The determination of the beta-carotene and vitamin A content of Montana butter from different areas at various seasons and after storage
by John L Brence

A THESIS Submitted to the Graduate Committee in partial fulfullment of the requirements for the degree of Master of Science in Dairy Industry at Montana State College
Montana State University

Abstract:
Fifteen samples of Montana butter produced in the Lewistown, Miles City, Havre, Ronan and Boseman areas in the late spring, fall and late winter seasons were studied, The micrograms of beta-carotene and vitamin A per gram of butterfat were determined on one sample from each area in each season by the method outlined by the Technical Committee on Vitamin Research on butter and the International Units per pound of butter (81 percent butterfat) was calculated from the beta-carotene and vitamin A values.

The beta-carotene and vitamin A values of the butter were determined on each sample from each area in each season, when fresh, after holding for 30 days at 40°F. and after storage for 90 days and for 180 days at 0°F.

The beta-carotene content of the fresh butters produced in the late spring was the highest of the three seasons studied, regardless of the area in which they were produced. She vitamin A content was more uniform in the spring season than in the other two seasons studied. This was attributed to the green grass pastures that were prevalent in all the areas in this season.

The calculated vitamin A potency of the spring butter averaged 22,830 International Units per pound. The ranges for the fall butter were from 20,611 to 15,908 International Units per pound and for the late winter butter from 25,498 to 15,504 International Units per pound.

The butters showed no appreciable changes in the beta-carotene or vitamin A values when stored at 0°F. for 90 days. When stored for 180 days at this temperature, the beta-carotene values were not noticeably changed, but the vitamin A tended to show decreases in value. This tendency was particularly marked in the butter samples produced in the fall season.

Butter held for 30 days at 40°F. showed no significant changes in the beta-carotene content, The vitamin A values were higher in most of the samples thus held.

THE DETERMINATION OF THE BETA-CAROTENE AND VITAMIN A CONTENT OF MONTANA BUTTER FROM DIFFERENT AREAS AT VARIOUS SEASONS AND AFTER STORAGE

by

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ABSTRACT

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INTRODUCTION

Butter is recognized as a food of high nutritive value, owing to the fact that it not only contains a large percentage of energy-producing milk fat, but is also a good source of vitamin A. In fact, nutritionists regard butter as the most important natural source of vitamin A in the average American diet, and list butter as one of the essential foods for adequate human nutrition.

While the composition of butter, with respect to its fat and moisture content, is regulated by State and Federal laws, no effort has been made to regulate or standardize its vitamin A content. Doubtless, this has been due to a lack of means for the accurate determination of the vitamin A content of butter.

In 1930, Moore (21) presented evidence to show that the colorless substance, vitamin A, was derived from a yellow plant pigment, carotene, the conversion taking place in the livers of herbivorous animals. It has further been shown by Karrer (24) that among the various forms of carotene, beta-carotene has the greatest vitamin A activity and that each molecule of beta-carotene is capable of giving rise to two molecules of vitamin A. Since then, it has become apparent that a determination of the vitamin A potency of butter, an animal product, necessitates the measurement of both vitamin A, the converted material, and beta-carotene, the unconverted precursor of vitamin A.

During the past fifteen years, a number of studies have indicated that the beta-carotene and vitamin A content of butter varies from time to time. Semb, Baumann and Steenbock (27) have definitely shown that the vitamin A content of butterfat varies seasonally, due to kind of feed consumed by the cow, and that different breeds of cows, consuming the same kind of feed, will secrete milk fat that contains different percentages of vitamin A. These investigators also report that cows in the early stage of lactation will produce milk fat with a higher vitamin A content than in the late stage of lactation.

While work has been done in a few states to determine the beta-carotene and vitamin A content of the butter they produce, no studies have been made on Montana butter. It would be of value commercially as well as from a nutritional standpoint to know the vitamin A potency of Montana butter, as produced in the different areas of the state during the various seasons, and after storage. If these facts were known, the nutritive value of Montana butter could be more fully evaluated and possibly some corrective measures could be advanced to produce butter of a more uniform beta-carotene and vitamin A content.

A national cooperative project for the determination of the vitamin A potency of commercial butter sold in the various states was approved by the directors of the Association of Land Grant Colleges in November 1941. The project was originally recommended by the Committee on Food and Nutrition of the National Research Council. The object was to ascertain the actual variation in the vitamin A values of commercial butters in towns and cities located in different regions of the United States.

In cooperation with the national project, this study was undertaken to determine the beta-carotene and vitamin A content of butter made in different areas of Montana in different seasons. The effect of holding the butter at different temperatures for different periods of time was also included in the study.

REVIEW OF LITERATURE

Booth, Kon, Dann and Moore (5) found that the total vitamin A activity of summer butterfat was about three times greater than that of winter butterfat. Semb, Baumann and Steenbock (27), reporting on five different breeds of dairy cattle, state that the vitamin A content of the milk fat varies seasonally with the feed. When the feed contained a relatively high carotene content the milk fat also contained a high beta-carotene and vitamin A content, and when the feed contained a low carotene content the resulting milk fat was also low in beta-carotene and vitamin A. Kemmerer and Fraps (14) determined the vitamin A potency of Texas butters and found some variation from month to month. They found the vitamin A content ranging from 42.6 International Units per gram of butter in April down to 31.0 International Units in October butter. Koehn (16) investigated the feasibility of winter pastures in Alabama and found that cows produced milk fat as high in vitamin A potency on winter pasture as they did on summer pasture. He compared the carotene content of the winter pasture grasses with that of the summer pasture grasses and found no significant difference.

From these reports it appears that season alone does not have an influence on the vitamin A potency of milk fat. There also seems to be a relationship between the carotene in the feed consumed by the cow in a particular season and the vitamin A potency of the milk fat.

Moore (22); Baumann, Steenbock, Beeson and Rupel (1); Gillam, Heilbron, Morton, Bishop and Drummond (10); and Loy, Hilton, Wilbur and Hauge (19) found that feed of high carotene content increased both the beta-carotene and vitamin A content of the resulting butter and that feeding a low carotene feed for prolonged periods of time decreased both the beta-carotene and the vitamin A. This is further supported by the work of Hilton, Wilbur and Hauge (11) who found that fresh luxuriantly growing pastures and good quality silages and hays are the principal sources of carotene in the rations of dairy cattle. Their study showed that cows on blue grass pasture produced milk fat of high vitamin A value, but when timothy hay was substituted for blue grass pasture, the vitamin A value of milk fat dropped markedly. However, when good quality alfalfa hay was substituted for the timothy hay the vitamin A potency of the milk fat was restored almost to that produced when the cows were on pasture. Treichler, Grimes and Fraps (30), using white and yellow corn as a source of carotene in the cows' ration, found that in 28 weeks the carotene content of the butterfat decreased 99 percent when white corn was fed and 97 percent when yellow corn was fed. White and yellow corn proved to be poor sources of carotene. When cows that had been depleted of vitamin A for 60 days were grazed on green pasture five hours a day for three days the vitamin A content of the milk fat increased almost to the normal level. After the three days on grass feed the vitamin A content of the butterfat varied only slightly with the increase in the number of days on pasture, but the beta-carotene content steadily increased up to 70 hours on pasture. Krauss and Washburn (17), determining the carotene content of green legumes to be made into silage and their loss of carotene in handling, found that by the time the alfalfa had reached the silo 27.7 percent of the carotene had been lost. Peterson, Bird and Beeson (25), investigating the A.I.V. silage as a source of carotene, determined that continuous feeding of A.I.V. silage during the winter months maintained a higher beta-carotene and vitamin A content in the milk fat than that produced on hay feeds. Hodgson, Knott, Murer and Graves (12) found, on a three year average, that field cured hay had 15 micrograms of carotene per gram of dry matter, grass silage 59, hay and silage 197, and pasture 259. When these rations were fed to Holstein cows they produced butterfats with an average beta-carotene content of 3.6, 6.5, 6.8 and 7.9 micrograms per gram, respectively.

These researches again indicate that there is a direct relationship between the carotene content of the feed fed to cows and the beta-carotene and vitamin A content of the butterfat produced.

The work of Sutton and Krauss (29) indicates that there are differences in the ability of breeds to transmit feed carotene into milk carotene. They found the beta-carotene content to be the highest in Guernsey, second highest in Jersey, next highest in Holstein and lowest in Ayrshire milk fat. The vitamin A content was the lowest in the Guernsey milk fat and highest in the Holstein milk fat. The total vitamin A potencies of the Holstein and Guernsey milk fats were approximately equal. The milk fat from Holsteins and Guernseys was significantly more potent than that from either the Ayrshire or Jersey breeds. Moore (22) concluded from his investigations that physiological factors determining the presence of carotene in the fat will vary with the breed and may vary between animals of the same breed. Watson, Bishop and Drummond (31) experimenting with Ayrshire and Shorthorn cows, noticed that Ayrshire cows subjected to similar feeds as the Shorthorn cows responded similarly in carotene production but at lower levels. Baumann, Steenbock, Beeson and Rupel (1) found that variations in either carotene or the vitamin A content of butterfat from individual cows of the same breed may deviate as much as 100 percent in amount, but that definite tendencies in breeds were dominant. They found that the carotene content was highest in Guernsey milk fat and lowest in Holstein and Ayrshire fats. They also found that the vitamin A was highest in Holstein and lowest in Guernsey butter. When butters from different breeds on the same rations were compared, inverse relationships between carotene and vitamin A were revealed. When carotene was high, vitamin A tended to be low, and when vitamin A was high, carotene tended to be low.

Dornbush, Peterson and Olson (9), in comparing various grades of Wisconsin market milks, found that Guernsey milk was low in vitamin A content but high in beta-carotene, particularly during the pasture season. The carotene content of the Guernsey milk was 17 micrograms per gram of milk fat in October, as compared with 9.9 micrograms per gram for an average of 345 other mixed breed samples for the months of September and October in the same year. Hilton, Wilbur and Hauge (11) summarized that when dairy cattle graze on the same pasture or are fed the same hay and silage ration there will be no significant difference in the total vitamin A value of the milk fat secreted by the different breeds. The above studies seem to confirm the fact that the milk fat produced by the different breeds of dairy cattle varies in beta-carotene and vitamin A content but the total vitamin A potency is about the same for all breeds.

Experimental evidence has been presented by Semb, Baumann and Steenbock (27); Dann (8); and Kemmerer and Fraps (14) that the milk produced in the early stages of lactation contains a higher carotene and vitamin A content than that produced in the later stages of the lactation period.

Some work has been done on the effect of storage, under different conditions, on the vitamin A potency of butters. Olson, Hegsted and Peterson (23) stored butter at 12°C. for three weeks, then held it at the freezing temperature for ten days and again stored it at 12°C. for 14 days. These storage periods did not result in any appreciable change in carotene and vitamin A values. Berl and Peterson (4); and Baumann and Steenbock (2), using lower storage temperatures, found that butter stored for six to eight months at a temperature ranging from 0°C. down to -23°C. did not depreciate in beta-carotene or vitamin A value. Crawford, Perry and Zilva (7) made biological determinations and found no loss in vitamin A potency of Australian and New Zealand butters stored from one to three years. The same investigators found a definite loss in the vitamin A activity in some English butter after storage for two years; however, the storage temperature of these butters was not given. In considering other storage conditions, Boschardt and Garrett (6) found that butter contaminated with copper or with a combination of copper and manganese resulted in the destruction of a considerable portion of the beta-carotene content of the butterfat upon storage. The investigators apparently did not determine the effect of the metals on the vitamin A stability.

Krukovsky, Ellis and Percy (18) observed that the development of oily-tallowy and tallowy flavors in milk fat was accompanied by corresponding losses in their carotene and vitamin A value, and concluded that the oxidation of the double bonds in the beta-carotene and vitamin A molecules destroyed their vitamin A activity. On the other hand, Shrewbury and Kreybill (28) and Wilkie (32) found that butter contains natural antioxidants which protect the carotene and vitamin A from the destructive action of oxygen and rays of the sun.

Baumann and Steenbock (2); and Kemmerer and Fraps (14) found that the acidity of cream before churning had no effect on the vitamin A activity of the butter. No difference was observed in vitamin A activity of butter made from sweet and sour cream. From these two studies it might be inferred that developed acidity of the cream or the amount of neutralizer used to standardize the acidity of the cream before churning does not have any effect on the vitamin A potency of the butterfat in the resulting butter.

The amounts of beta-carotene and vitamin A per gram of milk fat found by the various investigators in different parts of the United States varied over a wide range of values, as reported by Berl and

Peterson (4); Kemmerer and Fraps (14); Dornbush, Peterson and Olson (9); Koehn (16); Olson, Hegsted and Peterson (23); Hodgson, Knott, Murer and Graves (12); and Baumann and Steenbock (2). These scientists found that the beta-carotene content of milk fat ranged from 1.9 micrograms per gram of milk fat in March to 9.9 micrograms per gram in September. The vitamin A content ranged from 4.2 micrograms per gram in January to 28.8 micrograms per gram butterfat in August. The range of vitamin A for commercial butter was 35 to 50 International Units per gram of fat.

PROCEDURE

Source of the Butter Samples

After reviewing the varied results of studies made throughout the Nation, it seemed desirable to determine the vitamin A potency of Montana butter to see how the results compare with the results obtained in other parts of the United States. In order to obtain butter samples that would represent the different parts of Montana, the state was arbitrarily divided into five production areas, each of which was chosen to represent, as nearly as possible, feed and climatic conditions for that part of the state.

The area in Gallatin County surrounding Bozeman was selected to represent the south central part of the state. This is a typical irrigated area. The pasture forage consists mainly of mixed grasses and some legumes. The majority of the pastures are irrigated and produce green succulent feed from May until early fall, when the grass becomes mature and begins to dry. Alfalfa, cured under dry weather conditions, produces a hay of a green color which is the principal hay fed during the fall, winter and early spring seasons. The Holstein breed of cows predominates in this area. The cows freshen during the entire year, but probably the greatest number freshen in the spring.

The area in Lake County surrounding Ronan was selected to represent that part of the state lying west of the continental divide. Practically all of the agricultural section in this area is irrigated and nearly all of the cream produced in this area comes from the irrigated farms. The pastures, which are irrigated during the entire growing seasons, contain chiefly blue grass and white clover. The climate is somewhat milder and the growing season is a little longer than that of the Bozeman area. Not unlike the Bozeman area, the principal hay is high-grade alfalfa. The predominating breeds of dairy cattle in this area are Holstein and Guernsey. The cows freshen during the entire year, but probably the largest number freshen in the spring.

The Miles City area in Custer County represented the eastern part of the state, which is primarily a dry land area. There is some irrigation in the Yellowstone River Valley and in some of the tributary valleys where there is sufficient water. However, the majority of the farms are on dry land. The dry land pastures consist principally of native grass with a little sweet clover, while those on the irrigated farms consist of mixed grasses and some legumes. The native grass pastures on dry land are green and succulent in the spring and early summer, but in the late summer and the early fall the grass is generally dry and resembles bleached hay. The fall of 1943 was an exceptionally dry season so the cows were on very poor quality forage for a longer period than usual. It is the practice in this area to turn the cows on the stubble and corn stalk fields after the crops are harvested and there is very little green pasture feed available. The dry land hays consist of sweet clover and alfalfa, cereal hays, corn fodder, millet, sorghum and the native grass hay. On the irrigated sections the hay is principally alfalfa. The Holstein breed of cows predominates by a small margin; the rest are grade cows and some are of the beef type. Practically all of the cows in this area freshen in the spring.

Central Montana was represented by an area in Fergus County surrounding Lewistown, which consisted of dry land farms. The area has a higher annual rainfall than that of Miles City and growing conditions are somewhat more favorable, but the growing season is shorter. The pastures consist of native grass, which is green in the spring and summer but dry in the early fall. The hay crops are about the same as for the Miles City dry land section. The Holstein breed of cows predominates by a small margin; the rest of the milk cows are grades. The majority of the cows freshen in the spring.

The Havre area in Hill County was selected to represent the north central part of the state, which is primarily a dry land area. It receives about the same annual rainfall as the Miles City area but there is much less irrigation in this district. The pasture consists of native grass, which is succulent during the spring and early summer, but dries up in the late summer and fall and becomes bleached. After the crops are harvested in the fall the cows are turned on the stubble fields where there is practically no green feed. The hay in this area consists of native grass, cereal hays, millet and some alfalfa. The Holstein breed predominates by a narrow margin; the rest of the milk cows are grades. The majority of the cows freshen in the spring.

Conditions in the five areas selected to represent the state were a good cross section of the kinds of feeds and climatic conditions which prevailed in 1943, the year in which the study was made. The climatic conditions for the seasons studied were normal for the later winter and late spring, but the fall season was somewhat drier than usual. This resulted in a greater amount of dry fall pasture forage in the areas studied.

Sampling Procedure

Three times during the year a butter sample, consisting of two one-pound prints of commercial butter was obtained from a creamery in each of the five areas selected in the state. The samples were always accompanied by a complete pasteurization and churning record.

The first samples obtained from each area were from butter churned in the early part of April 1943 and represented late winter feeding conditions. The second samples obtained were from butter churned during the middle part of June 1943, and represented green grass pasture, or spring, feeding conditions. The third samples from each area were from butter churned during the middle part of October 1943, and represented dry pasture forage in dry land areas and some green feeds of greater maturity in the irrigated sections, or fall conditions.

When received, each one-pound print in the two pound sample was divided into half pounds, making four half-pound samples from each churning made in each area. One sample was analyzed at once for beta-carotene and vitamin A content. One sample was held in storage for thirty days at 40°F. and then analyzed. The other two samples were stored at 0°F., one being held for 90 days and the other for 180 days before analyses were made for their beta-carotene and vitamin A content. The temperatures of the refrigerators in which the butter samples were stored were held fairly constant.

The 10 gram sample used in each determination for the beta-carotene and vitamin A content of the butter was taken in the same manner in all cases. A slice of butter one centimeter thick was cut from one of the outside surfaces of the half pound sample and discarded. Then the sample to be tested was obtained by cutting a slice of butter one centimeter wide and one and one-half centimeters thick across the half pound sample next to the freshly cut surface. The sample cut in this manner had one side and two ends that had been exposed to the parchment wrapper and three sides that were fresh butter surface and weighed approximately 10 grams. The weight of the sample was adjusted to 10 grams ($\pm$ 0.01 g.) by adding to or removing butter from the fresh cut surface. This method of sampling was adopted because it was considered that it represented as nearly as possible the manner in which pounds of butter are divided for consumption in the home. The sample used for determining the fat and moisture contents was cut in the same manner as the sample for the beta-carotene and vitamin A determinations.

EXPERIMENTAL PROCEDURE

The chemical tests for the beta-carotene and vitamin A content of the butter were carried out in accordance with the method outlined by the Technical Committee on Vitamin Research on butter (20). Before beta-carotene or vitamin A values could be measured, it was necessary to calibrate the measuring instrument, a photoelectric colorimeter, with pure beta-carotene in a Skellysolve B solution, pure beta-carotene in a chloroform solution, and crystalline vitamin A alcohol in a chloroform solution, and plot a reference curve for each solution.

The Evelyn photoelectric colorimeter[1] was calibrated with pure beta-carotene[2] in both chloroform and Skellysolve B solutions. A series of 12 solutions of beta-carotene was prepared in duplicate containing 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, and 2.4 micrograms of beta-carotene per milliliter. The beta-carotene readings for both solvents were made using the 440 millimicron filter in the colorimeter. The center setting for the galvanometer, or the point at which the galvanometer needle had to be set before making a reading, was determined for each solvent. This blank reading was made by inserting a tube containing 10 milliliters of the pure solvent into the tube holder of the instrument and setting the galvanometer needle at 100. With the tube and solvent removed, the point at which the galvanometer needle

1. Manufactured by the Rubicon Company, Philadelphia, Pa.

2. Carotene (Provitamin A) obtained from the Eastman Kodak Co. (Number 3702) Beta-carotene 90% and Alpha-carotene 10%.

rested was the center setting for that particular solvent. Using the proper center setting on the galvanometer, the series of twelve solutions of the beta-carotene were read in the colorimeter to find the L 440 galvanometer readings corresponding to these carotene concentrations.

A reference curve for beta-carotene in Skellysolve B was constructed by plotting the 12 colorimetric L 440 readings, expressed as 2-log G values, on the abscissa, against the respective concentrations of beta-carotene in Skellysolve B solution, expressed as micrograms per milliliter on the ordinate. This curve is given in Figure 1 and was referred to in calculating the beta-carotene content in the samples of butter.

A factor was calculated from the reference curve for beta-carotene in Skellysolve B for the purpose of comparing this curve with curves plotted by other workers. This factor was calculated by finding the ratio for each solution read by dividing the micrograms of beta-carotene per milliliter of the Skellysolve B solution by the respective colorimetric L 440 readings, expressed as 2-log G values. The sum of these ratios was divided by the number of solutions read and the resulting quotient of 2.62 was the factor. This factor applied only to the galvanometer readings above 50^3 because the curve for galvanometer readings below 50^3 is not a straight line since the factor increased progressively with the additional concentration of beta-carotene. The factor 2.62 does not agree with the factor 2.93 found by Berl and Peterson (4) for galvanometer readings above 46^3 on the curve they plotted. These investigators calibrated the instrument they used

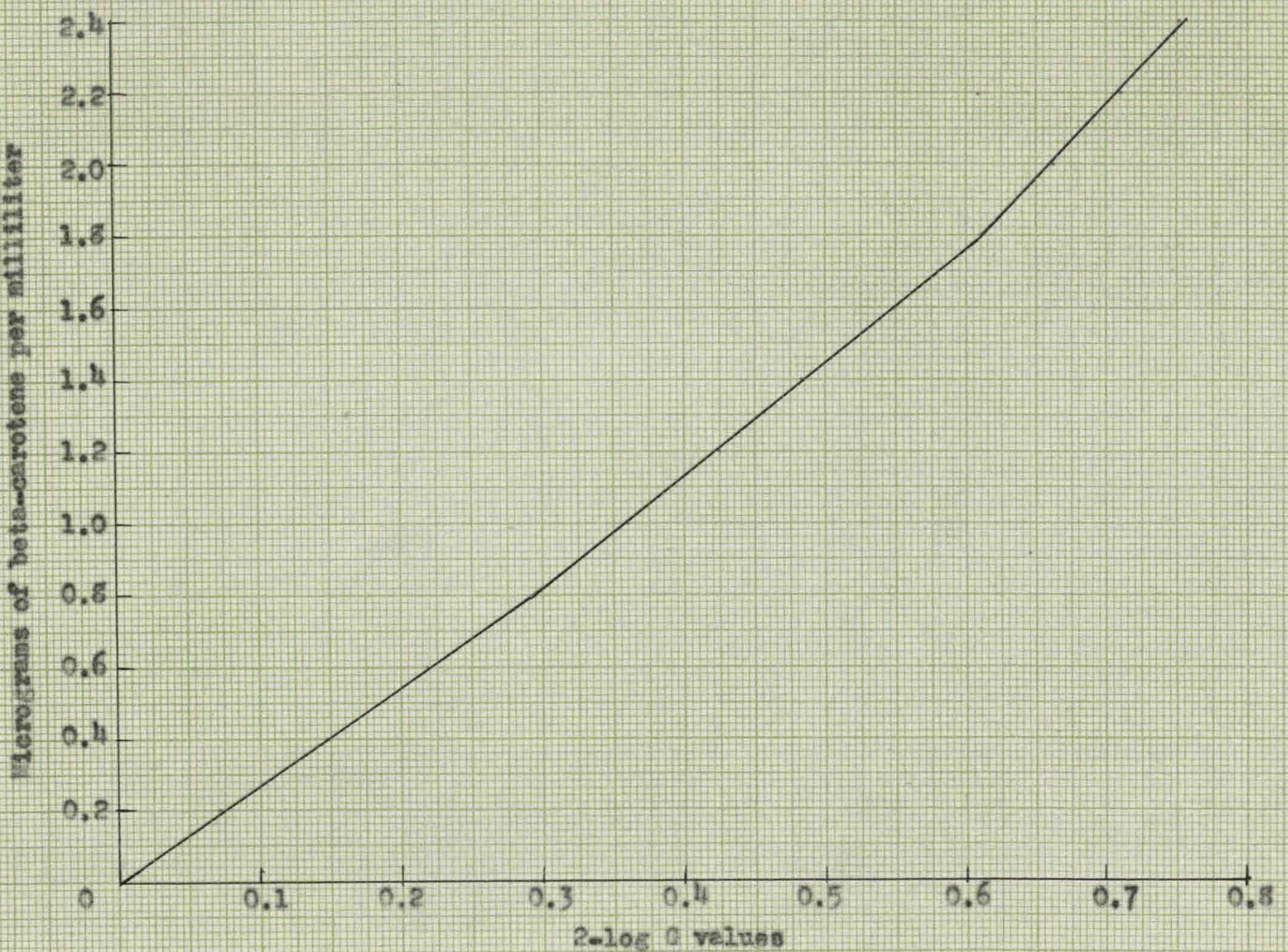

Figure 1. Reference curve for beta-carotene in Skellysolve B solution, constructed by plotting the colorimetric L 440 readings (expressed as 2-log G values on the abscissa) against the respective concentrations of beta-carotene in Skellysolve B solution (expressed as micrograms per milliliter on the ordinate)

with a beta-carotene material obtained from a different source, namely, the Distillation Products Inc., Rochester, N. Y. The difference between these two factors may possibly be due to a difference in the two beta-carotene materials used.

Figure 2 shows the reference curve for beta-carotene in chloroform solution, which was constructed in the same manner as the reference curve for beta-carotene in Skellysolve B solution. This curve was referred to in the calculation of the beta-carotene concentration in the vitamin A solutions.

The calibration of the photoelectric colorimeter for vitamin A was made by using crystalline vitamin A alcohol[3] in chloroform. A series of twelve solutions was prepared containing from 1 to 12 micrograms of vitamin A per milliliter of chloroform. A blank reading was made by mixing one milliliter of chloroform and 9 milliliters of antimony trichloride reagent to find the center setting on the galvanometer. The vitamin A readings were made with the 620 millimicron filter in the colorimeter.

After the instrument had been adjusted to the proper center setting a tube containing one milliliter of the prepared vitamin A solution was inserted into the colorimeter and 9 milliliters of antimony trichloride reagent was rapidly added from a pipette and the galvan-

3. Crystalline vitamin A alcohol, obtained from the Distillation Products Inc., Rochester, N. Y., Control K 118

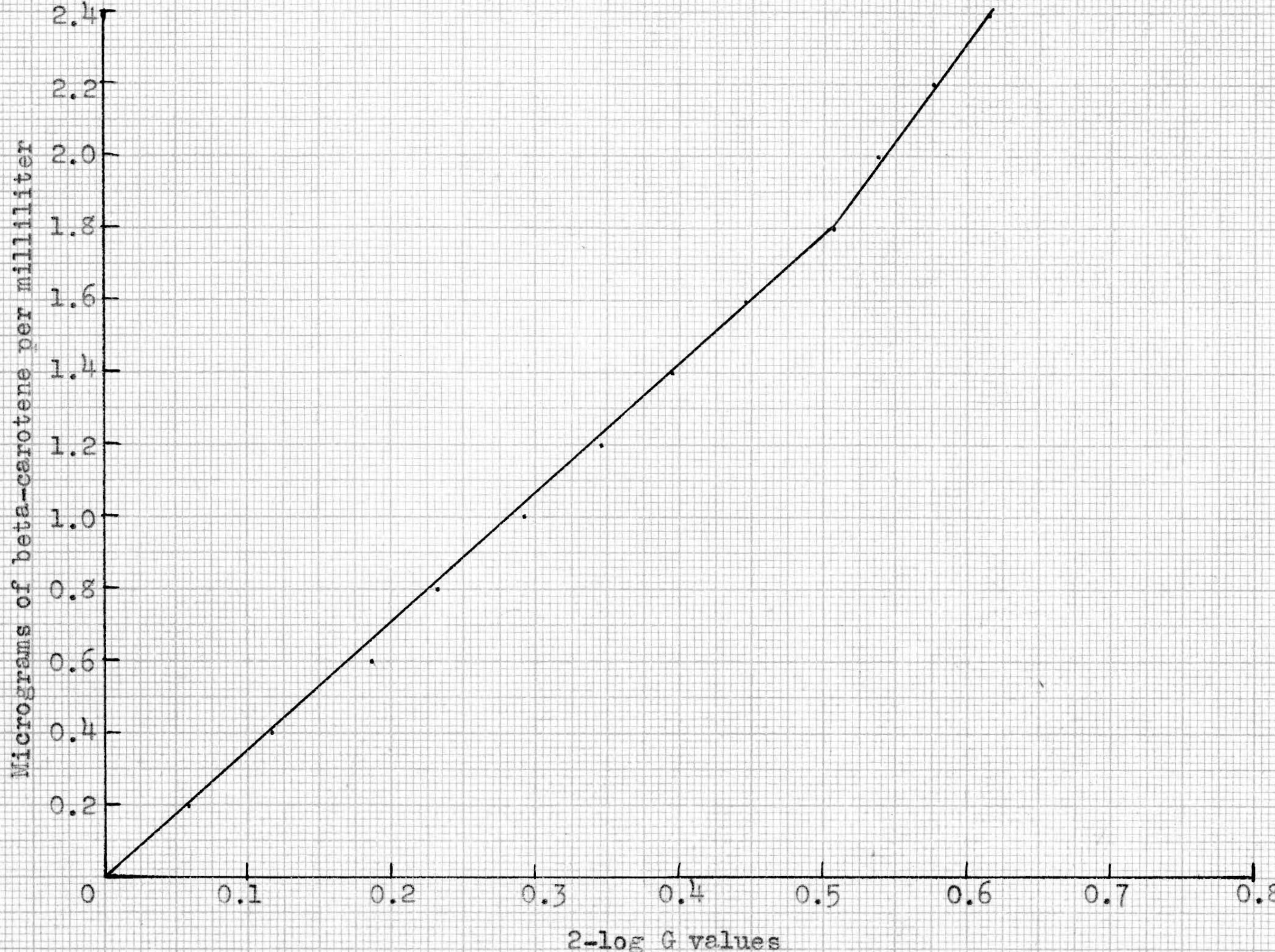

Figure 2. Reference curve for beta-carotene in chloroform solution, constructed by plotting the colorimetric L 440 readings (expressed as 2-log G values on the abscissa) against the respective concentrations of beta-carotene in chloroform solution (expressed as micrograms per milliliter on the ordinate)

ometer read at the point of temporary stability. This procedure was repeated for each of the twelve solutions. The reference curve for vitamin A was constructed by plotting the colorimetric L 620 readings, expressed as 2-log G values on the abscissa, against the respective concentrations of vitamin A alcohol in chloroform solution, expressed as micrograms per milliliter on the ordinate. (Figure 3)

A factor was calculated from this curve for the purpose of comparing it with the curves plotted by other workers. This factor was calculated by finding the ratio for each of the 12 solutions, by dividing the micrograms of vitamin A alcohol per milliliter of chloroform solution by the respective galvanometer L 620 readings, expressed as 2-log G values. The sum of these ratios was divided by the 12 readings, and the resulting quotient of 1.38 ± 0.04 was the factor. The factor 1.38 ± 0.04 compares favorably with the factor 1.39 ± 0.06 found by Baxter and Robeson (3) of the Distillation Products, Inc. laboratories. The factor 1.38 ± 0.04 did not compare so favorably with the factor 1.33 ± 0.06 found by Berl and Peterson (4), who used a crystalline vitamin A alcohol material obtained from the Distillation Products, Inc.

The following procedure for the determination of beta-carotene and vitamin A was carried out at room temperature and in subdued light.

A ten gram ($\pm$ 0.01 gram) sample of butter of known fat content was put into a 250 milliliter saponification flask equipped with a glass jointed reflux condenser and a mixture of five milliliters of saturated potassium hydroxide and twenty milliliters of absolute methanol was added. The butterfat in the mixture was saponified by heating in a

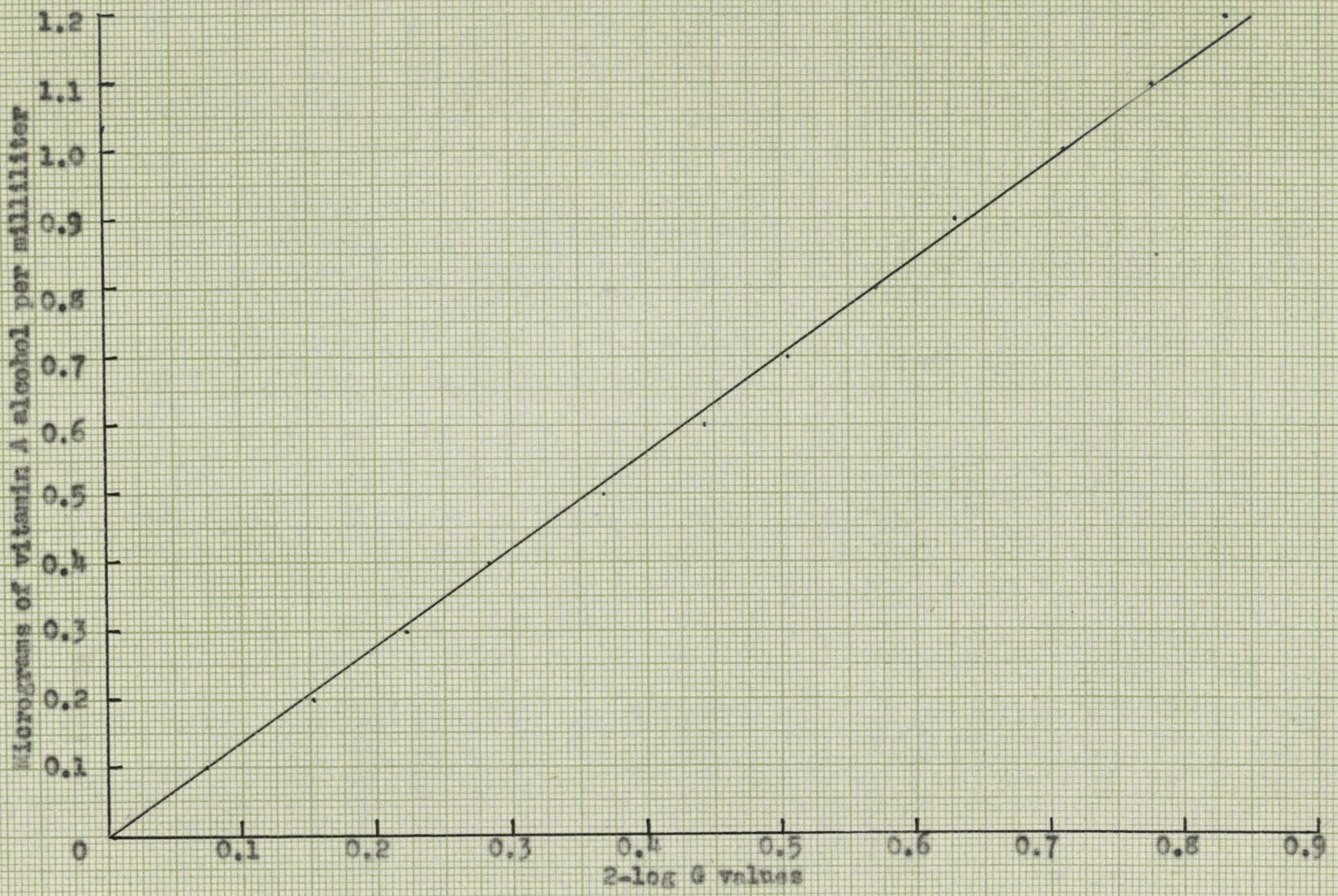

Figure 3. Reference curve for vitamin A, constructed by plotting the colorimetric L 620 readings (expressed as 2-log G values on the abscissa) against the respective concentrations of vitamin A alcohol in chloroform solution (expressed as micrograms per milliliter on the ordinate)

boiling water bath for ten minutes. The saponified material was diluted with 40 milliliters of distilled water and cooled to room temperature. This material was extracted three times with ether in a separatory funnel. The three ether extracts were combined and washed with distilled water until the washings were free from alkali. The washed ether extract was dried over anhydrous sodium sulfate and made up to a volume of 200 milliliters with ether. This solution was thoroughly mixed and divided into two equal parts.

One hundred milliliters of the ether solution was put into an evaporation flask and the ether removed under reduced pressure. The remaining residue was then dissolved with Skellysolve B and transferred to a separatory funnel. The carotene-Skellysolve B solution was extracted with 25 milliliter portions of 92 percent methanol until the methanol extractions were colorless. The carotene-Skellysolve B solution was then washed four times with distilled water and dried over anhydrous sodium sulfate. This solution was made up to 50 milliliters in volume and a ten milliliter portion was read, as described in the beta-carotene calibration procedure.

The other 100 milliliters of the ether extract was evaporated in the same manner as for the beta-carotene determination, except just as the evaporation was completed, the flask was immersed in a 70°C. water bath for 30 seconds to remove traces of moisture. The remaining residue was dissolved with a small solume of chloroform and the solution made up to ten milliliters in volume. One milliliter of the vitamin A solution was mixed with nine milliliters of a 22 to 25 percent antimony trichloride reagent and readings made as described in the vitamin A calibration procedure.

To calculate the micrograms of beta-carotene per gram of butter-fat from the galvanometer reading, it was necessary to correct the galvanometer reading for deviation from the standard. The corrected L 440 galvanometer reading was expressed as 2-log G value and this value read from the reference curve for beta-carotene in Skellysolve B (Figure 1). This gave the amount of beta-carotene in one milliliter of the butter sample solution. This value was then multiplied by ten to give the amount of beta-carotene present in one gram of butter, since ten milliliters of the solution were equal to one gram of butter. This value was divided by the percent of fat in the butter sample, which gave the micrograms of beta-carotene per gram of butterfat.

The micrograms of vitamin A per gram of butterfat were calculated from the corrected galvanometer reading expressed as 2-log G value and this read from the reference curve for vitamin A (Figure 3). The value found on the ordinate of the curve corresponding to the galvanometer reading gave the concentration of vitamin A per milliliter of the butter sample solution in micrograms. The value thus obtained included the vitamin A and the beta-carotene that was present in the vitamin A solution. To get the true vitamin A value, the original vitamin A reading was corrected for the beta-carotene content in the vitamin A solution.

This was done by reading a mixture of one milliliter of the vitamin