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TITLE A Study of the Influence of Certain Dietary Constituents on the Development of Dental Caries in Rats

AUTHOR Thomas Lester Canniff

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# A STUDY OF THE INFLUENCE OF CERTAIN DIETARY CONSTITUENTS ON THE DEVELOPMENT OF DENTAL CARIES IN RATS

by

Thomas Lester Canniff

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TABLE OF CONTENTS

TABLE OF CONTENTS

- - - -


| | | |
|---|---|---|
| INTRODUCTION | | 1 |
| HISTORICAL | | 4 |
| ETIOLOGY OF DENTAL CARIES | | 5 |
| REVIEW OF LITERATURE | | 10 |
| PART I | Development of the Caries Producing Diet. | 20 |
| | Experiment A | 25 |
| | Experiment B | 30 |
| | Experiment C | 35 |
| | Tables and Charts for Part I | 39 |
| PART II | The Effect of Vitamin D on the Experimental Production of Caries | 48 |
| | Experiment 1 | 49 |
| | Experiment 2 | 49 |
| | Experiment 3 | 50 |
| | Tables and Charts for Part II | 56 |
| Part III | The Effect of Feeding Various Carbohydrates on the Development of Caries | 62 |
| | Experiment 1 | 62 |
| | Experiment 2 | 66 |
| | Experiment 3 | 69 |
| | Experiment 4 | 70 |
| | Experiment 5 | 72 |
| | Experiment 6 | 75 |
| | Experiment 7 | 77 |
| | Experiments 8 & 9 | 80 |
| | Tables and Charts for Part III | 84 |
| BIBLIOGRAPHY AND LITERATURE CITED | | 102 |


PLATES

- - - - -


A. Diagram of Molars in Man and Rat 5a
B. Diagram of Molars Showing Scoring Areas 23a
C. Photograph of Carious and Non-Carious Molars 24a
D. Photograph of Graded Series of Mandibles 24b
E. Photograph of Graded Series of Mandibles 24c
F. Photograph of Graded Series of Mandibles 24d

# INTRODUCTION

# Introduction

Any study involving human beings and the control of their dietary habits presents at the very beginning certain difficulties and complications that are often insurmountable. One of the very significant factors is that the life span of the individual is too lengthy to be able to include the complete picture desired in some experimental studies. Control of dietary habits is extremely difficult unless the individuals participating are confined to an institution where there is little opportunity for indulging in food preferences. The cooperation of the subjects is not always what might be desired. Consequently, when feeding experiments are to be conducted, workers usually try to substitute experimental animals in which these difficulties can be eliminated. However, such selection must be made keeping in mind the fact that response to treatment should be reasonably comparable to the human response. Such a task is not always easy, for, differences in dietary habits, physiological structure, environment, and psychological factors may vary widely from one species to another.

The white, or albino rat has been a most satisfactory substitute for the human in many feeding experiments. They are prolific, thrive on a great variety of diets, have a short life span, and are small and easily handled. In the study of dental caries, all these factors are favorable.

The physiological structure of the rat molar cannot be said to be identical, but at least is comparable, and develops lesions that are very characteristic, and from an histological standpoint seem to be quite similar to carious lesions in human beings.

The purpose of the investigation was to study the experimental production of dental caries, and the influence of certain dietary factors on the development of such lesions in the albino rat. In this study, the work has been divided into three parts:

I. Development of the Caries Producing Diet.

This part of the work sought to establish an adequate diet (one on which the animals would have normal development, reproduction, and lactation), which would cause the development of dental caries in a certain definite period of time. Mechanical features were also considered.

II. The Effect of Feeding Certain Vitamin Supplements.

This part of the work sought to determine mainly whether a supplement of these vitamins had any appreciable effect in preventing or retarding dental caries.

III. The Effect of Feeding a Diet Containing High Levels of Certain Carbohydrates.

This part of the work was done to observe the effect of a high carbohydrate content of the diet on the production and development of carious lesions.

Carbohydrate was supplied in the form of Sucrose, Glucose, Lactose, and Corn Syrup.

# HISTORICAL

and

# REVIEW OF THE LITERATURE

## Historical and Review of the Literature

To write a discussion of the history of dental caries would practically be to write the history of the development of man. Dental caries is one of the oldest and commonest diseases of man. References to its existence have been traced back as far as the 22nd Century B.C.. The skull of a mummy in the British Museum, dating 2800 B.C., exhibits well marked signs of caries and other dental diseases. In the various records of man's progress and development, one finds quite frequent reference to the fact that dental caries occurred, no matter what the degree of civilization provided their diet included cooked starchy foods. Attempts to determine the cause of dental caries, or to find a method by which it might be prevented have, until recently, met with little success. Some of the earlier methods had to do with feeding certain naturally occurring substances, which would indicate that a few of the earlier scholars felt the disease to be one caused by a deficiency of some of the elements required by the body for the production of good bones and teeth.

It was not until the latter part of the 19th Century that any orderly method of study was suggested, although relatively little was accomplished beyond the accumulation of theories. Starting about 1920, several groups in this country and abroad began a series of studies on the causitive factors in dental caries. Since that time, the field

has proved to be a most prolific one, and much has been done in the way of clarifying the causes and treatment of the disease. One of the pioneer groups of this field was the Michigan Group Research on Dental Caries, headed by Dr. Russell W. Bunting (7). Their work has been most outstanding. Other groups such as the one headed by Drs. Klein and McCollum at Johns Hopkins University, by Dr. Rosebury and co-workers at Columbia University, and Dr. Hanke's group in the Chicago Dental Research Club of Chicago, Illinois, have done very noteworthy work in this field. In addition to such groups, there have been many outstanding individuals whose contributions have added materially to our knowledge and understanding of the disease. Among these workers, the names of Mellanby, the Agnews, Bodecker, Boyd, McBeath, and Macy should be mentioned, and there are many others. It is significant to the progress of research that this problem brought together people from many fields such as dentistry, physiological chemistry, nutrition, and bacteriology. With such a widely diversified group, much more thorough and successful work could be expected. A great deal has been accomplished but there is still much to be done before the problem begins to assume the nature of completion.

The Etiology of Dental Caries: In discussing the etiology and causes of dental caries, one should first consider the structure of the tooth and how a carious lesion develops. In the studies carried on with rats and reported in this

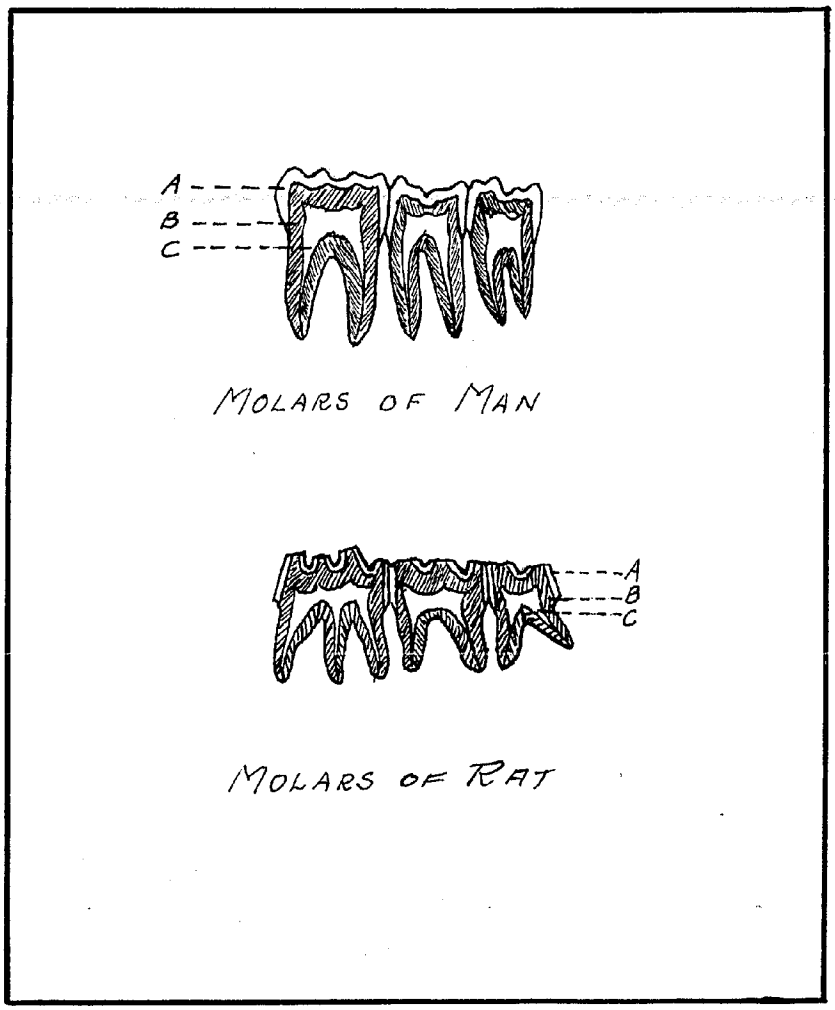


PLATE A

Diagrammatic Representation of Molars in Man, and Rats. A-- Enamel; B-- Dentin; C-- Pulp. Note the enamel covers entire crown in Man, while in Rats the Dentin is exposed at the molar cusps.

paper, we were mainly concerned with the structure and make-up of the molars. Caries of the incisors in rats is quite rare, and the experimental production of caries involves for the greater part the lower molars, and to a slight extent the upper molars. The structure of the tooth is divided into three main sections: the Enamel, the Dentin, and the Pulp. The enamel layer normally covers the outer exposed portion of the tooth, next comes the dentin, with the pulp comprising the innermost portion. A diagrammatic drawing of the lower molars of man and rat is shown in plate A.

This schematic comparison shows quite clearly the striking similarities and the significant differences in the structure of the molars of these two species. Note that the enamel layer in the molars of man covers the entire crown of the molar. In the rat, the dentin is exposed at the summit of the molar cusps. Occlusal fissures in the rat molar are relatively deeper and wider (actually they are similar in size in the two species). Rat molars approximate over a surface, rather than at a point as in human molars. This last feature may be held accountable for the relative infrequency of interproximal caries in rats (36).

In the development of a carious lesion, it is generally accepted that the process of excitation begins with the development of a bacterial colony on the surface of the tooth, which is frequently referred to as a plaque. This plaque is a thick, felt-like mass in which the micro-organisms are

imbedded. The bacteria in the plaque require food, and obtain it from the carbohydrate and albuminous materials which come in contact with them. Lactic acid is one of the conspicuous products formed in the bacterial fermentation of carbohydrates. The plaque prevents the excreted acid from being washed away, and, as a result, erosion of the tooth by acid begins to take place. The acid attacks the inorganic matter of the enamel, first the interprismatic cement substance between the enamel prisms or rods, later dissolving the transverse cement substance between the enamel globules. Thus, as the interprismatic cement substance is dissolved, crevices are formed by which the bacteria eventually reach the dentin. When the entire thickness of the enamel has been penetrated and the dentin is attacked, there is a change in the mode of progress of the decalcification. The process procedes along the junction of the enamel and dentin, as well as directly into the dentin. The enamel may now be attacked from its dentinal side as well.

When the dentin is attacked, we find a different chemical and anatomical structure to be acted upon. Beneath the enamel, the first layer of dentin is of such nature as to permit the bacteria to rapidly spread laterally in this zone. They also enter the dentinal tubules and penetrate by multiplication toward the pulp. A wedge shaped area of decay is produced. In most cases, decalcification precedes these

invasions.

In the rat molar, the dental caries developed are of the type known as fissure caries. They are the typical dental caries of molar fissures and are histologically similar to fissure caries in man, and establish the susceptibility of rats to dental caries. Four stages in the progress of these lesions have been described: (1) Penetration of the enamel through the enamel lamellae, or diffusely in the absence of lamellae; (2) spread at the dentinoenamel junction; (3) penetration of the dentin by infiltration of the tubules and disintegration of the dentin matrix; and (4) loss of surface continuity and formation of the cavity. These lesions can be distinguished from other destructive processes in rat teeth, one of which seems to result from the impact of dense food particles in teeth weakened by a rachitic condition, producing fracture through the cuspal dentin primarily, followed by secondary caries-like changes. The latter is not comparable to dental caries in man (35).

From the above discussion, one would be lead to believe that the formation of a bacterial plaque was essential for the production of dental caries. However, lodgement or impaction of food may serve equally well to provide conditions favorable for initiating tooth decay. Breese (5) says food lodgement is an indispensable factor in the production of caries. Sweets in their many forms, owing to their sticky nature, are the chief causes of food lodgement. Haber and co-workers (14) found in research in the Youth Movements in

Germany, that the incidence and progress of caries, also film and tartar formation, increase as the degree of mastication decreases. However, Rosebury and co-workers (32) found that fissure caries was caused primarily by food particles rich in carbohydrate that were impacted under strong masticating pressure, and that the particles not being subsequently dislodged furnished a pabulum for acid producing bacteria. These workers also found that the primary agent in fissure caries in rats (impaction) also causes the proximal type caries, so it may be that the etiology of both types follows an analogous pattern.

Klein and McCollum (22) believe the cause of macroscopic caries in rats to be due to (a) fracture of the molar cusps; (b) decay at the bottom of molar sulci (which the writer believes most frequently follows the impaction of food particles); (c) interproximal caries where food impaction has been frequently noted; and (d) a combination of all three factors. They found that the ingestion of a rachitogenic diet containing coarse corn meal gave carious lesions, whereas the same diet containing finely ground corn showed absence of caries.

Mellanby (31) questions whether or not caries begins as a process of decalcification, or as disintegration of protein, and states that there is no sound evidence as to the immediate cause of caries. This latter remark seems somewhat questionable in the light of all the work that has been done and the evidence that has been submitted.

The importance of heredity should probably be mentioned. Bunting and co-workers (7) believe inherited tendencies, or inherent individual characteristics in some cases are more important determining factors in caries than ordinary dietary conditions. Hunt and Hoppert (19) in their work on the role of inheritance in rat caries have been able to develop a resistant line and a susceptible line. Although the strains are probably not homozygous, they have quite definitely shown that the characteristics of resistance or susceptibility are inherited.

Review of the Literature: In reviewing the literature on Dental Caries, the first publication that should be mentioned is the review compiled for the Research Commission of the American Dental Association by the Advisory Committee on Research in Dental Caries. The theories on the cause of dental caries are numerous, but when stripped of the modifications elaborated by the many workers, they may be grouped into three principal categories: (1) Theories based on the structure of the teeth; (2) theories based on the dietary picture; and (3) theories based on the bacteriological picture. With this classification as a basis for the review, we may greatly simplify the mass of research that has been done.

For many years, caries was associated primarily with two factors--the tooth itself, and the hygienic condition of the mouth. It was felt that if, during the period of

growth and development of the teeth, the supply of calcium and phosphorous was adequate a good tooth structure was certain to follow. A sound, well calcified tooth was not supposed to decay if kept clean. However, we now find that our thinking must be somewhat revised. Mellanby says (31) that the type of tooth structure is related to diet during the development, and that there is a certain definite association between tooth structure and susceptibility to caries. Hypoplasia certainly does involve an inferior tooth structure, but this is far from necessarily a prime factor in caries. Cox (8) believes that enamel structure is a dominant factor in preventing initiation of caries. Gottlieb (13) feels that the degree of calcification is of some importance in the control and incidence of caries. Rosebury and co-workers (36) report that they found caries in rats that were healthy, well grown, and in teeth without appreciable structural defects.

In considering the hygienic condition of the mouth, one of the most important factors is the oral fluid or saliva. Certainly, the saliva would normally contain varied flora. For the bacteriological picture, it is advisable to wait until later in the review. Branson (4) states that the basic property underlying both immunity and susceptibility to caries is rendered comparatively simple when two attending local physiological factors, in addition to the bacteriological aspect, are clearly understood. One of these is the

ability of saliva under normal conditions to protect teeth effectively against caries. Koehne and Bunting (23) found that on a fine diet impacted food removed from the rat molars was cement-like and dry. If the impacted food was impermeable to the oral fluids, its bacterial decomposition could not take place. This substantiates Hoppert's theory as to why no caries resulted when rats were fed a fine diet (20). Further, Bunting and co-workers (7) found there was no consistent relationship between hardness or perfection and the state of mouth hygiene and the activity of caries. Florestano (10) postulates that the diastatic activity of the saliva, carried to all dental surfaces, is the main factor in the prevention of caries. Hanke (15) says the difference in caries-immunes and caries-susceptibles is due locally to the quality of their salivas. The buffering of saliva is thought by Hanke to be highly important. The Ann Arbor Group (7) has found no correlation demonstrable between amounts of salivary calcium, phosphorus, chlorides, pH, carbon dioxide capacity, total alkalinity, total solids, or ash and the activity of caries. Hanke (17) was unable to show any correlation between the pH of various regions of the mouth and dental caries.

The idea that dental caries was a disease of dietary deficiency is an old one. It was thought that certain inorganic constituents were required in order to lay down the necessary deposits in tooth and bone structure that made

them well formed and hard. Such elements as calcium and phosphorus were believed the most important because of the role they play in bone structure. To attribute this idea to any individual worker or group would be impossible--rather was it the opinion of the majority of workers. Naturally, then, these were two factors that were first studied.

Fleisch (9) says caries is a deficiency disease. Forshufvud (11) says caries is ordinarily an expression of disturbed metabolism, often due to inferior diet, but more frequently to excessive irregularity in diet and mode of living. However, there has been much work done that refutes the idea of caries as a deficiency disease. Rosebury from his work with rats (36) believes that dietary deficiency is not a determining factor in the etiology of fissure caries. Hanke (17) finds there is no apparent correlation between total calcium and soluble phosphate content of the blood serum and the incidence of dental caries. Further, he has stated that a superior diet of the average American child will not protect completely against caries, and that the juice of citrus fruits contains something (Vitamin C) required by a child to maintain oral health. LeFevre (26) has found that there is little difference in composition between fresh, human carious teeth, and sound ones, except in moisture content.

Lilly (27) believes that caries in rats is not

definitely related to a deficiency of any known food factor. McCollum and co-workers (30) think it not yet possible to name any one dietary deficiency as the specific cause of dental or oral disease, but do suggest that it may be a multiple or complex deficiency. They feel that any slight variation in the average American diet--which always so dangerously approaches the level of deficiency--might become active at any period of lowered resistance, or of physical or of nervous stress. McClendon (29) has shown that sound teeth contain more fluorine than carious teeth, and that the addition of from 5 to 22 parts per million of sodium fluoride to a fluorine deficient diet delayed the onset of caries. Bunting and co-workers (7) found there was no relationship between the intake of calcium and phosphorus, or acid/base dietary values and the activity of caries. They feel that there is no evidence that caries is primarily produced by malnutrition, or may be prevented by an adequate diet. The subject of the influence of carbohydrates should undoubtedly be mentioned as a dietary factor, but because of the mechanism through which it acts, mention will be made of it in the bacteriological phase of the discussion.

Bacteria have been definitely proved an important factor in the production of dental caries. Dr. Jay and co-workers of the Ann Arbor Group (7) have done outstanding work on this phase of the problem. They have found that the relative numbers of lactobacillus acidophilus in the

mouths of individuals with caries is relatively greater than in caries free individuals. Belding and Belding (2) consider caries to be a bacteriological disease subject to interpretation in terms of the laws of bacteriology and immunology. They view caries as a relatively specific disease occuring in those who partake of modern diets and have oral food retention areas. Rosebury and Karshan (33) have found aciduric bacteria, similar to L. acidophilus, to be normal inhabitants of the rat mouth, their presence being constant and independent of the feeding of human lactobacilli. Mellanby (31) suggests that certain bacteria play a part in the progress of caries, but regards questionably whether or not they initiate caries. Wessinger (37) believes caries due to localized action of various organic acids produced by mouth organisms from carbohydrates. He feels, however, that no single organism can be regarded as the sole etiologic factor, but that it is probably a symbiosis among many organisms that may account for the extremely rapid acid formation in the mouth. All the work on this phase of the problem has led to the use of the term B. odontolyticus in referring to those organisms which cause or contribute to the breakdown of dental tissue that is referred to as dental caries.

The role of carbohydrates then becomes quite apparent. Rosebury and co-workers (32) believe that carbohydrates in general enrich the acid yielding substrate, and that the

addition of sugar to a caries producing diet tended irregularly to increase the incidence of caries. Lilly (27) showed that high carbohydrate diets containing finely ground corn or corn starch would not initiate caries. Hanke (15) states that oral bacteria convert cane sugar and other carbohydrates into acid. Cox (8) found that fermentable carbohydrates promoted the enlargement of existing carious lesions, but that they had no effect with diets that initiate caries. Florestano (10) postulates that complex carbohydrates, under the salivary diastatic action, form simpler sugars required by aciduric micro-organisms for acid production. Branson (4) suggests that the perverting influence of refined carbohydrates--sugar especially--in breaking down the salivary defense thereby promotes fermentive processes, bacterial activity and propagation, thus virtually inviting caries development. Belding and Belding (2) state that increase in susceptibility to caries is due to a relative or absolute increase in the pathogenicity of oral flora and ensuing accelerated formation of acid, rather than by any change in the affected tooth that makes it less resistant to the action of caries producing acids. Sucrose seems to favor a shift in the nature of the normal oral flora resulting in a strain that will cause practically any of the carbohydrates consumed to be rapidly converted into acid. Cereals are rapidly fermented producing large amounts of acid. Thus, the simultaneous consumption of sugar and cereals, particularly in

refined forms, causes an increase in the relative and absolute streptococcic pathogenicity. This is probably the chief cause of the excessively high caries rates of civilized man. The sugar establishes the flora, whereas the cereals provide the chief source of acid for enamel decalcification. Bunting and co-workers (7) have found sugar to be a very important causitive factor in caries. A remarkably low degree of caries was found in children living on a diet low in sugar, even though the diet was deficient in calcium, phosphorus, and vitamin D. Active caries was induced by increasing the sugar intake while receiving a diet nutritionally adequate. Ingestion of a low sugar diet by children is conducive, as a rule, to freedom from caries.

If, as would be indicated from the amount of study, and as would seem very plausible, the incidence of caries is due to a bacterial activity, then the use of certain types of inhibitors might be valuable. Blayney (3) tested the effect of specific inhibitors of bacterial metabolic activities of bacteria on tooth surfaces. It was presumed these inhibitors would reduce the incidence of caries. This conclusion was tested using fluoride and iodo-acetate on the development of caries in the albino rat. Using a coarse particle diet which induces a high rate of caries, it was found that those animals whose diet contained the inhibitors showed a marked reduction in the incidence of carious lesions. Cultures from a small series of carious lesions showed both L. acidophilus and acid producing

streptococci with the latter in marked preponderance. Jay (7) states that an immunologic principle related to L. acidophilus has been demonstrated in the blood of caries free individuals, in whose mouth as a rule L. acidophilus does not exist, and when planted therein promptly disappears. It seems very probable that some of the most promising future work might well lie in the field of bacteriology.

In connection with the nutritional aspects of dental caries, the vitamins C and D seem to present the greatest interest. Vitamin D has long been felt to exert considerable influence on oral health, while vitamin D and its association with bone development, rickets, and calcification make it of importance. Brodsky and co-workers (6) found that massive doses of vitamin D, in addition to a well balanced hospital diet, resulted in marked diminution in the incidence of new carious lesions in some 200 children. Cox (8) and co-workers found increased caries immunity in young rats resulted after feeding haliver oil to mothers during pregnancy and lactation, in excess of amounts necessary to prevent rickets. Hanke (15, 18) found that vitamin C in preventing gingivitis prevents regions of stagnation which in turn lowers the incidence of caries. Lilly (27) reported that rats on vitamin A and B deficient diets developed no carious lesions, and that the addition of vitamin D had no effect on the incidence of caries. Rosebury and co-workers (32) report that the feeding of vitamin D reduced the incidence of caries without preventing it. Hoppert et al (20)

found that liberal additions of vitamins A, C, or D did not appreciably retard the decay of the teeth. This would point to a lack of direct relationship between these factors and the caries producing process.

# EXPERIMENTAL

## PART I

## DEVELOPMENT OF THE CARIES PRODUCING DIET

## Part I: Development of the Caries Producing Diet.

In the development of a caries producing diet, the first and most important factor to be considered is the nutritional value of the diet. In order that the picture of the cause of dental caries be not confused with a poor nutritional background, it was essential that the diet used should produce normal growth, reproduction, and lactation--the latter being one of the principal criteria in judging the adequacy of a diet. The second factor is of a mechanical nature, for it was found that apparently one of the essential requirements of a caries producing diet was that impaction of food particles in the teeth of the rat must precede the development of the lesions. This being the case, it was indicated that the physical nature of the diet--that is, the size of various particles in the diet--might contribute to the success of the diet in producing carious lesions.

The first factor was quite fully demonstrated in the work of a previous investigator, Dr. P. A. Weber, whose work along with some preliminary work of this study has already been published (20). A controversial factor appeared at this point concerning the cereal portion of the diet. The cereal portion of the diet was made up of yellow corn ground in a Wiley Mill using the coarse sieve. The ground corn was a composite mixture as far as particle size was concerned. As the cereal came from the mill, it was

composed of particles ranging in size and texture from that of flour fineness to coarse sand. A sample of this ground corn was passed through a series of graded sieves. 250 grams of this ground corn was found to grade as follows:

| | | | |
|---|---|---|---|
| Portion remaining on 20 mesh sieve... | 66 grams | 26.4% |
| " " " 40 " " ... | 110 " | 44 % |
| " " " 60 " " ... | 51 " | 20.4% |
| " passing through 60 " " ... | 15 " | 6 % |
| " lost (retained in sieves) ... | 8 " | 3.2% |
| TOTAL | 250 grams | 100.0% |

Some investigators felt that in feeding, the rats selected the finer portions of the ground corn, and discarded the coarser particles. In so doing, they were apt to upset the mineral balance found naturally occurring in the whole kernel of the corn. However, in the addendum to the publication by Hoppert, Webber, and Canniff (20), the possibility of caries being due to a deficiency of phosphorus was shown unlikely. The incidence of caries quite clearly appears to be independent of the phosphorus content of the diet. (See Table 1, taken from publication of Hoppert et al (20).)

To demonstrate the possible effect of particle size as a factor, a series of experiments was set up in which the cereal portion of the diet was supplied by the various fractions obtained by sifting the corn (ground in the Wiley Mill using the coarse sieve) through sieves of graded mesh as above mentioned.

Another point to be considered was the variability of the corn used. The corn kernel was found to vary widely,

TABLE I

Data showing lack of relationship between phosphorus content of the diet and incidence of caries.

| Diet | Parts | P | Ca | Ca/P ratio | Caries | Average of Femurs & Humeri |
|---|---|---|---|---|---|---|
| | | gram | gram | | per cent | |
| I. Cornmeal | 69 | 0.2001 | 0.0096 | | | |
| Wheat gluten | 20 | 0.0400 | 0.0156 | 0.263 | 90-100 | 43.5 |
| Whole-milk powder | 5 | 0.0420 | 0.0490 | | | |
| Crisco | 5 | - | - | | | |
| NaCl | 1 | - | - | | | |
| | 100 | 0.2821 | 0.0742 | | | |
| Same diet, with 1% $CaCO_3$ | | 0.2821 | 0.4742 | 1.68 | 90-100 | 50.4 |
| II. Oatmeal | 69 | 0.2994 | 0.0762 | | | |
| Wheat gluten | 20 | 0.0400 | 0.0156 | | | |
| Whole-milk powder | 5 | 0.0420 | 0.0490 | 0.41 | 0 | 45.5 |
| Crisco | 5 | - | - | | | |
| NaCl | 1 | - | - | | | |
| | 100 | 0.3436 | 0.1408 | | | |
| Same diet, with 1% $CaCO_3$ | | 0.3436 | 0.5408 | 1.57 | 0 | 51.9 |
| III. Stock Ration* coarse cornmeal | | 0.4302 | 0.3424 | 0.7 | 90-100 | 56.2 |

* Values given by Klein and McCollum.

from time to time, in moisture content, hardness, and other features. Thus, it seemed desirable to substitute for the corn some other cereal that would be more constant in nature, thus eliminating such variations. Rice seemed to possess the qualities desired, and a series of experiments was conducted to determine the effectiveness of this grain in the experimental production of caries. Rosebury and others had used rice and found it quite effective.

With these things in mind, the series of experiments in Part I was set up to clarify the following points:

A. The effect of particle size of the cereals used on the incidence of carious lesions.

B. The period in which the impaction of food particles was at a maximum, and so produced more caries.

C. The effectiveness of rice as compared with corn in the experimental production of caries in rats.

The feeding experiments in Part I, as well as Parts II and III, are all established on the same general plan. Young animals were used when weaned, at 21 to 28 days, having attained a weight of from 50 to 60 grams. They were placed on the experimental diets immediately or at such time as the plan of the experiment directed. The period of experimental feeding was from five to ten weeks depending on the information desired. At the end of the period, the animal was destroyed, the mandibles removed, cleaned, and filed for

scoring. If the upper molars showed any carious lesions, the maxillary plate was clipped from the skull and filed with the mandibles.

The caries score for each animal was computed by assigning a value to each lesion found, the score consisting of the sum total of these values in each animal. A rather arbitrary means of scoring was worked out as follows: Since caries seldom occur in the incisors, the plan of scoring was based on the molars (lower) which are six in number, three on the right and three on the left. Each side was scored separately and recorded. The uppers were scored together since there was seldom more than one cavity. The molars were numbered 1, 2, and 3 beginning at the front. The molar was divided into several areas as suggested by the frequency with which the lesions appeared in the molars. Plate B shows a diagram of the three molars and the designated areas in each molar. A pin point cavity was scored 1, a well defined medium cavity 2, a large cavity 3, this latter score being given as the cavity involved practically the entire area. Plates C, D, E, and F show a series of right mandibles with cavities of graded severity. Although such a method might lead to slightly different scores by various operators, the over-all pictures would be comparable and the averages would make the same conclusions possible. The method is simple, fairly rapid, and gives significant values. Examinations during the feeding period were made

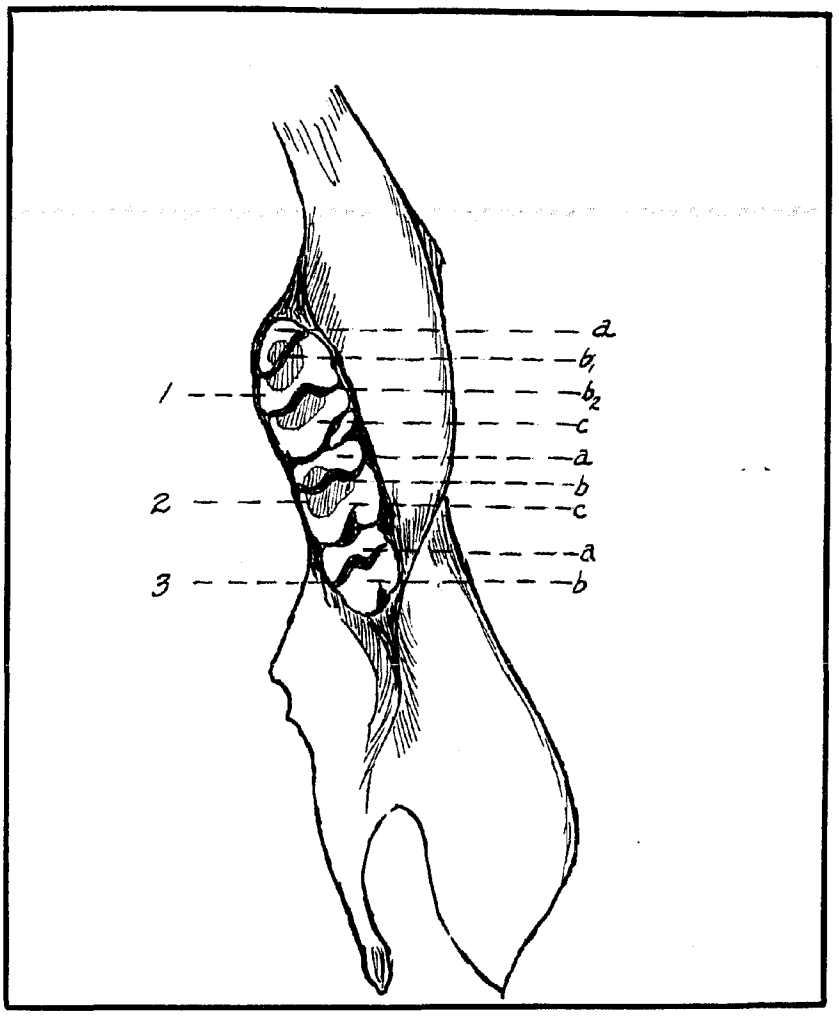


PLATE B

Diagram for Method of Scoring. Lesions are indicated by the red shaded areas. Score would be:

1:a-x,$b_1$-xx,$b_2$-xx; 2:b-xxx. Total = 8.

with the aid of a nasal speculum ground off at the tips. Such an instrument greatly facilitates the examination of the lower molar teeth of rats. The animals can be made to submit to such a gross examination without injury, and after learning to hold the animal properly, the examinations may be done quite rapidly. Two workers are most effective here, one to hold the animal, the other to make the examination.

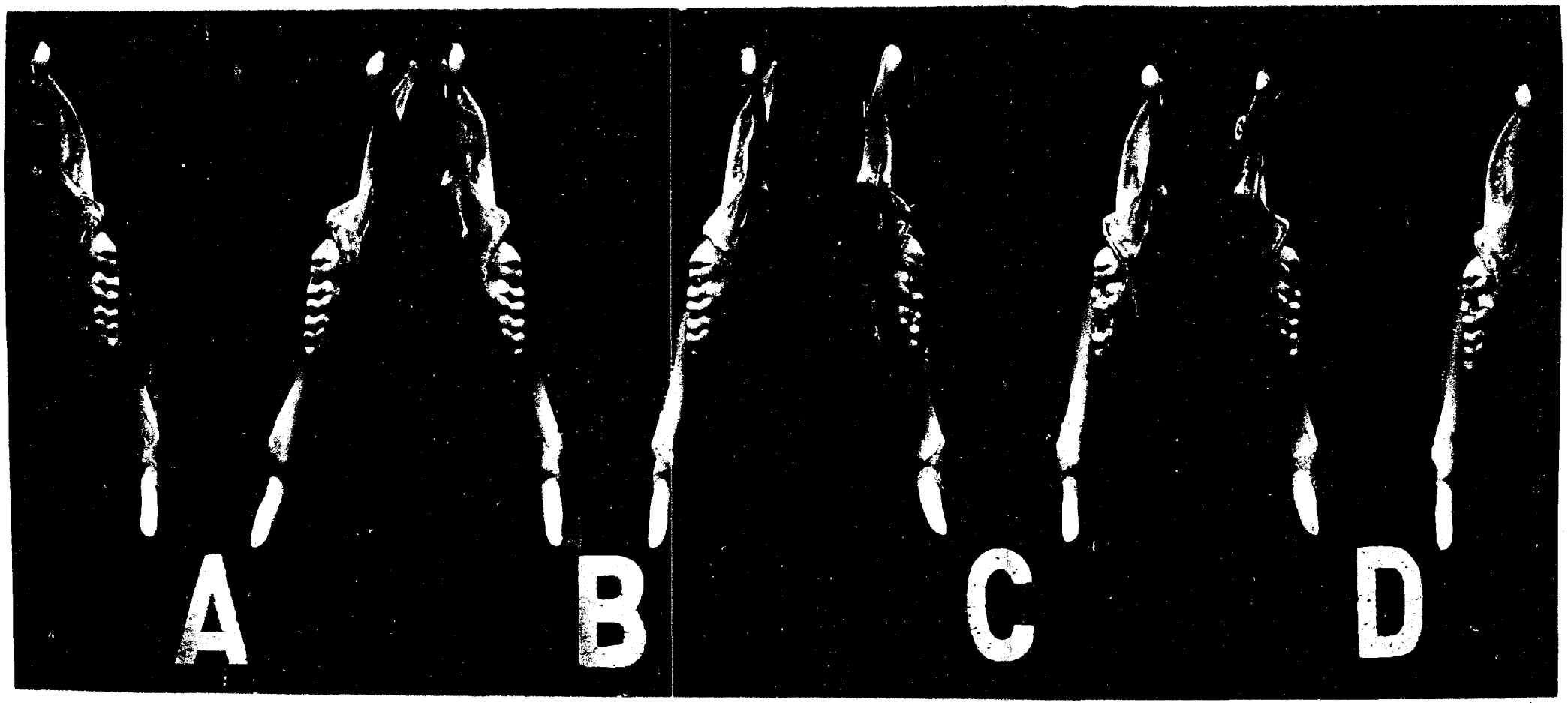


PLATE C

Mandibles of Four Rats.

A & B -- Fed Oatmeal Ration

C & D -- Fed Coarse Rice Ration

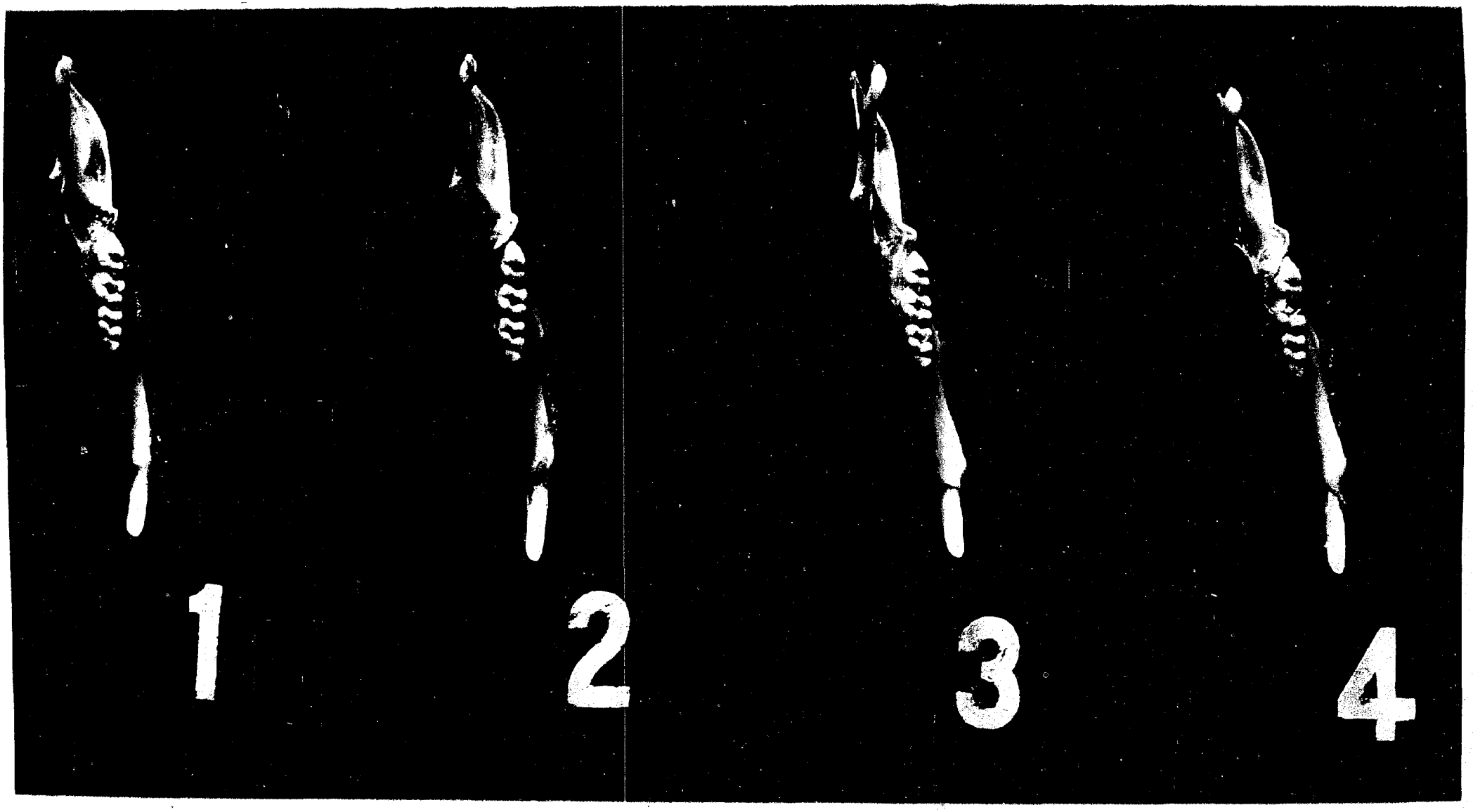


PLATE D
Right Mandibles Showing Carious Molars.
Numbers denote score of each mandible.

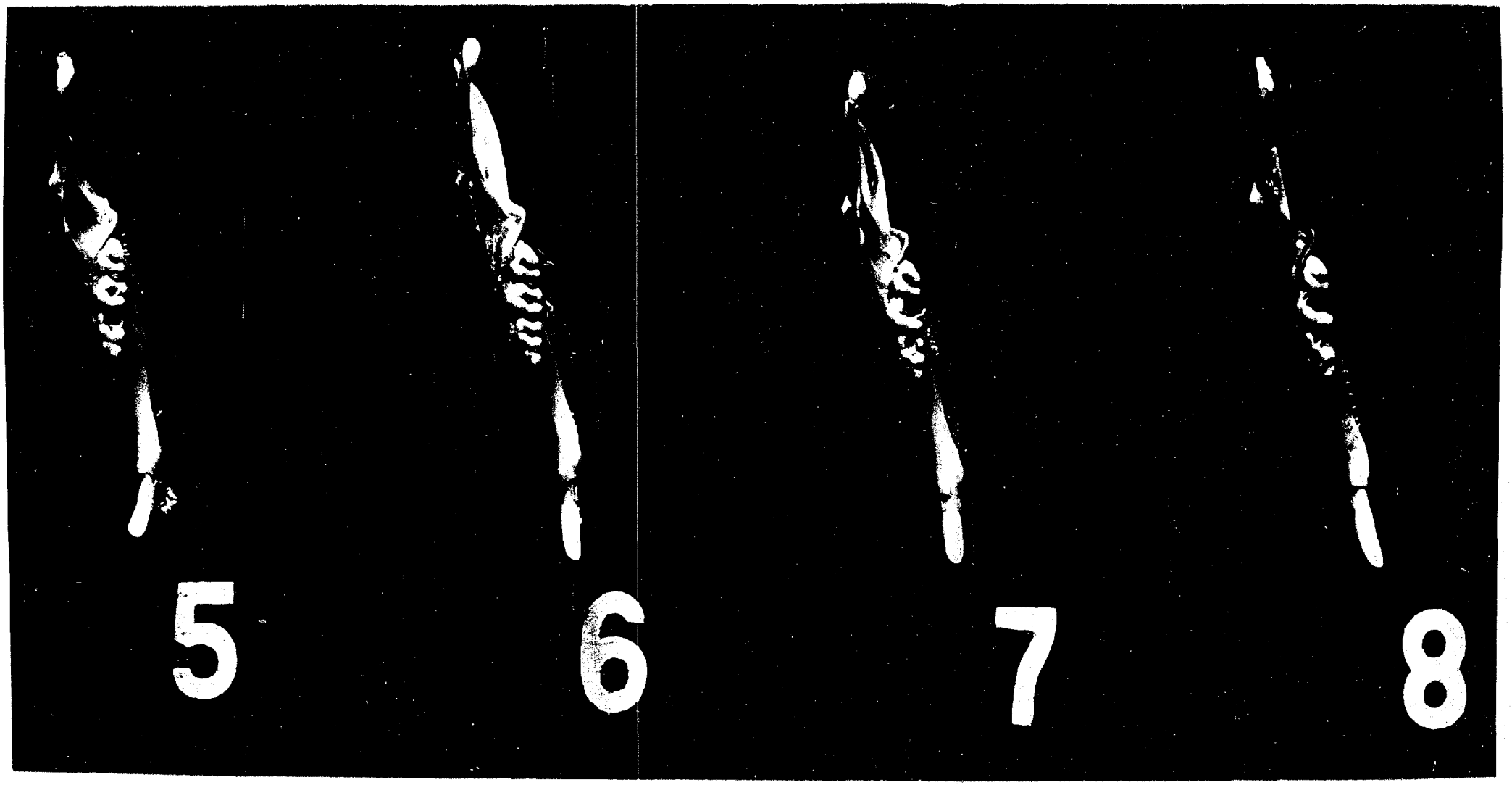


PLATE E
Right Mandibles Showing Carious Molars.
Numbers denote score of each mandible.

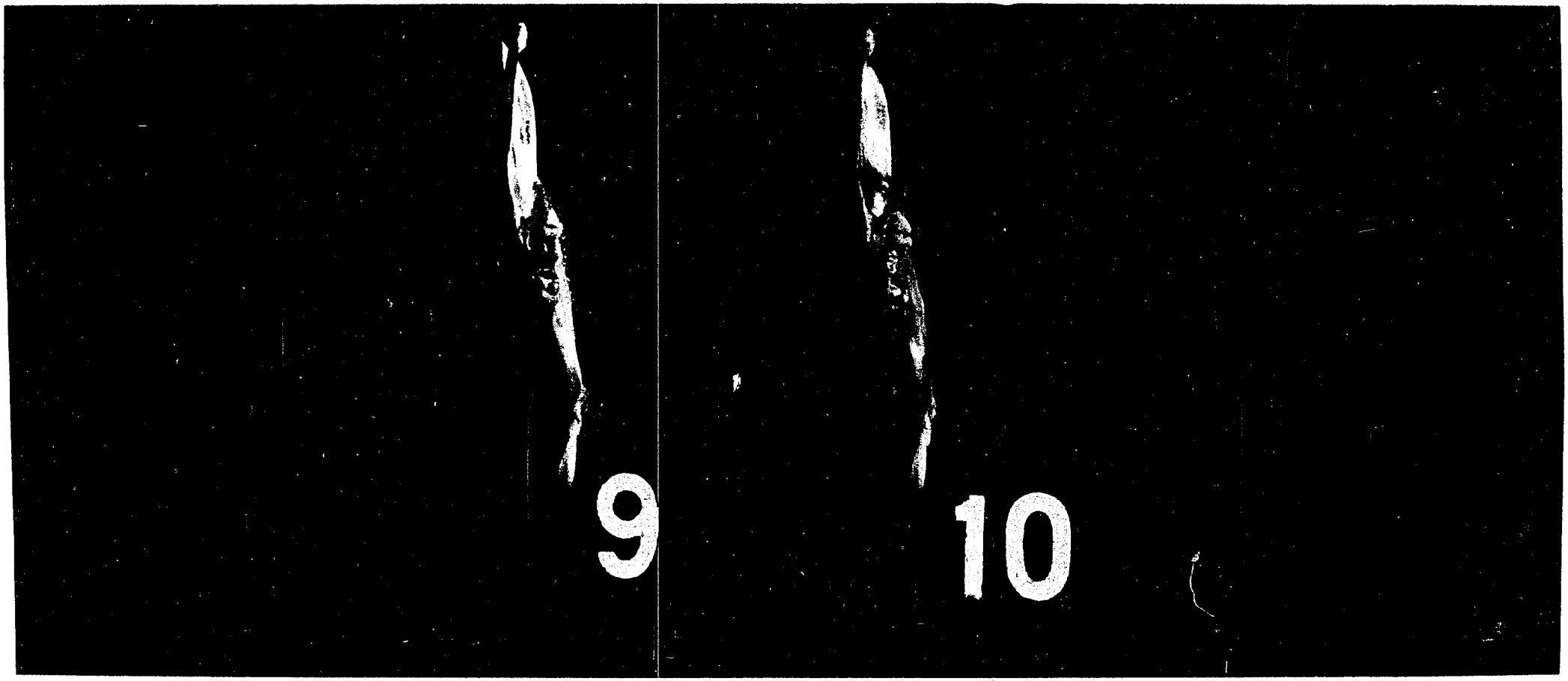


PLATE F
Right Mandibles Showing Carious Molars.
Numbers denote score of each mandible.

## A. The Effect of Particle Size of the Cereal on the Production of Carious Lesions.

Having determined quite conclusively that the carious lesions were due to the impactions of fairly coarse particles of corn in the sulci of the teeth, the following experiment was set up in which the cereal portion of the diet was made up of ground corn of varying degrees of particle size. It was felt advisable to learn something about the effectiveness of the relative particle size in the initiation of the carious lesion.

The corn used in the preparation of the rations was first ground in the Wiley Mill using the coarse sieve. This corn was then screened through a series of three graded sieves: 20 mesh, 40 mesh, and 60 mesh. This screening gave four fractions:

Fraction 1, the portion retained on the 20 mesh screen
Fraction 2, " " " " " 40 " "
Fraction 3, " " " " " 60 " "
Fraction 4, " " passing through the 60 mesh screen

The ration used was based on the following formula:

60% Cereal, 30% Whole Milk Powder, 6% Flax-seed Meal, 3% Alfalfa Meal, and 1% Sodium Chloride.

Using the above formula, six modifications were produced as follows:

1. The corn used was that as it came from the Wiley Mill.
2. The corn used was from Fraction 1 in the above table.
3. The corn used was from Fraction 2 in the above table.
4. The corn used was from Fraction 3 in the above table.
5. The corn used was from Fraction 4 in the above table.

6. The corn used was 2/3 Fraction 2 plus 1/3 Fraction 3 above.
7. The corn was replaced with ground oatmeal as the cereal.

A group of fourteen animals was selected at weaning, and two animals were placed on each of the above diets. The animals were fed the experimental ration for eight weeks. At the beginning of the fifth week, and each week thereafter, the animals were examined and the development and progress of the lesions was observed. At the end of eight weeks, the animals were destroyed, mandibles removed, cleaned, and scored. The scores appear in Table I-1, and the comparison of the scores is shown on Chart I-1.

## Results

From the chart and table of data, the following observations were made: The effectiveness of the diet in the production of dental caries is dependent on the particle size of the cereal. The group fed corn from Fraction 1 showed an average score that was lower than the controls fed the composite mixture, but higher than those fed diets containing the finer corn. The highest score was attained with diets containing corn retained by the 40 mesh sieve (Fraction 2), or with a mixture of the corn from Fractions 2 and 3 as described in modification 6. Animals fed on corn from Fraction 3, or 4 gave the lowest scores. The animals fed on the diet containing the oatmeal showed no caries whatever.

## Discussion

It is interesting to note that the largest particles are not the most effective in initiating caries, but rather the corn which passed through the 20 mesh sieve but was retained on the 40 mesh sieve (Fraction 2). This appears entirely reasonable for it has been shown that impaction must precede the formation of a carious lesion. The particles retained on the 20 mesh sieve were so large that the chance of impaction was materially reduced. That there was some impaction and resulting caries is probably due to the fact that as the molars performed their crushing action on the large particles, the particles were fractured, giving fragments of a size suitable for impaction in the sulci of the molars.

That the most severe caries should be produced with the corn of Fraction 2, or a mixture of Fraction 2 and 3, also seems reasonable. Particles of Fraction 2 were of a size that made impaction in the sulci much easier, much more frequent, and in all probability much faster. Since impactions were more numerous, there was a greater incidence of caries thus giving a higher score. In the case of rat no. 4x was found five distinct lesions, while in 14x, there were four. The mixture of Fractions 2 and 3, in the ratio of 2 to 1 respectively, showed the highest score and the most extensive impaction with nine lesions showing in rat 9x and five in 10x. From this it would logically be concluded that the particles more nearly

approaching in size the dimensions of the sulci of the teeth are the ones that are most effective in initiating the development of the carious lesions.

In the case of those animals whose diet contained only the fine corn impaction assumed an entirely different nature. This brings us to the diet containing the oatmeal which gives a comparable picture although a bit more at the extreme end of the scale. When a diet contains much fine material, we do have impaction but the effect obtained is very different from that obtained with the coarser cereals. Here the material is so fine that it packs into the sulci so solidly as to be practically impermeable to the saliva, whereas the coarse particles permit the free access of the saliva. In the review published by Koehne, Bunting, and Hadley (23) their findings corroborate those of this work. Thus if the material packed into the sulci of the lower molars is impermeable to the oral fluids, its bacterial decomposition will be impossible because of the lack of moisture. The impaction behaves much in the nature of a filling, keeping out the saliva and further impaction of the coarser particles. The impacted material is very dense and so firmly imbedded in the sulci that it may be removed only with difficulty with an explorer or sharp probe.

For experimental production of caries, then, it would seem that the composite mixture as it comes from the mill makes the most effective form of the cereal that may be used. While the total score may not be as great, it gives a truer

picture, and relieves one of the arduous task of sifting that is necessary in grading the cereal. Further, if there should be any possible disturbance in the distribution of the nutritional constituents brought about by sifting, the use of the composite mixture as it comes from the mill would for the greater part eliminate this factor. It must be kept in mind that there has been no attempt to establish any gradation in the caries producing effect of the diet. While ultimately it would be desirable to produce several diets whose caries producing effect might be established as slight, medium, and severe, in these and other preliminary experiments we have only sought a ration with high capacity for the production of caries.

## Summary and Conclusions

To summarize it might be stated that the particle size of the cereal portion of the diet is of definite significance in the experimental production of dental caries.

The particles should not be too large nor yet too small, but somewhere within the range comparable to the dimensions of the sulci of the molars.

The composite mixture of particle sizes as is obtained in a sample of corn coming directly from the Wiley Mill using the coarse sieve, seems to give the best over-all results.

B. To Determine the Period During Which Time Impaction is at a Maximum.

In this phase of the study, it was attempted to ascertain whether or not there was some particular period during the development of the animal when a maximum amount of impaction might take place. In this way, it was hoped that it could be determined at what age the animals showed maximal response to a caries producing diet, or whether there was sometime in the development of the teeth when their structure was particularly sesceptible or resistant to the impaction of food particles which caused the formation of carious lesions.

With this in mind a series of three experiments was conducted on the following plan. Litters of six or seven animals were selected, and at weaning were placed on the following diet:

60% Ground Oatmeal, 30% Whole Milk Powder, 6% Flax-seed Meal, 3% Alfalfa Meal, and 1% Sodium Chloride

One animal from each litter was placed on the caries producing diet which is the same as the above ration except that coarsely ground corn or rice was substituted for the oatmeal. At two week intervals, one animal from each litter was transferred to the caries producing diet for a period of eight weeks. At the end of the eight weeks of experimental ration feeding, the animals were destroyed, mandibles removed, cleaned, and filed for scoring. In case of lesions in the upper molars, the maxillary plate was also removed. At the

completion of the experiment, the "caries score" for each animal was calculated and recorded, and the results of the entire group compared. The distribution of animals used was as follows:

Experiment 1: 3 litters of 7 animals each; Corn as cereal
Experiment 2: 4 litters of 7 animals each; Corn as cereal
Experiment 3: 4 litters of 6 animals each; Rice as cereal

The scores of the group in Experiment 1 are found in Table III-3, with the comparison of the scores shown in Chart III-1. The scores of the group in Experiment 2 are found in Table III-2, with the comparison of the scores shown in Chart III-1. The scores of the group in Experiment 3 are found in Table III-1, with the comparison of the scores shown in Chart III-1. Average of Tables III-1, 2 & 3 are shown on Chart III-2.

## Results

From the data and comparison of the scores of the two series on corn, one finds that as the age of the animal increased, the caries score decreased. In the case of the two series on corn, the decrease seemed fairly consistent throughout. It will be noticed that in the last group there was a slight increase in score. The series on the rice diet in general showed a higher score with more severe caries, and there was one less group than on the corn diet. This brings in a point which was further studied in the next experiments, the relative effectiveness of corn as compared with rice in the caries producing diet. In all cases, whether the diet was corn or rice, the older animals showed

distinct wearing down of the molars.

## Discussion

From the caries scores it would seem that impaction takes place to a greater extent during the first two to four weeks after weaning. Further, as the animal matures and grows older the score decreases enough to lead one to believe that impaction is less. However, the fact that the last group in each series showed an increase in score would lead one to believe that there is a point reached when impaction again increases with a resulting increase in carious lesions. In view of a lack of a comparable group on the rice diet, no conclusions for that cereal are possible.

It would be extremely easy to leave the discussion at this point and draw only the simple conclusions stated above. There are, however, two significant points that must not be overlooked. The first point involves imp action of the fine diet that might have preceded the feeding of the coarse rice and corn diets. It will be recalled that in an earlier discussion on the nature of impactions on a fine diet, it was found that the fine material was very firmly packed into the sulci of the molars. This factor seems most likely to play an influential role in the present experiment. It is highly possible that as the period of feeding on the non-caries producing diet becomes longer, the sulci are eventually fairly well filled with the

impacted fine diet and thus reduces the frequency of impaction of coarse particles when the animal is placed on the caries producing diet. To say, then, that as the animal matures, the structure of the tooth changes making impaction less frequent may not be true. An attempt was made to examine carefully the teeth and see that there had been no impaction or development of caries on the oatmeal diet. However, in view of the difficulties encountered in removing impacted fine diet from molars, even after the mandibles had been removed, cleaned and dried, such attempts as were made to remove impacted material from the molars in situ in the live animal would not always be particularly effective.

The second point that should be considered is that in the older animals a marked erosion of the molars was noticeable. This erosion would tend to decrease the depth of the fissure, and at the same time expose more of the dentin of the tooth. The nature of caries in the last group of animals on the rice diet, and the last two groups on the corn diet appear to be of slightly different nature than in the earlier groups. It appears rather doubtful whether or not true fissure caries developed in these last groups. The work of Rosebury et al (36) most clearly defines the true fissure caries, and was identified most effectively by means of ground sections. Gross examination leads one to believe that in the last groups the type of caries was different due to the larger exposed area of dentin. To

establish this unquestionably ground sections would have to be made at fairly carefully chosen intervals. After the breakdown of the tooth structure has progressed far enough, it is practically impossible to differentiate between true fissure caries and other types of carious lesions.

## Summary and Conclusions

As the animal matures, a change from the non-caries producing diet to the caries producing one shows a decrease in the caries score.

The decrease in the caries score can be attributed to a decrease in frequency of impaction, but the decrease in the frequency of impaction may not necessarily be due to any change in tooth structure.

Other contributing factors are cited and their influence on the results obtained have been discussed.

The last groups show a slight increase in caries score, which may or may not be due to a type of caries other than true fissure caries.

## C. The Effectiveness of Rice as Compared with Corn in the Production of Experimental Caries.

As was mentioned previously, the use of corn in the caries producing diet did not always give consistent results--particularly as to the period of time required to develop carious lesions. This was thought to be due primarily to varying degrees of hardness and moisture content. For that reason, it was deemed advisable to try some of the other cereal grains. Both wheat and rice had been tried, and rice gave promise of being the best selection. Other workers, notably Rosebury, had used rice quite successfully. Accordingly, a comparison was made between the caries producing ration compounded with coarsely ground corn and the same ration compounded with coarsely ground rice. Both grains were ground in the Wiley Mill using the coarse sieve. As the work progressed further, and after it had been demonstrated that rice was a more suitable cereal, the grinding was done on a Hobart Feed Mill setting the adjustments of the burrs at $2\frac{1}{2}$. This gave a much more uniform grind and an average particle size somewhat smaller than from the Wiley Mill.

In this experiment, several groups were used but there is presented here only one of the more typical ones. A group of three litters giving a total of 19 animals was used. At weaning, a selection of 8 animals (4 males and 4 females) was made and these were placed on the caries producing diet containing the coarsely ground corn. The remaining 11 animals

were placed on the same diet in which the coarsely ground rice was used. The feeding was continued for five weeks, at which time 2 animals from the corn group and 3 from the rice group were destroyed. The mandibles were removed, cleaned, dried, and scored. Each week thereafter (i.e. at the 6th, 7th, and 8th weeks) a similar number was terminated and the caries score computed for each animal. The scores were then compared. The scores are found in Table II-1 and the comparison of the scores is shown on Chart II-1.

## Results

In the case of the rice group, the score shows that a more rapid production of caries was noticed at the end of the first five weeks than in the same period with corn. The scores for the entire rice group increase from an average of 2.5 up to 9 at the end of eight weeks. In the case of the corn, the scores run from 0 up to 6.5 at the end of eight weeks. The rice showed higher scores at the end of the 5th, 6th, and 8th weeks. The slight difference in the 7th week may have been due to differences in the animals.

## Discussion

The use of rice as the cereal portion of the caries producing diet instead of corn showed that the effects obtained were equally as reliable, and, in most cases, the response came earlier in the period of feeding. Animals fed the rice thrived and developed just as well, and the diet showed itself to be as adequate nutritionally as when the corn was used. Growth curves were not included in any of

the tables or charts of this paper as they contributed nothing of specific interest to the work, and when an animal failed to gain normally as shown by weight records, it was discarded. In preliminary work on the various diets, the animals were weighed each week to be sure that a normal gain was being made on the diet. Where diets failed to give the normal gain in weight, they were modified to remedy the deficiency, and the proved ration was the one used in the experiments reported here, There were very few instances where this happened, and specific mention will be made of such cases.

The main object of the portion of the work was to establish definitely that rice was as good or better than corn in the diet for production of experimental caries. It was found that rice produced caries more rapidly than corn; that is, that it caused the development of caries quite consistently in the 5th and 6th weeks while the corn produced caries about a week later. This was probably due to the fact that the average particle size of the rice was somewhat smaller than the corn, and thus impaction took place more readily and with greater frequency. Further, the physical characteristics of the rice kernel are apparently much less variable than with the corn kernel which would contribute greater consistency of results both with respect to time and severity of the lesions produced. On this basis, rice was used instead of corn in the subsequent experiments.

## Summary and Conclusions

Rations containing coarsely ground rice were found to produce experimental caries readily.

Caries were found to develop consistently and about a week earlier than with corn and were slightly more severe.

The particle size of the ground rice, as well as the more consistent physical characteristics of the rice, were undoubtedly responsible for the more rapid and somewhat more severe nature of the lesions produced.

It was concluded that rice was superior to corn in the caries producing diet.

TABLE I-1 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1x | F | --- | 3 | 2 | 5 | 6 | Stock Diet with coarse corn |
| 13x | F | --- | 3 | 4 | 7 | | |
| 2x | M | --- | --- | 1 | 1 | 4.5 | Fraction I; Corn retained on 20 mesh sieve |
| 3x | F | 1 | 5 | 2 | 8 | | |
| 4x | F | --- | 4 | 4 | 8 | 7.5 | Fraction II; Corn retained on 40 mesh sieve |
| 14x | M | --- | 3 | 4 | 7 | | |
| 5x | F | --- | 2 | 1 | 3 | 1.5 | Fraction III; Corn retained on 60 mesh sieve |
| 6x | F | --- | --- | --- | 0 | | |
| 7x | M | --- | --- | --- | 0 | 3 | Fraction IV; Corn passed through 60 mesh sieve |
| 8x | F | --- | 4 | 2 | 6 | | |
| 9x | F | --- | 3 | 6 | 9 | 8 | 2/3 Fraction II plus 1/3 Fraction III |
| 10x | F | 1 | 3 | 3 | 7 | | |
| 11x | F | --- | --- | --- | 0 | 0 | Oatmeal Control |
| 12x | F | --- | --- | --- | 0 | | |

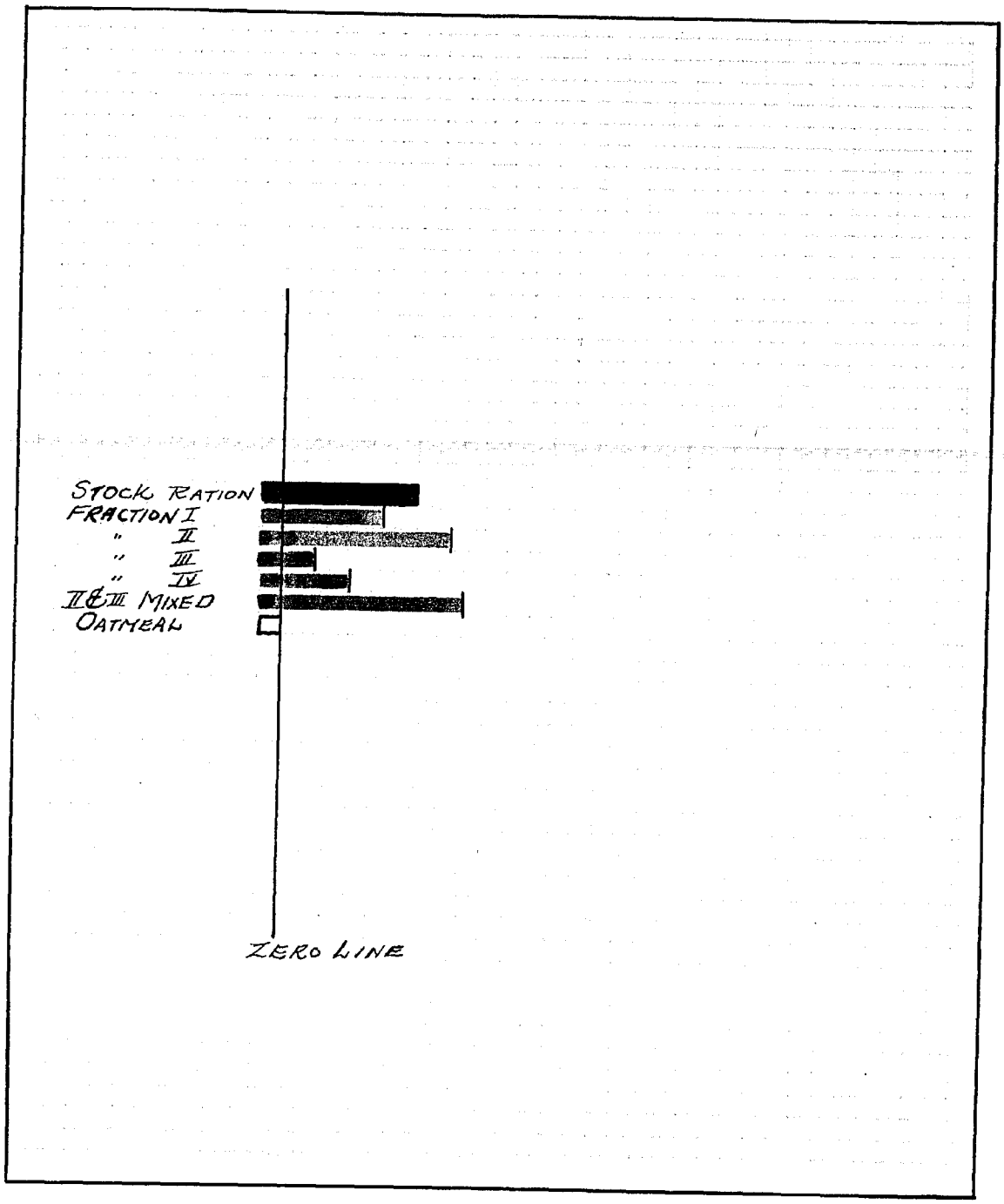


CHART I-1
From Table I-1; Graded Corn Series.

TABLE II-1 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks | |
|---|---|---|---|---|---|---|---|---|
| 1 | M | --- | --- | --- | 0 | 0 | 5 weeks | |
| 2 | F | --- | --- | --- | 0 | | | |
| 3 | M | --- | 1 | --- | 1 | 1.5 | 6 weeks | |
| 4 | F | --- | 2 | --- | 2 | | | Coarse Corn |
| 5 | M | --- | 4 | 4 | 8 | 6 | 7 weeks | |
| 6 | F | --- | 2 | 2 | 4 | | | |
| 7 | M | --- | 4 | 2 | 6 | 6.5 | 8 weeks | |
| 8 | F | --- | 3 | 4 | 7 | | | |
| 9 | M | --- | --- | --- | 0 | | | |
| 10 | F | --- | 1 | 2 | 3 | 2.3 | 5 weeks | |
| 11 | M | --- | 1 | 3 | 4 | | | |
| 12 | M | --- | --- | --- | 0 | | | |
| 13 | F | --- | 1 | 1 | 2 | 4 | 6 weeks | |
| 14 | F | --- | 6 | 4 | 10 | | | Coarse Rice |
| 15 | M | --- | --- | 1 | 1 | | | |
| 16 | F | --- | 3 | 3 | 6 | 5.3 | 7 weeks | |
| 17 | F | --- | 5 | 4 | 9 | | | |
| 18 | M | --- | 6 | 7 | 13 | 9 | 8 weeks | |
| 19 | F | --- | 3 | 2 | 5 | | | |

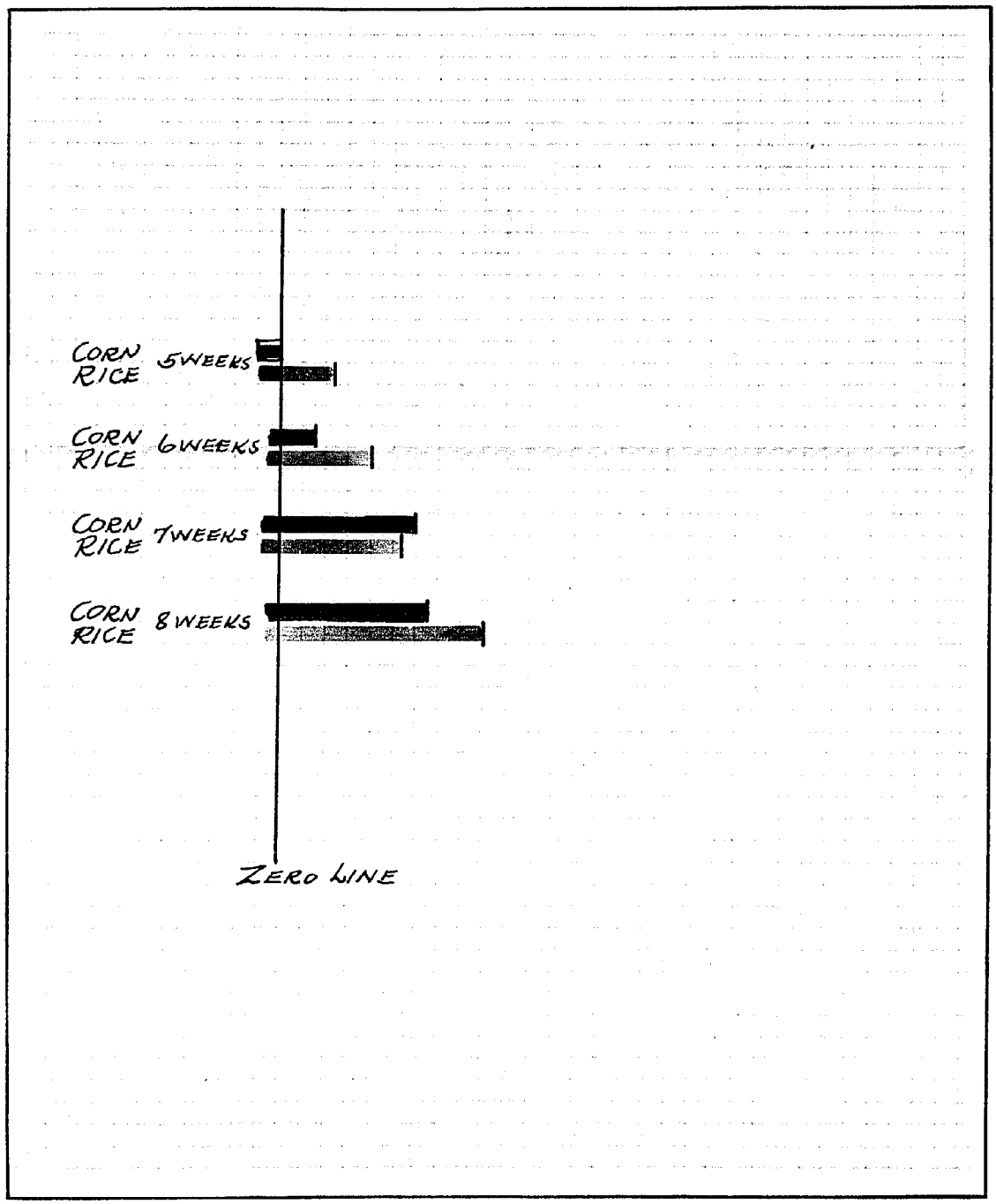


CHART II-2
From Table II-1; Comparative Action
of Corn and Rice.

TABLE III-1 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| | | | | | | | Place on Caries Producing Diet at beginning of: |
| 1 | M | --- | 4 | 5 | 9 | | |
| 2 | M | --- | 5 | 4 | 9 | 7.8 | 3rd week |
| 3 | F | --- | 3 | 3 | 6 | | |
| 4 | F | --- | 3 | 4 | 7 | | |
| 5 | F | --- | 4 | 3 | 7 | | |
| 6 | F | --- | 4 | 5 | 9 | 6.5 | 5th week |
| 7 | M | --- | 2 | 3 | 5 | | |
| 8 | M | --- | 3 | 2 | 5 | | |
| 9 | M | --- | 4 | 5 | 9 | | |
| 10 | M | --- | 4 | 3 | 7 | 7.5 | 7th week |
| 11 | F | 2 | 1 | 3 | 6 | | |
| 12 | F | --- | 4 | 4 | 8 | | |
| 13 | F | --- | 4 | 4 | 8 | | |
| 14 | F | --- | 3 | 5 | 8 | 6.8 | 9th week |
| 15 | M | 2 | 3 | 2 | 7 | | |
| 16 | M | --- | 2 | 2 | 4 | | |
| 17 | M | --- | 3 | 3 | 6 | | |
| 18 | M | --- | 1 | --- | 1 | 6.2 | 11th week |
| 19 | F | --- | 5 | 6 | 11 | | |
| 20 | F | --- | 3 | 4 | 7 | | |
| 21 | M | --- | 2 | 1 | 3 | | |
| 22 | M | --- | 2 | 1 | 3 | 3.8 | 13th week |
| 23 | F | --- | 2 | 3 | 5 | | |
| 24 | F | --- | --- | 4 | 4 | | |

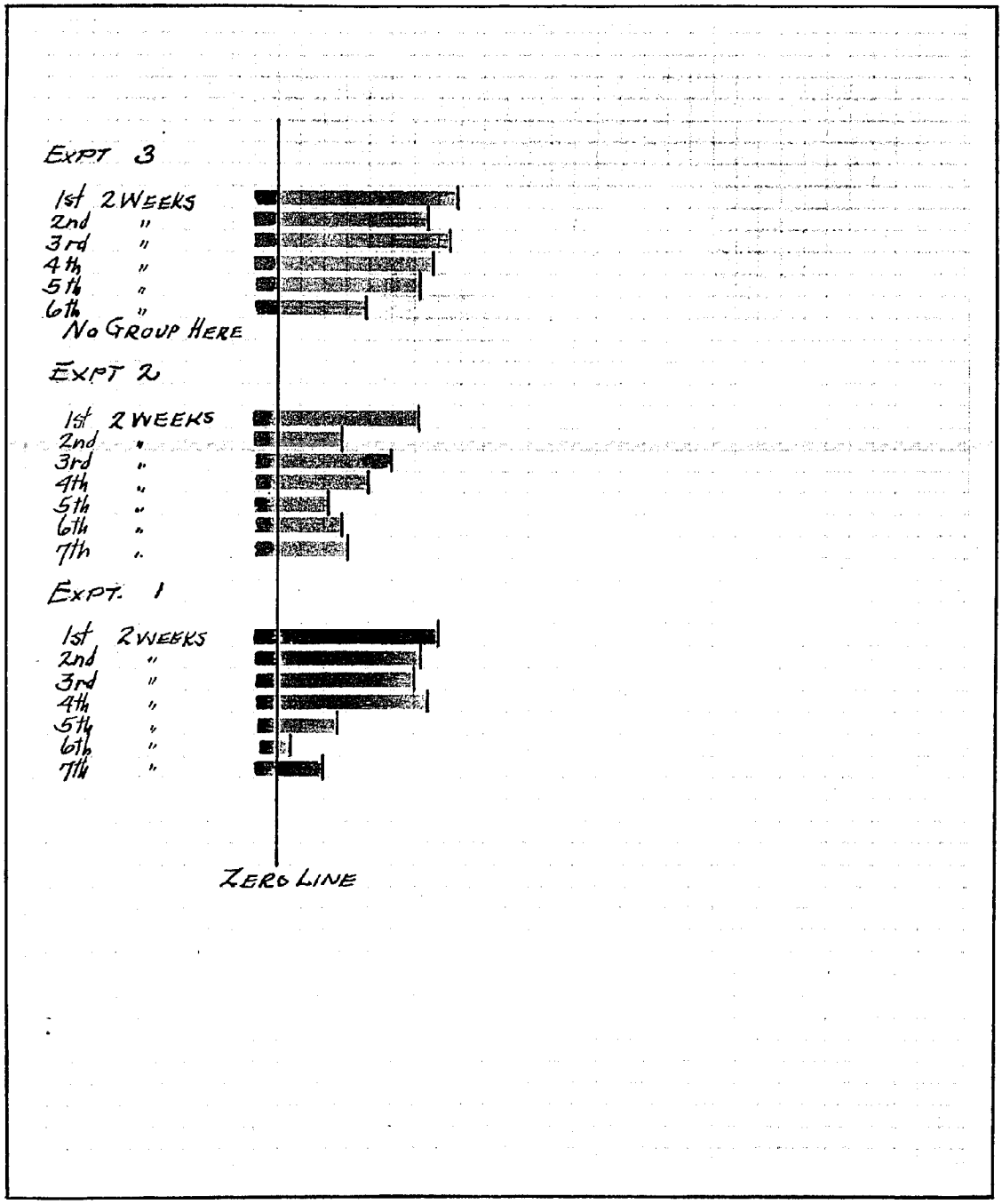


CHART III-1
From Tables III-1,2, & 3; Periods of Impaction.

TABLE III-2 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| | | | | | | | Place on Caries Producing Diet at beginning of: |
| 1 | M | --- | 2 | 2 | 4 | | |
| 2 | M | --- | 3 | 3 | 6 | | |
| 3 | F | --- | 5 | 4 | 9 | 6.2 | 3rd week |
| 4 | F | --- | 3 | 3 | 6 | | |
| 5 | F | --- | --- | 1 | 1 | | |
| 6 | F | 2 | 3 | 1 | 6 | | |
| 7 | M | --- | --- | --- | 0 | 2.8 | 5th week |
| 8 | M | --- | 2 | 2 | 4 | | |
| 9 | M | --- | 2 | 3 | 5 | | |
| 10 | M | --- | 4 | 3 | 7 | | |
| 11 | F | 2 | 2 | 4 | 8 | 5.0 | 7th week |
| 12 | F | --- | --- | --- | 0 | | |
| 13 | F | --- | 3 | 4 | 7 | | |
| 14 | F | --- | 1 | --- | 1 | | |
| 15 | M | 2 | 1 | 1 | 4 | 4.0 | 9th week |
| 16 | M | --- | 3 | 1 | 4 | | |
| 17 | M | --- | --- | --- | 0 | | |
| 18 | M | --- | 1 | --- | 1 | | |
| 19 | F | 1 | 2 | 1 | 4 | 2.2 | 11th week |
| 20 | F | --- | 2 | 2 | 4 | | |
| 21 | F | 1 | 1 | 3 | 5 | | |
| 22 | M | --- | 1 | 1 | 2 | | |
| 23 | M | 1 | 2 | --- | 3 | 2.8 | 13th week |
| 24 | M | --- | --- | 1 | 1 | | |
| 25 | F | --- | 3 | --- | 3 | | |
| 26 | F | --- | 3 | 3 | 6 | | |
| 27 | M | --- | --- | --- | 0 | 3.0 | 15th week |
| 28 | M | --- | 3 | --- | 3 | | |

TABLE III-3

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| | | | | | | | Place on Caries Producing Diet at beginning of: |
| 1 | M | --- | 3 | 3 | 6 | | |
| 2 | M | --- | 5 | 5 | 10 | 7.0 | 3rd week |
| 3 | M | --- | 3 | 2 | 5 | | |
| 4 | F | --- | --- | 3 | 3 | | |
| 5 | F | --- | 4 | 4 | 8 | 6.3 | 5th week |
| 6 | F | --- | 5 | 3 | 8 | | |
| 7 | M | --- | 2 | 2 | 4 | | |
| 8 | M | --- | 4 | 3 | 7 | 6.0 | 7th week |
| 9 | M | --- | 3 | 4 | 7 | | |
| 10 | F | --- | 4 | 4 | 8 | | |
| 11 | F | --- | 3 | 3 | 6 | 6.6 | 9th week |
| 12 | F | --- | 3 | 3 | 6 | | |
| 13 | F | --- | 1 | 4 | 5 | | |
| 14 | F | --- | 1 | 2 | 3 | 2.6 | 11th week |
| 15 | M | --- | --- | --- | 0 | | |
| 16 | F | --- | --- | --- | 0 | | |
| 17 | M | --- | 2 | --- | 2 | .66 | 13th week |
| 18 | M | --- | --- | --- | 0 | | |
| 19 | F | --- | 3 | --- | 3 | | |
| 20 | M | --- | 1 | 1 | 2 | 2.0 | 15th week |
| 21 | M | --- | --- | 1 | 1 | | |

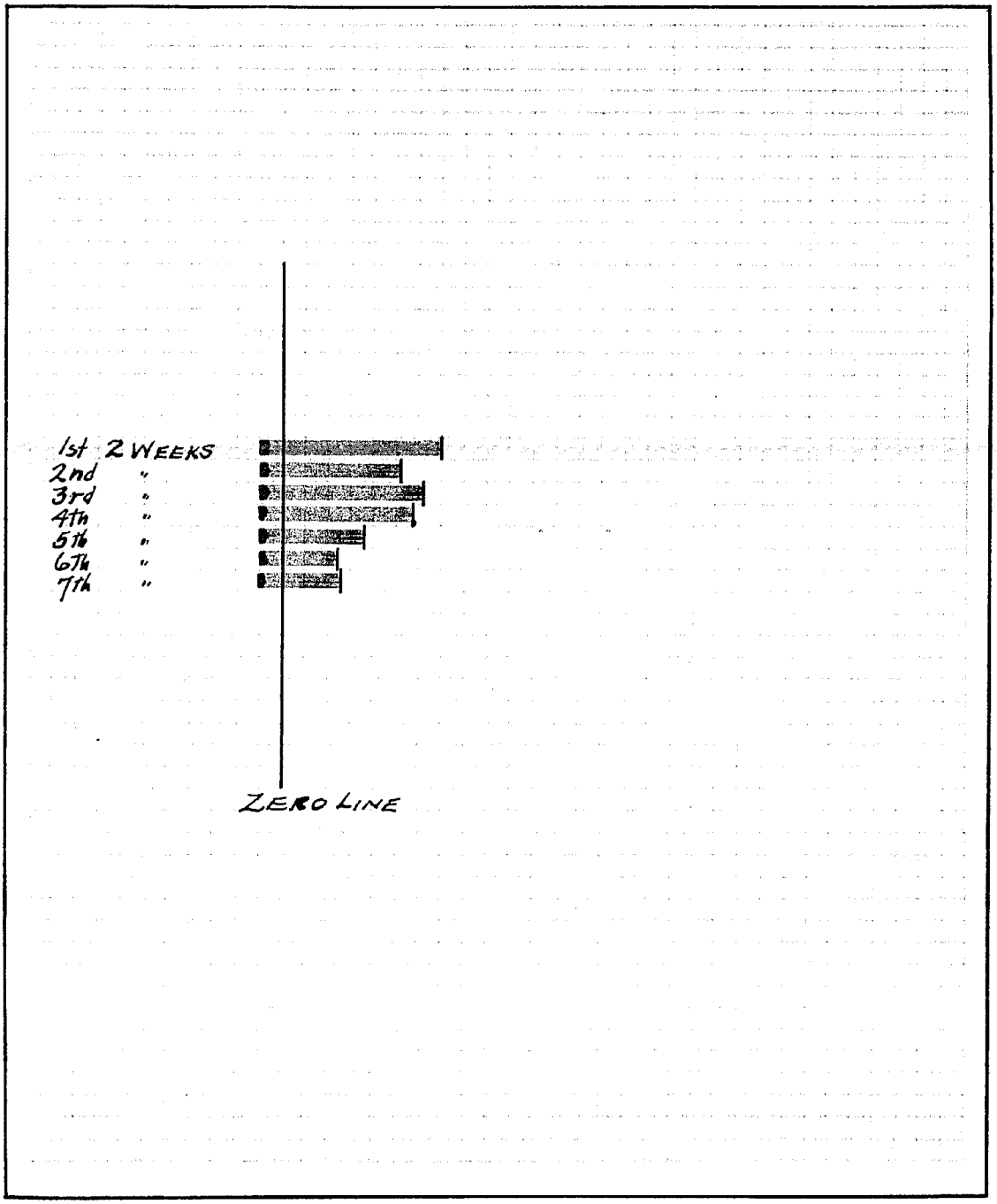


CHART III-2
Average of Tables III-1,2, & 3.
Periods Of Impaction.

# PART II

# THE EFFECT OF VITAMIN D ON THE EXPERIMENTAL PRODUCTION OF CARIES

## Part II: The Effect of Vitamin D on the Experimental Production of Dental Caries

The importance of Vitamin D in a diet has been quite conclusively demonstrated and its function as a regulator of calcium-phosphorus metabolism is well known. It controls the deposition of these minerals in the tissues, hence it may be considered an essential in the formation of normal bone and should play an important role in tooth formation and the maintainance of a normal tooth structure. Studies of blood calcium and phosphorus values in normal animals and in animals on experimental diets that Vitamin D effects the absorption and excretion of these two minerals as well as their utilization and deposition. It would appear, therefore, that Vitamin D is needed throughout life, the requirement being greatest during infancy, and next greatest during pregnancy and lactation. Calcium storage in the human fetus begins at about the fourth month, increasing thereafter, and becoming most rapid toward the end of pregnancy. If the calcium and phosphorus stored by the mother falls below the fetal demand, a severe drain on the tissue reserves of the mother will take place, which may account for the old adage, "for every child a tooth". The work of E. V. McCollum (*38) and of May Mellanby (*38) indicated that Vitamin D played a protective role in the development of caries, and while it did not prevent completely, it did reduce the incidence of caries.

There has been considerable controversy as to the role

and importance of this vitamin in the picture of dental caries. It was thought advisable to determine its effects, if possible, in the development or prevention of caries as produced in rats.

Many workers are of the opinion that the Vitamin D requirement of the rat is relatively much less than in the case of the human. To study the effect of this factor, a series of experiments was set up as follows:

Experiment 1. To study the effect of high Vitamin D backgrounds, the offspring of four virgin females were used. The females were started on the stock diet containing 3% irradiated dry yeast as a source of the vitamin. Feeding the diet was continued through pregnancy and lactation, and until the young were 5 weeks old. The young were than placed on the caries producing diet containing coarse rice as a cereal. The control group was composed of young animals of the same age selected from the stock colony. For the first five weeks, these controls were fed the stock diet containing 3% ordinary dry yeast. They too were then placed on the caries producing diet. Both groups were fed the experimental diet for a period of ten weeks. At the end of this period, the animals were destroyed, the mandibles removed, cleaned, scored, and filed. Scores of this group will be found in Table IV-1 and the comparison of the scores is shown on Chart IV-1.

Experiment 2. A second group of young from the females used above were placed on a duplicate experiment. Results of

this second experiment will be found in Table IV-2 and the comparison of the scores is found on Chart IV-1, with the combined comparison of the two groups on Chart IV-1.

Experiment 3. The third experiment was set up to determine the possible significance, if any, of the time at which the Vitamin D supplement was fed. The animals used in this experiment were offspring of 12 virgin females. Of the 12, only 10 produced litters which were reduced to 6 animals each in all cases but one, where 7 were used. Irradiated dry yeast was used as a source of Vitamin D. The supplement was fed each group for a certain period. The feeding plan was as follows:

Groups A & B: 2 females were fed the stock diet containing 3% irradiated yeast through pregnancy and lactation and until the young were five weeks old. At the beginning of the 6th week, the young, which made up Groups A and B, were placed on the coarse rice diet for 9 weeks. The period of feeding on the coarse rice diet was shortened here, as the lesions in Experiments 1 and 2 were rather severe for good scoring. The breakdown after the end of the eighth to ninth week seemed to be greatly accelerated possibly due to fracture of the shell-like structure that remained.

Groups C & D: 2 females were fed stock diet containing 3% irradiated yeast through pregnancy and lactation and until the young were 17 days old. Then, the stock diet containing plain dry yeast was substituted until the young were 5 weeks old. At the beginning of the 6th week, the young, which make

up Groups C and D, were placed on the coarse rice diet for 9 weeks.

Groups E & F: 2 females were fed the stock diet with 3% irradiated yeast through pregnancy and until the young were born. At birth of the young, the diet was changed to the stock diet with plain dry yeast. This was fed until the young were five weeks old, then changed to the coarse rice diet for 9 weeks. One of the females failed to produce a litter, so that this group is made up of young from the one female and is Group E.

Groups G & H: 2 females were fed the stock diet with plain dry yeast through pregnancy and until the young were born. The ration was then changed to the stock diet containing 3% irradiated yeast and fed until the group was 5 weeks old. The young, which make up Groups G and H, were then placed on the coarse rice diet for 9 weeks.

Groups I & J: 2 females were fed the stock diet containing plain yeast through pregnancy and until the young were 17 days old (this is about the earliest age at which the young will begin to eat the stock ration). The ration was then changed to stock diet containing 3% irradiated yeast and was fed until the young were 5 weeks old. At the beginning of the sixth week, the young were placed on the coarse rice diet which was fed for 9 weeks.

Groups K & L: 2 females fed stock diet with plain yeast through pregnancy, lactation, and until the young were 5 weeks old. Only one female produced a litter, the young

of which make up Group K, and which were placed on the coarse rice diet at the beginning of the sixth week, and continued for 9 weeks.

The results of this third experiment are found in Table IV-3, and the comparison of the scores is shown on Chart IV-2.

## Results

The results of Experiments 1 and 2 quite closely parallel each other. The average score for the group with the high Vitamin D background in Experiment 1 is 1.84, in Experiment 2, the score is 2.0. The control group in Experiment 1 shows a score of 9.7, while Experiment 2 is 11.4. This would definitely point toward a protective action when Vitamin D was fed prenatally at liberal levels.

In Experiment 3, the average scores run from 2.3 to 8.4. They are quite consistent for all except two groups, which when eliminated give an average range of 6.3 to 8. These two groups, i.e., Group E and Group K, gave abnormally low values and since there was only one litter in each they should probably be disregarded.

## Discussion

From the results of these experiments, the feeding of high levels of Vitamin D show some fairly significant results. In the case of Experiments 1 and 2, we see that there was a definite decrease both in incidence and in severity of caries. The controls on this group gave scores that were quite

comparable with results obtained in other similar experiments, and the wide difference in the two scores would certainly indicate that the Vitamin D does act as a protective factor in the development of caries.

In Experiment 3, no such clear cut evidence is found. The groups on the high Vitamin D levels, in fact, show a higher caries score than do the control groups. The purpose of the experiment was to determine, if possible, what period of feeding of the Vitamin D supplement produced the most effective protection against caries. The data are too limited to warrant the drawing of any definite conclusions.

In view of the results obtained with the first two experiments, it might be that a general statement could be made to the effect that high Vitamin D supplements fed the mother during periods of pregnancy and lactation would produce a protective action in the teeth of the young. The rapidity with which the teeth develop in the young rat is almost startling, and it is easily conceivable that any dietary imbalance might easily cause a hypoplasia that would go unnoticed under macro examination. It is certainly logical to believe that a well developed tooth of sound structure and calcification should be more resistant to caries than one which is the opposite in nature. In all probability, the pattern for the development of a carious lesion is the same in any tooth, regardless of its structure, development, or calcification. The difference would

surely be found in the rate of breakdown, the poorer structure going to pieces much more rapidly. From that standpoint then, Vitamin D can be of considerable value in the development of teeth of superior structure and calcification. This would seem to be the manner in which the vitamin exerts its protective action. Further, if such is the case the most important period for the feeding of a supplement would be that during which the teeth are being formed, that is, to the mother during pregnancy and lactation and to the young during the first five weeks after birth.

Some exploratory work was also done with Vitamin C supplements but the data gave no evidence to encourage further investigation. In studying such dietary factors as the vitamins, it must be kept in mind that the requirements of the rat are undoubtedly different than for humans, and further that the vitamin picture in the dietary is one of many factors and not of a single factor.

## Summary and Conclusions

Vitamin D appears to exert a definite protective action against dental caries when fed prenatally.

There was little or no evidence of any protective action when the Vitamin D was fed post-natally to the mother or to the offspring.

The most effective period for feeding supplements of the Vitamin D is to the female during pregnancy and lactation, and to the young during the first five weeks.

The protective effect is undoubtedly due to the fact that the Vitamin D permits the development of teeth of superior structure and calcification.

TABLE IV-1 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | M | --- | --- | --- | 0 | | |
| 2 | M | --- | --- | --- | 0 | | |
| 3 | M | --- | --- | --- | 0 | | |
| 4 | M | 3 | --- | --- | 3 | | |
| 5 | M | --- | --- | --- | 0 | | |
| 6 | M | --- | 1 | --- | 1 | | |
| 7 | F | --- | 1 | --- | 1 | 1.84 | Experimental Group |
| 8 | F | --- | --- | --- | 0 | | |
| 9 | F | --- | 1 | 2 | 3 | | |
| 10 | F | --- | 5 | 2 | 7 | | |
| 11 | F | --- | 4 | 3 | 7 | | |
| 12 | F | --- | 1 | --- | 1 | | |
| 13 | F | --- | 1 | --- | 1 | | |
| 14 | M | --- | 6 | 6 | 12 | | |
| 15 | F | --- | 6 | 5 | 11 | | |
| 16 | M | --- | 6 | 5 | 11 | 9.7 | Control Group |
| 17 | F | --- | 6 | 3 | 9 | | |
| 18 | F | --- | 5 | 2 | 7 | | |
| 19 | M | --- | 4 | 4 | 8 | | |

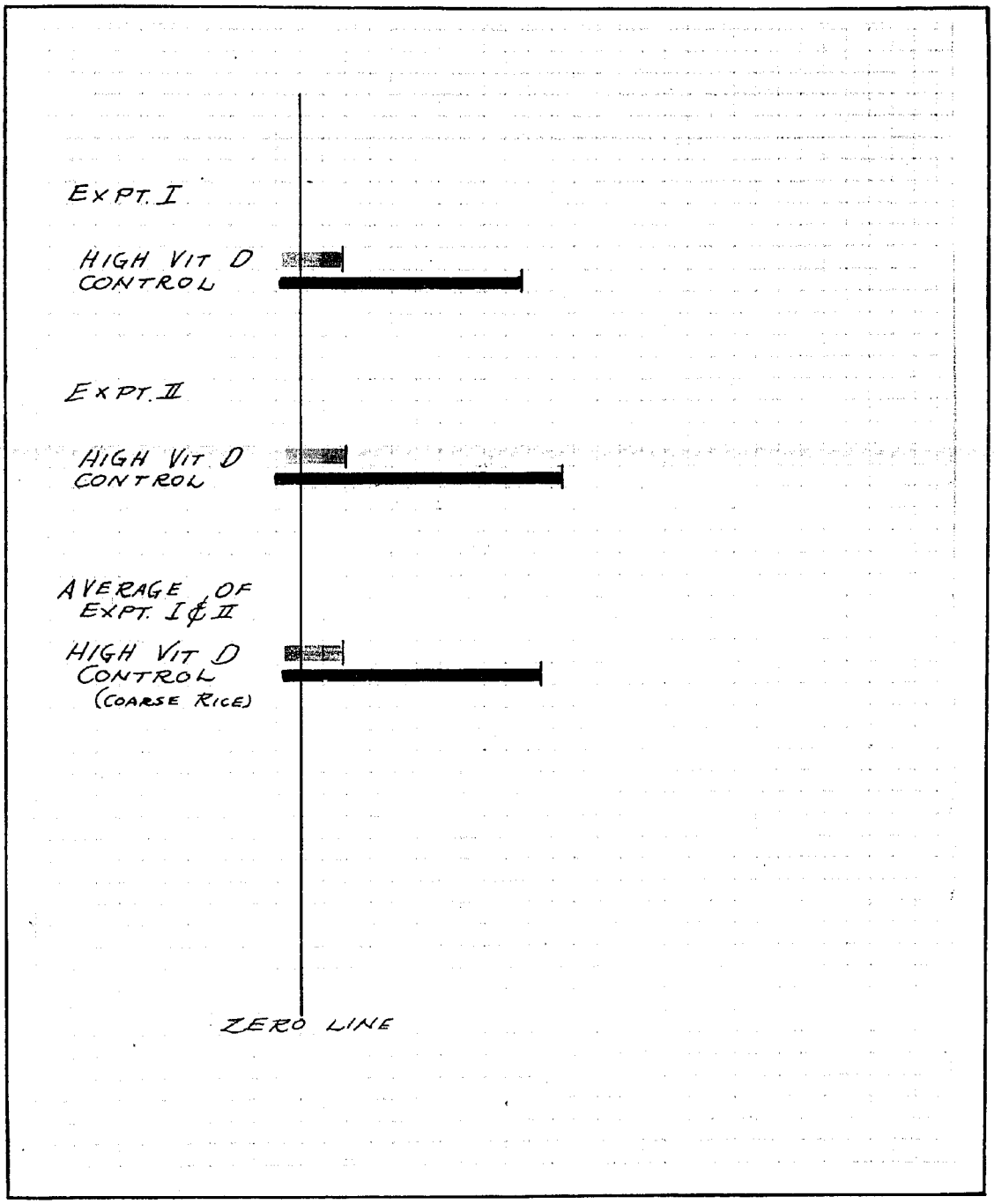


CHART IV-1
From Tables IV-1, & 2; High Vitamin D Background.

TABLE IV-2 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | --- | --- | 0 | | |
| 2 | F | --- | 1 | 3 | 4 | | |
| 3 | F | --- | 5 | 1 | 6 | | |
| 4 | F | --- | --- | --- | 0 | 1.7 | |
| 5 | F | --- | --- | --- | 0 | | |
| 6 | F | --- | --- | --- | 0 | | |
| 7 | F | --- | 1 | 1 | 2 | | |
| 8 | M | --- | 3 | 4 | 7 | | |
| 9 | M | --- | --- | 2 | 2 | | Experimental |
| 10 | M | --- | 1 | 3 | 4 | 4.25 | Group |
| 11 | M | --- | 1 | 3 | 4 | | |
| 12 | M | --- | 2 | --- | 2 | | |
| 13 | M | --- | --- | --- | 0 | | |
| 14 | M | --- | --- | --- | 0 | | |
| 15 | M | --- | --- | --- | 0 | | |
| 16 | M | --- | --- | --- | 0 | .9 | |
| 17 | M | --- | 2 | 1 | 3 | | |
| 18 | M | --- | 1 | --- | 1 | | |
| 19 | F | --- | 1 | 2 | 3 | | |
| 20 | F | --- | 1 | --- | 1 | | |
| 21 | F | --- | 2 | 1 | 3 | | |
| 22 | F | --- | --- | --- | 0 | 2.2 | |
| 23 | F | --- | 1 | 3 | 4 | | |

Average for all above = 2.4

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 24 | M | --- | 4 | 12 | 16 | | |
| 25 | M | --- | 5 | 5 | 10 | | |
| 26 | F | --- | 5 | 5 | 10 | | |
| 27 | F | --- | 4 | 3 | 7 | | Control Group |
| 28 | M | --- | 6 | 7 | 13 | | |
| 29 | M | --- | 6 | 6 | 12 | | |
| 30 | F | --- | 7 | 5 | 12 | | |
| 31 | F | --- | 8 | 3 | 11 | | |

Average for all above = 11.4

TABLE IV-3 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | M | --- | 6 | 5 | 11 | | |
| 2 | M | --- | 5 | 5 | 10 | | |
| 3 | F | --- | 5 | 4 | 9 | 8 | Group A |
| 4 | F | --- | 1 | 4 | 5 | | |
| 5 | F | --- | --- | 3 | 3 | | |
| 6 | F | --- | 5 | 5 | 10 | | |
| 7 | M | --- | --- | 1 | 1 | | |
| 8 | M | --- | 3 | 3 | 6 | | |
| 9 | M | --- | 5 | 5 | 10 | | |
| 10 | F | --- | 6 | 6 | 12 | 7.3 | Group B |
| 11 | F | --- | 3 | 2 | 5 | | |
| 12 | F | --- | 5 | 5 | 10 | | |
| | | | | | | Average of Group A & B | 7.3 |
| 13 | M | --- | 1 | 2 | 3 | | |
| 14 | M | --- | --- | --- | 0 | | |
| 15 | M | --- | 4 | 3 | 7 | 7.8 | Group C |
| 16 | F | --- | 5 | 7 | 12 | | |
| 17 | F | --- | 5 | 5 | 10 | | |
| 18 | F | --- | 7 | 8 | 15 | | |
| 19 | M | --- | 6 | 5 | 11 | | |
| 20 | M | --- | 6 | 4 | 10 | | |
| 21 | M | --- | 2 | 2 | 4 | | |
| 22 | M | --- | 4 | 5 | 9 | 7.8 | Group D |
| 23 | F | --- | 3 | 3 | 6 | | |
| 24 | F | --- | 5 | 2 | 7 | | |
| | | | | | | Average of Group C & D | 7.8 |
| 25 | M | --- | 2 | 3 | 5 | | |
| 26 | M | --- | --- | 1 | 1 | | |
| 27 | M | --- | --- | 1 | 1 | | |
| 28 | F | --- | 1 | --- | 1 | 3.5 | Group E |
| 29 | F | --- | 4 | 4 | 8 | | |
| 30 | F | --- | 2 | 3 | 5 | | |
| 31 | M | --- | 5 | 3 | 8 | | |
| 32 | M | --- | 2 | 3 | 5 | | |
| 33 | M | --- | 1 | 3 | 4 | | |
| 34 | F | --- | 4 | 5 | 9 | 7.5 | Group G |
| 35 | F | --- | 6 | 6 | 12 | | |
| 36 | F | --- | 5 | 2 | 7 | | |

Continued on next page

TABLE IV-3 (cont'd.) CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 37 | M | --- | 5 | 4 | 9 | | |
| 38 | M | --- | 5 | 5 | 10 | | |
| 39 | M | --- | 4 | 4 | 8 | | |
| 40 | M | --- | 4 | 5 | 9 | | |
| 41 | F | --- | 5 | 4 | 9 | 8.4 | Group H |
| 42 | F | --- | 3 | 3 | 6 | | |
| 43 | F | --- | 4 | 4 | 8 | | |
| | | | | | | Average of Group G & H 8 | |
| 44 | M | --- | 5 | 5 | 10 | | |
| 45 | M | --- | 3 | 3 | 6 | | |
| 46 | M | --- | 3 | 2 | 5 | | |
| 47 | F | --- | 3 | 5 | 8 | 7.5 | Group I |
| 48 | F | --- | 4 | 6 | 10 | | |
| 49 | F | --- | 3 | 3 | 6 | | |
| 50 | M | --- | 3 | 4 | 7 | | |
| 51 | M | --- | 5 | 4 | 9 | | |
| 52 | M | --- | --- | --- | 0 | | |
| 53 | F | --- | 2 | --- | 2 | 5.1 | Group J |
| 54 | F | --- | 1 | 2 | 3 | | |
| 55 | F | --- | 5 | 5 | 10 | | |
| | | | | | | Average of Group I & J 6.3 | |
| 56 | M | --- | 2 | 1 | 3 | | |
| 57 | M | --- | --- | 2 | 2 | | |
| 58 | M | --- | 1 | 1 | 2 | | |
| 59 | M | --- | 2 | 3 | 5 | 2.3 | Group K |
| 60 | F | --- | --- | 1 | 1 | | |
| 61 | F | --- | 1 | --- | 1 | | |

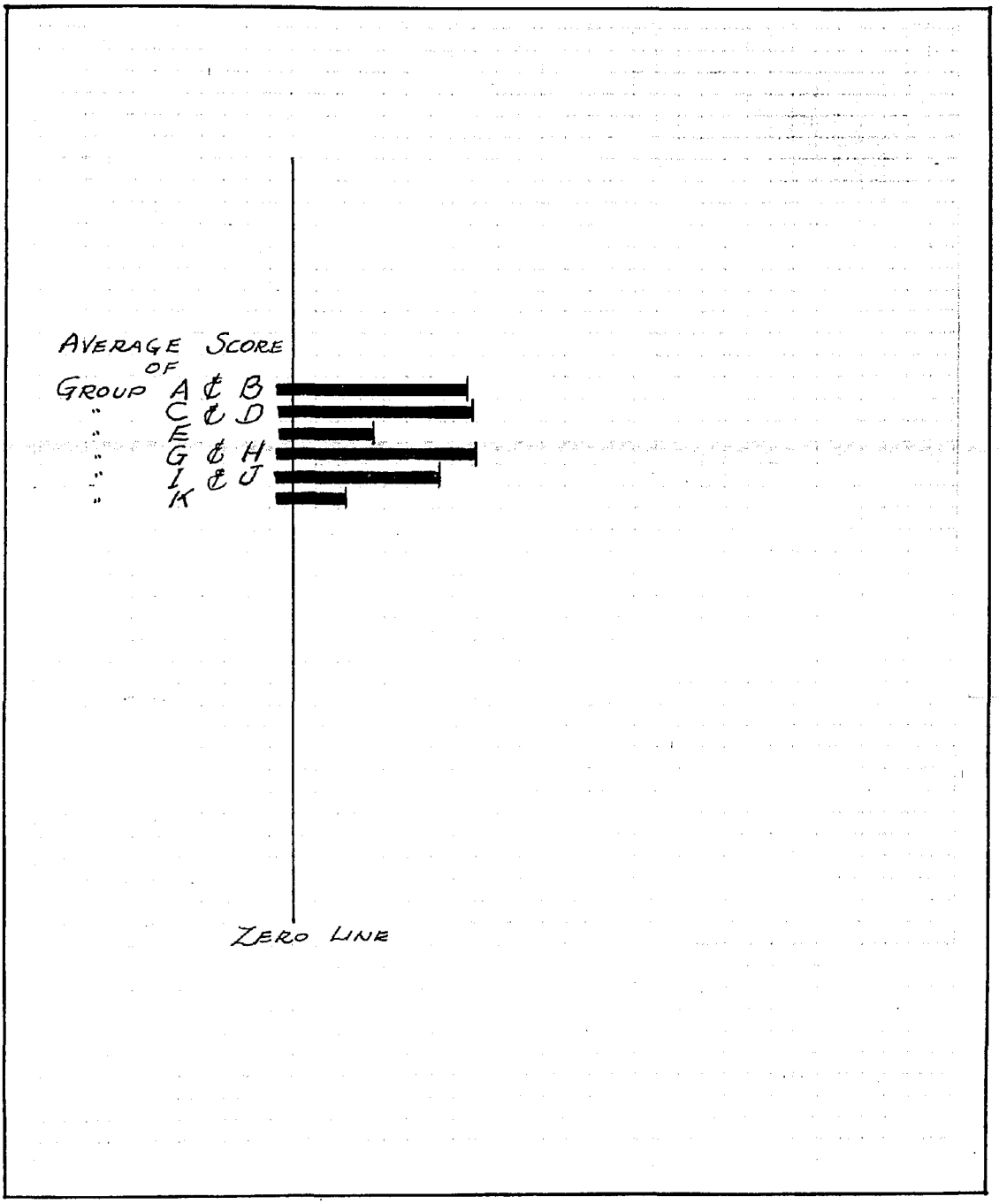


CHART IV-2
From Table IV-3; Most Effective
Period of Feeding Vitamin D.

# PART III

## THE EFFECT OF FEEDING VARIOUS CARBOHYDRATES ON THE DEVELOPMENT OF DENTAL CARIES

## Part III: The Effect of Feeding Various Carbohydrates on the Development of Dental Caries

The literature is replete with references to the significance of carbohydrates in the picture of dental caries. Practically every article written emphasizes the role of carbohydrates. Bunting and his group (7), Rosebury and co-workers (32), Belding and Belding (2), Hanke and co-workers (8), to mention only a few, have all pointed out the significance of carbohydrates. In only one instance, the work of Lilly (27), was it reported that there was no appreciable effect of carbohydrates on the development of caries. The possible explanation in this case may be that a fine diet was used which alone would fail to initiate carious lesions. Moderate use of sweets has long been the advice of the dental fraternity, and the work of Koehne (25) with a group of children has amply shown the effect of sugar on the incidence of caries.

Against this background, a series of experiments was planned to answer, if possible, several questions such as: What types of carbohydrates are most active in their influence on caries? Do all carbohydrates initiate caries, or is the effect of some simply that of extending the destruction of the tooth structure once caries has been initiated? Accordingly, the following series of experiments was set up:

EXPERIMENT NO. 1. The effect of substituting various

sugars for cereal. A group of 29 selected animals was placed at weaning (21-28 days) on the basal rice, or caries producing diet, containing: 60 parts coarsely ground rice, 30 parts whole milk powder, 6 parts flax-seed meal, 5 parts alfalfa meal, 3 parts dried yeast, and 1 part sodium chloride. At the end of five to six weeks, depending upon the development of lesions as determined by examination, the diet was changed to the experimental ration and feeding was continued for a period of 7-8 weeks, the experiment being terminated in the 8th week. The experimental rations used had the following composition:

| | Diet No. 1 | |
|---|---|---|
| Carbohydrate | 67% | (Sucrose, Glucose, or Lactose) |
| Casein (com'l.) | 18% | |
| Salt Mixture | 4% | (Modified Steenbock) |
| Dried Yeast | 5% | |
| Corn Oil | 5% | |
| Cod Liver Oil | 1% | |
| Total | 100% | |

In this experiment, the carbohydrate level was slightly higher than the cereal content. The animals were divided into eight groups with the distribution and rations as follows:

Group A - 4 animals (2 M, 2 F); Ration containing 67% Sucrose

Group B - 4 animals (2 M, 2 F); Ration containing 67% Glucose

Group C - 4 animals ( 2 M, 2 F); Ration was Carbohydrate Free Diet using 20% Flax-seed Meal
30% Alfalfa Meal
19% Com'l. Casein
30% Crisco
1% Sodium Chloride

Group D - 4 animals (2 M, 2 F); Ration containing 67% Lactose

Group E - 2 animals (2 F); Ration Coarse Rice Control Diet containing 66% Coarsely Ground Rice
30% Milk Powder Whole
3% Alfalfa Meal
1% Sodium Chloride

Group F - 4 animals (1 M, 3 F); Ration containing a combination of Sucrose and Oatmeal as follows:

30% Sucrose
27% Ground Oatmeal
18% Com'l. Casein
4% Salt Mix
5% Dried Yeast
5% Corn Oil
1% Cod Liver Oil

Group G - 4 animals (2 M, 2 F); Ration Oatmeal Control Diet as follows:

66% Oatmeal
30% Whole Milk Powder
3% Alfalfa Meal
1% Sodium Chloride

Group H - 1 animal (1 F); Ration containing Milk Powder as follows:

90% Whole Milk Powder
9% Alfalfa Meal
1% Sodium Chloride

At the beginning of the experimental period, there were four animals in Group E, but two died during the process of examination under ether anesthesia. However, the results with the coarse rice diet had been fairly well standardized

so that the two animals remaining served as sufficient basis for comparison. The caries scores of these animals are found in Table V-1, and the comparison of the scores is shown on Chart V-1.

## Results

Before the results are cited, it would be well to mention that the diets were apparently not as satisfactory as had been hoped. Although the animals were able to maintain weight, or to gain slightly, their general physical condition seemed to decline. The animals fed the high lactose diet, in particular, showed definite signs of a toxic effect. This necessitated lowering the level of carbohydrate in subsequent studies. For qualitative purposes, however, the results may be of some interest. The score of the rice controls with a value of 9 showed an average similar to that of other animals fed the same diet. The high score of the entire experiment was 9.4 for the average of the group on glucose. Sucrose gave a value of 6, lactose 2.5, carbohydrate-free 2, oatmeal and sugar combination 2, oatmeal basal 0, and high milk 0.

## Discussion

Because the diets were not entirely satisfactory, it is a bit difficult to draw any definite conclusions from this experiment. There are one or two points that may be mentioned, however. The 67% level of sugars in the experimental diets was obviously too high, particularly in the case of lactose, which is now known to be toxic at high intakes. Moreover, there does

not appear in this experiment to be any greater degree of caries on the high carbohydrate diets than was found with the coarse rice diet. All the control diets showed scores of around 2 or less which is no more than would be expected on the basis of the preliminary 5 weeks feeding on the caries producing diet. One difficulty is that it is not possible to assess accurately the condition of the teeth in live animals in the early stages of decay. The initiation of the carious lesion may take place some time before the lesion can be recognized by gross examination--indeed such must be the case. It is, therefore, easy to see that too much reliance cannot be placed on the results of this first experiment with the sugars.

EXPERIMENT NO. 2: The effect of substituting carbohydrates at a lower level, and their influence on dental caries. A group of 14 animals was placed on the rice basal diet as given in Experiment 1. At the end of five to six weeks, depending on the development of lesions as determined by examinations, the diet was changed to the experimental ration and feeding was continued for a period of 7-8 weeks, the experiment being terminated during the eighth week. The experimental diets used were based on the following formula:

Diet No. 2

| | | |
|---|---|---|
| Carbohydrate | 35% | (Sucrose, Glucose, or Lactose) |
| Casein (com'l.) | 20% | |
| Flax-seed Meal | 20% | |
| Alfalfa Meal | 9% | |
| Dried Yeast | 5% | |
| Crisco | 10% | |
| Sodium Chloride | 1% | |
| Total | 100% | |

Note that the carbohydrate level has been reduced from 67% to 35%, as compared to the diet of Experiment 1. The animals were divided into five groups with distribution and diets as follows:

Group A – 3 animals (2 M, 1 F); Ration containing 35% Sucrose

Group B – 3 animals (2 M, 1 F); Ration containing 35% Glucose

Group C – 3 animals (1 M, 2 F); Ration containing 35% Lactose

Group D – 3 animals (1 M, 2 F); Carbohydrate-free Diet as given in Experiment 1

Group E – 2 animals (2 M) ; Coarse Rice Control Diet as given in Experiment 1

The caries scores of these animals will be found in Table V-2, and the comparison of the scores is found on Chart V-2.

## Results

The high average score of the group is shown with sucrose giving a value of 10, followed by the rice control at 9.5, lactose at 10.3, glucose at 8.3, and the carbohydrate-free diet at 6. In this experiment, the animals on glucose showed a slight drop over the first experiment. Both sucrose and lactose showed results slightly higher than the control rice, but probably not significantly greater. The carbohydrate-free diet gave a score of six which was a little higher than expected.

## Discussion

From the results obtained, there are several points of

interest. It is apparent that when the animals were placed on the experimental rations, they must have attained a fairly uniform initiation of caries in the teeth, for the scores show fairly similar values. The usual score for animals on the caries producing diet is about 10, and in both Experiments 1 and 2, this value was maintained. The average scores of the animals on the sugars were 10.3, 10, and 8.3 which shows that even after the rice was taken away, the lesions continued to develop and at about the same rate. In the absence of both coarse cereal and sugars, the rate of dental deterioration was appreciably slower and was probably due to the small amount of rice left in the cavities at the time the rats were put on the several modified diets. The average scores in the second experiment were generally higher than those in the first. The principal purpose of the second experiment was to develop a ration nutritionally adequate and yet satisfactory for studying the decay of teeth in rats. In all subsequent experiments, the general proportions of this ration were used.

## Summary and Conclusions

A 35% level of sugars proved satisfactory for comparative studies of tooth decay in rats.

The modified ration gave better growth, weight, and a generally healthier experimental animal.

The results indicate in general that the lesions develop on the sugar containing diets at about the same rate as on the coarse rice or caries producing diet.

The carbohydrate-free diet gave a slightly higher score than was anticipated. This was probably due to the presence of rice in the cavities at the time the diets were changed.

EXPERIMENT NO. 3: The next step in the work with carbohydrates was to compare the relative effects of the cereal, if finely ground, with those of the various sugars. All the animals were fed the coarse rice ration (The simpler diet composed of 66% coarsely ground rice, 30% whole milk powder, 3% alfalfa meal, and 1% sodium chloride will be used from now on as the caries producing diet, and is referred to as the coarse rice diet or coarse rice control.) for a period of from 5 to 6 weeks depending on the condition of the teeth as determined by examination. As the carious lesions were initiated by the coarse rice ration the animals were changed to the experimental rations containing the various sugars, and finely ground rice. The fine rice diet was the same as the coarse rice diet given above, except that the cereal was very finely ground in a ball mill. The carbohydrate-free diet has been described in previous work.

A group of 19 animals was selected and at weaning was placed on the coarse rice diet. The rats were fed for 5 to 6 weeks depending on the initiation of caries. They were then placed on the experimental rations with the following distribution:

2 animals (2 M) continued on the coarse rice ration.
3 animals (2 M, 1 F) on the carbohydrate-free ration.
4 animals (2 M, 2 F) on the 35% sucrose diet.
4 animals (2 M, 2 F) on the 35% glucose diet.
4 animals (2 M, 2 F) on the 35% lactose diet.
2 animals (2 F) on the fine rice diet.

Experimental rations were fed for eight weeks. The caries scores of these animals will be found in Table V-3.

EXPERIMENT NO. 4 was a duplication of Experiment 3, and the caries scores of the animals in Experiment No. 4 are found in Table V-4. The scores of Experiments 3 and 4 have been combined to show the comparison of scores given on Chart V-3. The results and discussion of these two experiments will be considered together.

## Results

The average scores of the animals from both experiments find the coarse rice diet high with a value of 16.5, lactose, 11.4, sucrose, 10.4, glucose, 10.3, fine rice, 7, and the carbohydrate-free diet, 5.8. The animals on the coarse rice and fine rice rations in Experiment 3 were somewhat higher than expected, but fell to about their normally expected values in Experiment 4.

## Discussion

It was interesting to note that lactose gave the highest average of all the sugars. It is possible that the scores in this series were slightly higher due to the fact that the lesions produced by the coarse rice in the preliminary feeding period may have been somewhat more severe than previously. It would be expected from the work done by such workers as Rosebury et al (32), Hanke (15), Florestano (10), and Branson (4), we would expect to find sucrose most active with glucose following. However, little work has been done with

lactose, per se, and a final conclusion should probably be held in reserve until the results of some of the subsequent experiments have been reviewed. It would appear that the development of the lesion does continue on the diets containing the various sugars but not as rapidly as when the coarse rice is fed. With the fine rice, the lesions apparently slow down in their rate of development for the score is considerably lower than for any of the sugars.

This seemed a bit surprising in view of what happened with the coarse rice. Once having been initiated, it was thought that the fine rice would show at least as rapid a development of the lesions as was found with the sugars. Of course, one must always consider the possibility that the rapid rate with the coarse rice might be due in part to fracture inasmuch as the carious nature of the teeth rendered them more fragile, and less able to withstand the forces of mastication. Further, the fine rice may have exerted a filling action on the lesions which, though they had started to develop, might still have been small enough that when the fine rice became impacted it behaved as a filling keeping out oral fluids, thus retarding bacterial fermentation. It might be well to reserve final judgement on the effects of the fine rice until the data from additional animals may be scrutinized.

## Summary and Conclusions

Carious lesions continued to develop or enlarge under the influence of the various sugars.

The rate was, apparently, not as rapid as with the

coarse rice in the caries producing diet.

The effect of all three sugars was about the same, although lactose gave slightly higher scores than sucrose and glucose.

Fine rice showed lower results than were expected.

EXPERIMENT NO. 5: This experiment was a repetition of Experiments 3 and 4. This was done to check two questionable points that arose in the preceding work. It seemed desirable to determine whether or not lactose was consistent in producing a higher caries score, and whether or not the score produced on fine rice would be consistently lower than that of the sugars. In addition, a modification was made in that groups of animals were placed on the experimental rations without the preliminary 4 to 6 weeks period on the coarse rice diet.

A group of 81 animals was selected. These were taken at weaning (21-28 days), and were divided into three groups:

Group A: 4 units of four animals each. These units were placed on the experimental rations at once, having no preliminary feeding period on the coarse rice diet. From time of birth until about 28 days old they had access to the stock diet which contained some ground corn. The rations fed these four groups were: fine rice ration, 35% sucrose ration, 35% glucose ration, 35% lactose ration (rations described in Experiment 2). The experimental rations were

fed for a period of 14 weeks.

Group B: 6 units of ten animals each. These units were placed on the coarse rice diet for from 4 to 6 weeks for the initiation of carious lesions as determined by examination. When the lesions were found to have started, the animals were immediately transferred to the experimental rations for a period of 8 weeks. The rations used (given in Experiment 2) were as follows: coarse rice, fine rice, carbohydrate-free, 35% sucrose, 35% glucose, and 35% lactose.

Group C: 1 unit of five animals was placed on the coarse rice diet at weaning. This was fed for a period of six weeks, at the end of which time the animals were destroyed and the mandibles removed and scored as usual to give a standard of comparison for the animals on the experimental rations.

The caries scores for Group A are found in Table V-5a, for Group B and Group C in Table V-5b. The comparison of the scores for all three groups is shown on Chart V-4.

## Results

In the case of Group A the score for the fine rice was 7.2, for sucrose 3.5, for glucose 7.7, and for lactose 4.5. These scores were obtained with the animals having no preliminary feeding period on the caries producing diet.

The scores of the animals in Group B, which had a preliminary feeding period, were much higher and as follows:

fine rice 13, coarse rice 12.2, lactose 10.5, sucrose 10, glucose 7.7, and carbohydrate-free 8.9. It is interesting to note that fine rice gave the highest score, sucrose and lactose were about the same, while glucose gave a lower score than the carbohydrate-free diet.

The reference group, Group C, which was terminated after 6 weeks feeding on the coarse rice diet showed a score of 5.4. This will give an idea as to the average at the time of transfer to the experimental rations.

## Discussion

The results of Group A would lead one to believe either that the experimental rations would initiate carious lesions, or that some impaction had taken place on the stock diet, thus initiating caries which further developed on the experimental rations. It would appear the latter explanation was most valid. It is interesting to note that glucose gave the highest score in this group, while in the following group, it gave the lowest value. Fine rice also gave a high value.

The results of Group B were quite consistent and about what might be expected. It was thought the fine rice should show as great or greater effect in the development of caries after the lesions were once started, since the finely ground cereal would furnish a source of some carbohydrate and also a continuous supply of the aciduric organisms. Such proved to be the case. With the sugars, sucrose and lactose, the average scores were lower than either the fine or coarse

rice but were appreciably higher than the carbohydrate-free diet and also much higher than the score of the comparison Group C at 5.4. The score of the glucose ration was consistent from individual to individual but for some reason was low. It appears that there is a possibility of a decrease of the number and strength of the acid producing bacteria where the cereal is completely removed and a more or less pure form of carbohydrate substituted. Further, the production of acid by the bacteria present might be great enough, in the areas where impaction and lesions were found, to kill the bacilli. To exceed the maximum pH for existence of the bacteria at certain focal points of infection is not inconceivable.

## Summary and Conclusions

It may be concluded that a diet containing a high level of carbohydrate in the form of some sugar will cause the carious lesions to become enlarged.

The sugars alone are not likely to be the cause of the initiation of caries.

The cereal, whether fine or coarse, usually causes more severe lesions, since it not only furnishes a form of carbohydrate but also a source of the acid forming organisms.

Further work with the sugars would be of interest, particularly in light of the results with glucose.

EXPERIMENT NO. 6: This is a repetition of some of the previous experiments with slight modifications. A group of

48 animals was selected, and at the beginning of their 6th week, the animals were placed on the coarse rice diet for the initiation of caries. This preliminary period was from 5-6 weeks as determined by the examination of the molars. When teeth showed the initial development of caries, the animals were placed on the experimental ration which was fed for a period of 6 rather than 8 weeks. The 8 week period seemed to allow the destruction of the tooth to advance too far for good scoring. The distribution of animals was as follows:

(a) 8 animals continued on coarse rice ration.
(b) 10 animals on the sucrose ration.
(c) 10 animals on the glucose ration.
(d) 10 animals on the lactose ration.
(e) 10 animals on the carbohydrate-free ration.

The fine rice diet was eliminated from the series. At the end of the sixth week on the experimental ration, the animals were destroyed, the mandibles removed and scored as usual. Scores of these animals are found in Table V-6, with the comparison of the scores shown on Chart V-5.

## Results

The average scores obtained are as follows: coarse rice 11.6, sucrose 10, glucose 10.6, lactose 9.7, and carbohydrate-free 8. The scores are slightly lower than in previous work due to the shorter period on experimental rations, but on the whole seem to be more consistent.

## Discussion

The over-all averages of this group are more consistent

and form a good pattern. The glucose group showed a more normal response. The carbohydrate-free diet gave a score slightly higher than expected, but even so, the other scores are still from 2 to 3 points higher, which shows that lacking available carbohydrate the development of the carious lesions is slowed down. In this work, the three sugars showed scores closely grouped which would indicate that they all function in the same way and to about the same degree.

## Summary and Conclusions

The lesions develop more rapidly in the presence of carbohydrate in such form as sugars.

The most rapid development requires the presence of the cereal which furnishes carbohydrate and acid forming organism as well.

EXPERIMENT NO. 7: In this experiment, a group of 30 animals was selected. The group was divided into 4 units of 7 animals each, and 1 unit of 2 animals. They were 28-30 days old, and all were placed on the coarse rice (caries producing) ration until on individual examination caries could be detected. At this stage, the score being a minimum of 1 and a maximum of 3, the animal was placed on the experimental ration of its respective unit for a period of 8 weeks. The animals were destroyed at the end of the eighth week, the mandibles removed and scored as usual. The feeding plan was as follows:

Unit 1: 7 animals on carbohydrate free diet.
Units 2, 3 and 4: 7 animals each on the high carbohydrate diet with the following modification: 20% Flax-seed meal, 20% Casein (com'l.), 20% Alfalfa Meal, 5% Crisco, 4% Dried Yeast, 1% Sodium Chloride, and 30% Carbohydrate (Sucrose, Glucose, or Lactose). All ingredients were carefully blended before adding the carbohydrate, which was mixed in the last thing to avoid coating the sugars with the fat.
Unit 5: 2 animals as controls on the coarse rice.

The scores of the above animals may be found in Table V-7 with the comparison of the scores shown on Chart V-5.

## Results

The scores again conformed fairly well with those previously obtained. Glucose gave a value of 14.3, Sucrose 13.3, Lactose 12.3, Carbohydrate-free 9.6, and the 2 animals on the coarse rice, which served as controls, 15.5.

## Discussion

It is again seen that the carious lesions continue to enlarge on diets containing sugar, but the advance is not as rapid as when the animal is continued on the coarse rice diet. The average scores of all the animals on the sugar diets was 13.3, an increase in the average score of 3.7 over the carbohydrate free diet. This 13.3 average is 2.2 less than that of the animals on the caries producing diet. From these results, and those of preceding experiments, it would seem to have been definitely established that though the sugars do cause an increase in the severity of lesions that have been initiated, the lesions are more severe if

the animal is kept on the caries producing diet. The sugars may cause an increase in severity though they do not initiate the lesion.

A point might be made of the fact that it is highly probable, in the case of the rat at least, that the sugars themselves are not so harmful unless fed in a diet containing the caries producing factor, i.e. the coarsely ground cereal as rice or corn. If such a combination were fed the effect of the sugars superimposed on the coarse cereal would undoubtedly be a more drastic one. As has been mentioned before, the possibility of accurate and significant scoring diminishes after the breakdown in tooth structure has advanced beyond a certain point. Thus, a feeding experiment involving both factors would have to be checked by careful individual examinations. Otherwise, the scores would approach a maximum in every case, and in less time than the eight week period used when the foods in question were fed separately.

## Summary and Conclusions

It may be concluded that carbohydrate in the form of sugars causes the lesion to develop and become larger.

The development is fairly rapid and severe.

When sugars are included in the diet, the lesions are not as severe as when the caries producing diet is fed a similar length of time.

If the sugars were fed with the caries producing diet,

maximum severity of lesions and rapidity of breakdown in tooth structure might be expected.

EXPERIMENTS NO. 8 and 9: These two experiments were designed to give proof to the point mentioned in the discussion and summary of the preceding experiment. What would be the effect of feeding a combination of sugar with the caries producing diet?

To do this, a ration was devised which contained both coarse rice and sugars in the form of a corn syrup. The syrup was first mixed thoroughly with the coarsely ground rice so that the rice was quite effectively coated with the syrup. The syrup-rice mixture was allowed to dry, then crumbled, and the other ingredients added and mixed by hand. The formula of the ration is as follows:

| | | | | |
|---|---|---|---|---|
| 66 | parts | by | weight | coarsely ground rice |
| 30 | " | " | " | whole milk powder |
| 3 | " | " | " | alfalfa meal |
| 1 | " | " | " | sodium chloride |
| 10 | " | " | " | corn syrup (Dark Karo) |

It was also decided that it would be of interest to observe the effect of a high Vitamin D intake with such a ration. The procedure was as follows:

Experiment 8 was carried out with 16 animals 28-30 days old, which were divided into one group of 10 animals (4 M, 6 F) fed the corn syrup diet for 8 weeks, and one group of 6 animals (2 M, 4 F) fed the coarse rice diet for 8 weeks.

Experiment 9 was carried out with the offspring of 5 young females fed the regular stock diet containing 3%

irradiated yeast of high Vitamin D potency. The diet is reviewed here:

| | |
|---|---|
| 40% Ground yellow corn | 6% Alfalfa meal |
| 30% Oatmeal | 3% Irradiated yeast |
| 20% Whole milk powder | 1% Sodium chloride |

From the litters, 24 young were selected and at 28-30 days were divided and placed on the experimental rations as follows:

| | | |
|---|---|---|
| Group 1 - 5 animals | (3 M, 2 F) | Corn Syrup Ration |
| Group 2 - 8 animals | (4 M, 4 F) | Corn Syrup Ration plus 3% Irradiated Yeast |
| Group 3 - 8 animals | (4 M, 4 F) | Coarse Rice Ration plus 3% Irradiated Yeast |
| Group 4 - 3 animals | (3 F) | Coarse Rice Ration |

The experimental rations were fed for a period of 10 weeks.

Experiments 8 and 9 were terminated during the 10th week, when the animals were destroyed, mandibles removed and scored as usual. The scores of Experiment 8 are found in Table V-8, for Experiment 9 in Table V-9, with comparison of scores for both experiments being shown on Chart V-6.

## Results

The results were very much as anticipated and the scores for animals in Experiment 8 gave averages of 14.2 for the corn syrup ration against 12.3 for the coarse rice ration.

The results in Experiment 9 gave averages of 16.8 for the corn syrup ration, 15.9 for the same ration containing the irradiated yeast, 13.2 for the coarse rice ration containing the irradiated yeast, and 15 for the coarse rice

ration alone. The results are quite consistent and do indicate a protective action for the Vitamin D.

## Discussion

A ten weeks period was used in Experiment 9 for feeding the experimental rations inasmuch as all the previous experiments using carbohydrates had been preceded with a 4-6 week preliminary feeding on the caries producing ration. The differences were not as marked as was anticipated. It is entirely possible, however, that had the period of feeding been somewhat shorter, there would have been greater differences.

The action of the cereal as initiator and of the sugars as accelerator is quite plainly established. The results show that corn syrup acted as did the sugars in previous experiments causing lesions to become more severe, and that its effect superimposed on the action of the caries producing diet gave a score that was greater than would be obtained with either one alone. The results confirm the theories and observations of such workers as Bunting and co-workers, Rosebury, Belding and Belding, Cox, and many others. The protective action of the Vitamin D observed in previous work was again apparent.

The work done with the corn syrup ration indicates the desirability of a standardized caries producing diet. Such standardization would allow the application of statistical methods, and it would then be possible to evaluate the effect

of each factor. The availability of animals of uniform response would also be of assistance. This is largely a problem of selective breeding in which some progress has been made by Hunt and Hoppert who have developed strains of caries susceptible and caries resistant rats (19). The adoption of standardized procedures and improvement in accuracy, in selecting rats of approximately identical degree of decay initiated by the use of coarse rice, would materially aid in a more quantitative comparison of the materials studied in this research.

## Summary and Conclusions

It may be concluded that when a combination of sugar and cereal is fed, more severe lesions are produced than when these foods are used singly in suitable diets.

Under the conditions of the experiments performed, the influence of Vitamin D is protective rather than preventive.

TABLE V-1 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | M | --- | 6 | 7 | 13 | | |
| 2 | M | --- | 1 | 3 | 4 | | |
| 3 | F | --- | 1 | 1 | 2 | 6 | Sucrose |
| 4 | F | --- | 2 | 3 | 5 | | |
| 5 | M | --- | --- | --- | 0 | | |
| 6 | M | 2 | 6 | 7 | 15 | | |
| 7 | F | --- | 5 | 4 | 9 | 9.4 | Glucose |
| 8 | F | 1 | 5 | 7 | 13 | | |
| 9 | M | --- | 2 | --- | 2 | | |
| 10 | M | --- | 1 | --- | 1 | | |
| 11 | F | --- | 2 | 2 | 4 | 2 | Carbohydrate-free |
| 12 | F | --- | --- | 1 | 1 | | |
| 13 | M | --- | 2 | --- | 2 | | |
| 14 | M | --- | 1 | 1 | 2 | | |
| 15 | F | --- | 3 | 3 | 6 | 2.5 | Lactose |
| 16 | F | --- | --- | --- | 0 | | |
| 19 | M | --- | 3 | 4 | 7 | | |
| 20 | M | 2 | 5 | 4 | 11 | 9 | Coarse Rice |
| 21 | M | --- | 5 | 3 | 8 | | |
| 22 | F | --- | --- | --- | 0 | | |
| 23 | F | --- | --- | --- | 0 | 2 | Oatmeal and Sucrose |
| 24 | F | --- | --- | --- | 0 | | |
| 25 | M | --- | --- | --- | 0 | | |
| 26 | M | --- | --- | --- | 0 | | |
| 27 | F | --- | --- | --- | 0 | 0 | Oatmeal Basal |
| 28 | F | --- | --- | --- | 0 | | |
| 29 | F | --- | --- | --- | 0 | 0 | High Milk |

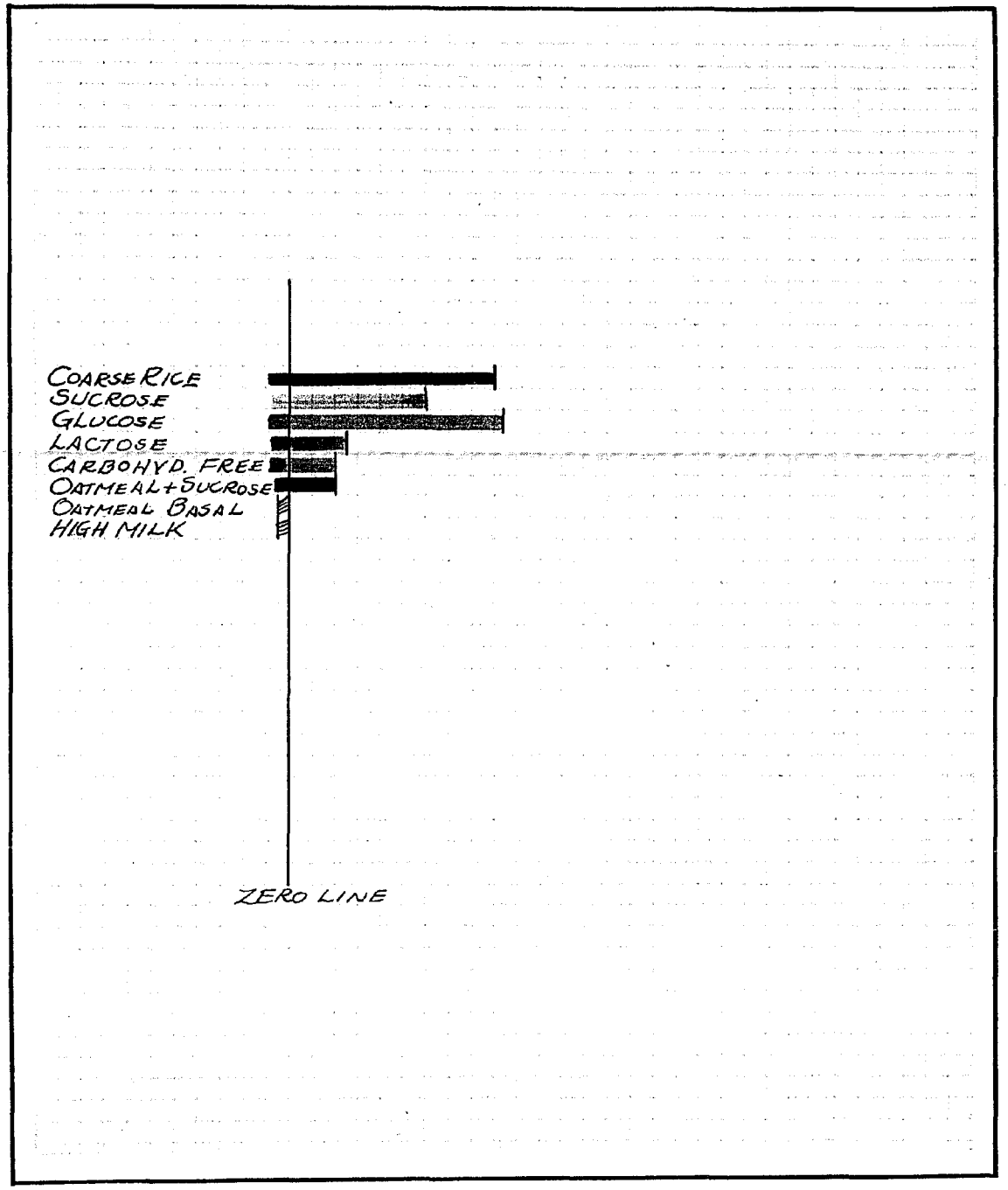


CHART V-1
From Table V-1; Preliminary Results
on High Sugar Diets.

TABLE V-2

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 2 | 4 | 6 | | |
| 2 | M | --- | 7 | 7 | 14 | 10 | Sucrose |
| 3 | M | --- | 6 | 4 | 10 | | |
| 4 | M | --- | 4 | 3 | 7 | | |
| 5 | M | --- | 4 | 4 | 8 | 8.3 | Glucose |
| 6 | F | --- | 6 | 4 | 10 | | |
| 7 | M | --- | 7 | 6 | 13 | | |
| 8 | F | --- | 5 | 5 | 10 | 10.3 | Lactose |
| 9 | F | --- | 5 | 3 | 8 | | |
| 10 | F | --- | 4 | 1 | 5 | | |
| 11 | F | --- | 2 | 3 | 5 | 6 | Carbohydrate-free |
| 12 | M | --- | 4 | 4 | 8 | | |
| 13 | M | --- | 4 | 3 | 7 | | |
| 14 | M | --- | 6 | 6 | 12 | 9.5 | Rice Control |

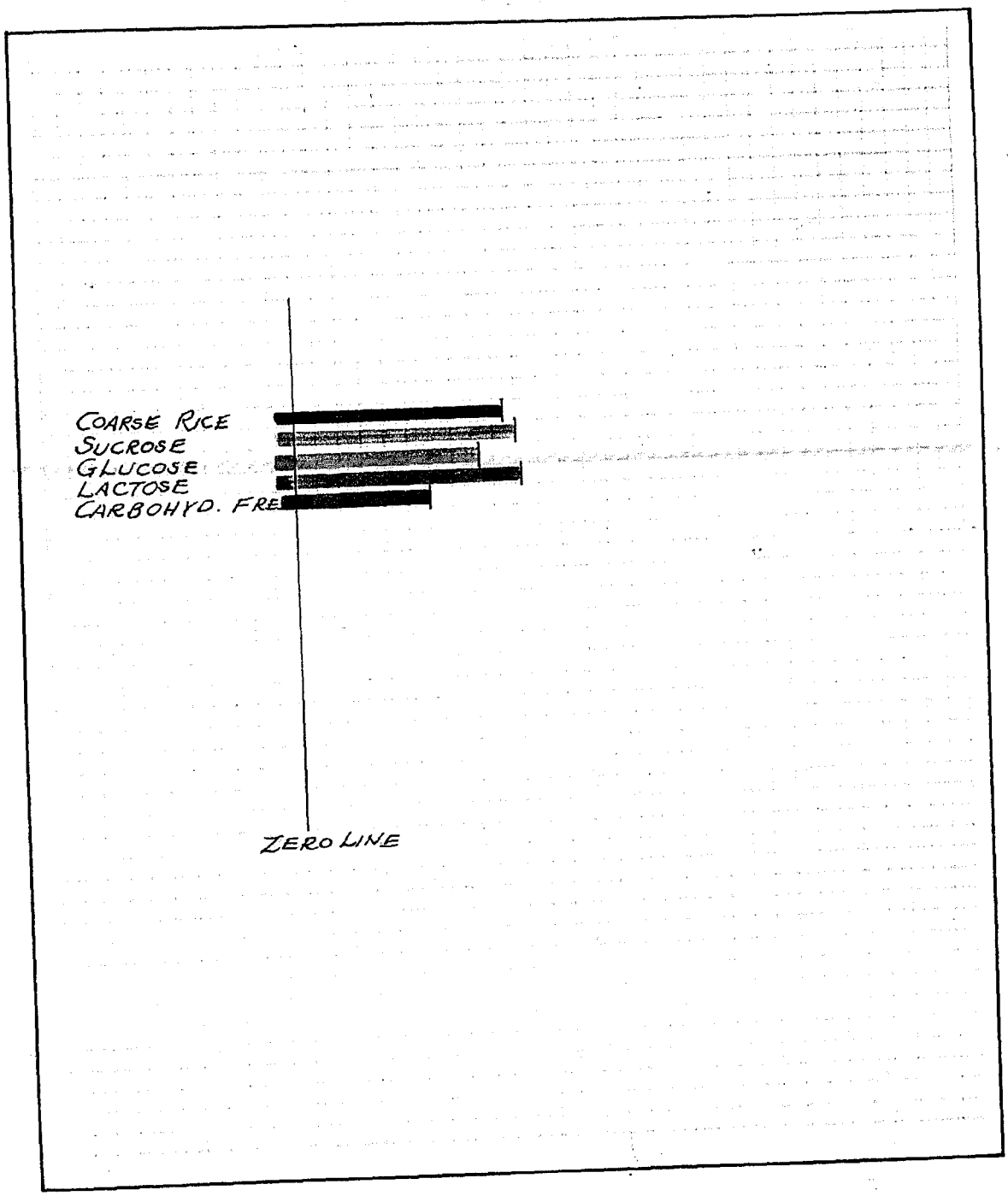


CHART V-2
From Table V-2; Feeding Various Sugars at 35% Levels.

TABLE V-3

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | M | --- | 9 | 9 | 18 | | |
| 2 | M | 3 | 8 | 10 | 21 | 19.5 | Rice Control |
| 3 | M | --- | 5 | 4 | 9 | | |
| 4 | M | --- | 3 | 4 | 7 | 8 | Carbohydrate-free |
| 5 | F | --- | 4 | 4 | 8 | | |
| 6 | M | --- | 6 | 5 | 11 | | |
| 7 | M | --- | 7 | 10 | 17 | | |
| 8 | F | --- | 4 | 5 | 9 | 13 | Sucrose |
| 9 | F | --- | 7 | 8 | 15 | | |
| 10 | M | --- | 5 | 4 | 9 | | |
| 11 | M | --- | 7 | 8 | 15 | | |
| 12 | F | --- | 3 | 6 | 9 | 13 | Glucose |
| 13 | F | --- | 10 | 9 | 19 | | |
| 14 | M | --- | 9 | 7 | 16 | | |
| 15 | M | --- | 10 | 7 | 17 | | |
| 16 | F | --- | 7 | 2 | 9 | 13.5 | Lactose |
| 17 | F | --- | 6 | 6 | 12 | | |
| 18 | F | --- | 6 | 6 | 12 | | |
| 19 | F | --- | 4 | 3 | 7 | 9.5 | Fine Rice |

TABLE V-4 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 3 | 3 | 6 | | |
| 2 | F | --- | 4 | 7 | 11 | 8.5 | Rice Control |
| 3 | F | --- | 2 | 2 | 4 | | |
| 4 | F | --- | --- | 1 | 1 | 2.5 | Carbohydrate-free |
| 5 | F | --- | 2 | 2 | 4 | | |
| 6 | M | --- | 5 | 3 | 8 | 6.6 | Glucose |
| 7 | M | --- | 4 | 4 | 8 | | |
| 8 | M | --- | 2 | 3 | 5 | | |
| 9 | M | --- | 2 | 2 | 4 | | |
| 10 | F | --- | 7 | 6 | 13 | 7.3 | Sucrose |
| 11 | F | --- | 5 | 4 | 9 | | |
| 12 | M | --- | 5 | 5 | 10 | | |
| 13 | M | 3 | 6 | 3 | 12 | | |
| 14 | F | --- | 5 | 4 | 9 | 9.2 | Lactose |
| 15 | F | --- | 2 | 4 | 6 | | |
| 16 | M | --- | 2 | 3 | 5 | | |
| 17 | M | --- | 2 | 2 | 4 | 4.5 | Fine Rice |

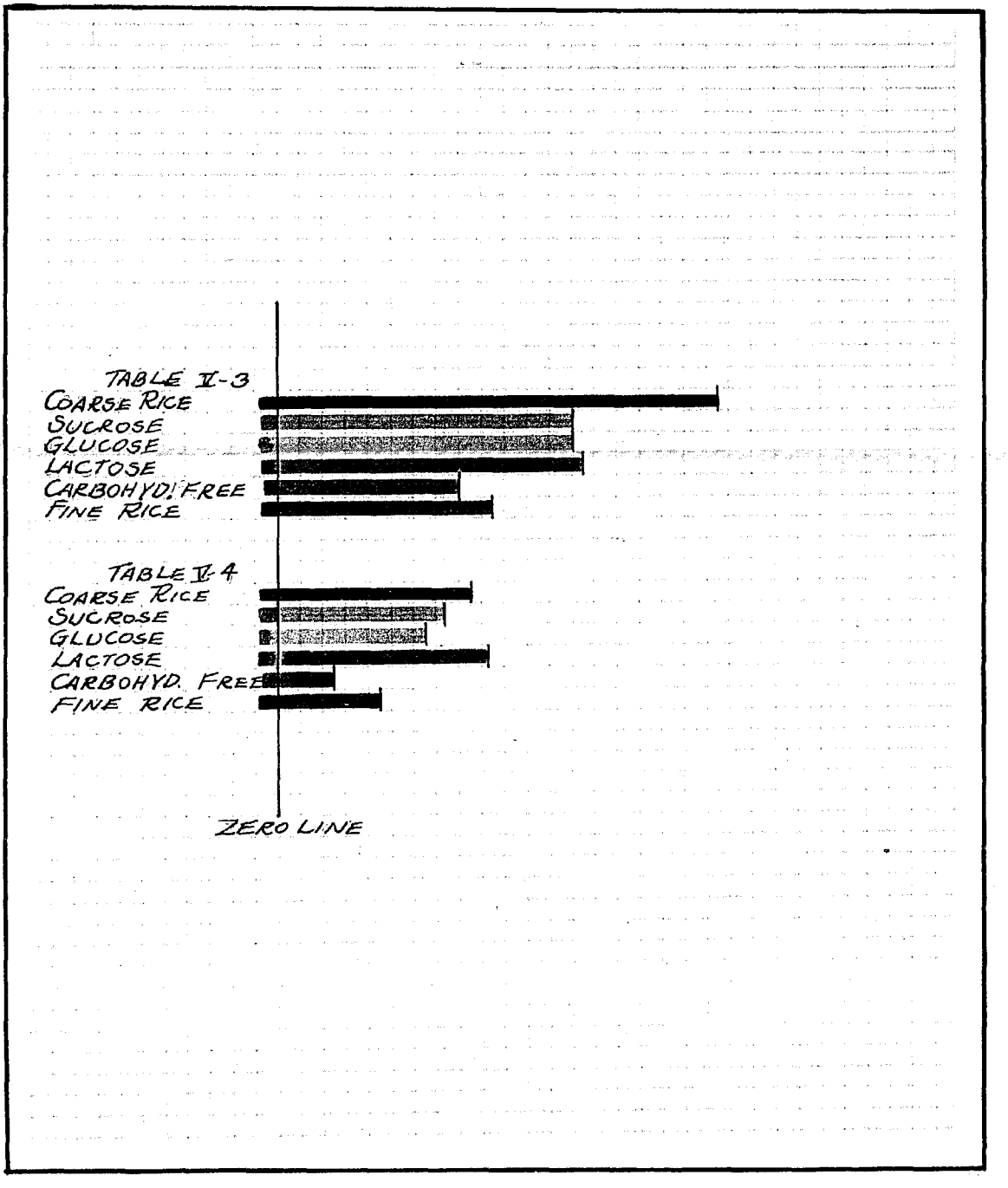


CHART V-3
From Tables V-3, & 4; Comparative Effects of Feeding Various Sugars, and Fine Rice.

TABLE V-5A CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 3 | 4 | 7 | | |
| 2 | F | --- | 3 | 3 | 6 | 7.2 | Fine Rice |
| 3 | M | --- | 4 | 3 | 7 | | |
| 4 | M | --- | 4 | 5 | 9 | | |
| 5 | F | --- | 2 | 3 | 5 | | |
| 6 | F | --- | 3 | 2 | 5 | | |
| 7 | M | --- | --- | --- | 0 | 3.5 | Sucrose |
| 8 | M | --- | 2 | 2 | 4 | | |
| 9 | F | --- | 5 | 5 | 10 | | |
| 10 | F | --- | 4 | 5 | 9 | 7.7 | Glucose |
| 12 | M | --- | 2 | 2 | 4 | | |
| 13 | F | --- | 6 | 2 | 8 | | |
| 14 | F | --- | --- | 2 | 2 | | |
| 15 | M | --- | 2 | 2 | 4 | 4.5 | Lactose |
| 16 | M | --- | 2 | 2 | 4 | | |

TABLE V-5B

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 17 | M | --- | 6 | 5 | 11 | | |
| 18 | M | --- | 7 | 6 | 13 | | |
| 19 | M | --- | 6 | 4 | 10 | | |
| 20 | M | --- | 5 | 7 | 12 | | |
| 21 | M | --- | 6 | 5 | 11 | | |
| 22 | M | --- | 4 | 7 | 11 | 13 | Fine Rice |
| 23 | F | --- | 10 | 9 | 19 | | |
| 24 | F | --- | 10 | 9 | 19 | | |
| 25 | F | --- | 7 | 5 | 12 | | |
| 26 | F | --- | 6 | 6 | 12 | | |
| 27 | M | --- | 4 | 4 | 8 | | |
| 28 | M | --- | 4 | 5 | 9 | | |
| 29 | M | --- | 10 | 8 | 18 | | |
| 30 | M | 3 | 7 | 6 | 16 | | |
| 31 | M | --- | 7 | 7 | 14 | | |
| 32 | M | --- | 5 | 5 | 10 | 12.2 | Coarse Rice |
| 33 | F | --- | 8 | 8 | 16 | | |
| 34 | F | --- | 5 | 6 | 11 | | |
| 35 | F | --- | 8 | 8 | 16 | | |
| 36 | F | --- | 2 | 2 | 4 | | |
| 37 | M | --- | 6 | 4 | 10 | | |
| 38 | M | 2 | 4 | 5 | 11 | | |
| 39 | M | --- | 5 | 6 | 11 | | |
| 40 | M | --- | 3 | 4 | 7 | | |
| 41 | M | --- | 3 | 5 | 8 | 8.9 | Carbohydrate-free |
| 42 | M | --- | 6 | 6 | 12 | | |
| 43 | F | --- | 4 | 3 | 7 | | |
| 44 | F | --- | 5 | 5 | 10 | | |
| 45 | F | --- | 4 | 4 | 8 | | |
| 46 | F | --- | 2 | 3 | 5 | | |
| 47 | M | --- | 2 | 3 | 5 | | |
| 48 | M | --- | 9 | 8 | 17 | | |
| 49 | M | --- | 4 | 2 | 6 | | |
| 50 | M | --- | 7 | 4 | 11 | | |
| 51 | M | --- | 6 | 4 | 10 | | |
| 52 | M | --- | 6 | 4 | 10 | 10 | Sucrose |
| 53 | F | --- | 5 | 9 | 14 | | |
| 54 | F | --- | 4 | 2 | 6 | | |
| 55 | F | --- | 3 | 4 | 7 | | |
| 56 | F | --- | 7 | 7 | 14 | | |

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TABLE V-5B (con't.) CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 57 | M | --- | 6 | 5 | 11 | | |
| 58 | M | --- | 5 | 7 | 12 | | |
| 59 | M | --- | 3 | 4 | 7 | | |
| 60 | M | --- | 2 | 2 | 4 | | |
| 61 | M | --- | 3 | 1 | 4 | 7.7 | Glucose |
| 62 | M | Died before completion | | | | | |
| 63 | F | --- | 2 | 2 | 4 | | |
| 64 | F | --- | 4 | 5 | 9 | | |
| 65 | F | --- | 5 | 3 | 8 | | |
| 66 | F | --- | 6 | 5 | 11 | | |
| 67 | M | --- | 4 | 4 | 8 | | |
| 68 | M | --- | 4 | 3 | 7 | | |
| 69 | M | --- | 4 | 6 | 10 | | |
| 70 | M | --- | 10 | 8 | 18 | | |
| 71 | M | --- | 5 | 7 | 12 | | |
| 72 | M | --- | 11 | 10 | 21 | 10.5 | Lactose |
| 73 | F | --- | 1 | 1 | 2 | | |
| 74 | F | --- | 2 | 3 | 5 | | |
| 75 | F | --- | 6 | 6 | 12 | | |
| 76 | F | --- | 5 | 5 | 10 | | |
| Control Group C: Terminate at 6 weeks for comparison | | | | | | | |
| 77 | M | --- | 3 | 4 | 7 | | |
| 78 | M | --- | 3 | 4 | 7 | | |
| 79 | F | --- | 2 | 1 | 3 | 5.4 | Coarse Rice |
| 80 | F | --- | 1 | 3 | 4 | | |
| 81 | F | --- | 3 | 3 | 6 | | |

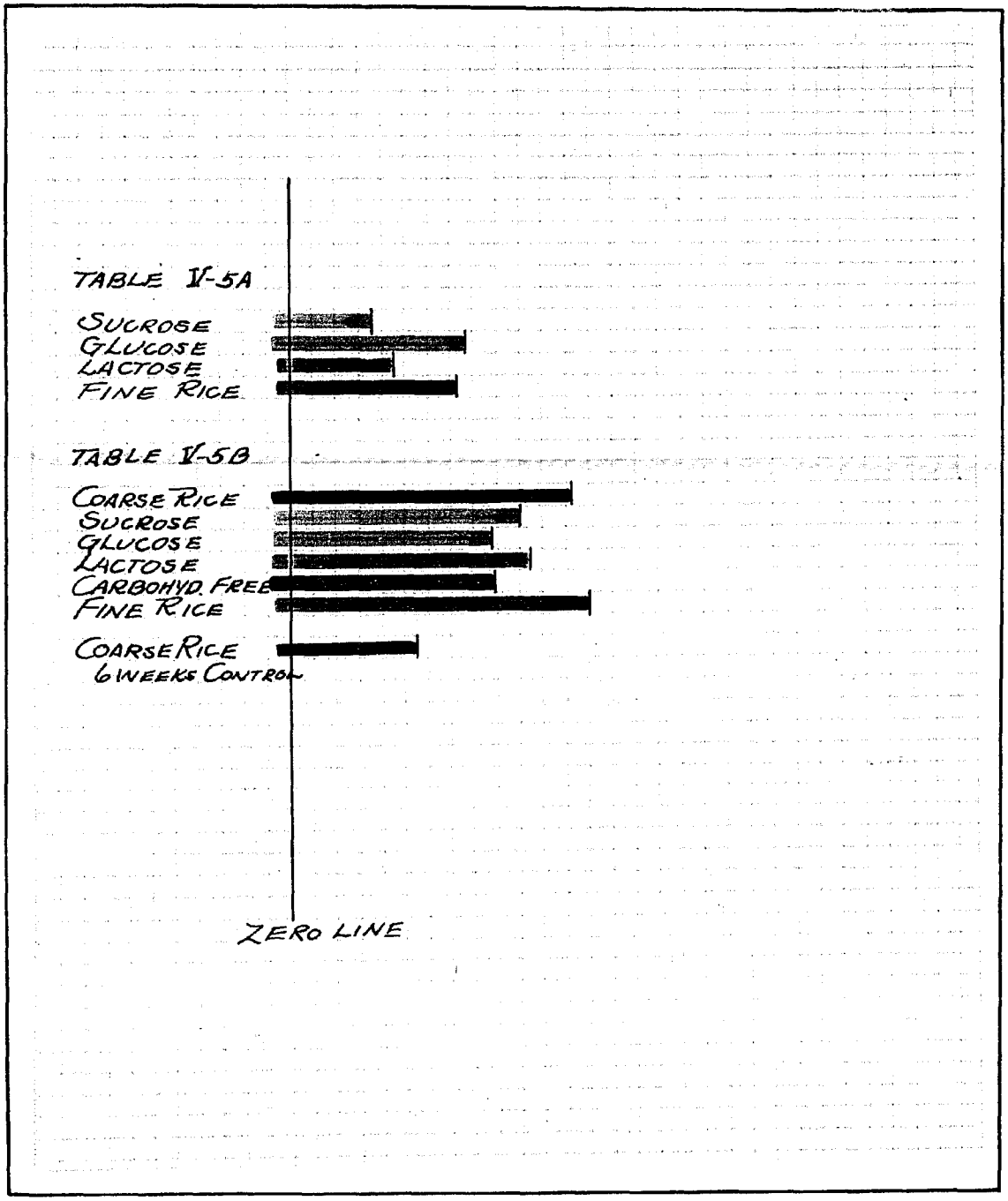


CHART V-4
From Tables V-5a, & 5b; Comparative Effects of Feeding Various Sugars, Fine Rice, and Coarse Rice.

TABLE V-6 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | M | --- | 6 | 7 | 13 | | |
| 2 | M | --- | 6 | 5 | 11 | | |
| 3 | M | --- | 5 | 7 | 12 | | |
| 4 | M | --- | 4 | 4 | 8 | | |
| 5 | F | --- | 6 | 6 | 12 | 11.6 | Coarse Rice |
| 6 | F | --- | 3 | 6 | 9 | | |
| 7 | F | --- | 6 | 8 | 14 | | |
| 8 | F | --- | 7 | 7 | 14 | | |
| 9 | M | --- | 6 | 6 | 12 | | |
| 10 | M | --- | 7 | 7 | 14 | | |
| 11 | M | --- | 5 | 6 | 11 | | |
| 12 | M | --- | 4 | 4 | 8 | | |
| 13 | M | --- | 5 | 5 | 10 | | |
| 14 | F | --- | 5 | 4 | 9 | 10 | Sucrose |
| 15 | F | --- | 2 | 2 | 4 | | |
| 16 | F | --- | 7 | 8 | 15 | | |
| 17 | F | --- | 3 | 3 | 6 | | |
| 18 | F | --- | 6 | 5 | 11 | | |
| 19 | F | --- | 5 | 5 | 10 | | |
| 20 | F | --- | 3 | 4 | 7 | | |
| 21 | F | --- | 7 | 11 | 18 | | |
| 22 | F | --- | 7 | 8 | 15 | | |
| 23 | M | --- | 4 | 4 | 8 | | |
| 24 | M | --- | 6 | 5 | 11 | 10.6 | Glucose |
| 25 | M | --- | 9 | 6 | 15 | | |
| 26 | M | --- | 1 | 3 | 4 | | |
| 27 | M | --- | 3 | 5 | 8 | | |
| 28 | M | Died before completion | | | | | |
| 29 | F | --- | 6 | 4 | 10 | | |
| 30 | F | --- | 6 | 7 | 13 | | |
| 31 | F | --- | 4 | 3 | 7 | | |
| 32 | F | --- | 3 | 4 | 7 | | |
| 33 | M | --- | 5 | 4 | 9 | | |
| 34 | M | --- | 5 | 4 | 9 | 9.7 | Lactose |
| 35 | M | --- | 5 | 5 | 10 | | |
| 36 | M | --- | 5 | 6 | 11 | | |
| 37 | M | --- | 2 | 6 | 8 | | |
| 38 | M | --- | 7 | 6 | 13 | | |

Continued on next page

TABLE V-6 (cont'd.) CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 39 | F | --- | 5 | 5 | 10 | | |
| 40 | F | --- | 4 | 3 | 7 | | |
| 41 | F | --- | 2 | 4 | 6 | | |
| 42 | F | --- | 3 | 6 | 9 | | |
| 43 | M | --- | 2 | 6 | 8 | | |
| 44 | M | --- | 9 | 6 | 15 | 8 | Carbohydrate-free |
| 45 | M | --- | 4 | 2 | 6 | | |
| 46 | M | --- | 3 | 4 | 7 | | |
| 47 | M | --- | 3 | 3 | 6 | | |
| 48 | M | --- | 3 | 3 | 6 | | |

TABLE V-7 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 5 | 4 | 9 | | |
| 2 | F | --- | 8 | 7 | 15 | | |
| 3 | F | --- | 3 | 4 | 7 | | |
| 4 | F | --- | 3 | 3 | 6 | 9.6 | Carbohydrate-free |
| 5 | F | --- | 4 | 4 | 8 | | |
| 6 | M | --- | 6 | 6 | 12 | | |
| 7 | M | --- | 5 | 5 | 10 | | |
| 8 | F | --- | 7 | 2 | 9 | | |
| 9 | F | --- | 7 | 5 | 12 | | |
| 10 | M | --- | 9 | 8 | 17 | | |
| 11 | M | --- | 8 | 9 | 17 | 13.3 | Sucrose |
| 12 | M | --- | 7 | 6 | 13 | | |
| 13 | M | --- | 5 | 8 | 13 | | |
| 14 | M | --- | 6 | 6 | 12 | | |
| 15 | M | --- | 9 | 10 | 19 | | |
| 16 | M | --- | 7 | 8 | 15 | | |
| 17 | F | --- | 5 | 6 | 11 | | |
| 18 | F | --- | 7 | 7 | 14 | 14.3 | Glucose |
| 19 | M | --- | 6 | 5 | 11 | | |
| 20 | M | --- | 9 | 10 | 19 | | |
| 21 | M | --- | 4 | 7 | 11 | | |
| 22 | F | --- | 7 | 8 | 15 | | |
| 23 | F | --- | 6 | 4 | 10 | | |
| 24 | F | --- | 5 | 7 | 12 | | |
| 25 | F | --- | 8 | 8 | 16 | 12.3 | Lactose |
| 26 | M | --- | 6 | 5 | 11 | | |
| 27 | M | --- | 6 | 7 | 13 | | |
| 28 | M | --- | 2 | 7 | 9 | | |
| 29 | M | --- | 7 | 10 | 17 | | |
| 30 | M | --- | 7 | 7 | 14 | 15.5 | Coarse Rice |

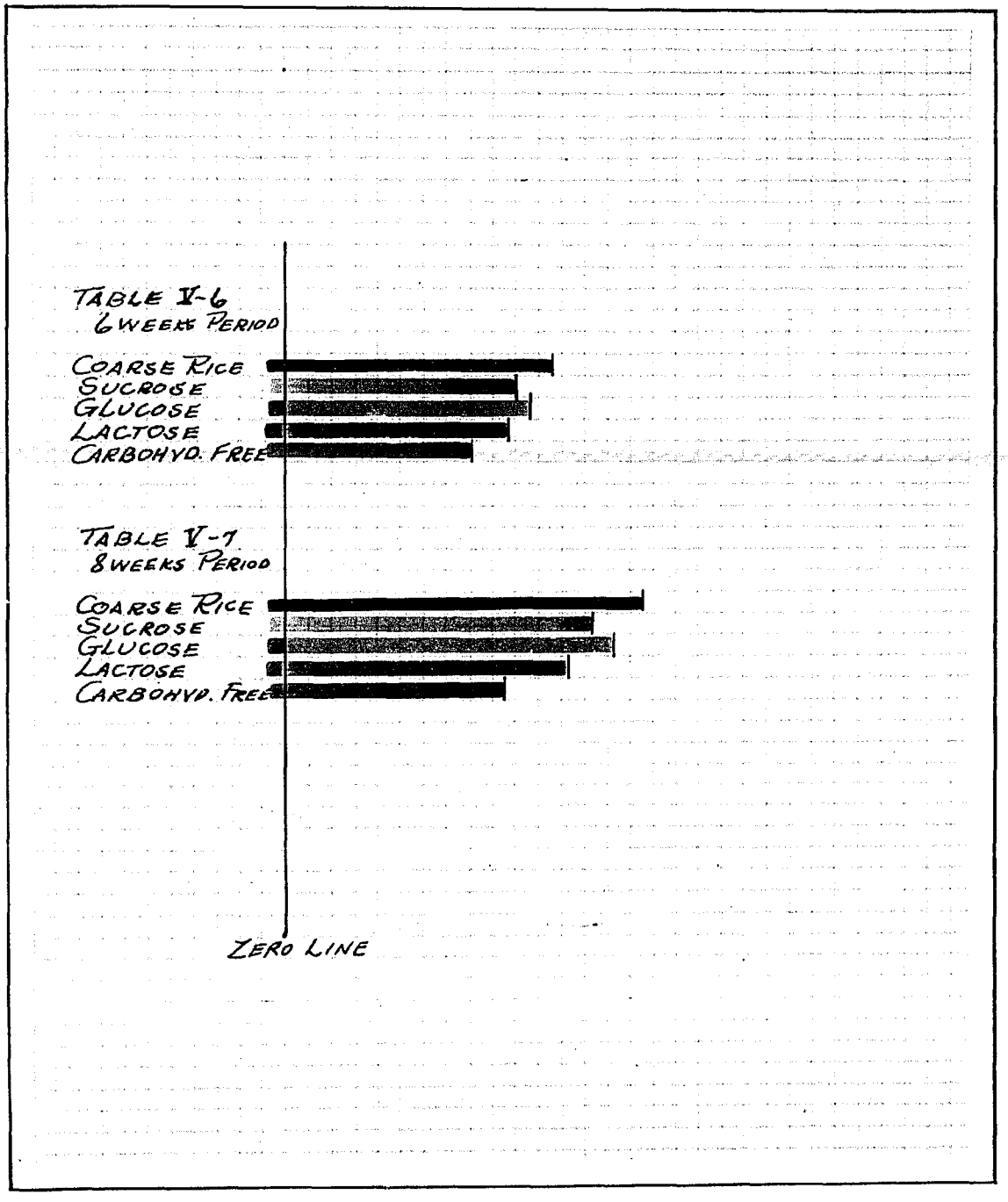


CHART V-5
From Tables V-6, & 7; Feeding Various Sugars for 6 and 8 week Periods.

TABLE V-8

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 6 | 8 | 14 | | |
| 2 | F | --- | 8 | 6 | 14 | | |
| 3 | M | --- | 6 | 8 | 14 | | |
| 4 | M | --- | 8 | 7 | 15 | | |
| 5 | F | --- | 6 | 4 | 10 | 14.2 | Corn Syrup Ration |
| 6 | F | --- | 4 | 7 | 11 | | |
| 7 | M | --- | 9 | 7 | 16 | | |
| 8 | M | --- | 9 | 7 | 16 | | |
| 9 | F | --- | 8 | 7 | 15 | | |
| 10 | F | --- | 8 | 9 | 17 | | |
| 11 | F | --- | 9 | 8 | 17 | | |
| 12 | F | --- | 6 | 5 | 11 | | |
| 13 | M | --- | 7 | 6 | 13 | 12.3 | Coarse Rice Only |
| 14 | M | --- | 5 | 6 | 11 | | |
| 15 | F | --- | 4 | 3 | 7 | | |
| 16 | F | --- | 8 | 7 | 15 | | |

TABLE V-9

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 9 | 10 | 19 | | |
| 2 | F | --- | 8 | 8 | 16 | | |
| 3 | M | --- | 7 | 7 | 14 | 16.8 | Corn Syrup Ration |
| 4 | M | --- | 9 | 7 | 16 | | |
| 5 | M | --- | 10 | 9 | 19 | | |
| 6 | F | --- | 6 | 8 | 14 | | |
| 7 | F | --- | 5 | 7 | 12 | | |
| 8 | F | --- | 5 | 7 | 12 | | |
| 9 | F | --- | 5 | 5 | 10 | | |
| 10 | M | --- | 9 | 10 | 19 | 15.9 | Corn Syrup Ration plus 1% Irradiated Yeast |
| 11 | M | --- | 10 | 10 | 20 | | |
| 12 | M | --- | 9 | 9 | 18 | | |
| 13 | M | --- | 11 | 11 | 22 | | |
| 14 | F | --- | 7 | 5 | 12 | | |
| 15 | F | --- | 7 | 5 | 12 | | |
| 16 | F | --- | 6 | 6 | 12 | | |
| 17 | F | --- | 6 | 6 | 12 | | |
| 18 | M | --- | 6 | 6 | 12 | 13.2 | Coarse Rice Ration plus 1% Irradiated Yeast |
| 19 | M | --- | 6 | 9 | 15 | | |
| 20 | M | --- | 6 | 5 | 11 | | |
| 21 | M | --- | 10 | 9 | 19 | | |
| 22 | F | --- | 10 | 7 | 17 | | |
| 23 | F | --- | 9 | 8 | 17 | 15 | Coarse Rice Ration |
| 24 | F | --- | 5 | 6 | 11 | | |

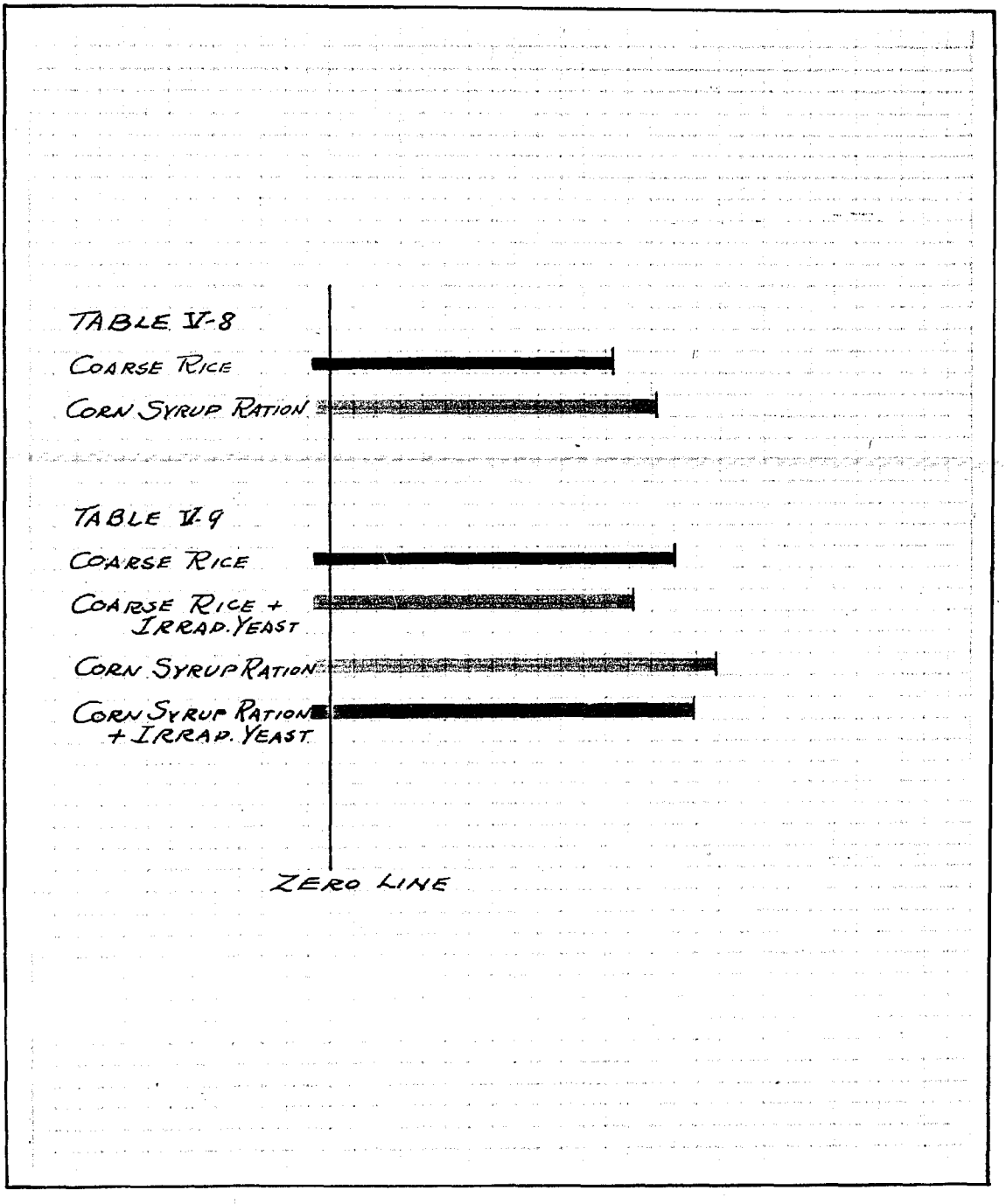


CHART V-6
From Tables V-8, & 9; Feeding Corn Syrup and Coarse Rice Simultaneously; Use of High Vitamin D Supplement.

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# BIBLIOGRAPHY AND LITERATURE CITED


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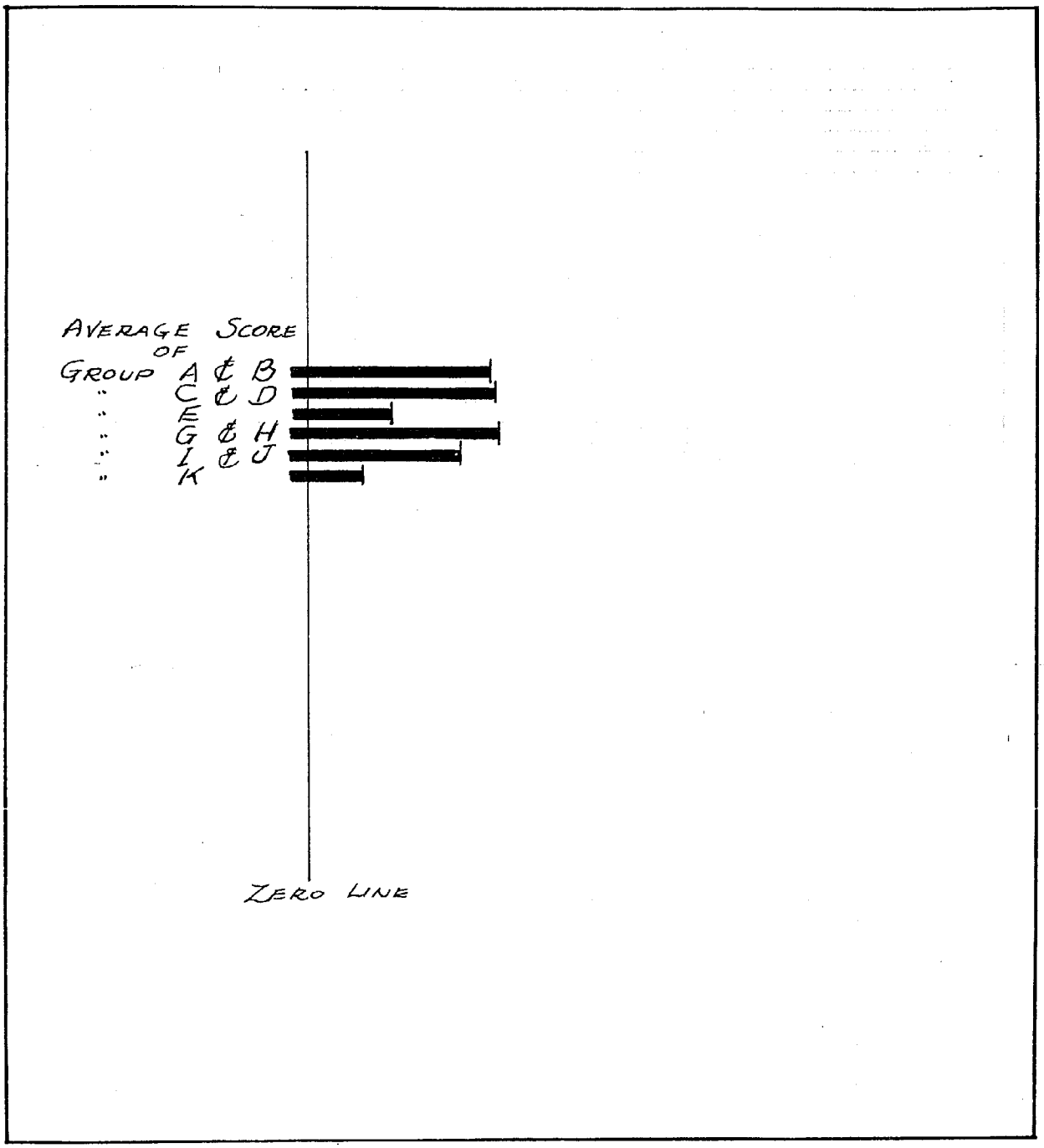


CHART IV-2
From Table IV-3; Most Effective
Period of Feeding Vitamin D.

# PART III

# THE EFFECT OF FEEDING VARIOUS CARBOHYDRATES ON THE DEVELOPMENT OF DENTAL CARIES

## Part III: The Effect of Feeding Various Carbohydrates on the Development of Dental Caries

The literature is replete with references to the significance of carbohydrates in the picture of dental caries. Practically every article written emphasizes the role of carbohydrates. Bunting and his group (7), Rosebury and co-workers (32), Belding and Belding (2), Hanke and co-workers (8), to mention only a few, have all pointed out the significance of carbohydrates. In only one instance, the work of Lilly (27), was it reported that there was no appreciable effect of carbohydrates on the development of caries. The possible explanation in this case may be that a fine diet was used which alone would fail to initiate carious lesions. Moderate use of sweets has long been the advice of the dental fraternity, and the work of Koehne (25) with a group of children has amply shown the effect of sugar on the incidence of caries.

Against this background, a series of experiments was planned to answer, if possible, several questions such as: What types of carbohydrates are most active in their influence on caries? Do all carbohydrates initiate caries, or is the effect of some simply that of extending the destruction of the tooth structure once caries has been initiated? Accordingly, the following series of experiments was set up:

EXPERIMENT NO. 1. The effect of substituting various

sugars for cereal. A group of 29 selected animals was placed at weaning (21-28 days) on the basal rice, or caries producing diet, containing: 60 parts coarsely ground rice, 30 parts whole milk powder, 6 parts flax-seed meal, 5 parts alfalfa meal, 3 parts dried yeast, and 1 part sodium chloride. At the end of five to six weeks, depending upon the development of lesions as determined by examination, the diet was changed to the experimental ration and feeding was continued for a period of 7-8 weeks, the experiment being terminated in the 8th week. The experimental rations used had the following composition:

| | Diet No. 1 | |
|---|---|---|
| Carbohydrate | 67% | (Sucrose, Glucose, or Lactose) |
| Casein (com'l.) | 18% | |
| Salt Mixture | 4% | (Modified Steenbock) |
| Dried Yeast | 5% | |
| Corn Oil | 5% | |
| Cod Liver Oil | 1% | |
| Total | 100% | |

In this experiment, the carbohydrate level was slightly higher than the cereal content. The animals were divided into eight groups with the distribution and rations as follows:

Group A - 4 animals (2 M, 2 F); Ration containing 67% Sucrose

Group B - 4 animals (2 M, 2 F); Ration containing 67% Glucose

Group C - 4 animals ( 2 M, 2 F); Ration was Carbohydrate Free Diet using 20% Flax-seed Meal
30% Alfalfa Meal
19% Com'l. Casein
30% Crisco
1% Sodium Chloride

Group D - 4 animals (2 M, 2 F); Ration containing 67% Lactose

Group E - 2 animals (2 F); Ration Coarse Rice Control Diet containing 66% Coarsely Ground Rice
30% Milk Powder Whole
3% Alfalfa Meal
1% Sodium Chloride

Group F - 4 animals (1 M, 3 F); Ration containing a combination of Sucrose and Oatmeal as follows:

30% Sucrose
27% Ground Oatmeal
18% Com'l. Casein
4% Salt Mix
5% Dried Yeast
5% Corn Oil
1% Cod Liver Oil

Group G - 4 animals (2 M, 2 F); Ration Oatmeal Control Diet as follows:

66% Oatmeal
30% Whole Milk Powder
3% Alfalfa Meal
1% Sodium Chloride

Group H - 1 animal (1 F); Ration containing Milk Powder as follows:

90% Whole Milk Powder
9% Alfalfa Meal
1% Sodium Chloride

At the beginning of the experimental period, there were four animals in Group E, but two died during the process of examination under ether anesthesia. However, the results with the coarse rice diet had been fairly well standardized

so that the two animals remaining served as sufficient basis for comparison. The caries scores of these animals are found in Table V-1, and the comparison of the scores is shown on Chart V-1.

## Results

Before the results are cited, it would be well to mention that the diets were apparently not as satisfactory as had been hoped. Although the animals were able to maintain weight, or to gain slightly, their general physical condition seemed to decline. The animals fed the high lactose diet, in particular, showed definite signs of a toxic effect. This necessitated lowering the level of carbohydrate in subsequent studies. For qualitative purposes, however, the results may be of some interest. The score of the rice controls with a value of 9 showed an average similar to that of other animals fed the same diet. The high score of the entire experiment was 9.4 for the average of the group on glucose. Sucrose gave a value of 6, lactose 2.5, carbohydrate-free 2, oatmeal and sugar combination 2, oatmeal basal 0, and high milk 0.

## Discussion

Because the diets were not entirely satisfactory, it is a bit difficult to draw any definite conclusions from this experiment. There are one or two points that may be mentioned, however. The 67% level of sugars in the experimental diets was obviously too high, particularly in the case of lactose, which is now known to be toxic at high intakes. Moreover, there does

not appear in this experiment to be any greater degree of caries on the high carbohydrate diets than was found with the coarse rice diet. All the control diets showed scores of around 2 or less which is no more than would be expected on the basis of the preliminary 5 weeks feeding on the caries producing diet. One difficulty is that it is not possible to assess accurately the condition of the teeth in live animals in the early stages of decay. The initiation of the carious lesion may take place some time before the lesion can be recognized by gross examination--indeed such must be the case. It is, therefore, easy to see that too much reliance cannot be placed on the results of this first experiment with the sugars.

EXPERIMENT NO. 2: The effect of substituting carbohydrates at a lower level, and their influence on dental caries. A group of 14 animals was placed on the rice basal diet as given in Experiment 1. At the end of five to six weeks, depending on the development of lesions as determined by examinations, the diet was changed to the experimental ration and feeding was continued for a period of 7-8 weeks, the experiment being terminated during the eighth week. The experimental diets used were based on the following formula:

| | Diet No. 2 | |
|---|---|---|
| Carbohydrate | 35% | (Sucrose, Glucose, or Lactose) |
| Casein (com'l.) | 20% | |
| Flax-seed Meal | 20% | |
| Alfalfa Meal | 9% | |
| Dried Yeast | 5% | |
| Crisco | 10% | |
| Sodium Chloride | 1% | |
| Total | 100% | |

Note that the carbohydrate level has been reduced from 67% to 35%, as compared to the diet of Experiment 1. The animals were divided into five groups with distribution and diets as follows:

Group A – 3 animals (2 M, 1 F); Ration containing 35% Sucrose

Group B – 3 animals (2 M, 1 F); Ration containing 35% Glucose

Group C – 3 animals (1 M, 2 F); Ration containing 35% Lactose

Group D – 3 animals (1 M, 2 F); Carbohydrate-free Diet as given in Experiment 1

Group E – 2 animals (2 M) ; Coarse Rice Control Diet as given in Experiment 1

The caries scores of these animals will be found in Table V-2, and the comparison of the scores is found on Chart V-2.

## Results

The high average score of the group is shown with sucrose giving a value of 10, followed by the rice control at 9.5, lactose at 10.3, glucose at 8.3, and the carbohydrate-free diet at 6. In this experiment, the animals on glucose showed a slight drop over the first experiment. Both sucrose and lactose showed results slightly higher than the control rice, but probably not significantly greater. The carbohydrate-free diet gave a score of six which was a little higher than expected.

## Discussion

From the results obtained, there are several points of

interest. It is apparent that when the animals were placed on the experimental rations, they must have attained a fairly uniform initiation of caries in the teeth, for the scores show fairly similar values. The usual score for animals on the caries producing diet is about 10, and in both Experiments 1 and 2, this value was maintained. The average scores of the animals on the sugars were 10.3, 10, and 8.3 which shows that even after the rice was taken away, the lesions continued to develop and at about the same rate. In the absence of both coarse cereal and sugars, the rate of dental deterioration was appreciably slower and was probably due to the small amount of rice left in the cavities at the time the rats were put on the several modified diets. The average scores in the second experiment were generally higher than those in the first. The principal purpose of the second experiment was to develop a ration nutritionally adequate and yet satisfactory for studying the decay of teeth in rats. In all subsequent experiments, the general proportions of this ration were used.

## Summary and Conclusions

A 35% level of sugars proved satisfactory for comparative studies of tooth decay in rats.

The modified ration gave better growth, weight, and a generally healthier experimental animal.

The results indicate in general that the lesions develop on the sugar containing diets at about the same rate as on the coarse rice or caries producing diet.

The carbohydrate-free diet gave a slightly higher score than was anticipated. This was probably due to the presence of rice in the cavities at the time the diets were changed.

EXPERIMENT NO. 3: The next step in the work with carbohydrates was to compare the relative effects of the cereal, if finely ground, with those of the various sugars. All the animals were fed the coarse rice ration (The simpler diet composed of 66% coarsely ground rice, 30% whole milk powder, 3% alfalfa meal, and 1% sodium chloride will be used from now on as the caries producing diet, and is referred to as the coarse rice diet or coarse rice control.) for a period of from 5 to 6 weeks depending on the condition of the teeth as determined by examination. As the carious lesions were initiated by the coarse rice ration the animals were changed to the experimental rations containing the various sugars, and finely ground rice. The fine rice diet was the same as the coarse rice diet given above, except that the cereal was very finely ground in a ball mill. The carbohydrate-free diet has been described in previous work.

A group of 19 animals was selected and at weaning was placed on the coarse rice diet. The rats were fed for 5 to 6 weeks depending on the initiation of caries. They were then placed on the experimental rations with the following distribution:

2 animals (2 M) continued on the coarse rice ration.
3 animals (2 M, 1 F) on the carbohydrate-free ration.
4 animals (2 M, 2 F) on the 35% sucrose diet.
4 animals (2 M, 2 F) on the 35% glucose diet.
4 animals (2 M, 2 F) on the 35% lactose diet.
2 animals (2 F) on the fine rice diet.

Experimental rations were fed for eight weeks. The caries scores of these animals will be found in Table V-3.

EXPERIMENT NO. 4 was a duplication of Experiment 3, and the caries scores of the animals in Experiment No. 4 are found in Table V-4. The scores of Experiments 3 and 4 have been combined to show the comparison of scores given on Chart V-3. The results and discussion of these two experiments will be considered together.

## Results

The average scores of the animals from both experiments find the coarse rice diet high with a value of 16.5, lactose, 11.4, sucrose, 10.4, glucose, 10.3, fine rice, 7, and the carbohydrate-free diet, 5.8. The animals on the coarse rice and fine rice rations in Experiment 3 were somewhat higher than expected, but fell to about their normally expected values in Experiment 4.

## Discussion

It was interesting to note that lactose gave the highest average of all the sugars. It is possible that the scores in this series were slightly higher due to the fact that the lesions produced by the coarse rice in the preliminary feeding period may have been somewhat more severe than previously. It would be expected from the work done by such workers as Rosebury et al (32), Hanke (15), Florestano (10), and Branson (4), we would expect to find sucrose most active with glucose following. However, little work has been done with

lactose, per se, and a final conclusion should probably be held in reserve until the results of some of the subsequent experiments have been reviewed. It would appear that the development of the lesion does continue on the diets containing the various sugars but not as rapidly as when the coarse rice is fed. With the fine rice, the lesions apparently slow down in their rate of development for the score is considerably lower than for any of the sugars.

This seemed a bit surprising in view of what happened with the coarse rice. Once having been initiated, it was thought that the fine rice would show at least as rapid a development of the lesions as was found with the sugars. Of course, one must always consider the possibility that the rapid rate with the coarse rice might be due in part to fracture inasmuch as the carious nature of the teeth rendered them more fragile, and less able to withstand the forces of mastication. Further, the fine rice may have exerted a filling action on the lesions which, though they had started to develop, might still have been small enough that when the fine rice became impacted it behaved as a filling keeping out oral fluids, thus retarding bacterial fermentation. It might be well to reserve final judgement on the effects of the fine rice until the data from additional animals may be scrutinized.

## Summary and Conclusions

Carious lesions continued to develop or enlarge under the influence of the various sugars.

The rate was, apparently, not as rapid as with the

coarse rice in the caries producing diet.

The effect of all three sugars was about the same, although lactose gave slightly higher scores than sucrose and glucose.

Fine rice showed lower results than were expected.

EXPERIMENT NO. 5: This experiment was a repetition of Experiments 3 and 4. This was done to check two questionable points that arose in the preceding work. It seemed desirable to determine whether or not lactose was consistent in producing a higher caries score, and whether or not the score produced on fine rice would be consistently lower than that of the sugars. In addition, a modification was made in that groups of animals were placed on the experimental rations without the preliminary 4 to 6 weeks period on the coarse rice diet.

A group of 81 animals was selected. These were taken at weaning (21-28 days), and were divided into three groups:

Group A: 4 units of four animals each. These units were placed on the experimental rations at once, having no preliminary feeding period on the coarse rice diet. From time of birth until about 28 days old they had access to the stock diet which contained some ground corn. The rations fed these four groups were: fine rice ration, 35% sucrose ration, 35% glucose ration, 35% lactose ration (rations described in Experiment 2). The experimental rations were

fed for a period of 14 weeks.

Group B: 6 units of ten animals each. These units were placed on the coarse rice diet for from 4 to 6 weeks for the initiation of carious lesions as determined by examination. When the lesions were found to have started, the animals were immediately transferred to the experimental rations for a period of 8 weeks. The rations used (given in Experiment 2) were as follows: coarse rice, fine rice, carbohydrate-free, 35% sucrose, 35% glucose, and 35% lactose.

Group C: 1 unit of five animals was placed on the coarse rice diet at weaning. This was fed for a period of six weeks, at the end of which time the animals were destroyed and the mandibles removed and scored as usual to give a standard of comparison for the animals on the experimental rations.

The caries scores for Group A are found in Table V-5a, for Group B and Group C in Table V-5b. The comparison of the scores for all three groups is shown on Chart V-4.

## Results

In the case of Group A the score for the fine rice was 7.2, for sucrose 3.5, for glucose 7.7, and for lactose 4.5. These scores were obtained with the animals having no preliminary feeding period on the caries producing diet.

The scores of the animals in Group B, which had a preliminary feeding period, were much higher and as follows:

fine rice 13, coarse rice 12.2, lactose 10.5, sucrose 10, glucose 7.7, and carbohydrate-free 8.9. It is interesting to note that fine rice gave the highest score, sucrose and lactose were about the same, while glucose gave a lower score than the carbohydrate-free diet.

The reference group, Group C, which was terminated after 6 weeks feeding on the coarse rice diet showed a score of 5.4. This will give an idea as to the average at the time of transfer to the experimental rations.

## Discussion

The results of Group A would lead one to believe either that the experimental rations would initiate carious lesions, or that some impaction had taken place on the stock diet, thus initiating caries which further developed on the experimental rations. It would appear the latter explanation was most valid. It is interesting to note that glucose gave the highest score in this group, while in the following group, it gave the lowest value. Fine rice also gave a high value.

The results of Group B were quite consistent and about what might be expected. It was thought the fine rice should show as great or greater effect in the development of caries after the lesions were once started, since the finely ground cereal would furnish a source of some carbohydrate and also a continuous supply of the aciduric organisms. Such proved to be the case. With the sugars, sucrose and lactose, the average scores were lower than either the fine or coarse

rice but were appreciably higher than the carbohydrate-free diet and also much higher than the score of the comparison Group C at 5.4. The score of the glucose ration was consistent from individual to individual but for some reason was low. It appears that there is a possibility of a decrease of the number and strength of the acid producing bacteria where the cereal is completely removed and a more or less pure form of carbohydrate substituted. Further, the production of acid by the bacteria present might be great enough, in the areas where impaction and lesions were found, to kill the bacilli. To exceed the maximum pH for existence of the bacteria at certain focal points of infection is not inconceivable.

## Summary and Conclusions

It may be concluded that a diet containing a high level of carbohydrate in the form of some sugar will cause the carious lesions to become enlarged.

The sugars alone are not likely to be the cause of the initiation of caries.

The cereal, whether fine or coarse, usually causes more severe lesions, since it not only furnishes a form of carbohydrate but also a source of the acid forming organisms.

Further work with the sugars would be of interest, particularly in light of the results with glucose.

EXPERIMENT NO. 6: This is a repetition of some of the previous experiments with slight modifications. A group of

48 animals was selected, and at the beginning of their 6th week, the animals were placed on the coarse rice diet for the initiation of caries. This preliminary period was from 5-6 weeks as determined by the examination of the molars. When teeth showed the initial development of caries, the animals were placed on the experimental ration which was fed for a period of 6 rather than 8 weeks. The 8 week period seemed to allow the destruction of the tooth to advance too far for good scoring. The distribution of animals was as follows:

(a) 8 animals continued on coarse rice ration.
(b) 10 animals on the sucrose ration.
(c) 10 animals on the glucose ration.
(d) 10 animals on the lactose ration.
(e) 10 animals on the carbohydrate-free ration.

The fine rice diet was eliminated from the series. At the end of the sixth week on the experimental ration, the animals were destroyed, the mandibles removed and scored as usual. Scores of these animals are found in Table V-6, with the comparison of the scores shown on Chart V-5.

## Results

The average scores obtained are as follows: coarse rice 11.6, sucrose 10, glucose 10.6, lactose 9.7, and carbohydrate-free 8. The scores are slightly lower than in previous work due to the shorter period on experimental rations, but on the whole seem to be more consistent.

## Discussion

The over-all averages of this group are more consistent

and form a good pattern. The glucose group showed a more normal response. The carbohydrate-free diet gave a score slightly higher than expected, but even so, the other scores are still from 2 to 3 points higher, which shows that lacking available carbohydrate the development of the carious lesions is slowed down. In this work, the three sugars showed scores closely grouped which would indicate that they all function in the same way and to about the same degree.

## Summary and Conclusions

The lesions develop more rapidly in the presence of carbohydrate in such form as sugars.

The most rapid development requires the presence of the cereal which furnishes carbohydrate and acid forming organism as well.

EXPERIMENT NO. 7: In this experiment, a group of 30 animals was selected. The group was divided into 4 units of 7 animals each, and 1 unit of 2 animals. They were 28-30 days old, and all were placed on the coarse rice (caries producing) ration until on individual examination caries could be detected. At this stage, the score being a minimum of 1 and a maximum of 3, the animal was placed on the experimental ration of its respective unit for a period of 8 weeks. The animals were destroyed at the end of the eighth week, the mandibles removed and scored as usual. The feeding plan was as follows:

Unit 1: 7 animals on carbohydrate free diet.
Units 2, 3 and 4: 7 animals each on the high carbohydrate diet with the following modification: 20% Flax-seed meal, 20% Casein (com'l.), 20% Alfalfa Meal, 5% Crisco, 4% Dried Yeast, 1% Sodium Chloride, and 30% Carbohydrate (Sucrose, Glucose, or Lactose). All ingredients were carefully blended before adding the carbohydrate, which was mixed in the last thing to avoid coating the sugars with the fat.
Unit 5: 2 animals as controls on the coarse rice.

The scores of the above animals may be found in Table V-7 with the comparison of the scores shown on Chart V-5.

## Results

The scores again conformed fairly well with those previously obtained. Glucose gave a value of 14.3, Sucrose 13.3, Lactose 12.3, Carbohydrate-free 9.6, and the 2 animals on the coarse rice, which served as controls, 15.5.

## Discussion

It is again seen that the carious lesions continue to enlarge on diets containing sugar, but the advance is not as rapid as when the animal is continued on the coarse rice diet. The average scores of all the animals on the sugar diets was 13.3, an increase in the average score of 3.7 over the carbohydrate free diet. This 13.3 average is 2.2 less than that of the animals on the caries producing diet. From these results, and those of preceding experiments, it would seem to have been definitely established that though the sugars do cause an increase in the severity of lesions that have been initiated, the lesions are more severe if

the animal is kept on the caries producing diet. The sugars may cause an increase in severity though they do not initiate the lesion.

A point might be made of the fact that it is highly probable, in the case of the rat at least, that the sugars themselves are not so harmful unless fed in a diet containing the caries producing factor, i.e. the coarsely ground cereal as rice or corn. If such a combination were fed the effect of the sugars superimposed on the coarse cereal would undoubtedly be a more drastic one. As has been mentioned before, the possibility of accurate and significant scoring diminishes after the breakdown in tooth structure has advanced beyond a certain point. Thus, a feeding experiment involving both factors would have to be checked by careful individual examinations. Otherwise, the scores would approach a maximum in every case, and in less time than the eight week period used when the foods in question were fed separately.

## Summary and Conclusions

It may be concluded that carbohydrate in the form of sugars causes the lesion to develop and become larger.

The development is fairly rapid and severe.

When sugars are included in the diet, the lesions are not as severe as when the caries producing diet is fed a similar length of time.

If the sugars were fed with the caries producing diet,

maximum severity of lesions and rapidity of breakdown in tooth structure might be expected.

EXPERIMENTS NO. 8 and 9: These two experiments were designed to give proof to the point mentioned in the discussion and summary of the preceding experiment. What would be the effect of feeding a combination of sugar with the caries producing diet?

To do this, a ration was devised which contained both coarse rice and sugars in the form of a corn syrup. The syrup was first mixed thoroughly with the coarsely ground rice so that the rice was quite effectively coated with the syrup. The syrup-rice mixture was allowed to dry, then crumbled, and the other ingredients added and mixed by hand. The formula of the ration is as follows:

| | | | | |
|---|---|---|---|---|
| 66 | parts | by | weight | coarsely ground rice |
| 30 | " | " | " | whole milk powder |
| 3 | " | " | " | alfalfa meal |
| 1 | " | " | " | sodium chloride |
| 10 | " | " | " | corn syrup (Dark Karo) |

It was also decided that it would be of interest to observe the effect of a high Vitamin D intake with such a ration. The procedure was as follows:

Experiment 8 was carried out with 16 animals 28-30 days old, which were divided into one group of 10 animals (4 M, 6 F) fed the corn syrup diet for 8 weeks, and one group of 6 animals (2 M, 4 F) fed the coarse rice diet for 8 weeks.

Experiment 9 was carried out with the offspring of 5 young females fed the regular stock diet containing 3%

irradiated yeast of high Vitamin D potency. The diet is reviewed here:

| | |
|---|---|
| 40% Ground yellow corn | 6% Alfalfa meal |
| 30% Oatmeal | 3% Irradiated yeast |
| 20% Whole milk powder | 1% Sodium chloride |

From the litters, 24 young were selected and at 28-30 days were divided and placed on the experimental rations as follows:

| | | |
|---|---|---|
| Group 1 - 5 animals | (3 M, 2 F) | Corn Syrup Ration |
| Group 2 - 8 animals | (4 M, 4 F) | Corn Syrup Ration plus 3% Irradiated Yeast |
| Group 3 - 8 animals | (4 M, 4 F) | Coarse Rice Ration plus 3% Irradiated Yeast |
| Group 4 - 3 animals | (3 F) | Coarse Rice Ration |

The experimental rations were fed for a period of 10 weeks.

Experiments 8 and 9 were terminated during the 10th week, when the animals were destroyed, mandibles removed and scored as usual. The scores of Experiment 8 are found in Table V-8, for Experiment 9 in Table V-9, with comparison of scores for both experiments being shown on Chart V-6.

## Results

The results were very much as anticipated and the scores for animals in Experiment 8 gave averages of 14.2 for the corn syrup ration against 12.3 for the coarse rice ration.

The results in Experiment 9 gave averages of 16.8 for the corn syrup ration, 15.9 for the same ration containing the irradiated yeast, 13.2 for the coarse rice ration containing the irradiated yeast, and 15 for the coarse rice

ration alone. The results are quite consistent and do indicate a protective action for the Vitamin D.

## Discussion

A ten weeks period was used in Experiment 9 for feeding the experimental rations inasmuch as all the previous experiments using carbohydrates had been preceded with a 4-6 week preliminary feeding on the caries producing ration. The differences were not as marked as was anticipated. It is entirely possible, however, that had the period of feeding been somewhat shorter, there would have been greater differences.

The action of the cereal as initiator and of the sugars as accelerator is quite plainly established. The results show that corn syrup acted as did the sugars in previous experiments causing lesions to become more severe, and that its effect superimposed on the action of the caries producing diet gave a score that was greater than would be obtained with either one alone. The results confirm the theories and observations of such workers as Bunting and co-workers, Rosebury, Belding and Belding, Cox, and many others. The protective action of the Vitamin D observed in previous work was again apparent.

The work done with the corn syrup ration indicates the desirability of a standardized caries producing diet. Such standardization would allow the application of statistical methods, and it would then be possible to evaluate the effect

of each factor. The availability of animals of uniform response would also be of assistance. This is largely a problem of selective breeding in which some progress has been made by Hunt and Hoppert who have developed strains of caries susceptible and caries resistant rats (19). The adoption of standardized procedures and improvement in accuracy, in selecting rats of approximately identical degree of decay initiated by the use of coarse rice, would materially aid in a more quantitative comparison of the materials studied in this research.

## Summary and Conclusions

It may be concluded that when a combination of sugar and cereal is fed, more severe lesions are produced than when these foods are used singly in suitable diets.

Under the conditions of the experiments performed, the influence of Vitamin D is protective rather than preventive.

TABLE V-1 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | M | --- | 6 | 7 | 13 | | |
| 2 | M | --- | 1 | 3 | 4 | | |
| 3 | F | --- | 1 | 1 | 2 | 6 | Sucrose |
| 4 | F | --- | 2 | 3 | 5 | | |
| 5 | M | --- | --- | --- | 0 | | |
| 6 | M | 2 | 6 | 7 | 15 | | |
| 7 | F | --- | 5 | 4 | 9 | 9.4 | Glucose |
| 8 | F | 1 | 5 | 7 | 13 | | |
| 9 | M | --- | 2 | --- | 2 | | |
| 10 | M | --- | 1 | --- | 1 | | |
| 11 | F | --- | 2 | 2 | 4 | 2 | Carbohydrate-free |
| 12 | F | --- | --- | 1 | 1 | | |
| 13 | M | --- | 2 | --- | 2 | | |
| 14 | M | --- | 1 | 1 | 2 | | |
| 15 | F | --- | 3 | 3 | 6 | 2.5 | Lactose |
| 16 | F | --- | --- | --- | 0 | | |
| 19 | M | --- | 3 | 4 | 7 | | |
| 20 | M | 2 | 5 | 4 | 11 | 9 | Coarse Rice |
| 21 | M | --- | 5 | 3 | 8 | | |
| 22 | F | --- | --- | --- | 0 | | |
| 23 | F | --- | --- | --- | 0 | 2 | Oatmeal and Sucrose |
| 24 | F | --- | --- | --- | 0 | | |
| 25 | M | --- | --- | --- | 0 | | |
| 26 | M | --- | --- | --- | 0 | | |
| 27 | F | --- | --- | --- | 0 | 0 | Oatmeal Basal |
| 28 | F | --- | --- | --- | 0 | | |
| 29 | F | --- | --- | --- | 0 | 0 | High Milk |

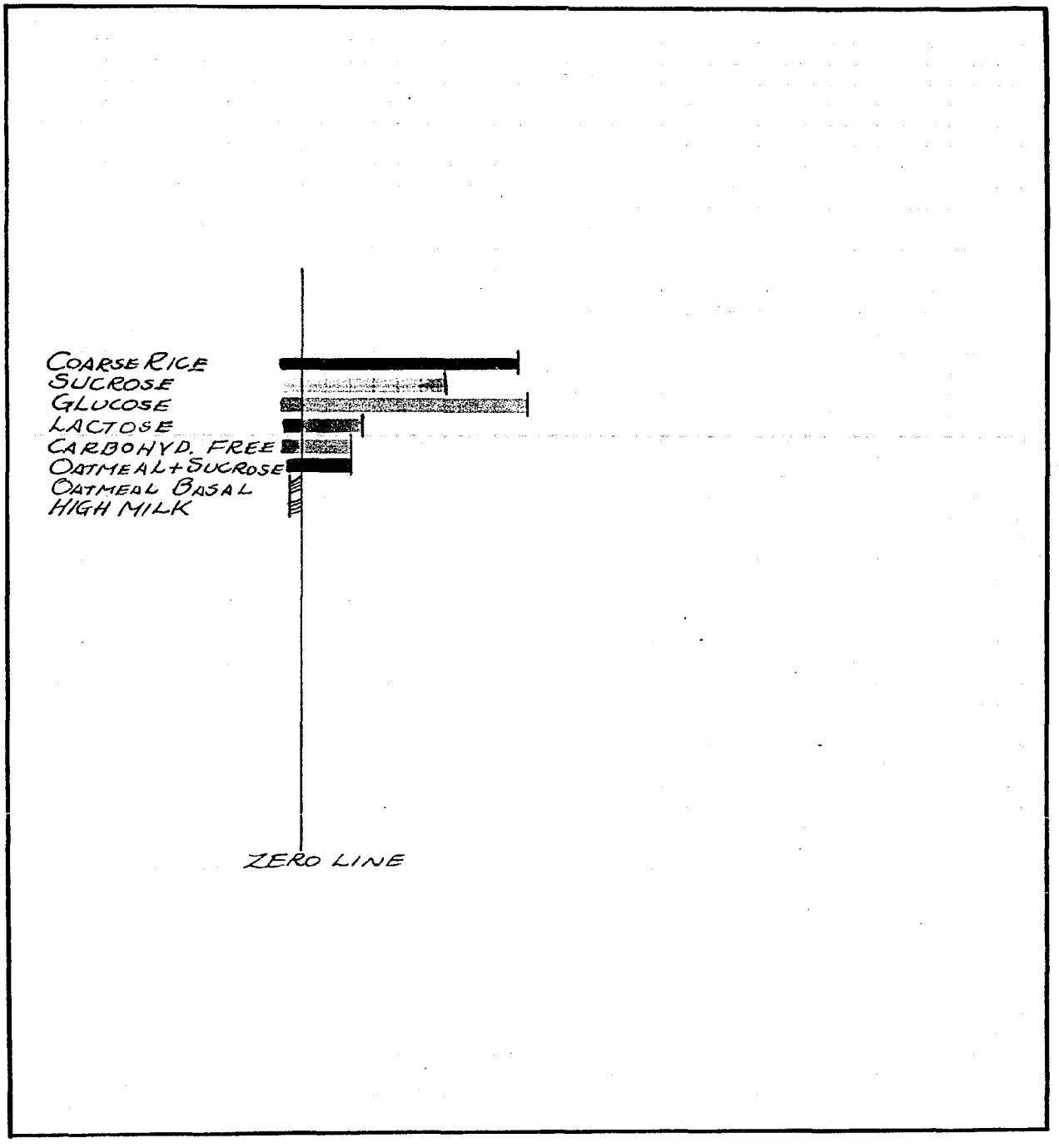


CHART V-1
From Table V-1; Preliminary Results
on High Sugar Diets.

TABLE V-2 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 2 | 4 | 6 | | |
| 2 | M | --- | 7 | 7 | 14 | 10 | Sucrose |
| 3 | M | --- | 6 | 4 | 10 | | |
| 4 | M | --- | 4 | 3 | 7 | | |
| 5 | M | --- | 4 | 4 | 8 | 8.3 | Glucose |
| 6 | F | --- | 6 | 4 | 10 | | |
| 7 | M | --- | 7 | 6 | 13 | | |
| 8 | F | --- | 5 | 5 | 10 | 10.3 | Lactose |
| 9 | F | --- | 5 | 3 | 8 | | |
| 10 | F | --- | 4 | 1 | 5 | | |
| 11 | F | --- | 2 | 3 | 5 | 6 | Carbohydrate-free |
| 12 | M | --- | 4 | 4 | 8 | | |
| 13 | M | --- | 4 | 3 | 7 | | |
| 14 | M | --- | 6 | 6 | 12 | 9.5 | Rice Control |

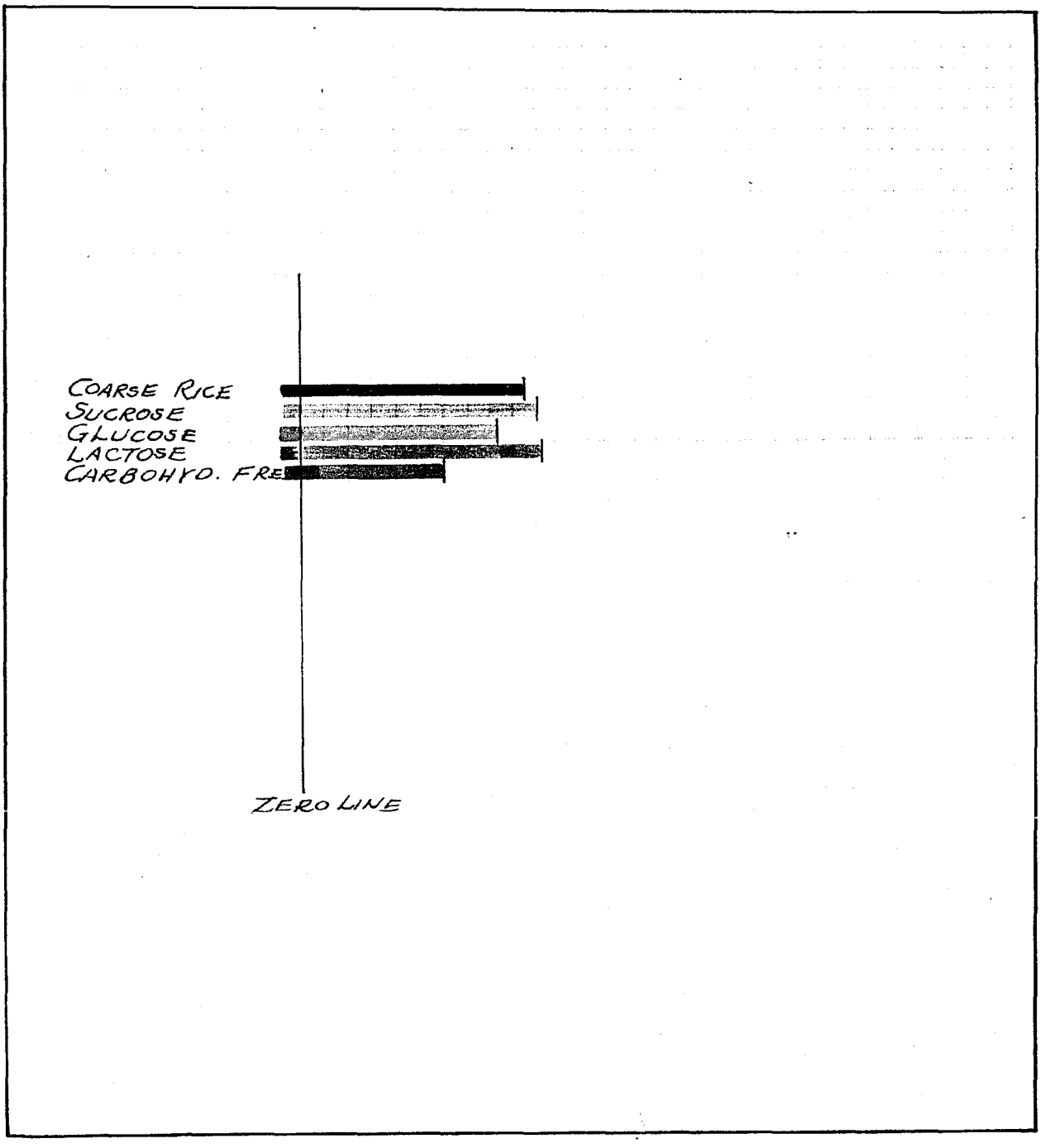


CHART V-2
From Table V-2; Feeding Various Sugars at 35% Levels.

TABLE V-3 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | M | --- | 9 | 9 | 18 | | |
| 2 | M | 3 | 8 | 10 | 21 | 19.5 | Rice Control |
| 3 | M | --- | 5 | 4 | 9 | | |
| 4 | M | --- | 3 | 4 | 7 | 8 | Carbohydrate-free |
| 5 | F | --- | 4 | 4 | 8 | | |
| 6 | M | --- | 6 | 5 | 11 | | |
| 7 | M | --- | 7 | 10 | 17 | | |
| 8 | F | --- | 4 | 5 | 9 | 13 | Sucrose |
| 9 | F | --- | 7 | 8 | 15 | | |
| 10 | M | --- | 5 | 4 | 9 | | |
| 11 | M | --- | 7 | 8 | 15 | | |
| 12 | F | --- | 3 | 6 | 9 | 13 | Glucose |
| 13 | F | --- | 10 | 9 | 19 | | |
| 14 | M | --- | 9 | 7 | 16 | | |
| 15 | M | --- | 10 | 7 | 17 | | |
| 16 | F | --- | 7 | 2 | 9 | 13.5 | Lactose |
| 17 | F | --- | 6 | 6 | 12 | | |
| 18 | F | --- | 6 | 6 | 12 | | |
| 19 | F | --- | 4 | 3 | 7 | 9.5 | Fine Rice |

TABLE V-4

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 3 | 3 | 6 | | |
| 2 | F | --- | 4 | 7 | 11 | 8.5 | Rice Control |
| 3 | F | --- | 2 | 2 | 4 | | |
| 4 | F | --- | --- | 1 | 1 | 2.5 | Carbohydrate-free |
| 5 | F | --- | 2 | 2 | 4 | | |
| 6 | M | --- | 5 | 3 | 8 | 6.6 | Glucose |
| 7 | M | --- | 4 | 4 | 8 | | |
| 8 | M | --- | 2 | 3 | 5 | | |
| 9 | M | --- | 2 | 2 | 4 | | |
| 10 | F | --- | 7 | 6 | 13 | 7.3 | Sucrose |
| 11 | F | --- | 5 | 4 | 9 | | |
| 12 | M | --- | 5 | 5 | 10 | | |
| 13 | M | 3 | 6 | 3 | 12 | | |
| 14 | F | --- | 5 | 4 | 9 | 9.2 | Lactose |
| 15 | F | --- | 2 | 4 | 6 | | |
| 16 | M | --- | 2 | 3 | 5 | | |
| 17 | M | --- | 2 | 2 | 4 | 4.5 | Fine Rice |

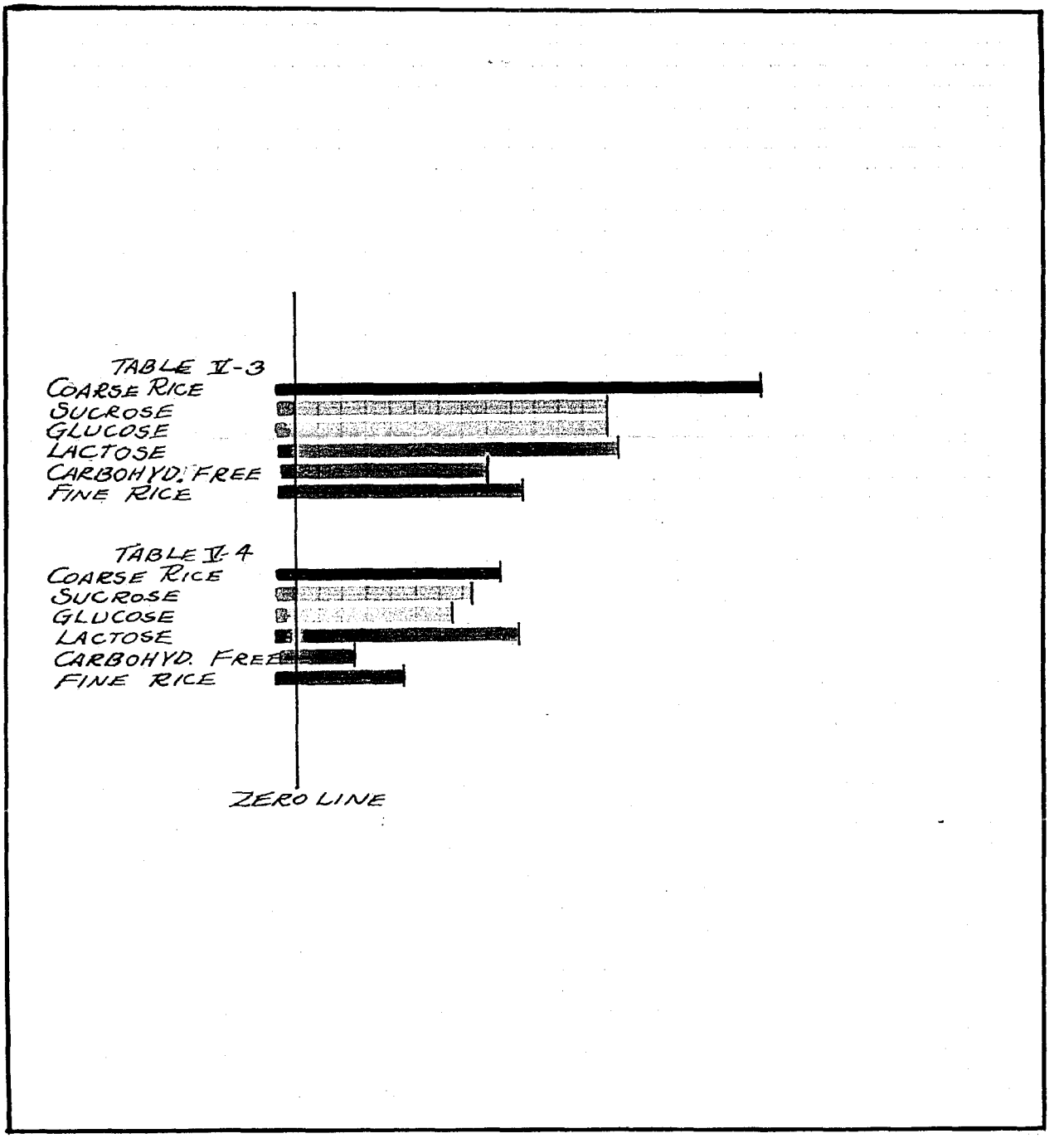


CHART V-3
From Tables V-3, & 4; Comparative Effects of Feeding Various Sugars, and Fine Rice.

TABLE V-5A

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 3 | 4 | 7 | | |
| 2 | F | --- | 3 | 3 | 6 | | |
| 3 | M | --- | 4 | 3 | 7 | 7.2 | Fine Rice |
| 4 | M | --- | 4 | 5 | 9 | | |
| 5 | F | --- | 2 | 3 | 5 | | |
| 6 | F | --- | 3 | 2 | 5 | | |
| 7 | M | --- | --- | --- | 0 | 3.5 | Sucrose |
| 8 | M | --- | 2 | 2 | 4 | | |
| 9 | F | --- | 5 | 5 | 10 | | |
| 10 | F | --- | 4 | 5 | 9 | 7.7 | Glucose |
| 12 | M | --- | 2 | 2 | 4 | | |
| 13 | F | --- | 6 | 2 | 8 | | |
| 14 | F | --- | --- | 2 | 2 | | |
| 15 | M | --- | 2 | 2 | 4 | 4.5 | Lactose |
| 16 | M | --- | 2 | 2 | 4 | | |

TABLE V-5B

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 17 | M | --- | 6 | 5 | 11 | | |
| 18 | M | --- | 7 | 6 | 13 | | |
| 19 | M | --- | 6 | 4 | 10 | | |
| 20 | M | --- | 5 | 7 | 12 | | |
| 21 | M | --- | 6 | 5 | 11 | | |
| 22 | M | --- | 4 | 7 | 11 | 13 | Fine Rice |
| 23 | F | --- | 10 | 9 | 19 | | |
| 24 | F | --- | 10 | 9 | 19 | | |
| 25 | F | --- | 7 | 5 | 12 | | |
| 26 | F | --- | 6 | 6 | 12 | | |
| 27 | M | --- | 4 | 4 | 8 | | |
| 28 | M | --- | 4 | 5 | 9 | | |
| 29 | M | --- | 10 | 8 | 18 | | |
| 30 | M | 3 | 7 | 6 | 16 | | |
| 31 | M | --- | 7 | 7 | 14 | | |
| 32 | M | --- | 5 | 5 | 10 | 12.2 | Coarse Rice |
| 33 | F | --- | 8 | 8 | 16 | | |
| 34 | F | --- | 5 | 6 | 11 | | |
| 35 | F | --- | 8 | 8 | 16 | | |
| 36 | F | --- | 2 | 2 | 4 | | |
| 37 | M | --- | 6 | 4 | 10 | | |
| 38 | M | 2 | 4 | 5 | 11 | | |
| 39 | M | --- | 5 | 6 | 11 | | |
| 40 | M | --- | 3 | 4 | 7 | | |
| 41 | M | --- | 3 | 5 | 8 | 8.9 | Carbohydrate-free |
| 42 | M | --- | 6 | 6 | 12 | | |
| 43 | F | --- | 4 | 3 | 7 | | |
| 44 | F | --- | 5 | 5 | 10 | | |
| 45 | F | --- | 4 | 4 | 8 | | |
| 46 | F | --- | 2 | 3 | 5 | | |
| 47 | M | --- | 2 | 3 | 5 | | |
| 48 | M | --- | 9 | 8 | 17 | | |
| 49 | M | --- | 4 | 2 | 6 | | |
| 50 | M | --- | 7 | 4 | 11 | | |
| 51 | M | --- | 6 | 4 | 10 | | |
| 52 | M | --- | 6 | 4 | 10 | 10 | Sucrose |
| 53 | F | --- | 5 | 9 | 14 | | |
| 54 | F | --- | 4 | 2 | 6 | | |
| 55 | F | --- | 3 | 4 | 7 | | |
| 56 | F | --- | 7 | 7 | 14 | | |

Continued on next page

TABLE V-5B (con't.) CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 57 | M | --- | 6 | 5 | 11 | | |
| 58 | M | --- | 5 | 7 | 12 | | |
| 59 | M | --- | 3 | 4 | 7 | | |
| 60 | M | --- | 2 | 2 | 4 | | |
| 61 | M | --- | 3 | 1 | 4 | 7.7 | Glucose |
| 62 | M | Died before completion | | | | | |
| 63 | F | --- | 2 | 2 | 4 | | |
| 64 | F | --- | 4 | 5 | 9 | | |
| 65 | F | --- | 5 | 3 | 8 | | |
| 66 | F | --- | 6 | 5 | 11 | | |
| 67 | M | --- | 4 | 4 | 8 | | |
| 68 | M | --- | 4 | 3 | 7 | | |
| 69 | M | --- | 4 | 6 | 10 | | |
| 70 | M | --- | 10 | 8 | 18 | | |
| 71 | M | --- | 5 | 7 | 12 | | |
| 72 | M | --- | 11 | 10 | 21 | 10.5 | Lactose |
| 73 | F | --- | 1 | 1 | 2 | | |
| 74 | F | --- | 2 | 3 | 5 | | |
| 75 | F | --- | 6 | 6 | 12 | | |
| 76 | F | --- | 5 | 5 | 10 | | |
| Control Group C: Terminate at 6 weeks for comparison | | | | | | | |
| 77 | M | --- | 3 | 4 | 7 | | |
| 78 | M | --- | 3 | 4 | 7 | | |
| 79 | F | --- | 2 | 1 | 3 | 5.4 | Coarse Rice |
| 80 | F | --- | 1 | 3 | 4 | | |
| 81 | F | --- | 3 | 3 | 6 | | |

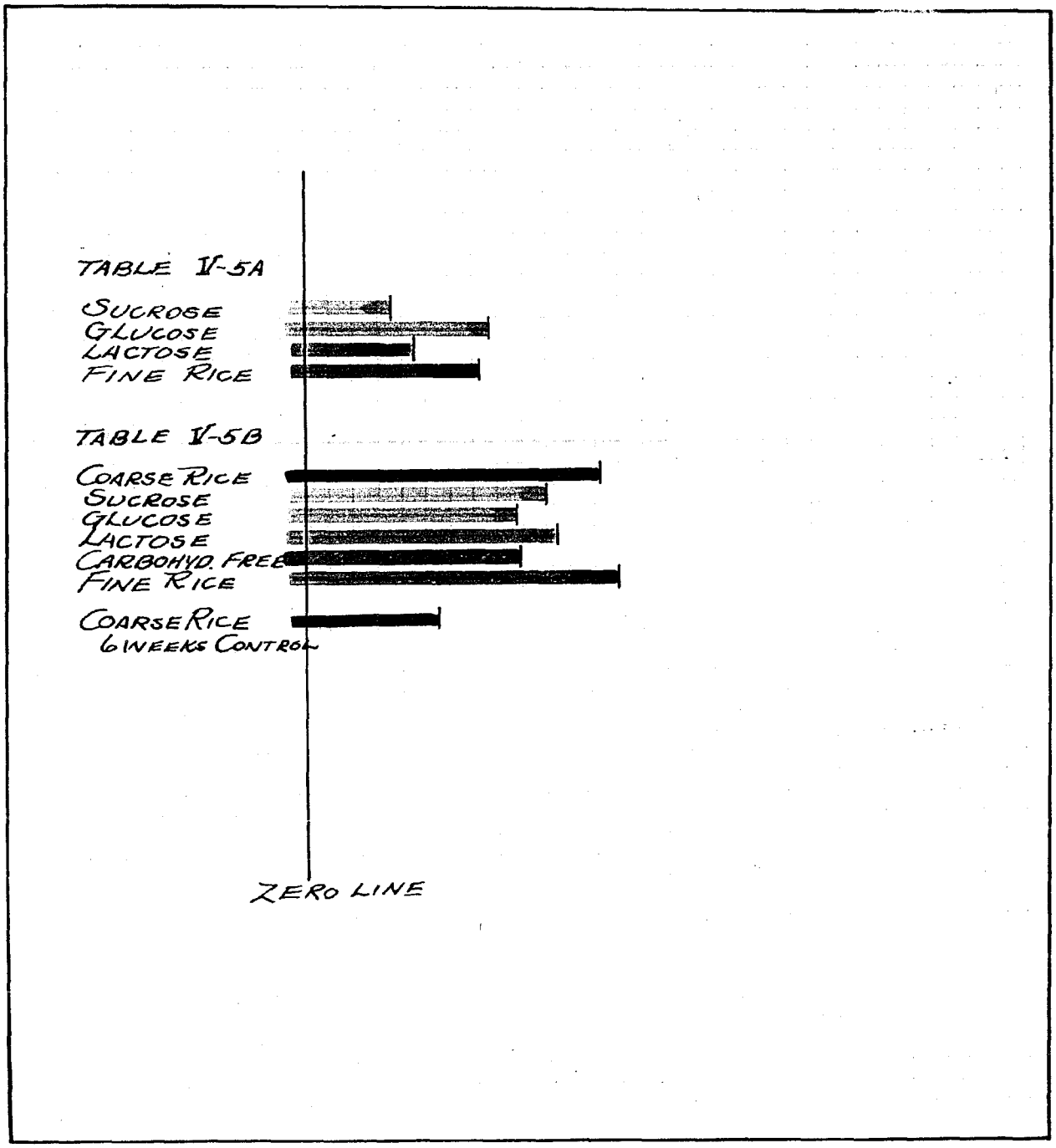


CHART V-4
From Tables V-5a, & 5b; Comparative Effects of Feeding Various Sugars, Fine Rice, and Coarse Rice.

TABLE V-6 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | M | --- | 6 | 7 | 13 | | |
| 2 | M | --- | 6 | 5 | 11 | | |
| 3 | M | --- | 5 | 7 | 12 | | |
| 4 | M | --- | 4 | 4 | 8 | | |
| 5 | F | --- | 6 | 6 | 12 | 11.6 | Coarse Rice |
| 6 | F | --- | 3 | 6 | 9 | | |
| 7 | F | --- | 6 | 8 | 14 | | |
| 8 | F | --- | 7 | 7 | 14 | | |
| 9 | M | --- | 6 | 6 | 12 | | |
| 10 | M | --- | 7 | 7 | 14 | | |
| 11 | M | --- | 5 | 6 | 11 | | |
| 12 | M | --- | 4 | 4 | 8 | | |
| 13 | M | --- | 5 | 5 | 10 | | |
| 14 | F | --- | 5 | 4 | 9 | 10 | Sucrose |
| 15 | F | --- | 2 | 2 | 4 | | |
| 16 | F | --- | 7 | 8 | 15 | | |
| 17 | F | --- | 3 | 3 | 6 | | |
| 18 | F | --- | 6 | 5 | 11 | | |
| 19 | F | --- | 5 | 5 | 10 | | |
| 20 | F | --- | 3 | 4 | 7 | | |
| 21 | F | --- | 7 | 11 | 18 | | |
| 22 | F | --- | 7 | 8 | 15 | | |
| 23 | M | --- | 4 | 4 | 8 | | |
| 24 | M | --- | 6 | 5 | 11 | 10.6 | Glucose |
| 25 | M | --- | 9 | 6 | 15 | | |
| 26 | M | --- | 1 | 3 | 4 | | |
| 27 | M | --- | 3 | 5 | 8 | | |
| 28 | M | Died before completion | | | | | |
| 29 | F | --- | 6 | 4 | 10 | | |
| 30 | F | --- | 6 | 7 | 13 | | |
| 31 | F | --- | 4 | 3 | 7 | | |
| 32 | F | --- | 3 | 4 | 7 | | |
| 33 | M | --- | 5 | 4 | 9 | | |
| 34 | M | --- | 5 | 4 | 9 | 9.7 | Lactose |
| 35 | M | --- | 5 | 5 | 10 | | |
| 36 | M | --- | 5 | 6 | 11 | | |
| 37 | M | --- | 2 | 6 | 8 | | |
| 38 | M | --- | 7 | 6 | 13 | | |

Continued on next page

TABLE V-6 (cont'd.) CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 39 | F | --- | 5 | 5 | 10 | | |
| 40 | F | --- | 4 | 3 | 7 | | |
| 41 | F | --- | 2 | 4 | 6 | | |
| 42 | F | --- | 3 | 6 | 9 | | |
| 43 | M | --- | 2 | 6 | 8 | | |
| 44 | M | --- | 9 | 6 | 15 | 8 | Carbohydrate-free |
| 45 | M | --- | 4 | 2 | 6 | | |
| 46 | M | --- | 3 | 4 | 7 | | |
| 47 | M | --- | 3 | 3 | 6 | | |
| 48 | M | --- | 3 | 3 | 6 | | |

TABLE V-7

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 5 | 4 | 9 | | |
| 2 | F | --- | 8 | 7 | 15 | | |
| 3 | F | --- | 3 | 4 | 7 | | |
| 4 | F | --- | 3 | 3 | 6 | 9.6 | Carbohydrate-free |
| 5 | F | --- | 4 | 4 | 8 | | |
| 6 | M | --- | 6 | 6 | 12 | | |
| 7 | M | --- | 5 | 5 | 10 | | |
| 8 | F | --- | 7 | 2 | 9 | | |
| 9 | F | --- | 7 | 5 | 12 | | |
| 10 | M | --- | 9 | 8 | 17 | | |
| 11 | M | --- | 8 | 9 | 17 | 13.3 | Sucrose |
| 12 | M | --- | 7 | 6 | 13 | | |
| 13 | M | --- | 5 | 8 | 13 | | |
| 14 | M | --- | 6 | 6 | 12 | | |
| 15 | M | --- | 9 | 10 | 19 | | |
| 16 | M | --- | 7 | 8 | 15 | | |
| 17 | F | --- | 5 | 6 | 11 | | |
| 18 | F | --- | 7 | 7 | 14 | 14.3 | Glucose |
| 19 | M | --- | 6 | 5 | 11 | | |
| 20 | M | --- | 9 | 10 | 19 | | |
| 21 | M | --- | 4 | 7 | 11 | | |
| 22 | F | --- | 7 | 8 | 15 | | |
| 23 | F | --- | 6 | 4 | 10 | | |
| 24 | F | --- | 5 | 7 | 12 | | |
| 25 | F | --- | 8 | 8 | 16 | 12.3 | Lactose |
| 26 | M | --- | 6 | 5 | 11 | | |
| 27 | M | --- | 6 | 7 | 13 | | |
| 28 | M | --- | 2 | 7 | 9 | | |
| 29 | M | --- | 7 | 10 | 17 | | |
| 30 | M | --- | 7 | 7 | 14 | 15.5 | Coarse Rice |

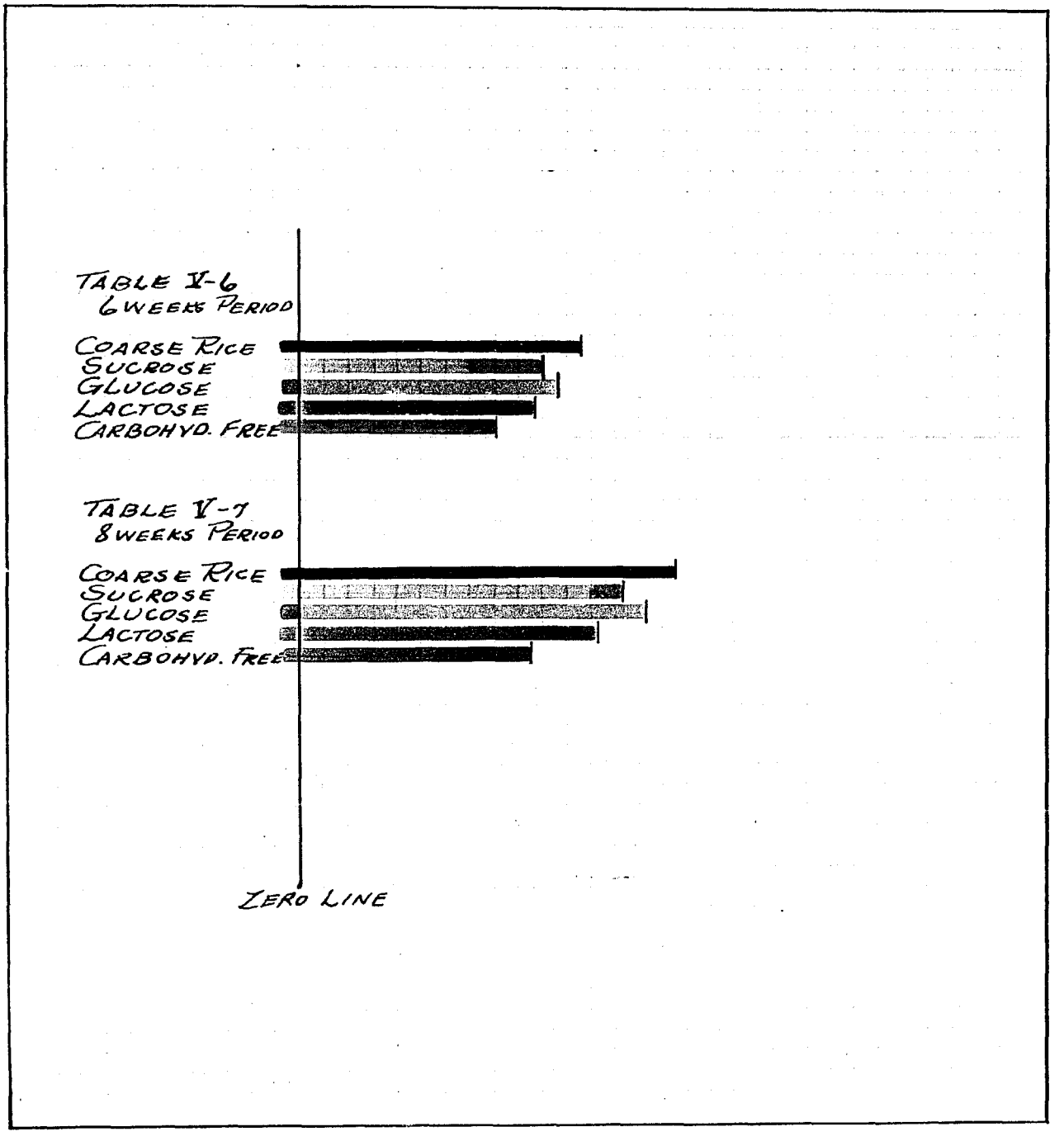


CHART V-5
From Tables V-6, & 7; Feeding Various Sugars for 6 and 8 week Periods.

TABLE V-8

CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 6 | 8 | 14 | | |
| 2 | F | --- | 8 | 6 | 14 | | |
| 3 | M | --- | 6 | 8 | 14 | | |
| 4 | M | --- | 8 | 7 | 15 | | |
| 5 | F | --- | 6 | 4 | 10 | 14.2 | Corn Syrup Ration |
| 6 | F | --- | 4 | 7 | 11 | | |
| 7 | M | --- | 9 | 7 | 16 | | |
| 8 | M | --- | 9 | 7 | 16 | | |
| 9 | F | --- | 8 | 7 | 15 | | |
| 10 | F | --- | 8 | 9 | 17 | | |
| 11 | F | --- | 9 | 8 | 17 | | |
| 12 | F | --- | 6 | 5 | 11 | | |
| 13 | M | --- | 7 | 6 | 13 | 12.3 | Coarse Rice Only |
| 14 | M | --- | 5 | 6 | 11 | | |
| 15 | F | --- | 4 | 3 | 7 | | |
| 16 | F | --- | 8 | 7 | 15 | | |

TABLE V-9 CARIES SCORE

| RAT NO. | Sex | Upper | Lower Rt. | Lower Lft. | Total | Average | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | F | --- | 9 | 10 | 19 | | |
| 2 | F | --- | 8 | 8 | 16 | | |
| 3 | M | --- | 7 | 7 | 14 | 16.8 | Corn Syrup Ration |
| 4 | M | --- | 9 | 7 | 16 | | |
| 5 | M | --- | 10 | 9 | 19 | | |
| 6 | F | --- | 6 | 8 | 14 | | |
| 7 | F | --- | 5 | 7 | 12 | | |
| 8 | F | --- | 5 | 7 | 12 | | |
| 9 | F | --- | 5 | 5 | 10 | | |
| 10 | M | --- | 9 | 10 | 19 | 15.9 | Corn Syrup Ration plus 1% Irradiated Yeast |
| 11 | M | --- | 10 | 10 | 20 | | |
| 12 | M | --- | 9 | 9 | 18 | | |
| 13 | M | --- | 11 | 11 | 22 | | |
| 14 | F | --- | 7 | 5 | 12 | | |
| 15 | F | --- | 7 | 5 | 12 | | |
| 16 | F | --- | 6 | 6 | 12 | | |
| 17 | F | --- | 6 | 6 | 12 | | |
| 18 | M | --- | 6 | 6 | 12 | 13.2 | Coarse Rice Ration plus 1% Irradiated Yeast |
| 19 | M | --- | 6 | 9 | 15 | | |
| 20 | M | --- | 6 | 5 | 11 | | |
| 21 | M | --- | 10 | 9 | 19 | | |
| 22 | F | --- | 10 | 7 | 17 | | |
| 23 | F | --- | 9 | 8 | 17 | 15 | Coarse Rice Ration |
| 24 | F | --- | 5 | 6 | 11 | | |

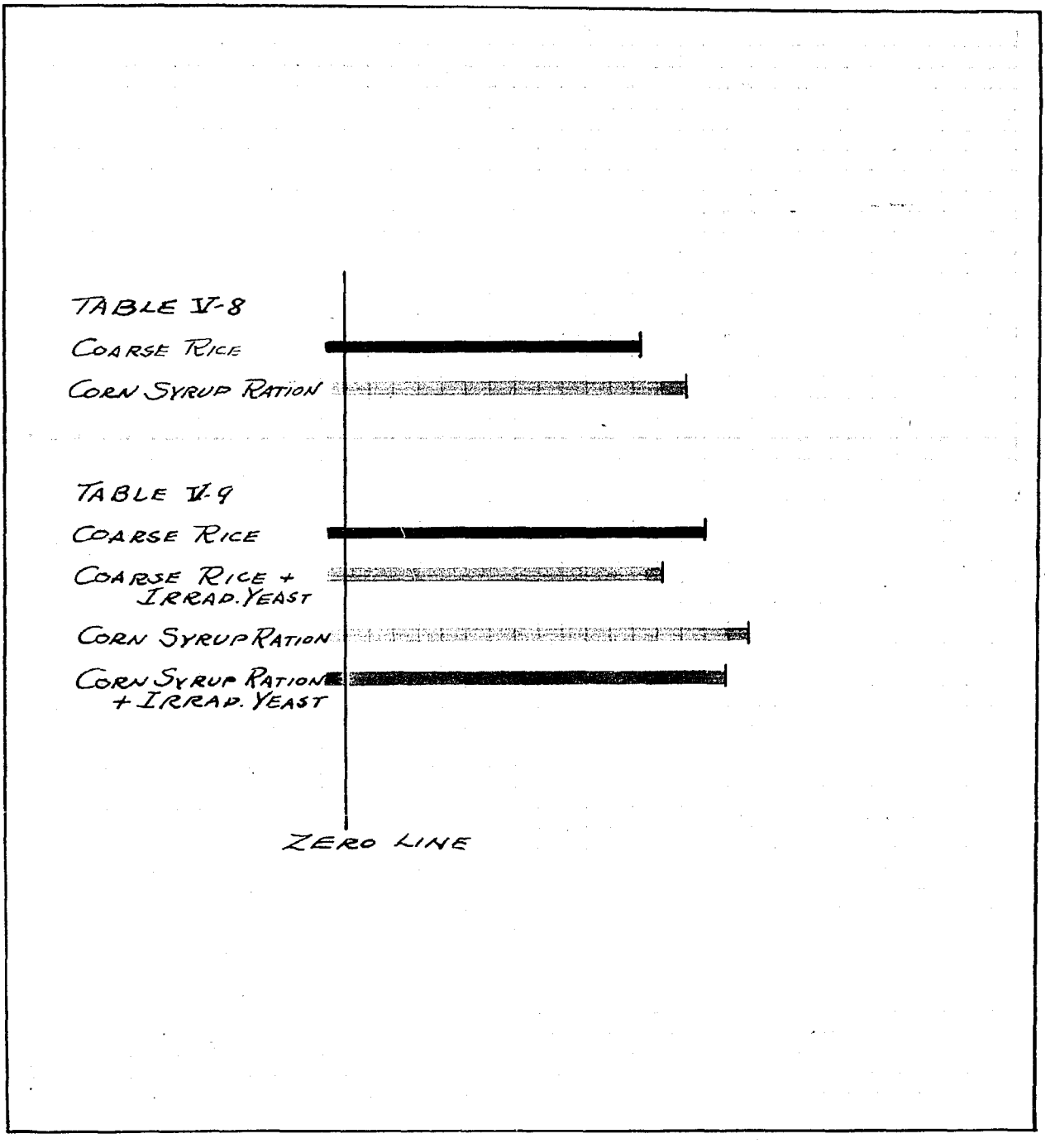


CHART V-6
From Tables V-8, & 9; Feeding Corn Syrup and Coarse Rice Simultaneously; Use of High Vitamin D Supplement.

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