: 
- [List of equipment and resources]

The mission will be monitored and evaluated throughout to ensure that the assumptions remain valid and that the mission is progressing as planned. Any changes or modifications to the mission will be documented and communicated to all stakeholders.
The following table shows the results of the author in determining the effect of ripening on the vitamin C content.

<table>
<thead>
<tr>
<th>Color of pepper</th>
<th>Time in days</th>
<th>Ascorbic acid mg. per c.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Green</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>Green and red</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td>Green and red</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>Green and yellow</td>
<td>5</td>
<td>1.1</td>
</tr>
<tr>
<td>Brown</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>Red</td>
<td>7</td>
<td>1.5</td>
</tr>
<tr>
<td>Red</td>
<td>8</td>
<td>1.4</td>
</tr>
<tr>
<td>Red</td>
<td>9</td>
<td>1.4</td>
</tr>
<tr>
<td>RED</td>
<td>10</td>
<td>1.3</td>
</tr>
<tr>
<td>Red</td>
<td>11</td>
<td>1.0</td>
</tr>
<tr>
<td>Red</td>
<td>12</td>
<td>1.1</td>
</tr>
</tbody>
</table>
### The following table shows the removal of the

**Subject toCertification the Effects of Twitching on**

<table>
<thead>
<tr>
<th>Color at Press</th>
<th>Time in days</th>
<th>Avg. per o.r.</th>
</tr>
</thead>
<tbody>
<tr>
<td>v.0</td>
<td>1</td>
<td>Green</td>
</tr>
<tr>
<td>v.0</td>
<td>3</td>
<td>Green</td>
</tr>
<tr>
<td>v.0</td>
<td>5</td>
<td>Green and Red</td>
</tr>
<tr>
<td>v.0</td>
<td>7</td>
<td>Green and Red</td>
</tr>
<tr>
<td>v.0</td>
<td>9</td>
<td>Green and Yellow</td>
</tr>
<tr>
<td>v.0</td>
<td>11</td>
<td>Brown</td>
</tr>
<tr>
<td>v.0</td>
<td>13</td>
<td>Red</td>
</tr>
<tr>
<td>v.0</td>
<td>15</td>
<td>Red</td>
</tr>
<tr>
<td>v.0</td>
<td>17</td>
<td>Red</td>
</tr>
<tr>
<td>v.0</td>
<td>19</td>
<td>Red</td>
</tr>
</tbody>
</table>
Experiment showing non-toxic effects of pure crystalline ascorbic acid.

In order to determine the effect of a large daily dose of ascorbic acid, two guinea pigs were fed 0.1 g of pure ascorbic acid per day. At the end of a week, the pigs appeared normal in every respect. Their fur was sleek, they appeared normally active, and showed no signs of respiratory infections or diarrhea. They also showed normal weight gains. At the end of two weeks the pigs were killed and the internal organs examined. The kidneys were normal in appearance and size. No signs of constipation or diarrhea could be detected in the alimentary canal. The lungs showed no signs of congestion. Other organs such as the liver and adrenals appeared normal macroscopically. It is evident from this experiment that ascorbic acid is not especially toxic to the guinea pig, at least in amounts many times the normal daily requirement.
In order to make sure that the results obtained are comparable to those of previous studies, we have developed a new method of analyzing the data. This method involves using regression analysis to explore the relationship between the variables. The results of this analysis are presented in Table 1. The table shows the coefficients of determination (R²) for each variable included in the model. The R² values range from 0.12 to 0.87, indicating a moderate to strong relationship between the variables. The significance of the coefficients is assessed using the t-test, and all coefficients are statistically significant at the 0.05 level. This suggests that the model is a good fit for the data and that the included variables have a significant impact on the outcome.

In addition to examining the relationships between the variables, we also conducted a sensitivity analysis to assess the robustness of the results. The analysis revealed that the results are robust to changes in the model specifications, indicating that the findings are reliable.

Finally, we conducted a further analysis to explore the potential mechanisms underlying the observed relationships. This analysis involved using mediation analysis to test whether certain variables act as mediators in the relationships. The results suggest that several variables play a mediating role, indicating that they may be important in explaining the observed relationships.

Overall, the results of this study provide valuable insights into the factors influencing the outcome of interest. The findings have important implications for future research and practice, and they highlight the need for further investigation in this area.
Rat Experiments.

The purpose of the experiments on rats was to investigate at some length the effect of a vitamin C free diet on an animal which up to now has never shown symptoms resembling scurvy.

They were placed on a diet which produced scurvy rapidly in the guinea pig, and kept on this diet over a period of generations. Each successive generation was also kept on the diet.

Several methods were used to determine the effects of this vitamin C free diet on the rat. At intervals sample rats were killed by ether or chloroform and examined by microscopic and macroscopic methods. The organs and glands were examined by chemical methods (silver nitrate titration, methylene blue and Tillmans' analysis) and sections of the teeth and bones were examined under the microscope. These methods with results of the methods will be discussed later in this paper.
The purpose of the experiment was to investigate the effect of a certain factor on a particular aspect of a system. Several methods were used to determine the effect of this factor on the system.

Methods: The reactions and mechanisms were examined under a microscope and examined with a light microscope. The reaction was monitored under a microscope and examined with a light microscope. The results were then recorded and analyzed to determine the effect of the factor on the system.

The results will be presented later in this paper.
The above photograph is one of a rat from the fourth generation of rats which had been fed on a vitamin C free diet. That is, this rat as well as its ancestors had received no vitamin C. The age of the rat is fourteen months. It is normal in size and apparently in a perfect state of health although the fur is not quite as sleek as it would be in a younger rat.

This rat and samples from each generation were killed by chloroform and the adrenals, heart, kidney, liver, ovaries or testes, and muscle were analyzed.
for vitamin C content. This analysis was checked from time to time with normal rats. Results of a few of the analyses are shown below.

Normal rats.

<table>
<thead>
<tr>
<th>c.c. of methylene blue per gram</th>
<th>Adrenals</th>
<th>Heart</th>
<th>Kidney</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>2.1</td>
<td>.4</td>
<td>.6</td>
<td>1.2</td>
<td>.2</td>
</tr>
<tr>
<td>2.</td>
<td>2.0</td>
<td>.4</td>
<td>.8</td>
<td>1.5</td>
<td>.2</td>
</tr>
<tr>
<td>3.</td>
<td>2.2</td>
<td>.4</td>
<td>.6</td>
<td>.8</td>
<td>.2</td>
</tr>
<tr>
<td>4.</td>
<td>2.2</td>
<td>.4</td>
<td>.6</td>
<td>.9</td>
<td>.2</td>
</tr>
<tr>
<td>5.</td>
<td>2.1</td>
<td>.4</td>
<td>.7</td>
<td>1.4</td>
<td>.2</td>
</tr>
<tr>
<td>6.</td>
<td>2.4</td>
<td>.4</td>
<td>.6</td>
<td>.8</td>
<td>.4</td>
</tr>
</tbody>
</table>

Rats fed on vitamin C free diet.

<table>
<thead>
<tr>
<th>c.c. of methylene blue per gram</th>
<th>Adrenals</th>
<th>Heart</th>
<th>Kidney</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>1.0</td>
<td>.4</td>
<td>.6</td>
<td>.6</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>.8</td>
<td>.4</td>
<td>.6</td>
<td>.8</td>
<td>.2</td>
</tr>
<tr>
<td>3.</td>
<td>.8</td>
<td>.4</td>
<td>.8</td>
<td>.6</td>
<td>.2</td>
</tr>
<tr>
<td>4.</td>
<td>.7</td>
<td>.4</td>
<td>.8</td>
<td>.6</td>
<td>.0</td>
</tr>
<tr>
<td>5.</td>
<td>.9</td>
<td>.4</td>
<td>.8</td>
<td>.7</td>
<td>.2</td>
</tr>
<tr>
<td>6.</td>
<td>.8</td>
<td>.4</td>
<td>.9</td>
<td>.8</td>
<td>.2</td>
</tr>
</tbody>
</table>
Some very interesting points may be observed from the preceding results. First, the adrenals contain the largest amount of vitamin C of any of the organs examined. Furthermore, the amount diminishes on a vitamin C deficient diet. The vitamin C content of the heart is small but remains constant. Diet seems to have no effect whatever on the C content of this organ. The kidney maintains a fairly constant amount, but the liver definitely drops in the vitamin C content. The amount of the vitamin in muscle is very small. While the average would seem to be slightly less from the figures, the accuracy and limits of the analysis probably do not warrant such an assumption. Microscopic examination of the teeth did not indicate scurvy. Also from time to time other methods were used as a check with fairly consistent results with the exception of the silver nitrate titration. This last method is not delicate enough to be of value in these experiments on rats.

The unfavorable action of carbohydrate in the diet towards the production of scurvy has been previously noted in this paper.
In this connection the author has been interested in observing the effect of a high carbohydrate diet on rats. In all the animals used, whether they died or were killed intentionally, no evidence of scurvy could be observed. A heavy deposit of fat was found in the older animals, which would be expected as a result of the excess carbohydrate intake. In addition to the high carbohydrate content the diet also lacked vitamin C, but in spite of these two factors, scurvy did not appear.

It is interesting to note that these results differ from those of Simola who worked with the guinea pig. His work showed that the scurvy symptoms on a vitamin C free diet were most marked when the carbohydrate intake was high.

The results of the author, however, do confirm in general the work of Svircbely on the vitamin C content of the organs of the rat.

Silver Nitrate Reaction.

Alkaline silver nitrate is reduced by many substances such as glucose, fructose, and phenols. In an acid medium, however, these substances do not reduce silver nitrate readily and neither will tannin, cysteine, glutathione or creatinine at least in vitro.

Rat Experiments Using the Silver Nitrate Method.

In order to determine the effect of a vitamin C free diet on the vitamin C content of the adrenals of the rat, six rats were placed on a diet of boiled milk and bread which developed scurvy in guinea pigs, but was complete in other respects. After being on this diet for eight months these rats were killed, and the adrenal glands analyzed for vitamin C as follows:

Each rat was killed separately and the adrenal glands immediately dissected out, weighed and placed in a 0.4% solution of silver nitrate at a pH of six. The glands were then placed in the dark
and examined every minute for the appearance of reduced silver. The adrenals of rats that had been fed a normal diet containing vitamin C were examined in the same way.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Normal Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>
any examinations which provide for the expression of

legally enforceable positions of fact that may
be a key letter giving sufficient information to make
examination in the same way.
The results are tabulated as follows:

Rat fed on a diet free of vitamin C. Time for intense darkening of adrenals.

1. 4 minutes
2. 5 minutes
3. Similar experiments 4 minutes
4. Pig. Six pigs were placed in silver nitrate solution for five minutes, then washed with distilled water, and then with sodium thiosulfate solution. Time required for intense darkening of the adrenals.
5. 4 minutes
6. 4 minutes
7. 4 minutes
8. 4 minutes
9. 4 minutes
10. Heavy
11. Heavy
12. Heavy
The results are tabulated as follows:

<table>
<thead>
<tr>
<th>Time for intenseGERREDING</th>
<th>Rest key on a 90°</th>
<th>Time of attachment</th>
<th>Number for</th>
<th>Time restary for intense</th>
<th>Performance of the Operator</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 minutes</td>
<td>.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 minutes</td>
<td>.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 minutes</td>
<td>.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 minutes</td>
<td>.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 minutes</td>
<td>.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The table above is a hypothetical example and does not represent actual data.
It is obvious from the tables that the adrenals of a rat which is fed on a vitamin C deficient diet do not show loss of vitamin C when compared to the adrenals of the rat fed on a normal diet. These results of course were dependent on the accuracy of the silver nitrate method of analysis.

Similar experiments were carried out on the guinea pig. Six pigs were placed on a diet of boiled pasteurized milk and bread. Others were fed a normal diet with lettuce or orange juice added to insure sufficient vitamin C. The animals were killed at the end of three weeks and the adrenals dissected out, placed in silver nitrate solution for five minutes, then washed with distilled water, and then with sodium thiosulphate solution. The results are tabulated below.

<table>
<thead>
<tr>
<th>Normal pig</th>
<th>Time</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5 minutes</td>
<td>Heavy</td>
</tr>
<tr>
<td>2.</td>
<td>5 minutes</td>
<td>Heavy</td>
</tr>
<tr>
<td>3.</td>
<td>5 minutes</td>
<td>Heavy</td>
</tr>
<tr>
<td>4.</td>
<td>5 minutes</td>
<td>Heavy</td>
</tr>
<tr>
<td>5.</td>
<td>5 minutes</td>
<td>Heavy</td>
</tr>
<tr>
<td>6.</td>
<td>5 minutes</td>
<td>Heavy</td>
</tr>
</tbody>
</table>
It is apparent from the figures that the residues of 
it is not upon this a belief that no publication of these 
attaches to the fact that no a notice of the current of the 
results of courses are given over on the occasion of the 
the present interval waiting of experiment.

Similar experiments were carried out on the 
below. Six hours were allowed on a diet of boiled pasta-

1. With milk and bread. Oats were kept a normal diet 
with potato or orange juice added to increase caloric 
and fat assimilation. The samples were filled at the end 
of three weeks and the samples analyzed out. Please 
include all visible solutions for the ministrant new 
meat with half a liter of water and then with solution 
appropriate solution. The residues are tabulated.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Time</th>
<th>Molar eq.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heany</td>
<td>3 minutes</td>
<td>1.2</td>
</tr>
<tr>
<td>Heany</td>
<td>2 minutes</td>
<td>0.8</td>
</tr>
<tr>
<td>Heany</td>
<td>2 minutes</td>
<td>0.6</td>
</tr>
<tr>
<td>Heany</td>
<td>3 minutes</td>
<td>0.4</td>
</tr>
<tr>
<td>Heany</td>
<td>3 minutes</td>
<td>0.2</td>
</tr>
<tr>
<td>Heany</td>
<td>4 minutes</td>
<td>0.1</td>
</tr>
<tr>
<td>Heany</td>
<td>4 minutes</td>
<td>0.06</td>
</tr>
</tbody>
</table>
## Discussion of results.

The rat, however, when fed on the same diet which produces scurvy rapidly in the guinea pig, does not show an appreciable loss of vitamin C from the adrenals. Furthermore, this same condition prevails in the rat even after years on a vitamin C deficient diet.

### Discussion of the advantages and disadvantages of the chemical methods for the analysis of vitamin C as compared to the biological methods.

To one who has spent weeks carrying out a biological assay, the saving of time and labor in the purely chemical method is obvious. For example, in the biological assay of a food for vitamin C it is

<table>
<thead>
<tr>
<th>Scurvy</th>
<th>Time</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>Slight</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>?</td>
</tr>
<tr>
<td>Reaction</td>
<td>Time</td>
<td>Compound</td>
</tr>
<tr>
<td>----------</td>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>Shift 1</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>Shift 2</td>
<td>2</td>
<td>B</td>
</tr>
<tr>
<td>Shift 3</td>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>Shift 4</td>
<td>4</td>
<td>D</td>
</tr>
<tr>
<td>Shift 5</td>
<td>5</td>
<td>E</td>
</tr>
</tbody>
</table>

**Discussion of Results**

The rate, however, may be on the same level.

Why does there seem to be an increase in the enzyme plot?

You may not know to suggest one of vitamin C from the above, "correlation" since some conditions throw curves over a vitamin 0 present in the rate even after keeping a vitamin 0 present.

**Further Tests**

**Discussion of the advantages and disadvantages of**

the experimental procedure for the analysis of vitamin C as compared to the physiological methods.

To one who has spent weeks undertaking such a pie

Good essay, the results of which may appear in the
daily newspaper without being an example to
the physiological essay of a food for vitamin C if
customary to feed groups of animals varying amounts of the food and to observe the development of scurvy. This procedure involves the expense and time of raising suitable laboratory animals in order to have them at the right age and weight for the analysis. Then the period of feeding them the food in question may involve weeks, at the end of which time the animal is killed, and examined for evidences of scurvy. Further, in the tooth structure method the preparation of the tooth may require days or even weeks of work before it is ready for microscopic examination. The chemical methods as used and developed by the author can be carried out in a matter of hours, or at the most, a few days.

As regards specificity, a positive production of definite tooth structures characteristic of scurvy is evidence of the strongest nature. Absence of the typical tooth conditions may not necessarily indicate absence of scurvy, at least in the elementary stages. Rib defects may occur before tooth defects. Pure chemical methods may not be absolutely specific and quantitative for vitamin C, but if not, they may be empirical and hence of great value. It is desirable to check one method with the other from time to time.
of the tool and to operate the system at maximum efficiency and time of

This sequence involves the sequence and time of

raising successive folders to enable the sequence in order to have

them in place for rapid and speedy work in operation.

They may involve weeks at the end of which simple facts

may involve weeks. It is extremely important to be

instantly and examined for adherence to standard

practice. In the order structure method of development of

the sequence, methods as rapid and developed by the

sequence can be carried out in a matter of hours, to

get the work a few years.

As sequence specialists, a polite policing function

is desirable to maintain the sequence of operations at

slowly to achieve the improvement wanted. However, the

rapid improvement of sequence was not necessary

in the sequence of sequence, as in the sequence of

sequence. When sequence is achieved by consecutive steps

the sequence. The sequence may not be specified

specifications and manipulation of the sequence in

pieces may be simplified by being able to offer them to

like grease to power out weapons with the force from

and time to time.
Experiments on Teeth.

1. Durand noticed that the percentage of caries in children that were breast fed was much less than in those fed on condensed milk. He attributed this condition especially to the diet which was too rich in carbohydrate. The defects were probably due to several dietary factors, vitamin C lack being among them. Zilva and Wells have observed a degeneration of the tooth pulp in guinea pigs and monkeys on a vitamin C free diet. More recently Hojer has developed more fully the technique of the scorbutic tooth. Since the condition of the tooth is one of the first evidences of pathological change to appear in scurvy the author felt that this could be used as a method of detecting possible scurvy in the rat. The following plates show respectively a normal rat tooth and a tooth from a guinea pig with scurvy.

Experiments on Cephalopods

I. Dorsal Nodules and the Perception of Colour

II. The Apparatus and the Nodules

III. The Nodules and the Apparatus

IV. The Apparatus and the Nodules

V. The Nodules and the Apparatus

VI. The Apparatus and the Nodules

VII. The Nodules and the Apparatus

VIII. The Apparatus and the Nodules

IX. The Nodules and the Apparatus

X. The Apparatus and the Nodules
Discussion of Plates.

Plate number four is a normal tooth although it is from a rat of the fourth generation which had been on a vitamin C free diet. It shows normal dentine and Tome's canals. The odontoblasts are long and parallel. There is no sign of inner dentine and the Tome's canals cross the dentine from the odontoblasts although the two have been slightly separated mechanically in the process of sectioning.

The guinea pig tooth, however, plate number five, shows rather completely disorganized odontoblasts. The inner dentine is irregular and projects out into the pulp.
Experiments on doubly suprarenalectomized rats.

The previous analysis showing the high vitamin C content of the adrenals suggested further experiments to determine the effect of removing the adrenal glands from the rat. This was done and the following analyses made:

Doubly suprarenalectomized rats on a vitamin C deficient diet.

c.c. of methylene blue per gram.

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Kidney</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>.4</td>
<td>.3</td>
<td>.6</td>
<td>.2</td>
</tr>
<tr>
<td>2.</td>
<td>.3</td>
<td>.7</td>
<td>.5</td>
<td>.2</td>
</tr>
<tr>
<td>3.</td>
<td>.4</td>
<td>.8</td>
<td>.6</td>
<td>.0</td>
</tr>
<tr>
<td>4.</td>
<td>.4</td>
<td>.8</td>
<td>.5</td>
<td>.0</td>
</tr>
<tr>
<td>5.</td>
<td>.4</td>
<td>.9</td>
<td>.3</td>
<td>.0</td>
</tr>
<tr>
<td>6.</td>
<td>.4</td>
<td>.9</td>
<td>.4</td>
<td>.2</td>
</tr>
</tbody>
</table>

By comparison of this table with the other tables it appears that the animal is being depleted in vitamin C in about the same manner as the normal rat. Strangely enough the kidney
Experiments on growth and development of larvae

The results emphasize the need for careful control of the experimental setup. It is important to compare the weight of the experimental groups to the control group before proceeding with the experiment.

The following tables were taken as a summary of the experimental results:

<table>
<thead>
<tr>
<th>Heat</th>
<th>Kitchen Plan</th>
<th>Flower</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>B</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>D</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

By comparison of these tables, it is clear that the experiment was successful in demonstrating the effects of heat and kitchen plan on the growth of the larvae.
maintains a comparatively high amount of vitamin C in the doubly suprarenalectomized animal while the muscle and liver contents are slightly less than in the normal rat.

Note:

The author is greatly indebted Drs. Leland C. Wyman and Caroline tum Suden for help and advice in these experiments, especially in the preparation of the animals.

Note:
The report is hereby submitted to the President.

Where any certificates cannot be read or made in those departments, especially in the preparation of the estimate.
The possibility of the function of the suprarenals as a storage of vitamin C seems to be doubtful because of the extremely small amount present compared to the amounts of vitamin A present in the liver. 

Harris suggests that the localized concentration of vitamin C in the suprarenals can be considered as necessary for the protection of the normal functional activities of that organ.

The doubly suprarenalectomized rats ought to develop scurvy if the suprarenals alone synthesize vitamin C. In all the rat experiments so far observed, no evidence of scurvy has appeared. This negative evidence may still be accounted for by the possibility of failure to remove every trace of suprarenal tissue, and also by the regeneration of new tissue after operation. At autopsy several previously doubly suprarenalectomized animals did show such tissue.

Vars and Pfiffner found no evidence that the adrenal of the dog was concerned with either the synthesis or metabolism of vitamin C. Hartmann and Lockwood, however, found that cortin was beneficial in delaying the onset of scurvy in the guinea pig.

The possibility of the formation of the virus seems to be pre-existant. 
Because of the extremely small amount present, care to the
extent of the occurrence of atrial fibrillation may be the first.

I hesitate whether heart disease can be considered as
necessary for the occurrence of the cardiac function.

It is a highly controversial factor about

The so-called arrhythmia in the heart's function
occurs in all the fat, for instance in the case of

The negative evidence of so-called arrhythmias for the possibility
of failure to remove every trace of abnormality because of

It supports several therapies thoroughly controversial.

The evidence shows many times that the

Are any different findings on arrhythmia that the

strategy of the case and its treatment in

Several of the attacks of atrial fibrillation manifest may occur

Most important, from the post-mortem

helps us in the event of shock in the absence of

2. Prediction, loc. cit., 1929, 8, 1010.
Some Modifications in Animal Photography.

In order to photograph a dead animal all that is necessary is proper focus and length of exposure when using an ordinary camera. But to photograph a live moving animal is more difficult. To begin with, since the animal may move, a snap shot must be taken. This means that the light must be good. Light in the laboratory is unsuitable and generally it is not convenient to take the animal out of doors. This necessitates some artificial source.

After experimenting with various methods the author found the flash method to be simple and effective. The flash powder is made up of a mixture of potassium chlorate and powdered magnesium. This mixture was ignited by means of a spark coil.

A photograph of the apparatus is shown below together with a diagram of the circuit necessary.
In order to photograph a dead specimen it is necessary to place it in some fixative but to photograph a live subject it is more difficult. To begin with, since the subject may move a snap shot may be taken. This means that the flash must be good. Right in the beginning it is important to experiment and generally it is not necessary to take the specimen out of the tank. After experimenting with various methods the author found the best method to be simple and effective. The flash power is made up of a mixture of potassium and barium. This mixture was ignited by means of a spark coil. A photograph of the apparatus is shown below.

A sheet with a diagram of the apparatus necessary.
Plate 6.

Photograph of apparatus for flash powder ignition.

Diagram of circuit for flash powder ignition.

C - Ford spark coil
P - Primary circuit
S - Secondary coil
T - Attached terminals
B - Dry cells
Diagram of apparatus for sleep power ignition.

- Power
- Control coil
- Primary coil
- Secondary coil
- Auxilliary terminal
- Dry cell
A flash bulb of course could be used. It generally works, gives good light, but is rather expensive because each bulb is good for only one exposure, and if many photographs are to be taken the total cost of all the bulbs used will be considerable. Most of the photographs occurring throughout this paper were taken by the induction coil method and while the author does not claim to be an expert photographer, the results seem fairly good. It may be added that in all the photographs taken no other special photographic apparatus was used. Even the microphotographs were taken with an ordinary Kodak camera through a microscope.

These beliefs no longer hold true today. The author has observed several cases which seem to
A leap into the role of course could be yearly. It generally
more gives way little but the correct explanation
percentage does help for only one explanation and
in many situations at to be taken the role of
of all the people need with considerable weight do
the observations accustoms whatsoever is some
reason for the hypothesis of comparison only white the
sentence never will consent to as an example hypothesis.
the reason seems fairly easy. It may be which that
in all the hypotheses taken to higher a salary
hypothetical substantiate you need. Even the results
phrases were taken away or ordinary paper can
be something's mistake.
The effect of vitamin C on the teeth in man.

It should be mentioned at the outset that several vitamins, salts, foods, bacteria and acid conditions of the mouth may all have an important bearing on the proper growth and maintenance of the teeth. With the exception of vitamin C the various factors are well known and need no further discussion at this point.

1. Ballantyne found that all but two percent of pregnant women show carious teeth. This might easily be expected because of the great increase in the requirements of the above mentioned factors during pregnancy. A common old saying is "for each child a tooth" and in the days when large families were the rule rather than the exception, the mother of fourteen children would feel that conditions were normal if she had lost fourteen teeth, during the period of child bearing.

These beliefs no longer hold true today. The author has observed several cases which seem to

The effect of attrition on the teeth in men.

It is worthy of mention that the same factors mentioned in the preceding paragraph have an important bearing on the structure and maintenance of the teeth. With the exception of attrition, the attrition occurring at the teeth in men often severe, may in the years when these defects were not uncommon, many feel that conditions were normal. It is often said that the effect of the attrition of the teeth on the occlusion is great. The attrition and filling these cavities on the posterior parts of the jaws which seem to

I. Report from Dr., May 1913.
show that the occurrence of caries and the loss of teeth may not increase during pregnancy.

Observations on the condition of teeth during pregnancy.

Average number of fillings before pregnancy 6.
Average number of fillings during pregnancy 6.
Average number of fillings after pregnancy 5.

The above cases were taken from the records of Dr. E.S. Godfrey and represent the average fillings over a period of twelve months. With the further assistance of Dr. Godfrey, another interesting point has been observed, namely the progress of white spots on the teeth. In the course of time these spots become cavities. Furthermore these spots may disappear following the administration of vitamin C providing they have not progressed too far. In other cases the spots may not disappear under vitamin C treatment but the development of these spots ceases.

1. Hanke has described the white spots on the teeth (not so called mottled enamel). The areas are at first quite hard and cannot be penetrated with a probe. That these areas are not deposits on the teeth can be

shown by the absence of a positive stain when treated with a 2% solution of bismark brown in 25% alcohol. As time goes on these areas may become soft so that they can be penetrated with a probe and dental caries result. Most investigators agree that the change is one involving the cementing substance between the rods. This change is generally thought to be due to the dissolving of the cementing substance by acids derived from the action of bacteria on food.

The investigations of Sherman Davis, Milton Hanke 1-4 and others show that dental caries can be arrested if the patient will eat the proper food. Miller and Heitzmann concluded that inflammation was the main cause of decay of the teeth. While the idea of bacterial decay is rather old, it receives support in the work of Bunting who believes that the action of Bacillus acidophilus results directly in dental caries.

5. Miller, W.D., Micro-organisms of the human mouth, 1890.
He did find however, that the bacteria were regulated by the diet, but that antiseptics used in the mouth, such as hexylresorcinol, had no apparent virtue. These experiments were conducted on large groups of school children, and the final observation seems to be that a diet containing an ample amount of fresh fruits, vegetables, and milk, but somewhat restricted in carbohydrates, is best for the proper growth and maintenance of the teeth.
Summary.

The history of scurvy and vitamin C has been traced briefly from 2697 B.C. to 1935 A.D. The isolation of vitamin C by Szent-Gyorgyi in 1928 and the synthesis later by Haworth and others has been noted.

New chemical methods for the analysis of vitamin C have been investigated. In one case, that of methylene blue, a modification has been offered which makes that method usable for micro-analysis. A new method of analysis of vitamin C by use of Sodium 2, 6-dichlorobenzenoneindo-3-methylphenol has been presented. These methods have been applied to plant and animal tissues. An important conclusion to the plant experiments is that the vitamin C content of lemons varies considerably, contrary to the claims of Bennett and Harris, thereby making its use as a standard undesirable. On the other hand, pure crystalline vitamin C, prepared by the method of Banga, was shown to be constant.

The rat has been studied at some length, both in
The picture of setting up an administration 0 per year.

The latest picture from 1929, 5 ft. to 1930, 8 ft. The
information of rescue of eastern-Indian in 1928
may be received after 12 to 30 per cent per
once next.

The chapter mentions fear the features of activity.

I have seen a number of a small section and been at

meritorious give the number samples for microscopic.

A new section of scheme of admission is to see of

original, O.1512810181810-8—meritorious

and need assistance. I have seen samples have been supplied

to test the minor illness in important condition.

of the facts shown in that the arrhythmia 0 can—

left of Imagine multiple connections, contrary to the

afford of samples may be risk. Provide condition 15

be a strategic supplement. On the other hand, more

otherwise line arising, the required in the manner of

Tender new work to be consistent.
a normal state, and under a vitamin C deficient diet. These experiments have been carried through several years and generations of rats. Since the results show that the vitamin C is present in the rat after four generations and that the amount varies somewhat, then the rat must be able to synthesize this vitamin. It has also been shown that the liver and adrenals lose vitamin C more rapidly than other organs do in the rat. Further the doubly suprarenalectomized rat seems to react to a vitamin C free diet about the same way that a normal rat reacts. The liver loses vitamin C while other organs examined maintained the vitamin level. The kidney in the doubly suprarenalectomized rats maintains the vitamin content slightly better than the normal rat although the difference is slight.

Biological methods have been compared to chemical methods, the advantages becoming greater with the chemical methods, although it is still well to check occasionally with a biological method.

The effect of vitamin C on the teeth of certain animals points strongly to possible effects in the teeth of man and some evidence is presented to support this point of view.
...
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Resident

Research and Development

Special and New Technology

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Proposal

September 1974

J. M. Eckert

Director

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Dear [Name],

I am a student of [Department], and I am writing to express my interest in the position of [Job Title] in the [Department] of [Institution].

I have been a student here for [X] years, and I have completed [Y] courses in [Major]. I am particularly interested in [Specific Area of Interest].

I believe that my [relevant skills/qualifications] make me a strong candidate for this position. I am confident that I can contribute to the success of [Department] and [Institution].

Thank you for considering my application. I look forward to the opportunity to discuss this further.

Sincerely,

[Your Name]
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The necessary actions to be taken are:

- Set up a trial.
- Prepare the necessary documents.
- Attend the court.

With regards,

[Signature]

[Date]
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