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THE EFFECTS OF CERTAIN DIETS ON THE  
TEETH OF THE ALBINO RAT WITH SPECIAL  
REFERENCE TO THE DEVELOPMENT  
OF DENTAL CARIES<sup>1</sup>

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PURPOSE AND AIM OF THIS STUDY

The problem of tooth hygiene, structure, decay, resistance to decay, its prevention, etc., has been one which has engrossed the attention of workers in various fields for many years. The dentist, the bacteriologist, the nutritionist, the pediatrician, the biochemist, and others have each made contributions to this interesting and important subject.

It was the purpose of this study to find the effects of certain diets on the development of teeth of the albino rat, and if possible to draw conclusions therefrom which might throw some light on the general problem of tooth hygiene, and the effects of diet on teeth and tooth preservation, as it may apply to the human species.

HISTORICAL

INCIDENCE OF CARIES IN ANCIENT TIMES

That caries is not a modern disease is adequately shown by studies in paleopathology and paleo-odontology. A few references will show how widespread this disease has been in historic and prehistoric times. I quote from Ruffer, the following: "Cariou human teeth from ancient remains have been discovered in so many places that it is legitimate to doubt whether there ever was an epoch when the human species was not cursed by toothache."

ENGLAND

"The population of England has certainly suffered from it (toothache) for thousands of years." Sixty-nine skulls from the Wiltshire tumuli from the stone age showed two carious teeth. In Northern England, in forty-four skulls, nine carious teeth were found. Six

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<sup>1</sup>Read before the Tennessee Academy of Science at the Nashville meeting, November 27, 1931.

carious teeth were found in thirty-two skulls of the bronze age. The incidence of caries among the Romans in England based on the examination of 133 skulls showed 28.67% carious. Seventy-six skulls of the Anglo-Saxons had fifteen carious teeth. The frequency of dental caries at present in the British Isles has been variously estimated from 86-98%.

## EGYPT

"From the earliest to present times, caries has attacked human teeth in Nubia and Upper and Lower Egypt. Both in Nubia and Egypt the ordinary form of caries is exceedingly rare in predynastic and protodynastic people, and among the poorer classes it never became common until modern times. Dental caries became common as soon as people learned luxury. In the cemetery of the time of the Ancient Empire excavated by the Hearst Expedition at the Giza pyramids, more than 500 skeletons of aristocrats of the time of the pyramid builders were brought to light, and in these bodies I found that tartar formations, dental caries, and alveolar abscesses were at least as common as they are in modern Europe today.

"At subsequent periods in Egyptian history the same thing is true—prevalence of caries among the wealthy classes and relative immunity among people who lived on a coarse, uncooked diet."

Ruffer's article is accompanied by many plates proving the validity of the above statements.

Investigations in the New World go to show that dental caries was also prevalent among its inhabitants.

## SOUTH AMERICA

Moodie examined skulls of Pre-Columbian Peruvians at the San Diego museum and found 6 with dental caries. MacCurdy made a study of the Pre-Columbian Peruvians of the Highlands and has listed the incidence of caries of the inhabitants.

## NORTH AMERICA

The study by Raymond C. Bentzen is typical of several that have been made on dental diseases among the Indians of North America. The teeth and jaws from a series of skeletons unearthed by the Jenk's Expeditions show that the Mimbres people possessed very hard teeth, due perhaps to the high calcium content of their food. Bentzen says that their diet consisted of coarse, natural foods which necessitated thorough chewing, thus giving plenty of exercise to the jaws. Prolonged mastication produced large, heavy jaws giving plenty of room for the teeth to erupt properly, but the teeth were rapidly worn down due to the gritty material in their food, and sometimes lost through exposure of the pulp and to apical abscesses.

Their teeth were fairly immune to caries through the enamel, but the calicular deposits caused recession of gums permitting contact of acid-forming bacteria at the cemento-enamel junction where 80%

of the caries started. Notwithstanding the fact that the Mimbres people observed no oral hygiene or care of the teeth, they were better fitted for thorough mastication of food than are the Americans of today.

#### HAWAII

A study published in 1927 shows that caries was a prevalent disease among the ancient Hawaiians.

The above references and quotations are sufficient to prove that caries is not a modern disease.

#### MODERN STUDIES AS TO THE INCIDENCE OF DENTAL CARIES

Leigh examined the crania of 324 modern Esquimaux from Greenland, Cumberland Sound, Point Barrow, St. Lawrence Island, St. Michael, Alaska, and the Siberian Coast, and found an incidence of but 1% caries.

Suk compared the eruption and decay of permanent teeth of whites and negroes. This report is of observations made on 1,008 sub-adult Zulus from all parts of Natal and Zululand. Of these 492 were males and 516 females, of different ages. The observations on whites were made on the children of several normal schools, on students of colleges, and on apprentices in different trades, in Prague, Bohemia. Results of observations show that white children have more than ten times as many decayed teeth as the negroes. At the age of eighteen years, there are only 10-15% of individuals among whites in large cities with a faultless set of teeth, while among Zulus 85-94% were found.

Campbell reports studies on 630 modern Australian aboriginal skulls of all ages. Thirty-seven of these were of children under twelve years of age, and showed no caries present. Modern inhabitants of Adelaide, Australia, show 94% carious teeth before the age of twelve. In the Australian aborigine, practically no caries was found in children. The slight incidence of caries found among middle aged skulls increased to 1% in skulls of aged aborigines.

Cooper's report showed an appalling dental condition of the children in the public schools of North Carolina. He says that 80% of all the school children enrolling in the public schools of North Carolina—at that time more than three-fourths of a million—were in dire need of correction for dental defects. It was found that not over 5% of this great number had ever visited a dentist's office for any purpose whatsoever.

A report by Rypins made on dental conditions in the pre-school children of Kansas City, Missouri, is most enlightening if not startling. Children in different districts all over the city were examined, and more than 25% of the children from birth to six years of age were found to have carious teeth. Between the ages of three to six, 44% were found carious. Of more than 8,000 children enrolled in the first grade each year, 5,000 have dental defects. Twenty-one

thousand teeth would require surgical treatment yearly to make the pre-school children of Kansas City dentally perfect upon entrance to school. This is a mighty economic and humanitarian problem.

Friel and Shaw reported on the dental conditions among the white children in the Union of South Africa. Six hundred children ranging from the ages of six to sixteen showed an incidence of 93.35% caries. Only 6.55% of these children had all sound teeth. There was an average of 4.82% carious teeth per individual.

#### THEORIES AS TO THE ETIOLOGY OF DENTAL CARIES

The etiology of dental caries—"the most prevalent of all human diseases"—has been the subject of discussion by many authors and many treatises from the earliest of times. Various have been the theories advanced.

Hippocrates, 465 B. C., attributed dental caries to "the stagnation of depraved juices" in the teeth. Galen, 131 A. D., thought it a form of inflammation, and later John Hunter (1778), Joseph Fox (1806), Thomas Bell (1831), and Boedeker (1866) were strong adherents to the inflammation theory of the cause of dental caries.

In both Europe and Asia, it was supposed by many that worms grew into teeth and gnawed away the substance. In China and Japan, the word used to designate carious tooth is "worm tooth." In parts of China, the modern method for treatment of toothache is to bore into the teeth "to let the worms out."

#### SOME GENERALLY ACCEPTED FACTS CONCERNING DENTAL CARIES

Bunting defines dental caries as "the process which leads to a disintegration of the teeth characterized by the formation of open lesions in the enamel, dentin, and cementum."

He says that the generally accepted facts concerning the process are:

1. Dental caries is a destruction of the hard substance of the tooth by a process, the initial stage of which is decalcification by acids.
2. The acids active in caries are not generally distributed in the saliva, but are localized and concentrated on certain areas of the tooth surfaces.
3. Carious lesions occur most frequently in the pits and fissures of the occlusal surfaces and on certain areas of the approximal, buccal and lingual surfaces of the teeth, at which locations there are opportunities for stagnation and the retention of foreign matter. They do not occur on smooth enamel surfaces that are frequently cleansed.
4. All initial lesions of caries contain acid-forming bacteria capable of producing and living in acids of sufficient potential to decalcify the enamel.
5. The hardness or softness of the teeth may affect the rate of progress and extent of caries but does not alone determine its occur-



rence. Caries, as a rule, runs a more rapid and extensive course in hypoplastic teeth than in the hard and well formed varieties, but instances commonly occur in which the poorest-formed teeth are wholly free from the disease.

6. Malhygiene of the mouth frequently favors the inception of dental caries and increases its activity, but alone does not determine its occurrence. Mouths that are habitually unclean are often wholly free from caries and, conversely, mouths that are scrupulously clean may be seriously affected by the disease.

7. The process of dental caries is related to and often determined by certain constitutional states and conditions of bodily health. The nature of these general influences and the manner in which they affect the course of this dental disease are not clearly understood at this time. The following bodily conditions are perhaps best known as systemic factors which either favor or oppose dental caries:

*Heredity.* There are strong evidences that the tendency toward dental caries or toward immunity to the disease may be transmitted from parent to child.

*Age.* Susceptibility to dental caries is clearly influenced by age. Incidence of the disease is known to be highest during the ages from seven to twenty years. After twenty years of age the tendency to caries is markedly decreased.

*Health.* It is frequently noted that severe onsets of dental caries follow attacks of general disease and disturbance of bodily health. During pregnancy caries may be unusually active. Children who are under-nourished or who are suffering from a general debility are usually especially prone to dental caries.

*Racial Influences.* Dental caries is more prevalent in certain races than in others. The natives of Africa, South America and the South Sea Islands, the Esquimaux and many other primitive peoples, are notably free from the disease, while those who live in the more civilized lands are extremely susceptible to it. There are evidences that the disease often increases in prevalence as people advance in the scale of civilization. It is also observed that, when persons migrate from a caries-free nation to a country in which it is prevalent, they and their progeny may later develop dental disease. There is no indication that dental caries is an endemic disorder or that it is induced by any climatic conditions. Diet is the only constant variable between immune and susceptible races which thus far has been recognized.

#### RECENT RESEARCHES ON DENTAL CARIES

While dental caries is by no means a modern disease, as shown by previous references, there is no period in history when such an interest in the problem has been shown as at present. With the advances made in the science of nutrition and knowledge of the function of the vitamins, and as the other elements of the diet become better known, scientific research has now more than ever before been

directed toward this problem. In the last ten years reports of the investigations of this subject by a wide variety of research workers have occupied a prominent place in the medical and dental literature. The popular press has given much space to the discussion of these research reports, indicating the appeal that the subject makes to the public. Because the attack on the problem is being made from various angles and the findings are fragmentary, different schools of thought have arisen giving rise to many conflicting statements as to the cause of this disease. The problem is a complex one, and it is inevitable that these differences must arise. At present, because of these divergent opinions and theories, the popular mind is confused and justly so. The author aims to review briefly the work of the more important workers in this field.

### (1) VITAMIN D AND DENTAL CARIES

Mrs. Mellanby worked with her husband studying the effects of rachitic diets on litters of puppies. She noticed abnormalities in the teeth of these puppies when fed on a diet consisting of separated milk, white bread, orange juice, sodium chloride, yeast and lean meat. When this deficient diet was supplemented with cod liver oil, whole milk, butter fat, or egg yolk, the teeth and jaws developed normally. The observed dental defects were:

1. Soft and poorly developed jaws and alveolar processes in which osteoid tissues often took the place of bone.
2. Teeth, chiefly lower incisors, crowded together.
3. A delay in eruption.
4. A delay in calcification of the enamel.
5. Deficient dentin.
6. The greater the rate of growth, the greater the defects when fat soluble vitamin D was deficient.

These are clearly hypoplastic changes in the dental structure. In a long series of articles over a period of ten years, Mrs. Mellanby tries to show the relationship between hypoplasia of the teeth and dental caries.

Histological studies of the teeth of dogs fed on adequate and inadequate diets show:

1. That good diets produced good enamel and dentin.
2. That after their formation teeth that are free from dental caries have a good grade of secondary dentin, and that teeth that are carious have a poor grade of dentin.

Mrs. Mellanby during recent years reports the results of dietary studies on children. She recommends that children be given diets which have been shown to produce perfectly formed teeth during the calcifying process, and also the continuing of the diet along similar lines after the teeth are calcified. She believes that Vitamin

D is the all-important factor in the calcifying of the teeth. She is opposed to and disagrees openly with those who at present place great stress on Vitamin C and its relation to caries.

She assumes that hypoplasia and caries always correlate, but does not explain why hypoplastic teeth often do not have caries. She reports no studies on the bacteriological factor and rather relegates this to the background, refusing to consider it seriously at all. In all her work she reports hypoplasia in puppies but no instances of caries. The author believes that this is due to the conical shape of the dog's teeth which prevents food substances from adhering to the teeth, and, although the causes of dental caries are present in the dog's mouth, prevents the occurrence of the disease. Mrs. Mellanby gives this no consideration whatever.

## (2) VITAMIN C AND DENTAL CARIES

Percy Howe, Director of the Forsythe Dental Infirmary in Boston, is representative of a class of investigators who feel that Vitamin C is the all-important factor in the cause of dental caries. His first studies were on the micro-flora of dental caries. Later, after the dietary experiments of Zilva and Wells who reported hypoplastic changes in the teeth of guinea pigs fed on scorbutic diets, Howe was attracted to the dietary factors causing dental changes. It seems that by means of a fat-free milk and oatmeal diet, Howe produced the first experimental caries in teeth. When he administered Vitamin C in the form of orange juice, the caries was arrested. This opened up an entirely new field of thought and study.

It is said that Howe produced in monkeys all of the dental diseases with which the dentist ordinarily meets by feeding diets deficient in the antiscorbutic factor. He gives no room to the antirachitic factor, Vitamin D, neither does he lay any stress on the bacteriological factor or to the shape of the teeth.

He suggests the following as preventive dietary measures for dental caries:

1. Liberal amounts of fresh natural foods which possess minerals and vitamins.
2. One pint of milk, which supplies the calcium needed for an individual.

He suggests the ingestion of a pint of orange juice daily.

Milton Theo Hanke of the University of Chicago has corroborated the findings of Howe on pulp and dentin changes and has made many beautiful histological specimens. Hanke does not, however, believe that Vitamin C is the all-important factor in dental caries but rather thinks that dentin and pulp changes, as well as those of the periodontal tissues, are profoundly affected thereby. He feels that Vitamin

C is rather a specific for the cure of gingivitis and pyorrhea rather than a specific for caries prevention.

### (3) MINERAL IMBALANCE AND DENTAL CARIES

C. J. Grieves, under the direction of E. V. McCollum at Johns Hopkins University, began a series of studies on calcium and phosphorus imbalance and dental development. Grieves, before his death, mentioned the possibility of endocrine disturbance as a factor in caries. H. Klein has continued the work of Grieves since his death, conducting studies on swine as experimental animals. During the last two or three years he has published several articles which seem to show that many dental changes are due to phosphorus and calcium imbalance.

### (4) HEALTH AND DENTAL CARIES

Harold F. Hawkins of the University of Southern California, has observed the relationship between dental caries, general health, and metabolic conditions. The eating of large amounts of cereals, pastry, and other acid-forming foods, is said to be highly correlated with caries. Those who habitually ate a more alkaline diet were said to be free from caries. He emphasized the relation between acidosis and caries and said that caries can be prevented only by building up a high alkaline reserve. He suggested a diet to furnish this reserve.

### (5) BACTERIA, FOOD AND DENTAL CARIES

Bunting, Professor of Dental Pathology in the Dental College of the University of Michigan, is attacking the problem of the cause of dental caries in a most thorough-going way. His group includes dentists, pathologists, bacteriologists, bio-chemists, and nutrition experts. For many years Bunting has held to the acid theory of the cause of dental caries. He says that the one factor which seems to be correlated with dental caries is the presence of *Bacillus acidophilus* in the saliva, in dental lesions, or in other parts of the oral cavity. Many thousands of patients' mouths at the Dental Clinic in Ann Arbor have been cultured for *Bacillus acidophilus*. Where the organism was present for any length of time, caries was said to develop. This bacillus may also be cultured from active carious lesions. Bunting believes that a high carbohydrate diet, especially one rich in sugar, lends itself more readily to fermentation by acidogenic organisms, thus starting the carious lesions in the teeth. He believes that this is especially true of irregularly formed teeth where occlusion is abnormal and food debris readily adheres between the teeth, being wedged in and not removed except by mechanical or bacterial action. Such tooth surfaces adjoining such retentive spaces are especially prone to caries.

For the last two or three years this group of workers has been conducting experiments with several groups of children under controlled dietary regimes, as is only possible in orphanages and other like institutions. His report is the most comprehensive yet made of

experiments along this line, with dental disease. The diet in these institutions was fortified with a quart of milk per day and large quantities of fresh fruit and vegetables, with only a minimum of sweets. After a period of many months, very few if any new carious lesions were observed. In a personal communication to the writer, he says, "In one institution over a period of one year and nine months among seventy-five children, we have found two pin-point cavities." These dietary studies have caused Bunting and his group to believe that while *Bacillus acidophilus* may be very much dependent upon constitutional factors, such as state of health and especially nutrition, tooth structure and hardness (or softness), are not important factors in dental caries because clinical findings show that many hypoplastic teeth are not carious. These workers stress the environmental factors which may lend themselves readily to impaction and subsequent fermentation of food debris, with bacterial action. These may be:

1. A high carbohydrate diet.
2. Irregularly arranged teeth.
3. States of disturbed metabolism and nutrition.

### SUMMARY OF HISTORICAL WORK

1. Dental caries is not a modern disease. This is indicated by many studies in paleopathology and paleodontology.
2. This disease has been wide-spread in prehistoric and historic times. At present it is the most prevalent of all human maladies.
3. Primitive peoples seem to have the lowest incidence of caries. With increasing civilization the disease increases.
4. There are several theories as to the cause of this disease.
  - (a) The inflammation theory.
  - (b) The worm theory.
  - (c) The electrical theory.
  - (d) The acid theory.
  - (e) The theory of metabolic disturbance (Nutritional):
    - (1) Vitamin C deficiency.
    - (2) Vitamin D deficiency.
    - (3) Calcium phosphorus imbalance.
    - (4) A combination of metabolic disturbances.
5. Present day investigation is attacking the problem from a variety of angles of approach. The reports of these investigators are still fragmentary, and various schools of thought have arisen.
6. Studies of this subject are still in their infancy. When all factors have been considered by competent investigators, our concept of the disease, *dental caries*, bids fair to be more complete than at the present time.



7. The rat may be used to advantage as an experimental animal in the study of dental caries.

#### EXPERIMENTAL

Various investigators report the occurrence of dental caries in the monkey, guinea pig, and rat. May Mellanby showed the effect of high cereal diets on the teeth of litters of puppies. She states that comparable results have been found in the rat. To observe the effect of certain diets on the teeth of the rat, presumably with the occurrence of caries, and to find a method for producing this disease

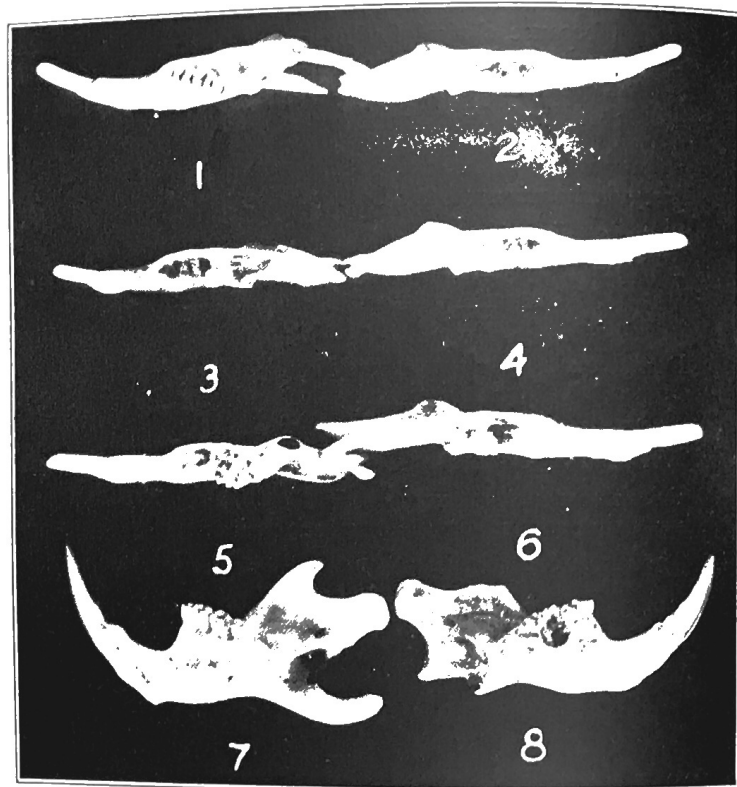


Fig. 1. Mandibles from Rats Receiving Stock Ration. Nos. 1 and 7 are normal; all others show caries in different degrees of severity.

that it might be more thoroughly studied with its various causative factors, was the purpose of this study. The method of attack was to feed high cereal diets comparable to those which May Mellanby found to produce hypoplasia in puppies.

#### PROCEDURE

*Animals Used.* Animals used were the Albino and Piebald strains of rats of known heredity, whose parents were raised and maintained

on a stock ration consisting of, 60 per cent whole yellow corn meal, 30 per cent whole milk powder, 6 per cent linseed oil meal, 3 per cent alfalfa leaf meal, 1 per cent sodium chloride. A total of sixteen series, with some 275 animals, was used.

*Care of Animals.* These animals, weighing from 40-60 grams each and averaging twenty-eight days of age, were placed in circular screen cages ten to twelve inches in diameter and twelve inches in height. The cages were of the raised-bottom type, thus keeping the animals from access to the feces. They were placed on granite pans whose bottoms were covered with removable paper. The cages were cleaned twice per week. The ration was placed in containers so devised as to keep the rats from scratching out but a minimum of the ration. Distilled water in drinking tubes was given daily to

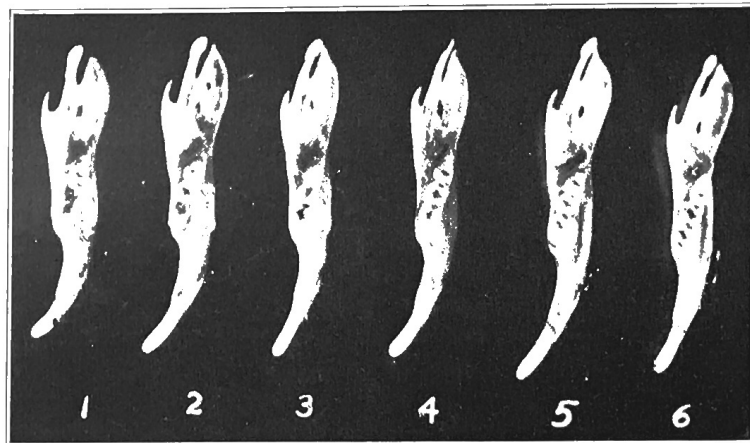


Fig. 2. Mandibles from Rats Receiving Corn Meal in Different Degrees of Fineness. Nos. 1, coarse corn meal; 2, corn meal retained by a 20-mesh sieve; 3, corn meal retained by a 40-mesh sieve; 4, corn meal retained by a 60-mesh sieve; 5, corn meal passing through a 60-mesh sieve; 6, ground oatmeal.

the animals on the experimental ration. Two or three rats of different litters were placed in the same cage, and compared with litter mates on the same diets or variations of the diet. Negative and positive controls were used when necessary and advantageous. The rats were weighed weekly, and weights recorded.

*Observations on Animals.* Animals after being on the ration for from six to twenty-two weeks were etherized, their mandibles and skulls were removed and kept for further observations. The humeri and femurs of many of the animals were removed and, after continuous extraction with hot alcohol for a seventy-two hour period, were ashed and the percentage of ash determined. A technique for the examination of the teeth of living rats was devised allowing ob-

servations of the teeth to be taken at regular intervals, showing clearly the time of the occurrence of the disease and the rate of the progress of the same. Macroscopical and microscopical studies were made of the teeth.

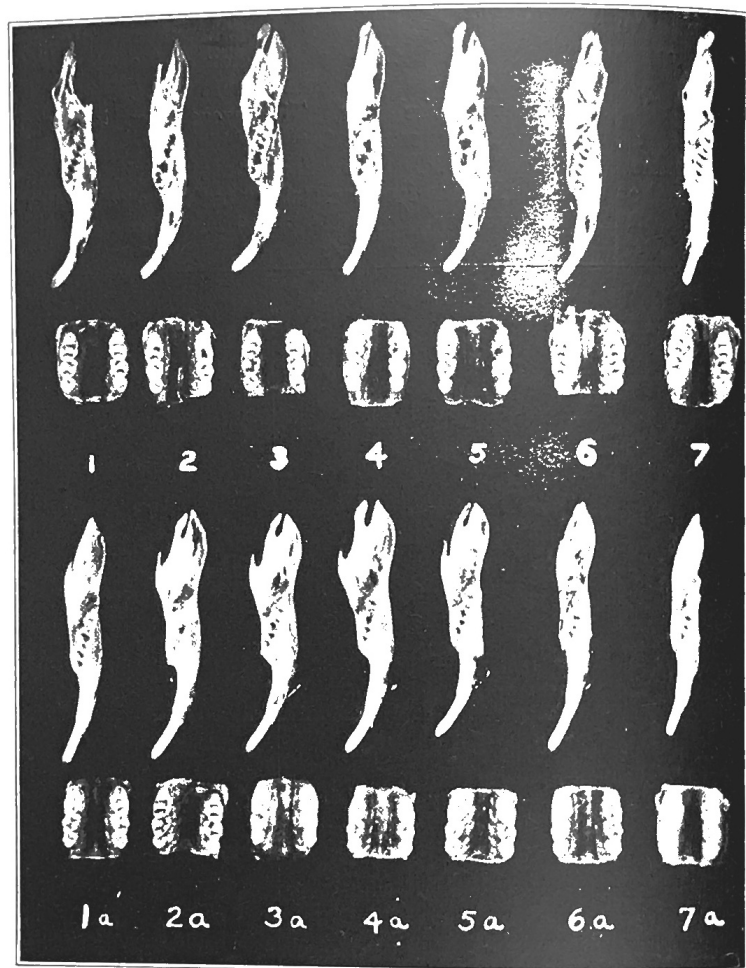


Fig. 3. Comparison of Corn with Rice and Hard Wheat in the Production of Dental Caries in Rats. Nos. 1-7 are from rats with calcium carbonate in the diet; Nos. 1a-7a from rats without the calcium carbonate. Nos. 2-5 are upper molars showing distinct cavities. Nos. 1 and 1a, oatmeal; 2-3 and 2a-3a, corn; 4-5 and 4a-5a, rice; 6-7 and 6a-7a, wheat.

*Preparation of Diets.* The grains, as the yellow corn and oats, were medium-finely ground in our own mill. The vegetables used were bought fresh and dried at a temperature which maintained as

far as possible the vitamin content. After drying they were finely ground in a Wiley mill.

The various constituents of the diet were weighed and mixed thoroughly. The rations were placed in the specially prepared cups and kept constantly before the animals. Cod liver oil and orange juice were pipetted and administered directly to the individual animals. Some animals were irradiated twice weekly under a mercury vapor light at a distance of fifteen inches for two minutes.

#### DESCRIPTION OF RESULTS

The basal diet selected for the initial studies was characterized by its high cereal (oatmeal), low calcium content, which certain investigators have found to produce poor skeletal as well as poor dental development. It was hoped that a high incidence of dental caries might accompany the condition of poor dental development. Animals on the basal diet showed decidedly subnormal growth and low ash

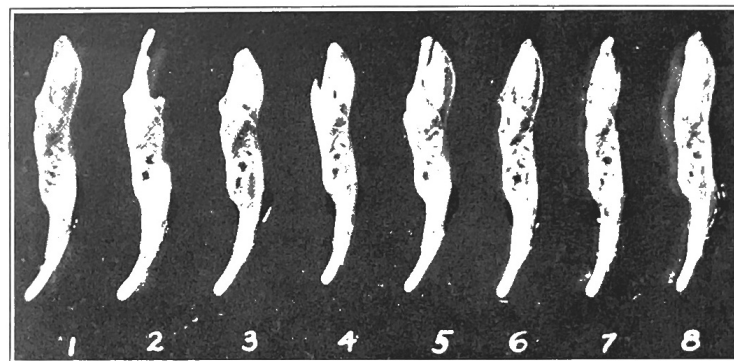


Fig. 4. Mandibles of Rats Receiving Stock Rations with Supplements of Various Dietary Factors. Nos. 1, oatmeal; 2, coarse corn meal; 3, coarse corn meal plus 1% cod liver oil; 4, coarse corn meal plus 1% calcium carbonate; 5, coarse corn meal plus 1% tricalcium phosphate; 6, coarse corn meal plus 1cc. orange juice daily; 7, coarse corn meal plus 15-20 grams fresh cabbage daily; 8, coarse corn meal plus 15-20 grams fresh carrots daily.

content of the bones. Ash determinations on the humeri and femurs gave the following results with the basal diet: humeri, 38.6%; femurs, 35.0%; as compared with humeri, 50.0%; femurs, 50.0%, on normal animals fed the stock ration. That the teeth of the animals on the basal ration were abnormally soft was apparent by the excessive wearing away of both the lower and upper incisors, the latter showing characteristic cupping. In no case was there the slightest indication of dental caries.

The inclusion of vegetables improved somewhat the growth and bone development of the experimental animal, although growth was still far below normal. Again no caries was observed. The addition of calcium carbonate, of cod liver oil, or the exposure of the animal

to ultra-violet irradiation resulted in practically normal growth and bone development. Caries was not observed in these animals.

When the basal diet was supplemented by various sugars, such as sucrose, glucose, and lactose, in amounts up to 20% of the ration, there was still no indication of dental decay. Dental caries, however, was observed simultaneously in animals in which the basal oatmeal diet was modified by replacing the oatmeal with potatoes (boiled, dried, and ground) and in the case of the animals on the stock ration. Inasmuch as the occurrence of dental caries on the stock ration was



Fig. 5. Photomicrograph of Longitudinal Section of Carious Mandibular Molars. One is impacted with food.

more surprising, it was decided to study the stock ration thoroughly to determine the cause of the development of dental caries in this ration.

In view of the emphasis placed upon certain dietary factors such as Vitamins A, C, and D, for proper dental development, the stock ration was supplemented by what was considered liberal amounts of these factors, supplied as cod liver oil and orange juice. These

factors, however, had no influence whatsoever in preventing the appearance of caries.

The addition of calcium carbonate or of tricalcium phosphate failed also to have any effect in preventing dental decay. Glucose and sucrose added to the extent of 20% to the diet did not seem to increase the severity of the carious lesions. In order to make observations on as many animals as possible, use was made of a large number of animals belonging to the stock colony. Of 140 animals examined, 127 or approximately 90% had teeth in various stages of decay.



Fig. 6. Photomicrograph from Ground Section of Teeth after Imbedding in Georgia Rosin. Note that by this method enamel is preserved. The dark-stained areas in the dentin show the softening effect of caries.

Since caries was observed with such regularity in animals on the stock ration, it was desirable to know how early dental decay began. Accordingly, two series of young animals after weaning were placed on the stock ration, and animals were selected from these and killed at weekly intervals from six weeks and onward. Dental decay was found as early as six weeks, although in some cases there was no evidence of caries in animals killed as late as ten or eleven weeks.

Inasmuch as this method did not permit observations of the progress of the caries to be made, it was felt imperative to devise

some means of examining the teeth in the living animals. Accordingly animals were anesthetized and strapped on a rack.

With this improved technique, further observations were made as to the time of the first appearance of dental caries. At this time it was observed that particles of food were impacted in the interstices of the lower molars and in the carious lesions. On closer observation these particles proved to be corn meal. From this it appeared that the impacted corn meal might be the determining factor in the production of caries in the rats.

From observations previously made, indicating that in diets containing oatmeal as the sole cereal caries never developed, it seemed desirable to replace the corn meal by oatmeal, as well as by feeding definite mixtures of these cereals. The results of these series indicate in a striking way that, as the percentage of corn meal is increased, the incidence of caries followed in almost mathematical exactness. Equally striking was the total absence of caries when oatmeal alone was used in the diet.

Corn meal of 40 mesh fineness and that passing the 60 mesh was used. In the ration in which corn meal finer than 60 mesh was used, no caries was found. This was also true of the ration in which oatmeal was substituted for the corn meal. Corn meal ground to 40 mesh produced caries but of less severity than regular stock ration corn meal.

#### DISCUSSION

Disturbed nutritional balance, as lack of mineral or vitamin content, has been reported as a factor in the cause of dental decay in experimental animals. It has been assumed that poor skeletal and dental development accompany each other. Investigators do not agree as to what nutritional factor is most important, some holding that fat soluble Vitamins A and D are most important. Still another group is quite as certain that Vitamin C is the only determining factor. Still others believe it to be a combination of several nutritional factors. In this experiment, caries did not develop in diets known to be inadequate as evidenced by very poor growth and soft teeth. In striking contrast, a high incidence of caries has been produced in a diet adequate in all the nutritional factors known.

In all the nutritional studies that have been reported, the physical characteristics of the diet used seem to have been totally disregarded. While it is not the purpose of this discussion in any way to minimize nutritional factors, attention is called to another factor, namely: the mechanical or physical nature of the diet. This has received little or no attention.

That particles of corn meal, and other foods of a like consistency and size, impact in the teeth of rats, has been clearly proven by many observations on dead and living animals. In cases where food impactions have been noted in living animals, later caries has been

observed in the same spots. In the diets used in this experiment, the foods which were of the consistency to impact most readily in the teeth were medium or finely ground corn meal, dried potatoes, and medium-finely ground wheat. These particles were also of such size that they lent themselves readily to mechanical impaction in the interstices of the teeth, later to lead to fermentation, acid production, decalcification of the enamel and open carious lesions of the enamel and dentin. Bacteriological studies made on the mouths of these rats showed the presence of acidogenic micro-organisms. The histological specimens show impactions of vegetable tissue in the carious lesion. It is believed that no changes from within the tooth, as of the tooth pulp, odontoblastic layer of cells, change in dentin, etc., from those of normal teeth can be seen in the histological studies made.

Oatmeal and finely ground corn, being of a softer and finer consistency, do not have the physical characteristics which might lend themselves to mechanical impaction, and therefore the absence of dental caries in animals on these diets.

The contour of the molars of the rat lends itself readily to the impaction of food particles of the proper consistency and size. When these particles are of a kind to be potential sources of fermentation, it is believed that caries will be produced. Bunting calls attention to the fact that while dental hypoplasia has been repeatedly reported in puppies by May Mellanby, yet no dental caries has been observed. This he believes to be due to the contour of the teeth, and even suggests the impossibility of dental caries in the dog due to the shape of the teeth. In clinical practice caries has been observed in practically the same location on the same teeth in children of the same family who have inherited a similar dentition.

It has been emphasized over and over again by dental clinicians that carious lesions occur most frequently in the pits and fissures of the occlusal surfaces and on certain areas of the approximal, buccal, and lingual surfaces of the teeth, at which locations there are opportunities for stagnation and retention of foreign matter. They do not occur on smooth enamel surfaces that are frequently cleansed.

It is possible that the beneficial effects to the teeth reported by workers in human nutrition, when large quantities of vegetables, fruit and fruit juices are ingested, may be due to the cleansing effect of those constituents of the diet as well as to the strictly chemical or nutritional factors. Diets upon which dental improvement has been observed, and those in which dental decay has been prevented, are those in which the physical characteristics are such as to have a cleansing effect on the teeth. When large quantities of fruits, vegetables, and milk made up the bulk of the diet, the intake of carbohydrate is lowered, thus giving less opportunity for impaction of potentially fermentative food elements. This seems to be a factor overlooked by workers in this field of investigation.

Dental caries thus far observed and reported by McCollum (54), Bracco (73), Bunting (74), Marshall (75), and Knowlton (76) have



always been in the mature animal. Marshall believes the caries observed in these adult animals to be comparable to the caries of adult humans; and that active dental caries, while present in adult human beings, is recognized as preeminently a disease of childhood. Caries in this experiment has been found regularly in immature animals, advanced caries being observed in individuals but six weeks on the diet.

It is believed that a method of experimentally producing caries in the immature rat has been found. This makes possible a study of the chemical, histological, and bacteriological changes of the teeth of such animals. This should be most welcome to investigators interested in these phases of the problem.

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## ADDENDUM

Since this paper was read before the Tennessee Academy of Science, developments have come that cause us to present the following:

*Was the production of caries in our rats due to a deficiency of phosphorus in the diet?*

In recent comment by Klein and McCollum,<sup>1</sup> exception was taken to the explanation that caries in our work<sup>2</sup> was caused by the retention of particles of corn meal by the teeth, with subsequent bacterial action;

TABLE 1

*The Lack of Relationship Between Phosphorus Content of Diet and Incidence of Caries*

DIET	PARTS	P	CA	CA/P RATIO	CARIES	AVERAGE OF FEMURS AND HUMERI
		Gram	Gram		Per Cent	Per Cent
I. Corn meal.....	69	0.2001	0.0096	0.263	90-100	43.5
Wheat gluten.....	20	0.0400	0.0156			
Whole-milk powder.....	5	0.0420	0.0490			
Crisco.....	5					
NaCl.....	1					
	100	0.2821	0.0742			
Same diet, with 1 per cent CaCO <sub>3</sub> ..		0.2821	0.4742	1.68	90-100	50.4
II. Oatmeal.....	69	0.2994	0.0762	0.41	0	45.5
Wheat gluten.....	20	0.0400	0.0156			
Whole-milk powder.....	5	0.0420	0.0490			
Crisco.....	5					
NaCl.....	1					
	100	0.3436	0.1408			
Same diet, with 1 per cent CaCO <sub>3</sub> ..		0.3436	0.5408	1.57	0	51.9
III. Stock ration*..... (Coarse cornmeal)		0.4802	0.3424	0.7	90-100	56.2

\*Values given by Klein and McCollum.

and that the fineness of the meal was an important factor in the production of caries. Klein and McCollum suggest that the caries in our experiments was due rather to a deficiency of phosphorus in the diet. While it is true that the corn meal passing through a 60-mesh screen was richer in phosphorus (owing to removal of some of the

<sup>1</sup>Klein and McCollum: *Science*, 74: 662; December 25, 1931.

<sup>2</sup>Hoppert, Webber, and Canniff: *Science*, 74: 77, July 17, 1931.