

STUDIES ON THE DESTRUCTION OF VITAMINS DURING THE PASTEURIZATION
OF MILK

BY

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I INTRODUCTION

As the pasteurization of milk has become a general practice, it is increasingly important to know what, if any, adverse effect pasteurization has upon the nutritive properties of milk. Careful experimental work has shown that the only significant, adverse effect of pasteurization, from the nutritive standpoint, was the partial destruction of vitamin C and vitamin B₁ (33) and that these deficiencies of pasteurized milk could be readily overcome by properly supplementing the diet.

The general purpose of this investigation was to study the factors affecting the destruction of vitamins during the pasteurization of milk and, if possible, to find a method of pasteurization in which this destruction would be kept at a minimum.

II. VITAMIN C

A. Introduction:

Of the nutritive factors of milk, vitamin C is probably the most sensitive to the processes of oxidation and is the factor that is most readily destroyed during pasteurization. Although it has been recognized that bottle fed infants should receive some additional vitamin C supplement, the fact remains that if the original quantity of vitamin C were retained in the milk until the time of feeding, then a considerable fraction of the requirement for this vitamin would be supplied. Furthermore the economic conditions of recent years have made it difficult for underprivileged families to properly supplement the diet with anti-scorbutic foods. Thus the problem of preventing the destruction of vitamin C during the pasteurization and storage of milk is one of considerable practical importance.

Many investigations have been made on the destruction of vitamin C during the pasteurization of milk^(7, 17, 55, 67, 71). The general agreement is that from 25 to 50% of the original vitamin C content is destroyed during pasteurization by the "holder" method but an even more significant fact is that storage of the pasteurized milk results in a further rapid destruction of the vitamin. In recent years several attempts have been made to prevent this destruction. Swope⁽⁶²⁾ pasteurized milk in an atmosphere of CO₂ with good results. However, this method is objectionable due to the carbonated flavor imparted to the milk. When the work was repeated on a commercial scale,⁽⁴⁶⁾ vitamin C was still destroyed even if pasteurized in an atmosphere of CO₂, N₂ or in a partial vacuum. Guthrie, Hand and Sharp⁽¹²⁾ have

proposed a denaturation of the milk to prevent destruction of the vitamin and give evidence in support of their method. However this method does not seem to be adaptable to the "holder" process of pasteurization, which is the prevailing method at the present time.

Flash pasteurization has been found to have little or no destructive action on the vitamin C in milk (49, 67, 71). Woessner et. al. (71) state that the advantage of the flash pasteurization process in preserving the vitamin C content of milk seems dependent on the fact that the destruction of the ascorbic acid appears to be more dependent on the time of heating than on the temperature itself.

Many investigations have been carried out on the effect of visible and ultraviolet light on the vitamin C in milk (28, 48, 60). However, Guthrie et. al. showed that light had very little effect on the vitamin provided that all the air was excluded. This indicated that the light acted as a catalyst in the oxidation of vitamin C.

The literature described above has indicated that the destruction of vitamin C in milk is an oxidative process which is catalyzed by heat, light, and certain metals especially copper.

Milk freed from oxygen has been heated for three hours at 145°F after the addition of 0.1 mg. / litre of dissolved copper, with no appreciable oxidation of reduced ascorbic acid. (15). The mechanism of the catalytic oxidation of vitamin C has been given by Barron, DeMaio and Klemperer (2). The reduced ascorbic acid is oxidized to dehydroascorbic acid while the catalyst is being reduced. The reduced catalyst is then reoxidized by atmospheric oxygen, the hydrogen peroxide formed being split into water and oxygen. The addition of

amino acids retarded the catalytic action of copper, presumably owing to complex salt formation. It, therefore, appeared as though the destruction of the vitamin could be most efficiently prevented by removing all the air from the milk before pasteurization and keeping the milk free of air until bottled and capped. It was the purpose of this part of the investigation to determine whether this could be accomplished on a laboratory and commercial scale.

B. Method of Analysis of Vitamin C in Milk:

Chemical methods of analysis of vitamin C have, at the present time, almost completely replaced the older biological assays. The results obtained using chemical method of analysis of the vitamin in milk have been shown to agree with the biological assays (26). The normal healthy cow secretes vitamin C in its milk only in the reduced form (29). Contact with atmospheric oxygen soon transforms part of the vitamin into the reversibly oxidized form known as dehydroascorbic acid. This form of the vitamin is biologically active and any chemical method should allow for its determination as well as the vitamin in the reduced form. The precise nutritional effectiveness of dehydroascorbic acid is not known (38). In this investigation, two methods of analysis were used. In the pasteurization experiments on the laboratory scale, only the vitamin C in the reduced form was analyzed, a titration method being used. In the experiments on pasteurization on a commercial scale, both the ascorbic and the dehydroascorbic acids were analyzed by a colorimetric method.

(1) Titration Method:

Sharp (36) reports that he finds no mention in the literature

of any substance in milk other than ascorbic acid which might be reduced by 2-6-dichlorophenolindophenol and that the presence of proteins apparently did not affect the reaction. The rapid method for the determination of reduced ascorbic acid in milk as reported by Sharp (57) was tried but was discarded due to the difficulty in determining the end point. The method finally adopted was as follows: To 25 ml. of milk were added 5 ml. of 8% trichloroacetic acid and the precipitated proteins removed by filtration on a Buchner funnel. The filtrate was then titrated with a solution of sodium 2-6-dichlorophenolindophenol which had been freshly standardized against crystalline ascorbic acid (57). It is now realized that this method may give values that are slightly lower than the true value of reduced vitamin C in milk but this small discrepancy should not affect the interpretations of the effects of pasteurization.

(2) Colorimetric Method:

The literature contains numerous procedures for the determination of ascorbic acid in milk but only very few for the important, equally biologically active dehydroascorbic acid (71). Lon and Watson (28) have suggested a procedure for the estimation of dehydroascorbic acid in milk but Woessner, Elvehjem and Schuette (69, 70) have shown that the use of a photoelectric colorimeter is essential for such a determination because it eliminates the interference due to other substances which are formed when the milk is treated with hydrogen sulfide. Their method, which was used in this investigation, is specific for ascorbic and dehydroascorbic acids. The apparatus, general techniques and calculations were identical with those des-

scribed by Mindlin and Butler (40). The correction for the turbidity of the solution was carried out according to Bessey (4).

G. The Effect of Pasteurization and Storage on the Vitamin C Content of Milk

(1) Laboratory Experiments:

A gallon sample of fresh raw milk was obtained from the receiving station of the Virginia Polytechnic Institute Creamery. This was divided into 5 portions which were treated in the following manner:

1. Raw
2. Pasteurized
3. Pasteurized in presence of copper
4. Vacuum - Pasteurized
5. Vacuum - Pasteurized in presence of copper

Pasteurization was carried out in 1 litre Erlenmeyer flasks and kept at 143-145°F. for 30 minutes in a water bath with occasional stirring. The copper was introduced by placing two bright clean pennies in the milk just prior to pasteurization. After pasteurization the milk was immediately chilled in ice water and stored in the dark at 36°F. Samples were withdrawn and analyzed for vitamin C by the titration method previously described.

The vacuum-pasteurization was carried out in the apparatus shown in Fig. 1. The suction was applied before placing the flask in the water bath. The pressure was low enough so that the milk started boiling at about 85°F. This boiling soon freed the milk of all of its dissolved air. The pressure was gradually increased allowing the temperature of the milk to increase and was finally adjusted so that the milk boiled gently at a temperature of 143-145°F. This was continued for 30 minutes. The water bath was kept at 148°F. and it

required about 20 minutes to get the milk to pasteurizing temperature. The results of this experiment are given in Table I.

TABLE I

Effects of Air and Copper on the Destruction of Vitamin C in Milk During Pasteurization and Storage

Milk Sample	Vitamin C (mg/litre)		Initial % loss due to Pasteurization
	Initial	Stored 28 hours	
Raw	10.4	4.8	--
Pasteurized	7.3	1.2	33
Pasteurized - Copper	1.3	1.2	88
Vacuum - pasteurized	9.8	5.8	9
Vacuum - pasteurized - copper	10.4	5.0	0

These results clearly indicate the catalytic action of copper and also show that in the absence of air, copper had no destructive action on the vitamin C content of milk.

(2) Commercial Pasteurization:

The first step in this investigation was to determine accurately the effect of standard commercial pasteurization and storage of milk on the ascorbic and dehydroascorbic acid content. The raw milk samples were taken from a 200 gallon stainless steel pasteurizing vat just before heating and placed in half pint bottles. The milk was then pasteurized 31 minutes at 144°F., cooled over a surface cooler and bottled as usual in the bottling machine. These bottles were stored in the dark at 35°F. along with the raw milk samples. Vitamin C analyses were made at stated intervals by the colorimetric method described

above.* The results are given in Table II.

TABLE II

The Effect of Pasteurization and Storage on the Ascorbic and Dehydro-ascorbic Acid in Milk

Kind of Milk	Time Stored Hours	Vitamin C (mg./litre)		
		Reduced	Oxidized	Total
Raw	0	20.2	3.8	24.0
	30	12.9	8.6	21.5
	60	8.8	--	--
Vat Pasteurized (144°F. for 31 min)	0	15.5	1.0	16.5
	30	2.1	10.4	12.5
	60	0.0	--	--

(3) Vacuum Pasteurization on a Commercial Scale:

The apparatus was set up as in Fig. II. The pasteurizer was a 200 gallon capacity "glass-lined" vat, ** so designed that pasteurization could be carried out in a partial vacuum. The milk was heated and kept at pasteurizing temperature by continuous circulation through the heat exchanger. The temperature could be controlled by adjusting

*An Evelyn photoelectric colorimeter was used (Serial Number 6182). This colorimeter was used in all the subsequent colorimetric determinations in this investigation.

**Obtained through the courtesy of the Pfaunder Co., Rochester, N. Y.

the rate of flow of steam and cold water to the outer pipes of the heat exchanger. The holding vat was of stainless steel and the surface cooler was of tinned copper. The pipe lines were the ordinary "sanitary pipe-line" commonly used by the dairy industry. No attempt was made to exclude any copper. Before starting the heating, the vacuum pump was turned on and the pressure reduced to about 100 millimetres. The milk started boiling at 125°F. which was attained after about 10 minutes. The pressure was then gradually increased until the temperature of the milk rose to 145°F. The milk was kept boiling gently for 30 minutes, pumped up to the holding vat and cooled over a surface cooler. Samples were taken for analyses of vitamin C at three points; (a) raw milk from the vat just before pasteurization, (b) pasteurized milk from the top holding vat before cooling and (c) milk after passing over the surface cooler. The samples were collected in half pint bottles and stored in the dark at 35°F. Analyses for vitamin C were made after various storage intervals. It would have been desirable to have determined both the reduced and oxidized forms of the vitamin but at this point the supply of nitrogen gas was exhausted and only the reduced ascorbic acid could be determined. As a control, a batch of milk was pasteurized in the same way as described above except that no vacuum was applied. The results are shown in Tables III and IV and in Fig. III.

Table III

Effect of Vacuum-Pasteurization on the Ascorbic and Dehydroascorbic Acid Content of Milk

Kind of Milk	Vitamin C (mg/litre)		
	Reduced	Oxidized	Total
Raw	19.4	5.4	24.8
Vacuum-Pasteurized 145°F. for 30 min.	18.6	4.4	23.0
Vacuum-Pasteurized 145°F. for 30 min. and passed over surface- cooler.	18.9	5.2	24.1

Note:-(1) Milk was stored 2 hours before analysis

(2) Estimated accuracy of determinations - 0.5 mg/l.

Table IV

Effect of Pasteurization and Storage on the Reduced Ascorbic Acid
Content of Milk

Kind of Milk	Hours of Storage			
	2	20	50	70
	mg/l	mg/l	mg/l	mg/l
Raw	19.4	17.6	15.0	15.3
Pasteurized 145°F. for 30 min.	11.5	1.5	—	—
Vacuum-Pasteurized 145°F. for 30 min.	18.6	16.5	10.6	8.8
Vacuum-Pasteurized 145°F. for 30 min. and surface cooled	18.9	13.3	4.2	0.6

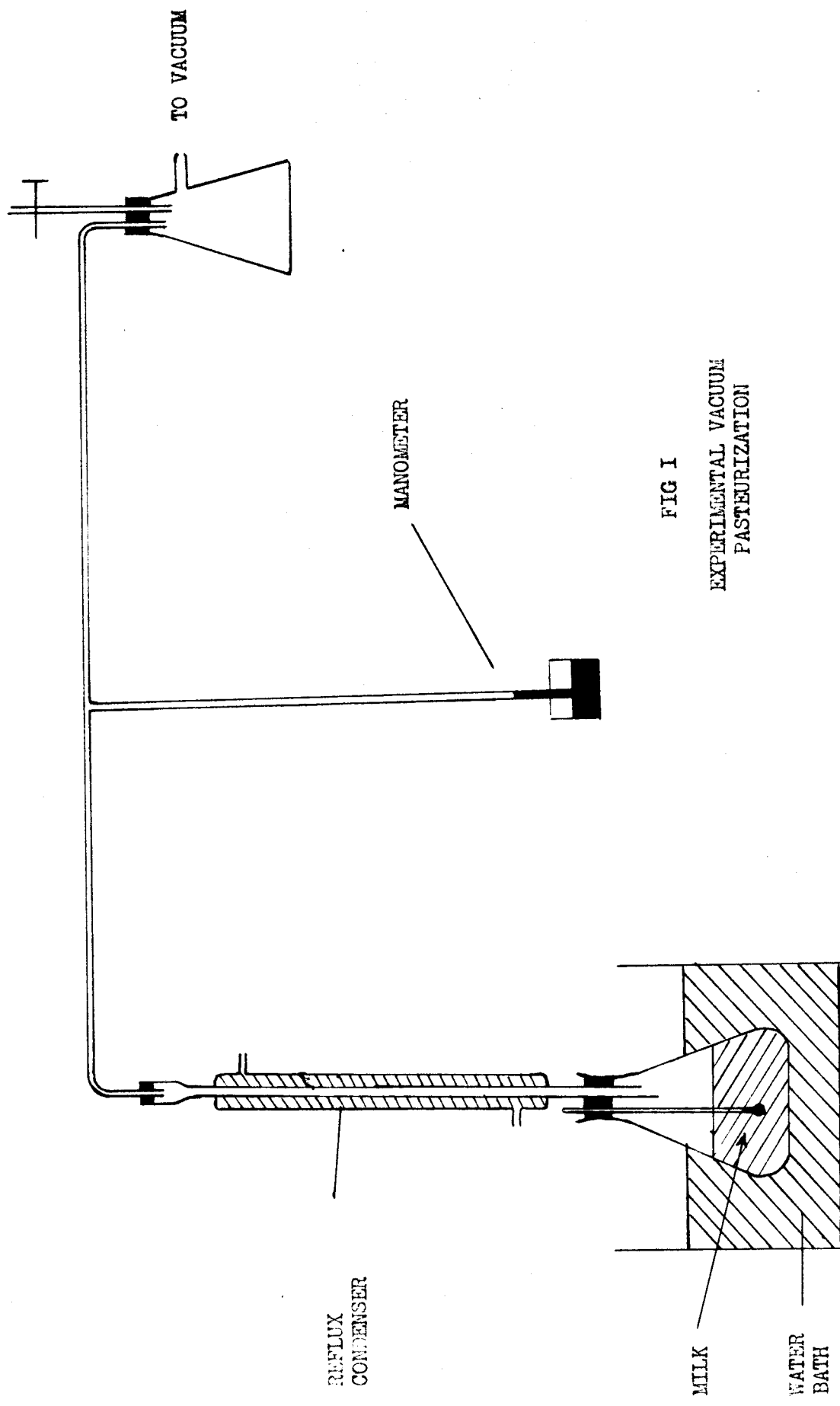


FIG I
EXPERIMENTAL VACUUM
PASTEURIZATION

FIG II
APPARATUS FOR COMMERCIAL
VACUUM-PASTEURIZATION

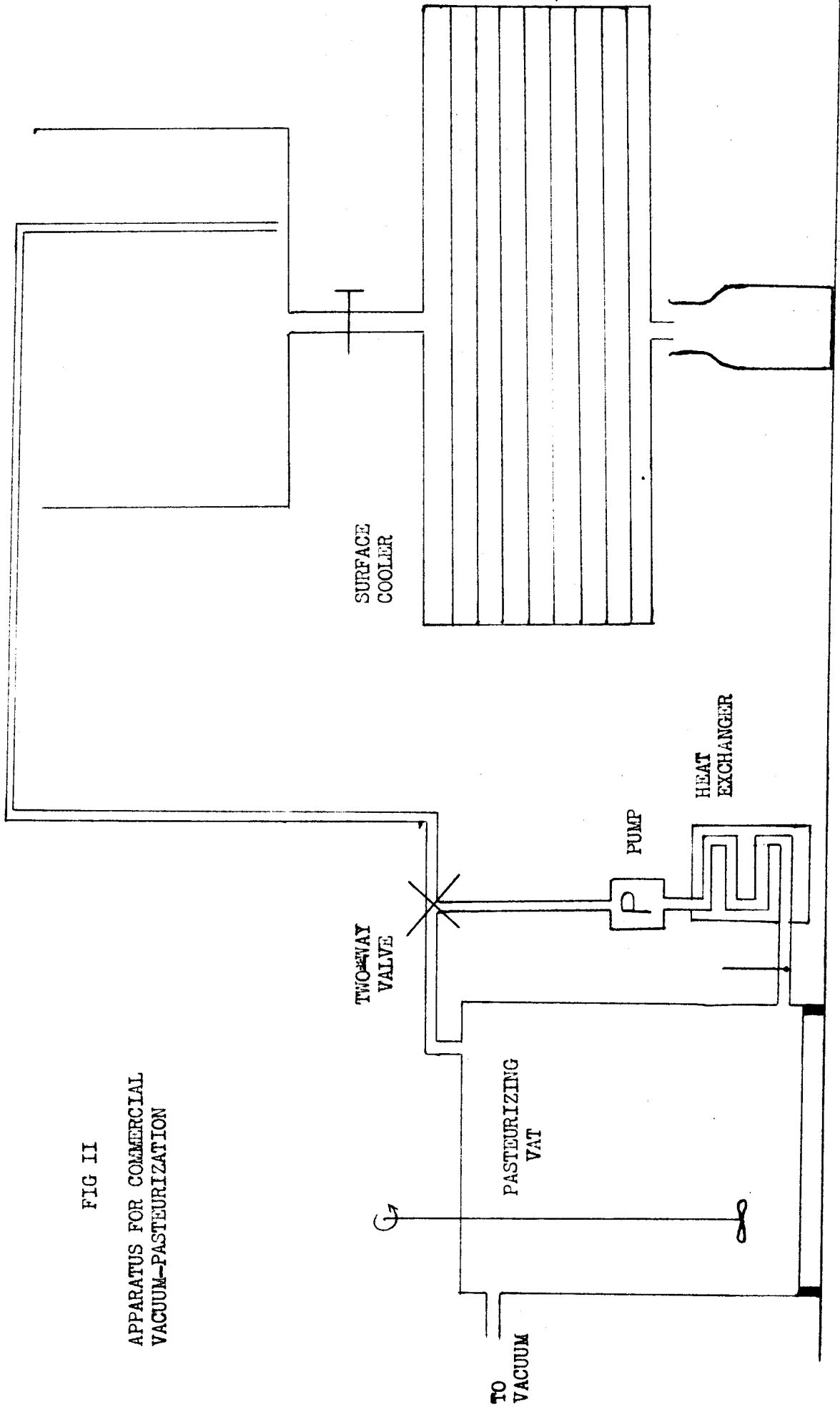


Fig. III.--Effect of Pasteurization on the Reduced Form of Vitamin C in Milk.

I Raw Milk

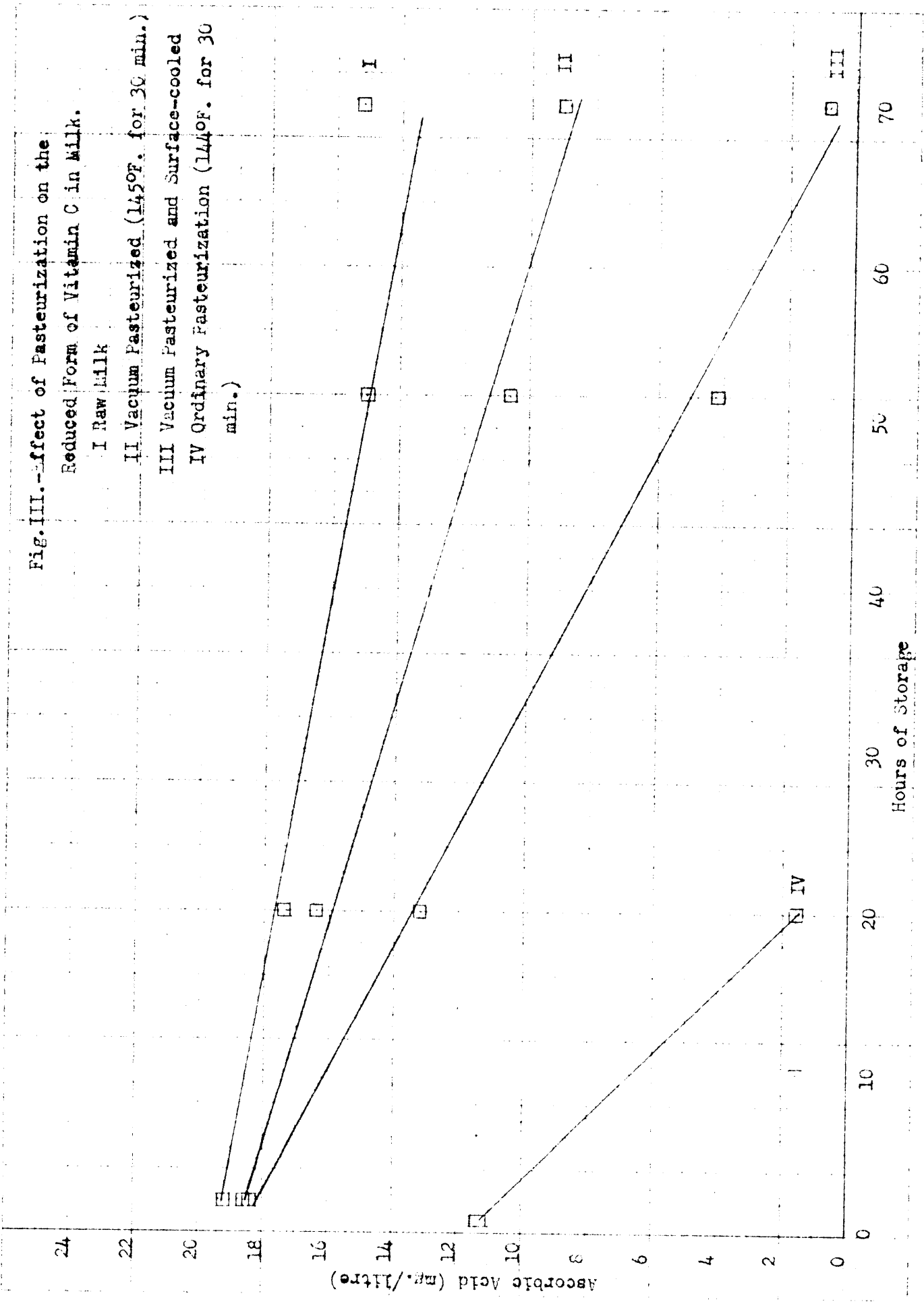
II Vacuum Pasteurized (145°F. for 30 min.)

III Vacuum Pasteurized and Surface-cooled

IV Ordinary Pasteurization (144°F. for 30 min.)

Ascorbic Acid (mg./litre)

Hours of Storage



III. VITAMIN B₁

A. Introduction:

The first problem that naturally arose in the investigation involving vitamin B₁ in milk was to find a method of quantitative assay of this vitamin in milk. A search of the literature revealed that the only methods which had been developed were the biological methods and the thiochrome assay. The animal assay had the advantage of well established techniques but also had the disadvantage of a prolonged period required to prepare animals for the test. The limited time of storage of milk and the possibility of destruction of the vitamin during storage rendered this method especially unsuitable to an investigation of this kind where the effects of various methods of pasteurization were to be studied. The thiochrome assay required the use of a fluorometer which was not available. Thus the major problem of this investigation developed into that of adapting one of the existing chemical, or biochemical, methods of analysis of vitamin B₁ to the quantitative assay of this vitamin in milk.

B. Methods of Analysis of Vitamin B₁:

(1) Biological Assay:

Up to the present time the biological method of testing for the presence of the antineuritic vitamin has been the most satisfactory approach to the assay problem, despite the many objectional features inherent in this particular form of testing (1). The animals which have been mostly used are the pigeon and the rat. With pigeons, three methods have been mainly used:

(a) Protective Methods - This involves an estimation of the daily ration of a particular foodstuff which is sufficient to prevent neuromuscular symptoms in birds, when added to a vitamin B₁-free diet. Such a method has recently been elaborated by Jukes and Heitman (22).

(b) Weight Maintenance Methods - These methods lack reproducibility with birds due to the chance presence in the diet of varying amounts of unknown factors.

(c) Curative Methods - The minimal dose required to affect a cure of severe symptoms of polyneuritis is determined. The method is not very accurate.

In rat experimentation the main tests devised have been those concerned with growth and with the cure of polyneuritic symptoms. Again three methods may be used:

(a) Growth Methods - This method involves the determination of the minimum ration of a foodstuff required for growth and maintenance when added to a diet complete in all other essential constituents. The degree of accuracy is high and the sensitivity is about one microgram of the crystalline vitamin (41).

(b) The Curative Methods and the Bradycardia Method give comparable results and are applicable for wide varieties of substances having different degrees of activity.

It is sufficient to state that the inherent disadvantages in the biological methods render them unsuitable for an investigation of this kind. The more rapid and, in many cases, more accurate chemical and biochemical methods showed more promise.

(2) Biochemical Assays:

(a) Catatorulin Assay - This method is based on the increase in oxygen uptake observed in a pyruvate substrate as a result of adding vitamin B₁ to avitaminous pigeon's brain. Peters concluded that the test, in spite of difficult techniques, may still prove useful because it is fairly sensitive, reacting to less than 0.2 micrograms of vitamin B₁, and because unlike the other biochemical tests, it has a low sensitivity to the phosphoric esters (44).

(b) Fermentation Method - In the presence of a suitable sugar-salt buffer mixture, vitamin B₁ caused a pronounced stimulation in the rate of alcoholic fermentation. The method has been developed by Schultz, Atkin and Frey (54) but was not suitable for this investigation due to the complicated apparatus required.

(c) Bacterial Metabolism Method - Silverman and Berkman (59) described a method which is based on the increased anaerobic pyruvate metabolism of vitamin B₁-deficient cells of *Propionibacterium pentosaceum* occurring on the addition of the vitamin. Care must be exercised since the organisms may be "trained" to synthesize the vitamin. It is the opinion of this author that the techniques involved are too difficult and the apparatus too complicated for the method to have wide application. It was definitely not suitable for this investigation.

(d) Cocci Growth Method - Knight (27) has demonstrated that under suitable conditions, the growth of *Staphylococcus aureus* is proportional to the amount of vitamin B₁ in the medium. It was thought that this method might be suitable for the determination of

the vitamin B₁ in milk since the growth of the cocci could be determined by the turbidity produced in the medium. Several attempts were made to prepare the basal medium of West and Wilson (56) which contained a solution of amino acids obtained by the acid hydrolysis of casein. In every case the resulting medium was of a light brown color which interfered somewhat with the use of the Evelyn Colorimeter for measuring the turbidity. Also the introduction of the milk rendered the solution turbid to the point that the "blank" reading was so high as to make the method inaccurate. A further difficulty was that the stock culture of *Staphylococcus aureus* grew moderately well in the basal medium without addition of vitamin B₁. This was probably due to traces of the vitamin in the constituents, although C. P. chemicals were used. The possibility of carrying over some of the vitamin in the inoculum is, however, not to be ignored. The use of this method was therefore discarded.

(e) Fungus Growth Method - Schopfer (52) in 1935 has shown that the fungus *Phycomyces blakesleeanus* does not grow in a medium containing no vitamin B₁ and that within certain limits, the amount of growth of the fungus is directly proportional to the concentration of the vitamin. A method of estimating the vitamin B₁ content of wheat germ was also proposed, with results comparable to the rat-growth method (53). Since that time the problem has been further investigated by workers in this country (5, 34, 50). The method of analysis of the vitamin by means of the fungus *Phycomyces blakesleeanus* has been developed by Bonner and Erickson (5) who showed that the fungus can utilize a mixture of the pyrimidine and thiazole

components of the vitamin as well as the vitamin per se and that such mixtures have quantitatively the same activity as the vitamin itself. The fungus can also use mixtures of certain substituted thiazoles and pyrimidines which are closely related to the components of thiamin. The activity of these vitamin analogs is slight compared to that of vitamin B₁ or its components. That this fungus can synthesize thiamin from the pyrimidine and thiazole fractions could not be demonstrated by these authors but was shown to take place by Leonian & Lilly (35). Palei (43) has shown that the results obtained from the action of vitamin B₁ preparations on *Phycomyces* agreed with the biological experiments on pigeons. He also pointed out that the determination of the vitamin in foodstuffs by this method is doubtful since the changing amounts of organic acids and other unknown factors introduced can affect the development of *Phycomyces*. Lilly (36) has reviewed the literature on this subject and has pointed out that by using a suitable selection of test fungi it is possible to determine the following, semi-quantitatively at least: (a) the thiamin thiazole, (b) the thiamin pyrimidine, (c) both thiamin components together and (d) thiamin per se. The exact methods however have not yet been exactly worked out. Previous work has been mainly centered on the biological considerations of the thiamin and other requirements of the fungi and, as far as this author could determine, no practical methods have been developed for the vitamin B₁ assay of foodstuffs. In the following experiments these principles were applied to the assay of vitamin B₁ in milk so that a method of checking the results of the chemical assays would be available.

Experimental Procedure and Data:

The nutrient medium of Bonner and Erickson (5) was prepared as follows:

Dextrose - - - - - 100 gms.
L-Asparagin + + - - - 4.0 gms.
MgSO₄ · 7H₂O - - - - - 0.5 gms.
KH₂PO₄ - - - - - 1.5 gms.
Water - - - - - 1 litre

Twenty-five ml. portions of this medium were pipetted into 125 ml. Erlenmeyer flasks and autoclaved for 15 minutes at 15 pounds pressure. The stock culture of *Phycomyces blakesleeianus*[†] was cultivated on Difco Potato Dextrose Agar. Two drops of a spore suspension of the fungus, made by transferring a trace of growth to 5 ml. of sterile water, was used as the inoculum. Luxuriant growth of the fungus was obtained in this medium, without further additions of thiamin, even after 15 successive transfers via spore suspensions. This indicated that the medium contained some thiamin as an impurity. The asparagin was the first component to be suspected but recrystallization from water was not effective. Fresh reagents were then obtained^{**} and the asparagin recrystallized three times from water.

[†]Obtained through the courtesy of Dr. Virgil Greene Lilly, Dept. of Plant Pathology and Bacteriology, West Virginia University.

^{**}L-Asparagin C.P. Phensteil
Glucose - Bacto grade. Difco Co.
Mineral salts - C.P. Sakers Analyzed

Care was taken not to introduce any extraneous thiamin. In this experiment amounts of thiamin ranging from 0.02 to 3.0 micrograms were added per 25 ml. of medium in 150 ml. Erlenmeyer flasks. One-half and one ml. of raw skim milk were also added to the medium in other flasks. The pH of the medium was sufficiently low that no destruction of the vitamin should have occurred during the subsequent autoclaving. The culture of the fungus on potato dextrose agar was transferred once into this, ^{presumably} thiamin-free medium and the inoculations into the flasks containing added milk and vitamin B₁ were made from the growth of this culture via a spore suspension. After incubating 12 days at room temperature (approximately 23°C) the flasks were sterilized by autoclaving and the mycelia of two duplicate flasks were dried at 100°C in a vacuum oven for 4 hours and weighed. The results are given in Table V. It is evident from this table that the presence of added thiamin had very little effect on the amount of growth of the fungus. In all cases there was very little aerial mycelium which was abundant on the agar slants. It appears as though the basal medium still contained traces of thiamin or that some necessary technique in the cultivation of the fungus was not correctly carried out by this investigator. However, every care was taken to duplicate the work of the original authors (5) and the causes for these negative results are unknown to this author. It may be possible that this particular strain of *Phycomyces blakesleeanus* did not require thiamin in its metabolism but this is unlikely. The problem warrants further investigation.

Table V

Effect of Thiamin on the Growth of *Phycomyces Blakesleeanus*

Thiamin in Micrograms per 50 ml.	Wt. of Mycelium in mg.
0.0	117
0.055	129
0.09	131
0.27	147
0.90	127
1.80	136
6.0	121
1 ml. milk	117
2 ml. milk	160

(3) Chemical Methods of Analysis of Vitamin B₁:

(a) Qualitative Tests - Villela and Leal (64) have found, in the course of investigations on blood phosphate fractions by the Fiske and Subbarow technique, that vitamin B₁ gives an intense blue color with ammonium molybdate in sulfuric acid solution and aminonaphthosulfonic acid. Their work was repeated in our laboratory using the following procedure: To 100 micrograms of thiamin hydrochloride* in aqueous solution were added 5 ml. of 2.5% ammonium molybdate in 3N H₂SO₄ and also 1 ml. of 0.25% L-amino-2-naphthol-4 sulfonic acid containing Na₂SO₃ and NaHCO₃ (11). According to the original investigators, a blue color should be formed which is due to the thiamin. No such color was obtained in this experiment after allowing the solution to stand for 10 minutes. This investigator agrees with the suggestion made by Youngburg (72) that the blue color observed by Villela and Leal was probably due to impurities. Thus the vitamin does not interfere in phosphorus determinations.

According to Raybin (47) an orange color, extractable by chloroform is immediately developed when about 1 mg. of vitamin B₁ in a few ml. of saturated borax solution is treated with alcoholic 2,6 dibromoquinonechloroimide. The procedure recommended is as follows:

"1 mg. of thiamin is dissolved in a few ml. of a borax solution of pH approximately 9.6 and to it is added a drop of an alcoholic solution of the reagent. The color develops at once, gradually increasing in intensity. It appears as though the thiazole portion is responsible for the color with the reagent. Many amines, phenols and derivatives interfere."

*All of the vitamin B₁ used in this work was crystalline Thiamine Hydrochloride C. P. Merck.

Experimental: The reagent was prepared by dissolving 0.2 gm. of 2,6-dibromoquinonechloroimide (Eastman) in 50 ml. of 95% ethyl alcohol. This yielded a yellow solution which turned brown after standing several days at room temperature. It will be referred to as the "Reagent". Two ml. of a saturated solution of borax were added to 10 ml. of water containing 50 micrograms of vitamin B₁. This rendered the solution alkaline to phenolphthalein. Four tenths of a ml. of "Reagent" were added. A violet color was produced which turned brown on standing. A blank test was also made and it was found that the borax solution alone produced a similar color, of the same intensity as measured by the colorimeter. That this color was due to the action of the alkaline nature of the borax solution was shown by adding varying amounts of the "Reagent" to $N/10$ Na OH. The colors ranged from light yellow to orange and, as the amount of "Reagent" was increased, to a brownish red color. This color was not extracted by chloroform allowing for the possibility of separating the interfering action of the borax. The presence of the borax appeared to be necessary since no reaction took place when the vitamin solutions were adjusted to the same pH with sodium hydroxide.

Ten ml. of a saturated solution of borax containing 0.5 mg. of vitamin B₁ were pipetted into a colorimeter tube. One-tenth of a ml. of the "Reagent" was added and the solution was allowed to stand for 4 hours. The violet color, which immediately formed, later turned a brownish-red. The color was then extracted with 7 ml. of chloroform. After settling, the color of the bottom chloroform layer could be read in the colorimeter without removing the aqueous layer. It was found

that the chloroform layer in the tube containing the vitamin B₁ had a yellow color while that of the "blank" tube, containing only borax, was colorless. Further experiments however showed that, under the conditions described, the reaction was not quantitative.

No further information could be obtained about the work of Raybin and thus the use of this method was abandoned. With our present knowledge about this reaction, its adaptation to the assay of vitamin B₁ in milk was improbable due to the large amount of the vitamin required.

(b) The Thiochrome Assay:

The pale yellow-blue fluorescent compound now known as thiochrome was first discovered by Barger who oxidized vitamin B₁ with alkaline ferricyanide. Following up this work, Jansen (20, 21) devised a procedure for the chemical determination of vitamin B₁ which depended on the quantitative oxidation of the vitamin to the fluorescent thiochrome, extraction with isobutanol, and estimation of the intensity of the fluorescence by comparison in a standardized photoelectric fluorometer. Many other substances interfered in this reaction, which has been the subject of many modifications.

Cerecedo and Hennessy (6) introduced the use of synthetic zeolites for the isolation of the vitamin from foodstuffs, and later these same authors (15) showed that materials interfering in the thiochrome method could be eliminated by the use of a base-exchanging zeolite. Milk was one of the foodstuffs which they analyzed by this method but it is now known that the method they used gives low results. Chemical determinations of the vitamin B₁ in milk by the thiochrome method

have been carried out by Bertagni (5) and especially by Kon, Houston, and Thompson (32). The latter authors made an extensive comparison of the thiochrome and biological methods of estimation of the vitamin B₁ in milk. Statistical methods were applied to the analyses of the vitamin in raw, spray-and-roller-dried, evaporated and sterilized milk. Good agreement between the two methods was found, especially at lower levels of milk feeding. The apparent potency of all milks as measured biologically was found to be systematically higher when milk was fed at a high level than when it was fed at a low level. The authors explain the discrepancy between fluorometric and biological assays at high levels of milk feeding as due to a deficiency of some essential factor in the basal diet, but they point out that the beneficial effect of the major constituents of milk cannot be ruled out.

The rapidity and accuracy of the thiochrome method as applied to the analysis of milk made it especially desirable for an investigation of this kind. It was not used in this work since a fluorometer was not available.

(c) The Formaldehyde - Azo Reaction

This is one of the modifications of the Ehrlich-Pauly reaction in which the vitamin is coupled with a diazotized amino compound to give a dyestuff. This reaction was described and developed by Minnersley and Peters (24, 25) in which diazotized sulfanilic acid is added to an alkaline carbonate mixture and to which is added, after a suitable interval, the vitamin solution treated with a drop of formaldehyde to stabilize the pink color against fading due to oxidation.

Traces of metals interfere by reducing the pinkness of the solution. This method has been used in a preliminary investigation of the vitamin content of foodstuffs in which parallel results were obtained with biological assay. Although the method showed promise of being useful for the assay of vitamin B₁ in milk, it was not used in this investigation.

(d) Melnick and Field Method:

The method which seemed the most suitable for the determination of vitamin B₁ in milk was that described by Melnick and Field,⁽³⁸⁾ and which was successfully applied by them to the determination of the vitamin in urine,⁽³⁹⁾ and other natural sources including yeast, rice polishings, wheat germ and liver. The method is based on the reaction between thiamin and diazotized p-amino acetophenone in alkaline solution to produce an insoluble red pigment. The preparation of the reagent and its specificity for the 4-methyl-5-beta-hydroxythiazole portion of the thiamin molecule has been described by Preblud and McCollum^(46a).

C. Experimental:

This phase of the investigation deals with the attempt to adapt the Melnick-Field method to the quantitative analysis of vitamin B₁ in milk.

(1) Use of Zeolite to Obtain Vitamin B₁ Concentrates:

The technique of absorption on synthetic zeolite and subsequent elution was used in preparing concentrates of Vitamin B₁ for analysis.

The apparatus and procedure used was nearly identical to that described by Melnick and Field, ⁽³⁸⁾ being modified only in a few minor details. The actual procedure at first adopted was as follows:

To 900 ml. of milk were added 150 ml. of 8% trichloroacetic acid which brought the milk to a pH of 4.3. After standing for 5 minutes, with occasional stirring, the proteins were filtered out and 175 ml. aliquots of the filtrate were taken for analysis. These aliquots were passed through the zeolite^{*} column and the absorbed vitamin eluted with 10 ml. of 25% K Cl at pH 2 in the exact manner as described by Melnick and Field ⁽³⁸⁾. Five ml. of the eluate were pipetted into a 50 ml. cream-test bottle. This was followed by the addition of an equal volume of a 95% ethyl alcohol solution containing 5 mg. of phenol per ml. One drop of broms thymol blue indicator was added and, while a fine stream of nitrogen gas bubbled through the solution, a 1 N NaOH solution was added dropwise until a faint but positive blue color appeared. Usually one or two drops were sufficient. Ten ml. of the Prebluda-McCollum reagent ^(46a) were then immediately added and the bottle stoppered and allowed to stand for 15 to 18 hours at room temperature. Eight ml. of xylene were then added and the mixture vigorously shaken. Five ml. of a solution of sulphuric acid^{**} were added carefully, the bottles tightly stoppered and shaken for 15 to 20 seconds. After the addition of 10 ml. of 25% NaOH solution the shaking was repeated for another period of 15 to 20 seconds. Water

*Obtained through the courtesy of the Permutit Co., New York City.

**33 ml. of H₂SO₄ (sp. gr. 1.84) diluted with distilled water to 100 ml.

was added to bring the xylene layer up into the neck of the bottle which was then centrifuged for several minutes. At least 6 ml. of the xylene layer were pipetted off into a colorimeter tube and the color was compared in the colorimeter with a standard similarly treated. For the standard solutions, 1 ml. of several solutions containing varying amounts of thiamin (0 to 50 micrograms) were added to 4 ml. aliquots of blank KCl eluates (10 ml.). The blank eluate is essential, not owing to any lack of specificity, but because the amount of pigment recovered from permutit eluates varies from 85 to 95% of the theoretical. The percentage recoveries are consistent for eluates from the same permutit filter.

This procedure was used for several determinations of the vitamin B₁ content of pasteurized milk, adding known amounts of pure vitamin B₁ to some samples in order to determine if quantitative recoveries could be obtained. The results of these analyses varied from 13 to 22 micrograms of vitamin B₁ per 100 ml. of milk which is lower than is to be expected for a good grade of milk. Also recoveries of added vitamin varied from 50 to 70% of the theoretical. Further experiments indicated the reasons for these discrepancies. It was found that the zeolite did not completely absorb all of the vitamin B₁ from the milk filtrates. The elution of the vitamin from the zeolite by the KCl was not quantitative. This took place only when using milk filtrates since consistently good results were obtained when solutions of the crystalline vitamin were concentrated. The reason for this was due to the presence of the relatively large amounts of inorganic salts in milk, which "saturated" the zeolite, and interfered in the ab-

sorption and elution of the vitamin. These interfering substances may be eliminated by a preliminary extraction of the milk filtrates with a selective solvent such as benzyl alcohol, but the procedure is time consuming and it was thought advisable to find another method of concentrating the vitamin.

(2) Superfiltrol as an Absorbant of Vitamin B₁:

Emmett, Peacock and Brown (9) have confirmed the findings of Melnick and Field (56) that their method gives quantitative results. In a later paper (10) these authors described the use of superfiltrol as a simple method for the concentration of thiamin solutions. Their procedure was adapted to the analysis of milk in the following manner:

Five-tenths of a gram of superfiltrol* were added to a 56 ml. aliquot of a protein-free milk serum at pH 8 and the mixture shaken occasionally for one hour or longer. After centrifuging, the clear liquid was carefully poured off. To the absorbate was added 5 ml. of water and an equal volume of a 95% ethyl alcohol solution containing 5 mg. of phenol per ml. One drop of broms thymol blue indicator was added, and, while a fine stream of nitrogen gas bubbled through the mixture, a 1 N NaOH solution was added dropwise until a blue color appeared. Ten ml. of fresh Prebluda-McCollum reagent was added, mixed thoroughly, allowed to stand at room temperature for two hours, or longer, filtered on a small Hirsch filter and washed with a few ml. of water. The paper and absorbate were then transferred to a dry

*Obtained from the Filtrol Corp., Los Angeles, California.

centrifuge tube and 8 ml. of 95% ethyl alcohol added. The tube was stoppered, shaken to dissolve the pigment and centrifuged. Six ml., or more, of the solution were pipetted into a colorimeter tube and the color compared with a standard similarly treated. Filter No. 520 was used.

(a) Calibration of the Colorimeter:

The colorimeter was calibrated with solutions containing known amounts of vitamin B₁. These standard solutions were prepared by diluting a stock solution of the vitamin. This stock solution was prepared by dissolving 50 mg. of crystalline thiamin hydrochloride, accurately weighed, in 95% ethyl alcohol and diluting to 100 ml. A drop of acetic acid was added to make sure that the solution was acid. It was kept in sealed small bottles in the ice cream hardening room at a temperature of -4°F . Once a bottle was opened the remainder of its contents were discarded. The standard solutions were diluted to about 60 ml. with distilled water which had been brought to a pH of 4.5 with HCl. The procedure was identical to that described above for milk. The results of this calibration are given in Table VI and plotted in Fig. IV. However, as a standard procedure, a standard solution of vitamin B₁ was included in each set of experimental determinations in order to check the technique. This standard could then be used to determine the constant K, which was used to calculate the vitamin content of the unknown, according to the formula:

$$C = K (2 - \log O)$$

$$= Kl$$

where C = Concentration of vitamin B₁

O = Galvanometer reading

K = Constant to allow for amount
of sample and dilutions.

L = Density of color

$$= 2 - \log O$$

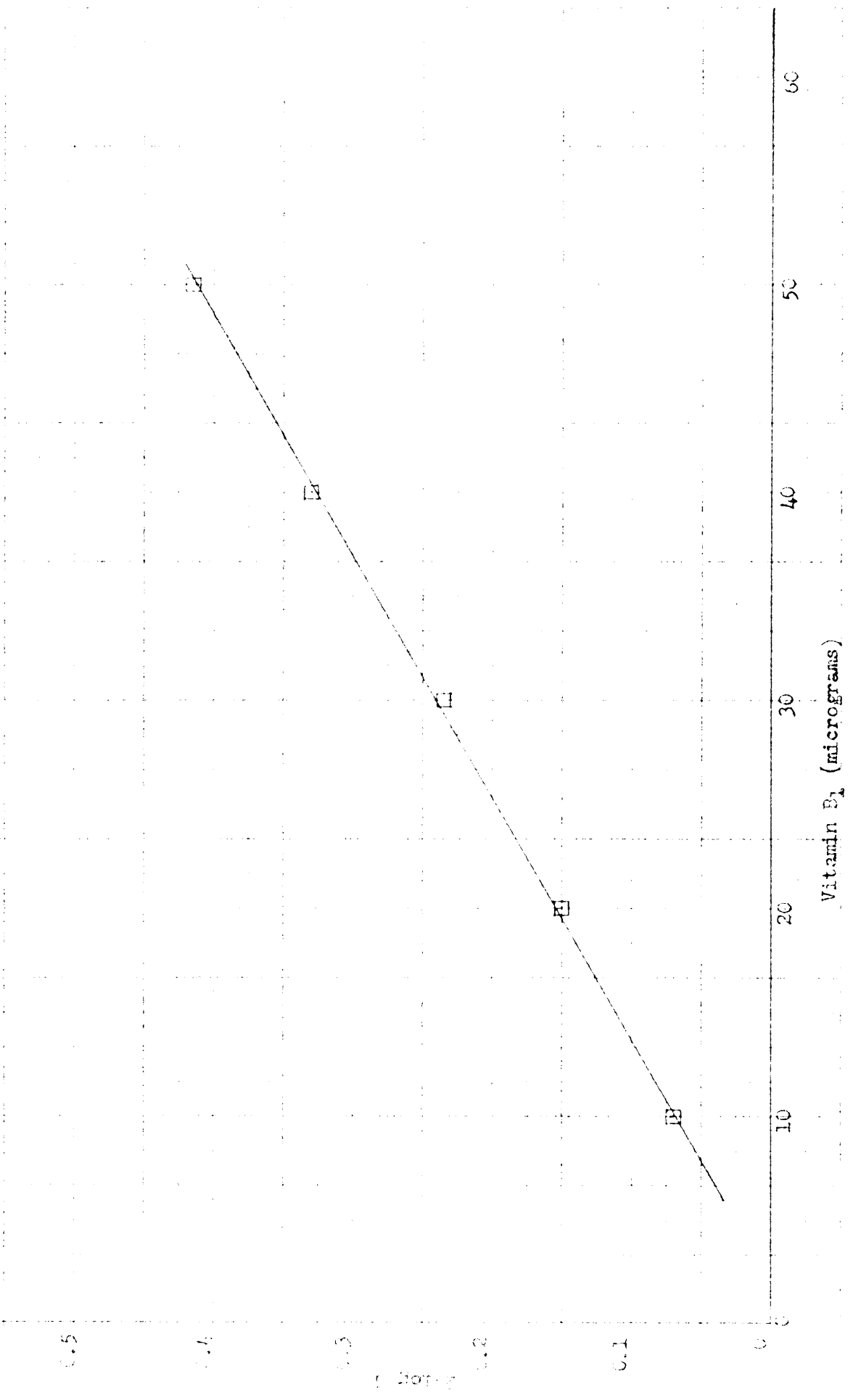
This method was applied to samples of raw milk, after precipitation and filtration of the proteins. Duplicate aliquots checked to about 5% and the recovery of 10 micrograms of added vitamin B₁ to an aliquot was 8.7 micrograms. The milk was found to contain 21.6 micrograms of thiamin per 100 ml. This value does not represent the total amount of the vitamin in the milk since this method does not determine the vitamin which exists in the esterified form. The following experiments were carried out to determine the total vitamin B₁ in milk.

Table VI

Calibration of Photoelectric Colorimeter for the Analysis of
Vitamin B₁ in Milk

Vitamin B ₁ (micrograms)	G	L	K
0	100	0	—
10	84 ²	0.073	137
20	69 ⁰	0.155	129
30	58 ²	0.232	129
40	46 ³	0.330	121
50	38 ²	0.414	121

Fig. IV.-Calibration Curve for Vitamin B₁
Analysis Using Superfiltrol as an
Absorbant



(b) Free and Combined Vitamin B₁ in Milk:

Lohmann and Schuster (37) reported that thiamin may exist in nature as the phosphoric ester to constitute cocarboxylase, the coenzyme of carboxylase. Cocarboxylase has biological activity corresponding to free thiamin (37). The chemical method of analysis of the vitamin does not determine the vitamin which exists in the esterified form. Milk is known to contain part of its vitamin B₁ activity in the form of cocarboxylase (32) and thus for the determination of the total amount of the vitamin in milk, incubation with takadiastase is necessary.

To an aliquot portion of protein free milk "serum" at pH 8 was added dropwise a solution of 10% NaOH until the pH was approximately four. This is the optimum pH for the action of the enzyme. Two drops of JHC1g and 0.5 gm. takadiastase* were added and incubated 8 hours at 37°C. The vitamin was then absorbed on superfiltrol and analyzed as previously described. It was noted that the superfiltrol also absorbed the riboflavin from the milk but further experiments with synthetic thiamin and riboflavin showed that the latter did not interfere in the reaction. The following results were obtained:

<u>Sample</u>	<u>Vitamin B₁ (micrograms)</u>
65 cc. Milk Serum Aliquot	10.5
65 cc. Milk Serum Aliquot + 10 microgram thiamin	19.6
65 cc. Milk Serum Aliquot after takadiastase incubation	16.2
1 gm. Takadiastase	0.0

*Parke Davis & Co.

Further experiments indicated that pure vitamin B₁ which was added to an aliquot of protein-free milk serum could be quantitatively recovered, within the limits of the experimental errors of about 5%. However when the pure vitamin was added to the milk before the precipitation of the proteins, quantitative recoveries were not obtained. In the calculations of the vitamin B₁ in milk from the results on aliquots of milk serum it was first assumed that the distribution of the vitamin between the protein precipitate and the filtrate was in the ratio of their respective volumes. The above described and other experiments have shown that this assumption was incorrect and that some vitamin B₁ was in some way attached to the protein. This is in agreement with the work of Kon et al (18, 32) who showed that an appreciable amount of the vitamin B₁ in milk is combined with the protein and which is not split off by repeated extraction with trichloroacetic acid. Thus in order to obtain quantitative results it was found necessary to enzymatically hydrolyze the milk proteins to soluble products. For purely mechanical considerations, it was found necessary to remove the fat and thus all further experiments were carried out on skim milk.

(C) Total Vitamin B₁ in Milk:

The method which was finally adopted for the analysis of the total vitamin B₁ in milk is as follows: To 75 ml. of fresh skim milk (pH=6.8) were added 10 ml. of NHCl containing 0.4 gms. of pepsin.* The pH of the mixture was 2.1. After shaking thoroughly, 2 drops of CHCl₃ were

*Pfansteil Co., Pepsin 1/5000

added and the mixture was incubated for 48 hours at 37°C.* Four-tenths of a gram of fresh pepsin were added after the first 24 hours. The addition of 5 ml. of KNaOH brought the pH to 4.0. Five-tenths of a gram of takadiastase were then added and the mixture was further incubated for 6 hours. At this point only a small residue of undigested protein remained which was filtered off and washed with about 5 ml. of water. The vitamin B_1 in the filtrate, which to the best of our present knowledge, contains all of the original vitamin in the milk, was then analyzed in the manner previously described. The following are several typical results:

<u>Sample</u>	<u>Vitamin B_1 micrograms/100 ml.</u>
Raw Milk + Pepsin Incubation	20.4
Raw Milk + Pepsin + Takadiastase Incubation	36.0
Recovery of 50 micrograms of added thiamin	52.6

This method appears to be suitable for the analysis of the vitamin B_1 in milk but further work should be carried out to compare the results of this method with those obtained by the thiochrome and biological assays.

*45°C. would have been more suitable but no incubator was available at this temperature.

D. The Effect of Pasteurization on the Vitamin B₁ Content of Milk:

The original purpose of this part of the investigation was to determine the extent of the destruction of the vitamin during pasteurization and also whether this destruction could be prevented by the method of vacuum-pasteurization as described in the section on vitamin C. However, after the work on the chemical method of assay of the vitamin had been under way for several months, the publication of Houston et al ⁽¹⁹⁾ was received which showed that only 10% of the vitamin was destroyed by commercial pasteurization of the milk. This small amount of destruction would hardly warrant the adoption of the method of vacuum pasteurization.

Several experiments were started to confirm the work of Houston⁽¹⁹⁾ and to study the effect of vacuum pasteurization and storage of the milk on the vitamin B₁ content. However, some unknown experimental error was made in the preparation of the Prebluda-McCollum reagent since the latter did not react with the vitamin. Lack of time prevented the repetition and continuation of these experiments.

IV. RIBOFLAVIN (VITAMIN B₂)

It is well known that riboflavin resists autoclaving at hydrogen ion concentrations within the range in which the pH of milk normally falls (33). Thus it is to be expected that pasteurization would have no effect on the riboflavin content of milk. This has been shown to be correct by many investigators (14, 19, 33, 68). Sterilization of the milk by autoclaving had no effect on the riboflavin content (30, 19). These results indicate that further investigations on the effect of pasteurization on the riboflavin content of milk are not needed.

Chemical methods of analysis of riboflavin in milk are based on the fluorescent properties of the vitamin (14, 61, 68). These methods are relatively accurate and compare favorably with the refined methods of biological assay (16). These methods, however, require the use of a fluorometer, a delicate and expensive instrument, which is not available in most laboratories. Emeric developed a colorimetric method for the determination of riboflavin in milk (3). It was thought advisable to calibrate the photoelectric colorimeter for this method and to test it on several samples of milk. The calibration was carried out with purified riboflavin (C.P. Merck) which was dissolved in and appropriately diluted with 20% methyl alcohol. All operations involving riboflavin were carried out in subdued daylight since the vitamin is known to be sensitive to light. The results are given in Table VII which also shows that the riboflavin solutions are stable in the light in which the experiments were carried out. The density of the color due to riboflavin is directly proportional to concentra-

tion of riboflavin and is illustrated in Fig. V. In the analysis of riboflavin in milk the latter is treated with $KMnO_4$ which is then removed by an excess of H_2O_2 . This treatment was shown to have no effect on pure solutions of riboflavin in 20% alcohol.

TABLE VII

Calibration of Photoelectric Colorimeter for the Determination of Riboflavin in Milk

Riboflavin*	G_1	G_2	G_{mean}	L	G_3	G_4
0.00	100	100	100	0.000	100	100
0.05	87°	87'	87°	0.060	87'	-
0.08	79 ^S	79 ^S	79 ^S	0.098	79 ^S	79 ^S
0.10	64°	65 ^S	65 ^S	0.198	64°	65 ^S
0.15	51'	51 ^S	51'	0.291	51°	51 ^S

*Riboflavin concentration in mg. per 25 ml. of 20% methyl alcohol.

G_1, G_2 - Galvanometer readings

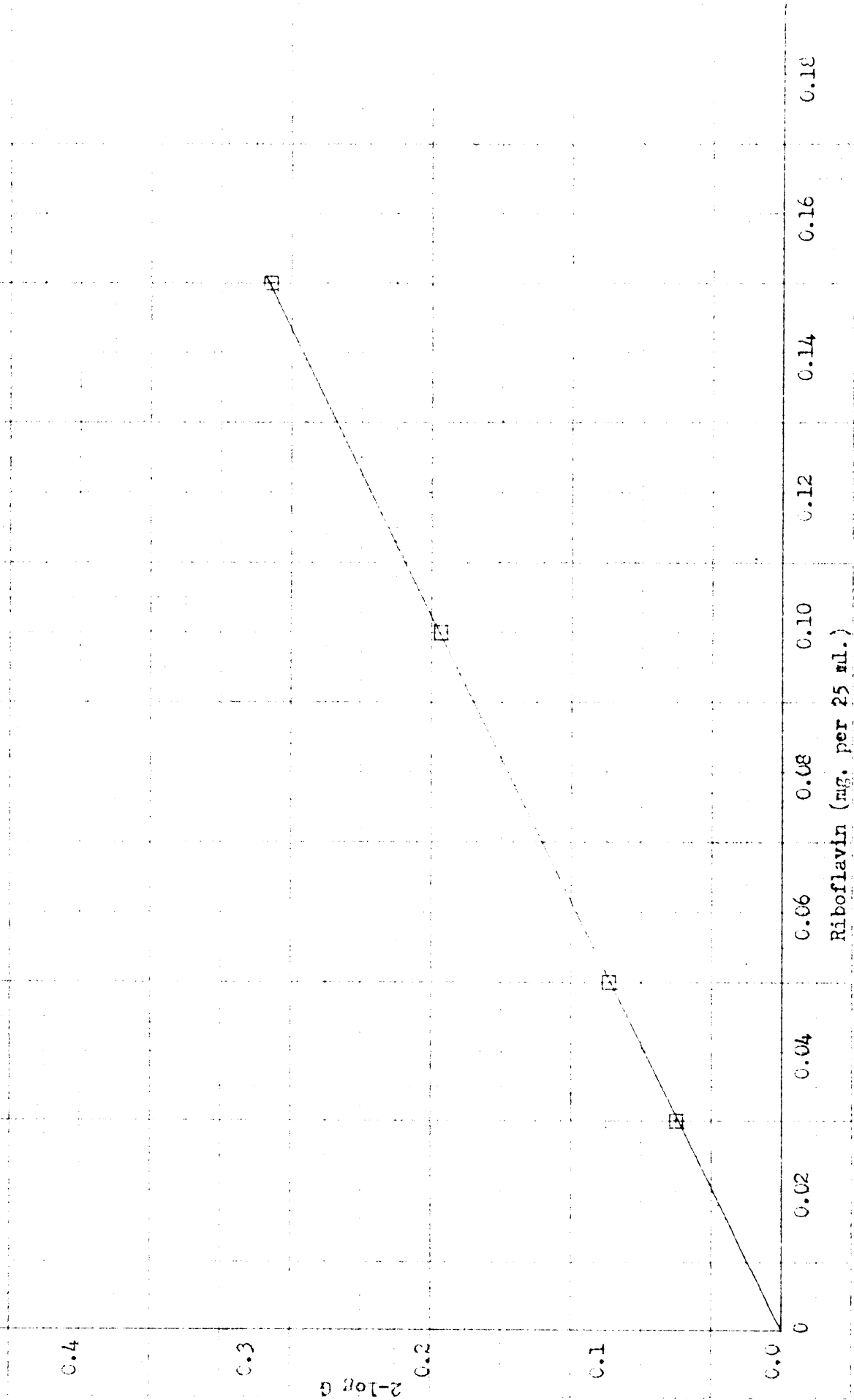
L = $2 - \log G_{mean}$

G_3 = Galvanometer reading after the riboflavin solutions had been allowed to stand 10 minutes in subdued daylight

G_4 = Galvanometer readings after the riboflavin solutions were treated with $KMnO_4$ and H_2O_2

Filter No. 440

FIG. V.—Calibration of Evelyn Photoelectric Colorimeter
for the Determination of Riboflavin in Milk



The procedure for the determination of riboflavin in milk is as follows: To 50 ml. of skim milk were added slowly and with constant stirring 50 ml. of methyl alcohol and the solution was held at 60°C. for 15 minutes. After cooling to room temperature, 0.1 ml. of glacial acetic acid was added and the mixture was diluted to 100 ml. with methyl alcohol. After shaking, the precipitate was allowed to settle for 15 minutes and then removed by filtration. Seventy ml. of the filtrate were concentrated under vacuum to about 20 ml. and 0.5 ml. glacial acetic acid and 1 ml. of 4% $KMnO_4$ were added. The solution was shaken and allowed to stand 1 minute. One ml. of 3% HgO_2 was added to remove excess $KMnO_4$, the solution was filtered and the filtrate was diluted to 25 ml. with methyl alcohol. The yellow color was determined with the colorimeter using filter No. 440.

A sample of fresh pasteurized milk from the Dairy of the Virginia Polytechnic Institute was found to contain 1.60 mg. of riboflavin per litre. In a series of 400 determinations Hand ⁽¹⁴⁾ has shown that the normal riboflavin content of milk varied from 1.20 to 3.40 mg. per litre.

V. VITAMIN A AND CAROTENE

A. Methods of Analysis:

Early studies have shown that the growth of experimental animals is proportional to the vitamin A content of the diet. It was also shown that vitamin A is concerned with the prevention and cure of specific pathological conditions. The assay of vitamin A by the growth method was introduced as early as 1920 and has since been refined and standardized until it is possible to obtain results with it that have as high a degree of accuracy as can be expected of procedures in which animals are used as reagents (42).

Chemical and physical methods of analysis include the color test with antimony trichloride and spectrographic or absorption methods. The latter require special instruments which are very expensive and long experience with spectrographic measurements are required in order to obtain accurate results. Antimony trichloride reacts with both vitamin A and carotene to give a blue color, the intensity of which obeys Beer's law. By the use of suitable filters and the Evelyn photoelectric colorimeter it is possible to analyze for carotene and vitamin A in mixtures of the two. This method appeared to be the most suitable for the determination of vitamin A value of milk. The Evelyn photoelectric colorimeter was calibrated with solutions of pure carotene* in petroleum ether. The yellow color due to the carotene was read directly on the colorimeter using filter No. 440. The results are given in Table VIII and plotted in Fig. VI.

*90% beta; 10% alpha - Made by the S. M. A. Corporation

TABLE VIII

Calibration Curve for Carotene

Carotene*	G ₁	G ₂	G _{mean}	L
0	100	100	100	0.00
1.0	92 [']	91 ^o	91 ²	0.039
2.0	83 ³	83 ³	83 ³	0.077
3.0	75 ²	75 [']	75 [']	0.123
4.0	69 ²	69 ^o	69 ³	0.163
5.0	63 ²	63 ^o	63 [']	0.199

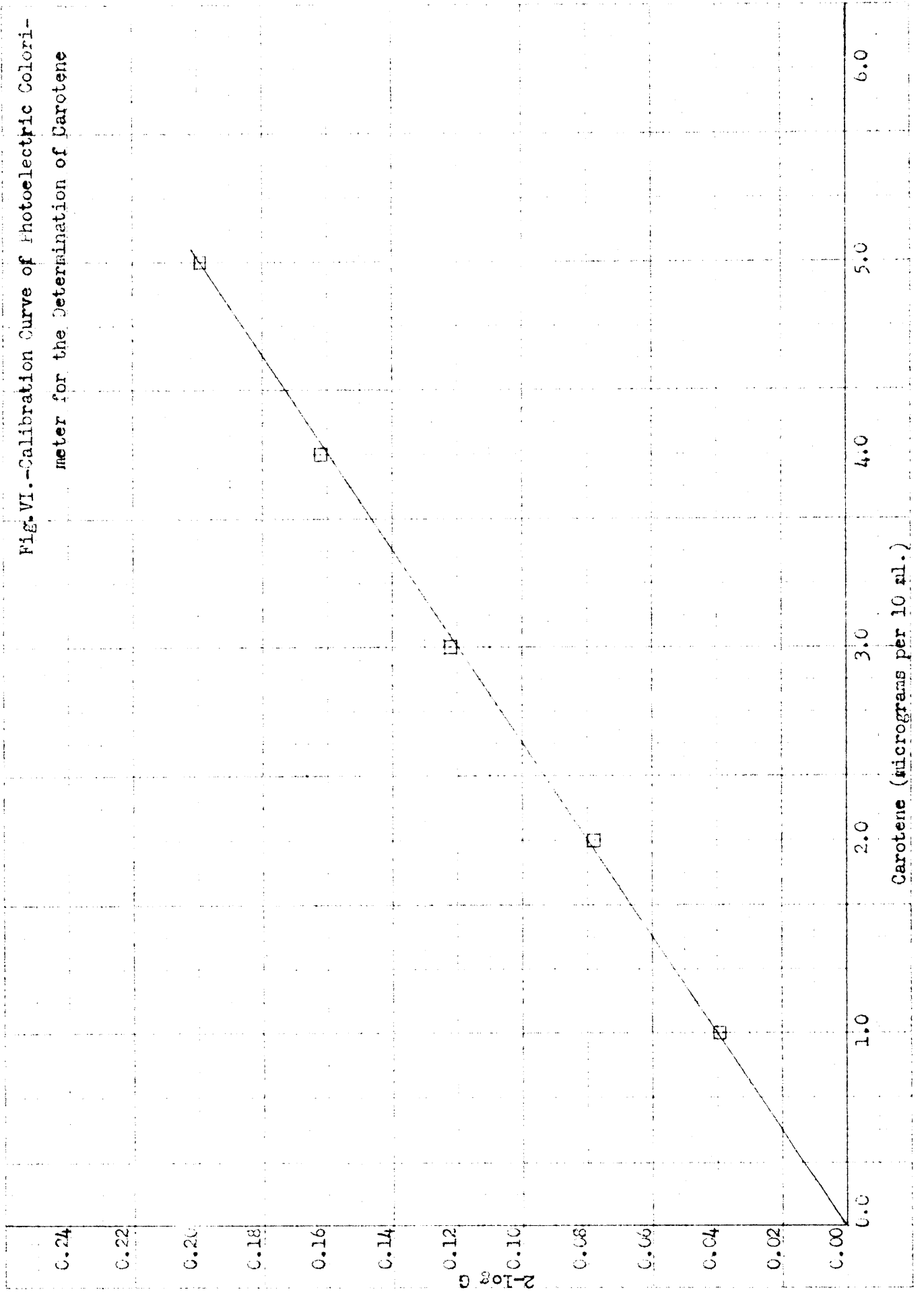
*Carotene concentration in micrograms per 10 ml. of petroleum ether.

G = Galvanometer reading

L = Density of color

= $2 - \log G_{\text{mean}}$

Fig. VI.--Calibration Curve of Photoelectric Colorimeter for the Determination of Carotene



This calibration curve may be used in the direct colorimetric determination of carotene. For a mixture of vitamin A and carotene, the vitamin A may be calculated by the formula⁽²³⁾

$$L \text{ value of vitamin A} = \frac{0.0525 \left\{ L_{620} - (0.14 L_{440}) \right\}}{A}$$

where L_{440} = Density of the yellow color due to carotene. Filter No. 440

L_{620} = Density of the blue color due to carotene and vitamin A. Filter No. 620

A = Constant to account for weight of sample and subsequent dilutions

Since vitamin A solutions are colorless, the vitamin does not interfere in the direct colorimetric determination of carotene.

B. Effect of Pasteurization:

Indirect evidence and knowledge of the heat stability of vitamin A have led to the popular belief that this vitamin is only slightly affected by pasteurization, if at all. Krauss, Erb and Washburn⁽³⁸⁾ studied the growth curves of rats fed raw and pasteurized milk as supplements to a vitamin A free diet and concluded that no destruction of vitamin A resulted from heating milk at 145°F. for 30 minutes in a closed container. Pratt⁽⁴⁵⁾ showed that nickel or manganese did

not catalyze the destruction of vitamin A during pasteurization. Kon⁽³¹⁾ showed that neither carotene nor vitamin A is affected by pasteurization and that the addition of copper did not influence the results. The vitamin A assays were carried out by animal feeding and spectrophotometric methods. Kon and Henry⁽³⁰⁾ showed that no carotene or vitamin A is destroyed during the commercial sterilization of milk and it has also been shown that irradiation of the milk had no effect on this vitamin⁽³¹⁾.

These results appear conclusive and this phase of the problem was not further investigated.

VI DISCUSSION

The results shown in Tables II and III indicate the changes in the distribution of ascorbic and dehydroascorbic acid that take place during the pasteurization and storage of milk. During the storage of raw milk, only a small percentage of the total vitamin C is destroyed but at the same time a large proportion of the reduced ascorbic acid is oxidized to dehydroascorbic acid. Vat pasteurization immediately destroys all of the dehydroascorbic acid and some of the reduced form. On storage of the pasteurized milk the oxidation to dehydroascorbic acid proceeds at a more rapid rate than in raw milk.

Pasteurization of milk in the absence of air had very little effect on the two forms of vitamin C. However, during the storage of this milk it was noted that the ascorbic acid content decreased slowly. In milk that had been passed over a surface cooler, this decrease was more rapid. This decrease in the amount of ascorbic acid in the milk was due to the exposure of the milk to the air after pasteurization, which was not prevented with the techniques used.

In the experiments on commercial vacuum pasteurization the milk was heated by continuous circulation through a heat exchanger. This is undesirable for general commercial practice and could be eliminated by the installation of a rotating heating coil inside the vat. The most desirable equipment for a commercial plant would be a vat equipped for pasteurization at a pressure low enough so that the milk would boil gently at 144°F . After pasteurizing, the milk may be cooled by

passing through a tubular cooler when it may be bottled in a vacuum bottler so that the milk would not be exposed to the air at any time. From the results obtained in this investigation, it appears evident that if the milk is pasteurized in this manner, it will reach the consumer with all or very nearly all of the vitamin C that was present when it first arrived at the plant. If pasteurization in the absence of air is carried out then it becomes possible to enrich the milk by the addition of synthetic ascorbic acid, without any losses. A milk high in vitamin C content would be valuable from many standpoints and would be commercially feasible.

Since the completion of this work, a milk-deaerator has been put on the market (63) which prevents destruction of vitamin C and also prevents the development of oxidized flavors. This method is well adapted to use with the short hold, high temperature process of pasteurization. It cannot be used efficiently in conjunction with the "holder" method which is the method most widely used at the present time. This milk-deaerator would thus supplement the method of vacuum pasteurization providing a valuable new asset to the dairy industry.

It might also be mentioned that the method of vacuum-pasteurization described in this work should also prevent the development of oxidized flavor, as well as remove the volatile "feed" flavors, but further investigation in this direction is necessary.

VII. CONCLUSIONS

(1) A survey of the literature revealed experiments which showed conclusively that there is no destruction of vitamin A, carotene or riboflavin when milk is pasteurized at 145°F. for 30 minutes.

(2) Ten to fifteen per cent of the vitamin B₁ and 20 to 40 per cent of the vitamin C are destroyed during pasteurization by the holder method. Storage of pasteurized milk results in a further rapid destruction of vitamin C.

(3) Destruction of vitamin C is prevented, even in the presence of copper, if milk is pasteurized in the absence of air.

(4) Milk pasteurized and bottled in the absence of air would retain all, or very nearly all, of the original vitamin C, even after storage for several days. This process appears to be commercially feasible.

(5) A chemical method of vitamin B₁ analysis has been adapted to the assay of this vitamin in milk.

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