THE RIBOFLAVIN CONTENT OF LIQUID AND POWDERED SKIM MILK

by

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INTRODUCTION

Milk is known as a valuable source of riboflavin, probably the most valuable in the human diet. Studies have been made of the riboflavin content of liquid whole milk but comparatively few values are available for either liquid or dried skim milk. As the use of skim milk has become increasingly prevalent, especially in low cost diets, and as dried milk is being more widely used in bakeries and food service institutions, it is desirable to secure additional figures for the riboflavin content of this food, both in the liquid and dried forms.

REVIEW OF LITERATURE

Riboflavin was identified as a part of the Vitamin G (B_2) complex in 1933. The substances which before that time had been variously designated as ovoflavin, lactoflavin, or various other flavins, were found to be identical and the name riboflavin was accepted. This term is appropriate in that it indicates the chemical composition of riboflavin, which is composed of a sugar called ribose and a flavin, a yellow pigment containing nitrogen. Recently Street and Reeves (1940) have presented convincing evidence that the yellow fluorescent pigment formed by the tubercule bacillus is identical with riboflavin.

Riboflavin is water soluble and is insoluble in ordinary fat solvents. It is stable to heat, to most oxidizing agents

and to strong mineral acids, but is sensitive to alkali. It is also sensitive to light and undergoes an irreversible decomposition on irradiation with ultra violet or visible light.

Riboflavin is essential to the growth and development of the growing body and to an optimal nutritional state at any age. This is true of both animals and human beings. Digestive disturbances, nervousness, skin disorders, and abnormal eye conditions result from a diet low in riboflavin. A high level of riboflavin in the diet tends to produce a more buoyant state of health, greater freedom from disease, especially infectious diseases, and a longer period of vitality before the onset of old age.

It is widely distributed in animal and vegetable tissues but generally in small amounts. Milk, liver, yeast, and green leafy vegetables have been found to be good sources of this vitamin. Booher (1938) stated that in milk at least 90 per cent of the riboflavin is in a free dialysable form, which facilitates chemical measurement, but in most other materials, it occurs in combinations of high molecular weight.

Until recently the Bourquin-Sherman unit was used to express amounts of this vitamin present in foods and other substances. This unit of measurement was originally defined as that amount of the test food which when fed to a standard test animal previously depleted according to the prescribed technique, would produce an average gain of three grams per week over an eight-week period. However, results varied widely with different workers. Undoubtedly the purity of the riboflavin

used as a standard and the differences in growth rates found among animal colonies have been responsible for much of the variation in the Bourquin-Sherman values of foods studied. It is possible that the differences in the amount of riboflavin required, depending on the size of the test animal used, have also influenced the values.

Since the pure form of this vitamin has been available to use as a standard, riboflavin has been measured in terms of weight, usually micrograms, and the use of Bourquin-Sherman units has been discontinued. Bessey (1938) reported that one Bourquin-Sherman unit was equal to two 2.5 micrograms of riboflavin. Kramer et al. (1939) found that one Bourquin-Sherman unit was equal to about 3.6 micrograms of the riboflavin (Lactoflavin PX grade) used by them, and Kunerth and Riddell (1938) used the same value. Booher (1938), in reviewing various figures, stated that Kuhn estimated one Bourquin-Sherman unit to be equal to seven micrograms of riboflavin, and that Von Euler found the value of one Bourquin-Sherman unit to be two micrograms of riboflavin. Booher, herself, stated that in her opinion three micrograms of riboflavin was equal to one Bourquin-Sherman unit and that the discrepancies in the data available were probably due to the completeness of prevention of coprophagy and the use of different diets in experimental work. Sherman (1941) stated that what was formerly called a "Bourquin-Sherman unit of Vitamin G" is equal to about 2.5 micrograms of riboflavin.

The riboflavin content of milk has been a source of interest for some time. Sherman (1941) reported the riboflavin content

of fresh whole milk as 195-240 micrograms per 100 grams. Kunerth and Riddell (1938) have estimated fresh whole milk to contain 1,950 micrograms of riboflavin per quart. Lunde, Kringstad, and Olsen (1939) found 2.4 micrograms per gram of milk, and Kunerth et al. (1937) reported 2.0-2.7 micrograms per gram. Milk samples from individual cows were analyzed by Kramer, Gardner and others (1938) and were found to have from 1.7-2.4 micrograms per gram, and a pooled sample of herd milk, 2.0 micrograms of riboflavin per gram. Kramer et al. (1939) reported the average riboflavin value for all milks tested by them to be 2.1 micrograms per gram with a range of 1.6--2.8. Javillier (1940) stated that cow's milk contained 800-3,000 micrograms of riboflavin per liter. Hand and Sharp (1939) reported the riboflavin content of 400 samples of milk estimated flucrometrically as .60-3.42 mg. per liter of milk. Cox (1939), in estimating the riboflavin content of various livers, found 900-2,255 Bourquin-Sherman units per 100 grams, and stated that the mean of the test livers was 10-20 times as high in riboflavin value as the milk with which it was compared.

Levine and Remington (1937), using Sherman's early value (1932) of 0.40-0.75 units of riboflavin per gram of milk, calculated dry whole milk to contain 3.3-6.3 units per gram. Bourquin (1929) reported that dried milk powder was twice as rich in Vitamin G as whole wheat and only one-fourth as rich as autoclaved baker's yeast. Guha (1931), in investigating Vitamin B_2 of "glaxo" milk, stated that the powder was active only in a dose as large as 0.4 grams. Heiman and others (1937),

working on the relative amounts of flavin in dried skim milk and dried whey found that the whey was 10-20 per cent richer in flavin than the dried skim milk. Jukes (1937), as a result of a biological assay with chicks, found that dried skim milk contained ten units of lactoflavin per gram, with one unit representing six micrograms of lactoflavin. However, he stated that the purity of the lactoflavin used was not known, and five micrograms daily produced a slightly better growth response in rats than is required for one Bourquin-Sherman unit. On that basis, the values reported would be expected to be high. Levine and Remington (1937) reported 5.28 Bourquin-Sherman units per gram of dried whole milk. Jukes and Richardson (1938) found values of from 7-9 units of riboflavin per gram of dried whole milk and of from 8-14 units per gram of dried skim milk, with one unit here representing 2.3 micrograms of riboflavin. Hodson and Norris (1939), by flourometric determinations, found 17.6-22.2 micrograms of riboflavin per gram of dried skim milk.

In addition to the biological method, which has been used most extensively, chemical methods have been utilized for riboflavin determinations. Both colorimetric and fluorometric procedures have been used although Kemmerer (1940), in his report on riboflavin, stated that most collaborators agree that fluorometric rather than colorimetric methods are likely to give better results. Colorometric methods are accurate but they can be used only if colored materials which often accompany riboflavin are absent, as they interfere with the determination. Fluorescence measurements are more specific for it is thought

that no other substance present in the frequently used acetone extract of the natural products gives a green fluorescence (Hand, 1939). Light filters may be used to screen out a blue or violet fluorescence which is sometimes observed. In addition, riboflavin occurs in milk in an amount convenient for measurement of fluorescence, but too small for ordinary direct colorimetric determinations. Concentrations as small as 0.1-4 mg. per liter may be detected by the fluorometric method while the range for the colorimetric method is from 4-40 mg. per liter.

Emmerie (1937) has developed a colorimetric method for riboflavin determination and has modified it so that it can be used for milk. Sullivan and Norris (1939) measured the light absorption by the use of filters and a photoelectric cell, and a similar method was used by Hodson and Norris (1939). Supplee, Ansbacher, Flannigan, and Hanford (1936) and Whitnah, Kunerth and Kramer (1937) compared visually the fluorescence of unknown and standard riboflavin solutions. Hand and Sharp (1939) estimated the riboflavin content of milk by measuring with a photoelectric cell the intensity of the fluorescence of an acetone filtrate, and Hand (1939) determined riboflavin content by comparing the fluorescence of an acetone filtrate with that of a cube of uranium glass which had previously been calibrated against solutions containing known amounts of riboflavin.

METHOD OF PROCEDURE

Biological

A modification of the Bourquin-Sherman biological method, which has been shown by Booher and Blodgett (1933), Booher, Blodgett and Page (1934), Bisbey and Sherman (1935), and Bessey (1938) to be reliable for the quantitative estimation of the riboflavin content of foods, was used. This method was described recently by Van Duyne (1940).

Albino rats from Wistar Institute stock were used as subjects for this experiment. The stock colony was fed the following basal diet suggested by Sherman and Crocker (1922):

Dried whole milk	1 part
Ground whole wheat	2 parts
Sodium chloride	2% of weight of wheat

Cod liver oil was added to the diet in the ratio of 30 grams of cod liver oil to 1000 grams of wheat. Also, lettuce was fed two or three times per week and the rat mothers were given ground meat twice a week for three weeks before and one week after gestation.

This diet and water were supplied ad libitum until the young rats were approximately 28-29 days old and weighed 45-55 grams each. At this time groups of 5-6 rats were placed in cages which had raised wire floors, and were fed the riboflavin-deficient diet used by Van Duyne (1940), which consisted of:

Casein (Labco vitamin-free)	18%
Osborne and Mendel salt mixture	4%

Butterfat	8%
Cod liver oil	2%
Labco rice polish concentrate	2%
Cornstarch	66%

The riboflavin-free diet and water were supplied ad libitum until the rats were depleted of their body store of riboflavin as shown by a cessation of weight gain. At the end of the first week they were harnessed to prevent coprophagy, and put into individual cages with raised screen floors. The harnesses were similar to those described by Page (1932) but were made from directions supplied by Munsell.[1] The animals were weighed weekly for two weeks and then daily until depleted.

When the weight of the rats remained stationary for three days or when there was a decrease in weight, the animals were considered ready for experiment. At this time they were divided into eight groups.

Groups 1 and 3 were given 20 ml. per week of liquid skim milk in addition to the basal diet. This was measured with a pipette and was fed in three feedings during the week. The liquid milk samples were frozen and stored until the time of their use, as Grayson (1935) has shown that no loss of Vitamin G occurs if milk remains frozen during storage.

Groups 2 and 4 received weighed samples of dry skim milk equivalent to the weight of the samples of liquid milk used. A determination of the total solids in each liquid milk sample was made and the amounts for the samples of dried milk calculated accordingly. The weight of dry milk corresponding to

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[1]Personal communication

20 ml. of liquid milk was 1.9 grams of Sample 2 and 1.2 grams of Sample 4. These amounts were weighed on an analytical balance and stored in a refrigerator until the time of use.

The milk[2] to be tested was obtained from Kansas creameries. The liquid samples were taken from the storage tanks of the cold unpasteurized milk which was to be dried, and corresponding samples of the same lot of milk were obtained after drying. The spray process was used in the drying procedure.

In drying Sample No. 2 (dried milk corresponding to liquid milk No. 1) the milk was heated to 180° F. and held for 30-45 minutes. The spray room temperature was 185° F. The Gray Jensen process of drying was used for sample No. 4. The milk was subjected to temperatures of 180-200° F. for 30 minutes.

Groups 2, 3, and 4, which served as the positive controls, received in addition to the diet daily supplements of 2.5, 5, and 7.5 micrograms of synthetic riboflavin[3] respectively. This was dissolved in distilled water in a concentration suitable for feeding ½-1 ml. of the liquid daily. It was fed to the rats by having them lick the drops from the tip of a syringe graduated to one one-hundredth of a ml.

Group 8, which served as a negative control, received only the basal riboflavin-free diet.

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[2]Samples No. 1 and 2 supplied by the Bennett Creamery Company, Ottawa, Kansas. Samples No. 3 and 4 supplied by the Hiawatha Dairy Products Company, Hiawatha, Kansas.

[3]Secured from Merck and Company, Inc., Rahway, New Jersey.

Records of the amount of food consumed were made for all groups and water was supplied ad libitum. The test period was four weeks in length and weighings were made weekly. Ten or more rats were used to test each milk sample. In as far as possible one rat from each litter was placed on the dried milk supplement, one on the liquid milk supplement, one on each of the three levels of riboflavin supplements, and one served as a negative control. The number of males and females in each group was as nearly equal as possible.

A curve of response to the supplements of known amounts of riboflavin was made, using the average weight gains of the animals over the four-week period. The amount of riboflavin in each milk sample was then measured by using this curve as a curve of reference and locating on the graph the amount of riboflavin corresponding to the average gain produced by that sample.

Chemical

Evidence that fluorometric methods of riboflavin determinations are applicable to materials such as milk has been presented by Supplee, Ansbacher, Flannigan, and Hanford (1936). In this study the rapid method described by Whitnah, Kunerth and Kramer (1937) was used.

A trichloroacetic acid extract of the milk was prepared by adding 15 ml. of ten per cent trichloroacetic acid to 10 ml. of milk, letting it stand 30-60 minutes, and centrifuging five minutes at about 2000 r. c. f. The solution was then neutralized

with sodium hydroxide, using methyl orange as an indicator, and diluted until its fluorescence could be compared with standard riboflavin solutions of known concentrations. These standard solutions were made from the same riboflavin solution as that used for the supplemental feedings in the biological assay. They were made to contain 0.06-0.12 micrograms of riboflavin per ml. as the fluorescence of concentrations weaker than 0.06 may be detected but is difficult to estimate with any degree of accuracy, and concentrations stronger than 0.12 micrograms of riboflavin are easily underestimated in value. The readings were made in a dark room in the light of an Eveready Fluoray lamp and the concentration of riboflavin in the milk sample was calculated on the basis of the dilution made.

RESULTS AND DISCUSSION

The choice of a basal diet was important in this assay as it was imperative that it be free of riboflavin and yet contain adequate amounts of the rest of the B complex vitamines. "Labco" casein was used in accordance with the findings of Supplee, Flannigan, Hanford and Ansbacher (1936) that lactoflavin is a contaminant of many commercial purified or vitamin-free caseins, but that Labco, by its extensive preparation, is rendered vitamin-free. In the diet used the parts of the B complex, other than riboflavin, were furnished by a rice polish concentrate instead of the whole wheat extract of the original Bourquin-Sherman diet, as Clarke et al. (1940) found that rice polishings concentrate is a more satisfactory source of the other members of the B vitamins than the extract of wheat. El Sadr, Macrae, and Work (1940) have criticized experimental diets using either whole wheat extract or rice polish concentrate as a source of the B complex, believing that adequate amounts of all B_2 vitamins, other than riboflavin, are not supplied. Van Duyne (1940), however, obtained an excellent correlation between graded amounts of riboflavin fed and the subsequent growth response of rats on a diet in which the B complex, other than riboflavin, was furnished by rice polishings. In the present experiment her diet was used.

A survey of the data from the biological assay is given in Table 1.

Table 1. Growth responses of rats fed the riboflavin-deficient diet with and without supplements.

Supplement	Sample number	Amount fed per day	Number of animals	Average weekly gains (gm.) 1	2	3	4	Average total gain (gm.)
Liquid skim milk	1	3.3 ml.	12	8	7	5	6	26
Dry skim milk	2	0.3 gms.	13	9	6	6	7	28
Liquid skim milk	3	3.3 ml.	11	7	8	9	5	29
Dry skim milk	4	0.2 gms.	11	5	6	8	8	27
Riboflavin	5	2.5 micrograms	15	5	4	4	4	17
Riboflavin	6	5.0 micrograms	15	5	6	9	8	28
Riboflavin	7	7.5 micrograms	15	5	11	11	11	38
None	---	---	23	1.0	1.2	-0.3	0.0	1.9

At least 11, and in most cases 12-15 rats were used to test any one supplement. There were 23 rats in the group which served as a negative control. At the end of the four-week test period the negative control animals had just about maintained original weight, the average gain being 1.9 grams. In addition to stunted growth, they showed some other evidences of riboflavin deficiency. Their fur was short and wooly and lacked the long guard hairs and the luster present in that of the normal rat. Lesions about the nostrils and on the wrists were almost universally present and occasionally mouth lesions as well. In some cases the eyeballs were sunken and a scaliness occurred around the eyes. Some of these effects may have been produced by mechanical injury from cages or harnesses, but as the conditions cleared up when the animals were transferred to a good

diet following the experimental period, they must have been due largely to the riboflavin deficiency of the diet. Bessey and Wolbach (1939) have also reported sunken eyeballs in the rats which they maintained on a diet deficient in riboflavin.

The growth responses of the rats that had received riboflavin were in accord with the amounts of the supplement given, although the weight gains were not strictly proportional. In rat growth methods the delicacy of the assay depends upon the fact that a small amount of the supplement fed will produce a relatively large growth response. Since Van Duyne had found that from 2-8 micrograms of riboflavin fed six times per week had resulted in greater increments in growth than lower or higher levels, the riboflavin supplements in this experiment were fed at daily* levels of 2.5, 5, and 7.5 micrograms. With the exception of the highest level, these same amounts had served satisfactorily to produce a reference curve in experimental work of Kramer et al. (1939), Ansbacher et al. (1936), and Cox (1939).

In this experiment the average gain for the four-week period of those animals on the 2.5 microgram level was 17 grams. Those receiving 5 micrograms daily during the test period gained an average of 28 grams, and those on the 7.5 microgram level showed an average gain of 38 grams. A curve of response to these graded amounts of riboflavin was made by plotting the average weight gains of these three groups of animals. (Fig. 1)

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*"Daily", as used in this experiment, refers to six times per week in accord with the Bourquin-Sherman and Van Duyne methods.

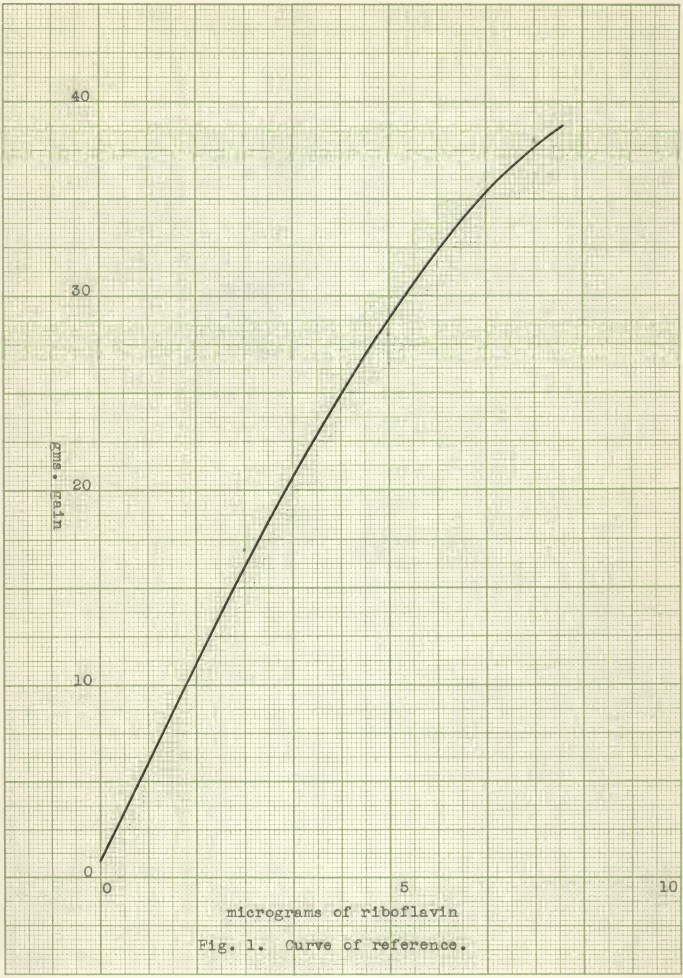

Fig. 1. Curve of reference.

The growth response of these rats receiving the graded amounts of riboflavin formed a typical curve. However, the actual weight gains of the animals in response to comparable riboflavin levels are larger than those reported by Van Duyne (1940). On the other hand, they are not as large as those reported by El Sadr (1940). The larger response may have been due in part to the different basal diet which El Sadr used, and also to the fact that he fed riboflavin supplements seven instead of six days per week.

The appearance of the animals receiving riboflavin also corresponded to the level at which the supplement was fed. Many of those animals of the 2.5 microgram level showed some signs of riboflavin deficiency such as wrist lesions and short wooly fur. Those receiving 5 micrograms of riboflavin daily showed fewer and less pronounced actual signs of deficiency, but grew at a subnormal rate. The appearance of the rats that received 7.5 micrograms of riboflavin daily approached that of the normal rat. Their fur was lustrous and contained many long guard hairs; their eyes were bright; there was no dermatitis evident, and they grew to a large size.

The appearance of the animals that received milk supplements approximated that of those receiving 7.5 micrograms of riboflavin daily. In no case were there evidences of dermatitis. The eyes were bright and a deep pink, and the fur of these animals, with few exceptions, was long and lustrous. These rats showed average gains over the four-week period within the range of growth produced by the riboflavin supplements. Liquid skim milk sample

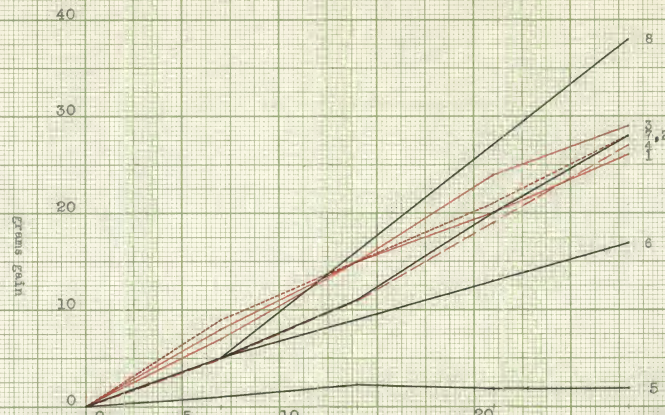

Fig. 2. Growth curves of animals receiving riboflavin and skim milk.

No. 1 produced an average gain of 26 gm. in the animals that received it. The corresponding milk after drying (dry skim milk sample No. 2) produced an average gain of 28 gm. The rats receiving liquid skim milk sample No. 3 gained an average of 29 gm. during the test period and those receiving the corresponding dried milk (dry milk sample No. 4) showed an average gain of 27 gms.

All groups made good weight gains from week to week. (Fig. 2).

Using the curve of response as a curve of reference (Fig. 1) the riboflavin content of each milk sample was calculated, as shown in Table 2.

Table 2. Riboflavin content of milk obtained by biological assay.

Supplement	Sample number	Average gain (4 weeks)	Estimated riboflavin per gram of sample
Liquid skim milk	1	26	1.27
Dry skim milk	2	28	15.79
Liquid skim milk	3	29	1.47
Dry skim milk	4	27	22.90

These values of the riboflavin content of milk agree well with those found by other workers, no value being outside of the reported range.

Figures for the riboflavin content of liquid skim milk are not available. Munsell (1940), in summarizing all data, has reported skim milk to be an "excellent" source of riboflavin, but has given no definite riboflavin values.

Riboflavin content of milk has been reported in the literature in various ways. For purposes of comparison it is desirable to express all values in a common unit. This has been done by estimating from the author's data the value of the riboflavin content in terms of micrograms per gram [4] (Table 3).

Table 3. Riboflavin content of liquid whole milk.

Investigator	Micrograms per gram
Javillier (23)	0.76--2.9
Sherman (32)	1.0 --1.9
Kunerth et al. (30)	2.0 --2.7
Kramer et al. (28)	1.7 --2.4
Kramer et al. (27)	1.6 --2.8
Kunerth and Riddell (31)	2.0
Lunde, Kringstad, and Olsen (33)	2.4

The results of the present assay for riboflavin in liquid skim milk seem entirely in line with former data, as the values of 1.27 and 1.47 micrograms of riboflavin per gram of milk fall within the range of reported figures. As riboflavin is water soluble it would be expected that liquid skim milk would contain slightly more of this vitamin than liquid whole milk. However, this difference would be small and could be lost in the wide range reported for whole milk.

The riboflavin values of the dried skim milk as determined from the biological assay are also in accord with previously reported values. Jukes (1937) found 60 micrograms of riboflavin

[4] For converting Bourquin-Sherman units to micrograms the relationship suggested by Sherman was used--1 B. S. unit = 2.5 micrograms. For converting units of volume to units of weight, the average specific gravity of milk, 1.032, was used.

per gram of dried skim milk but it is apparent from his report that this value was about twice as high as would be expected due to the degree of purity of the lactoflavin used. Later in an experiment with Richardson (1938) he reported 18.4-32.2 micrograms per gram. The values obtained in the present experiment of 15.79 and 22.90 are within their range.

A comparison of the riboflavin content of the two samples of liquid milk and the corresponding dried milk shows that there was no evidence of destruction of riboflavin during the drying process. Little information is available as to loss of riboflavin during drying. Henry et al. (1939) stated that, as determined by biological assay, Vitamin G was not destroyed during the drying of milk, but suffered some loss during an evaporating process. In the same study, riboflavin was measured fluorometrically by two methods and the results indicated a higher riboflavin value in the raw than in the dried or in the evaporated milks. However, there is no evidence that this was due to heat treatment because the milk sources were not identical.

Davis and Norris (1934) studied the effect of the manufacturing process (Merrell-Soule spray, Gray-Jensen spray, and open-roller) upon the Vitamin G content of dried skim milk and reported that there was no measurable destruction of this vitamin during drying. The liquid and dried milk samples used by them were from the same tanks of milk to insure the same original vitamin potency. In another series of experiments they found no destruction of Vitamin G in skim milk prepared by the spray or roller methods even when the milk was scorched, neutralized,

or made alkaline and held at high temperatures.

The general opinion seems to be that heat is not responsible for a loss of riboflavin in milk. Krauss (1933), in comparing raw and pasteurized milks, stated that the heat treatment did not destroy any Vitamin G originally present in raw milk, and added, "This is to be expected because it is known that Vitamin G resists autoclaving at hydrogen-ion concentrations within the range in which the pH of milk usually falls." Javillier (1940) stated that stabilization of milk by homogenizing, preheating, sterilizing, etc., has no appreciable effect on the activity of Vitamin B_2. Houston, Kon and Thompson (1940) found that riboflavin is not adversely affected by pasteurization and that it is sufficiently heat stable to withstand the drastic treatment of sterilization of milk. It would be expected, then, that loss of riboflavin would not occur during drying and the results of the present experiment bear out these studies.

Results of the chemical assay of riboflavin are shown in Table 4.

Table 4. Riboflavin content of skim milk obtained by fluorometric assay.

Supplement	Sample number	Estimated riboflavin per gram of sample micrograms
Liquid skim milk	1	1.25
Dry skim milk	2	17.98
Liquid skim milk	3	1.32
Dry skim milk	4	21.04

Hand and Sharp (1939) have reported values estimated fluorometrically* of 0.58-3.3 micrograms of riboflavin per gram of liquid whole milk. Whitnah et al. (1937) found 1.3* micrograms per gram of whole milk. The riboflavin value of the liquid skim milk estimated by fluorometric measurements in the present study was 1.25 micrograms of riboflavin per gram for Sample No. 1 and 1.32 for Sample No. 3. The riboflavin content of the dried milk samples obtained from the chemical assay were 17.98 and 21.04 micrograms per gram of dried skim milk. These are in accord with the values estimated fluorometrically by Hodson and Norris (1939) who reported 17.6-22.2 micrograms of riboflavin per gram of dried skim milk.

A comparison of the riboflavin content of milk determined by the biological and chemical methods is shown in Table 5.

Table 5. Comparison of riboflavin values obtained from biological and chemical assays.

Supplement	Sample number	Estimated riboflavin in micrograms per gram Biological	Chemical	Difference in value between methods
Liquid skim milk	1	1.27	1.25	1.5
Dry skim milk	2	15.79	17.98	13.0
Liquid skim milk	3	1.47	1.32	10.7
Dry skim milk	4	22.90	21.04	8.5

Agreement between the two methods of determination was good. In general the values obtained by the fluorometric method were lower than those resulting from the biological assay. The maximum difference between the riboflavin values as calculated

*Calculated from author's data.

by the two methods was 13 per centwith an average difference of 8.4 per cent. Using the same chemical method of assay and comparing with the Bourquin-Sherman method of biological assay, Kramer et al. (1939) reported a maximum difference of 25 per cent with an average difference of ten per cent. They, too, found that the fluorometric method gave consistently lower readings.

Henry, Houston and Kon (1940) compared results of El Sadr's biological method of assay with those of Emmerie's fluorometric method and reported good agreement when the milks were fed at levels supplying up to ten micrograms daily. For a higher level the agreement was not so satisfactory. Hodson and Norris (1939) compared results of their fluorometric method of assay with a microbiological method and a photometric method and found good agreement among the three.

It is evident from the results that both the liquid and dried skim milk used in this study were good sources of riboflavin. According to tentative standards released in April, 1941, by the Committee on Foods and Nutrition of the National Research Council, the daily requirement of riboflavin in the diet is 3 mg. for an average man and 2.8 mg. for an average woman. Thus, one quart of skim milk will furnish approximately one-half of the daily requirement of riboflavin.

SUMMARY AND CONCLUSIONS

The riboflavin content of two samples of liquid skim milk and the corresponding two samples of dried skim milk (spray

process was determined by both biological and chemical assays. The Van Duyne modification of the original Bourquin-Sherman method was used for the in vivo method and the rapid method of Whitnah et al. was employed for the fluorometric determinations.

Results of the two methods were in agreement with a maximum difference of 13 per cent and an average difference of 8.4 per cent. Biological values for the riboflavin content of liquid skim milk were 1.27 and 1.47, and for dried skim milk were 15.79 and 22.90 micrograms per gram. On this basis one quart of skim milk would supply approximately 44 per cent of the daily riboflavin requirement. No destruction of riboflavin in the liquid milk occurred in these samples during drying.

The results of this study indicate that:

1. Both liquid and dried skim milk are good sources of riboflavin.

2. There is no destruction of riboflavin in milk during drying (spray process).

3. Both the biological and the fluorometric methods used in this study are satisfactory for measuring the riboflavin content of either liquid or dry skim milk.

4. One quart of either liquid skim milk or reconstituted dried skim milk supplies about 44 per cent or approximately one-half of the 3 mg. which has been recommended as the daily requirement of riboflavin.

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LITERATURE CITED

(1) Ansbacher, S., Supplee, G. C. and Bender, R. C.
Lactoflavin, a necessary growth-promoting factor.
Jour. Nutr. 11:401-409. 1936.

(2) Bessey, Otto A.
Vitamin G and synthetic riboflavin. Jour. Nutr.
15:11-15. 1938.

(3) Bessey, O. A., and Wolbach, S. B.
Vascularization of the cornea of the rat in riboflavin
deficiency, with a note on corneal vascularization in
Vitamin A deficiency. Jour. Expt. Med. 69:1-12. 1939.

(4) Bisbey, Bertha, and Sherman, H. C.
Experiments upon the extraction and stabilities of
vitamin B (B_1) and of lactoflavin. Jour. Biol. Chem.
112:415-420. 1935.

(5) Booher, Lela E.
Chemical aspects of riboflavin. Amer. Med. Assoc.
Jour. 110:1105-1111. 1938.

(6) Booher, L. E., and Blodgett, H. M.
The concentration and probable chemical nature of
vitamin G. Jour. Biol. Chem. 102:39-46. 1933.

(7) Booher, L. E., Blodgett, H. M. and Page, J. W.
Investigations of the growth-promoting properties
of vitamin G concentrates. Jour. Biol. Chem.
107:599-605. 1934.

(8) Bourquin, Anne
Experiments on quantitative determination of vitamin
G. Dissertation. New York. Columbia Univ. 20 p.
1929.

(9) Clarke, M. F., Lechycka, M. and Cook, C. A.
Biological assay of riboflavin. Jour. Nutr. 20:
133-144. 1940.

(10) Cox, Hazel S.
Biological assay of riboflavin content of beef, calf,
lamb, mutton and pork liver. Unpublished thesis.
Kans. State Col. of Agr. and Appl. Sci. 25 p. 1937.

(11) Davis, H. J., and Norris, L. C.
The effect of process of manufacture upon the vitamin G content of dried skim milk. Cornell Sta. Rpt. 47:119. 1934.

(12) El Sadr, M. M., Macrae, I. F. and Work, Elizabeth.
The estimation of riboflavin. II. A new biological method. Biochem. Jour. 34:601-612, 1940.

(13) Emmerie, A.
The chemical determination of flavin (vitamin B_2) in urine, liver, and milk. Congr. intern. tech. chim. ind. agr. Compt. rend. Ve. Congr. 1:57. 1937.

(14) Grayson, R. V.
Freezing fresh milk. Is this method of preserving a solution to the surplus problem? Milk Dealer 24:30-32. 1935.

(15) Guha, B. C.
Investigations of vitamin B_2. Biochem. Jour. 25: 945-950. 1931.

(16) Hand, D. B.
Determination of riboflavin in milk by photoelectric fluorescence measurements. Indus. Engin. Chem. Analyt. Ed. 11:306-309. 1939.

(17) Hand, D. B., and Sharp, P. F.
The riboflavin content of cow's milk. Jour. Dairy Sci. 22:779-783. 1939.

(18) Heiman, Victor, and Carver, J. S.
Relative flavin (Vitamin G) content of dried skim milk, dried whey, and dried buttermilk. Poultry Sci. 16:434-437. 1937.

(19) Henry, K. M., Houston, J. and Kon, S. K.
The estimation of riboflavin in milk. II. Comparison of fluorometric and biological tests. Biochem. Jour. 34:601-612. 1940.

(20) Henry, K. M., Houston, J., Kon, S. K., and Osborne, L. W.
The effect of commercial drying and evaporation on the nutritive properties of milk. Jour. Dairy Res. 10:272-293. 1939.

(21) Hodson, A. Z., and Norris, L. C.
A flourometric method for determining the riboflavin content of foodstuffs. Jour. Biol. Chem. 131:621-630. 1939.

(22) Houston, J., Kon, S. K., and Thompson, S. Y.
The effect of commercial pasteurization and sterilization on the Vitamin B_2 and riboflavin content of milk as measured by chemical methods. Jour. Dairy Res. 11(1):67-70. 1940.

(23) Javillier, M.
Milk and the resupplying of man with vitamins. Compt. rend. acad. agr. France, 26:391-399. 1940.

(24) Jukes, Thomas H.
Biological assay of lactoflavin with chicks. Jour. Nutr. 14:223-233. 1937.

(25) Jukes, T. H., and Richardson, G. A.
Assays of riboflavin and the filtrate factor in certain milk products. Jour. Agr. Res. 57:603-610. 1938.

(26) Kemmerer, A. R.
Report on riboflavin. Assoc. Off. Agr. Chem. Jour. 23:346-351. 1940.

(27) Kramer, M. M., Cickman, R. M., Hildreth, M. D., Kunerth, B. L., and Riddell, W. H.
The riboflavin value of milk. Jour. Dairy. Sci. 22:753-759. 1939.

(28) Kramer, M. M., Gardner, Inez, Kunerth, B. L., and Riddell, W. H.
Vitamin G (riboflavin) content of colostrum and milk of cows determined biologically. Jour. Agr. Res. 56:232-238. 1938.

(29) Krauss, W. E., Erb, J. H., and Washburn, R. G.
Studies on the nutritive value of milk. Ohio Agr. Expt. Bul. 518:1-33. 1933.

(30) Kunerth, B. L., Kramer, M. M., Dickman, R. M., and Whitnah, C. H.
Biological assay of milk with lactoflavin as a standard for vitamin G. Kans. Acad. Sci. Trans. 40:297-299. 1937.

(31) Kunerth, B. L. and Riddell, W. H.
Milk as a source of riboflavin. Kans. Acad. Sci. Trans. 41:231-232. 1938.

(32) Levine, Harold, and Remington, Roe E.
Vitamin G content of some foods. Jour. Nutr. 13:525-542. 1937.

(33) Lunde, G., Kringstad, H., and Olsen, A.
Über die bestimmung des lactoflavins in naturprodukten. Hoppeseyleis Ztschr. 260:141-147. 1939.

(34) Munsell, Hazel.
Vitamins and their occurrence in foods. Milbank Memorial Fund Quarterly, 18:311-344. 1940.

(35) Page, J. W.
An improvement in experimental method for investigation of vitamin G. Soc. Expt. Biol. and Med. Proc. 30: 87-88. 1932.

(36) Sherman, H. C.
Chemistry of food and nutrition. New York. Macmillan, 611 p. 1941.

(37) Sherman, H. C.
Chemistry of food and nutrition. New York. Macmillan, 614 p. 1932.

(38) Sherman, H. C., and Crocker, Josephine.
Growth and reproduction upon simplified food supply. III. The efficiency of growth as influenced by the proportion of milk in the diet. Jour. Biol. Chem. 53:49-52. 1922.

(39) Street, H. R., and Reeves, R. E.
Occurrence of riboflavin in tubercule bacillus. Soc. Expt. Biol. and Med. Proc. 44:641. 1940.

(40) Sullivan, R. A., and Norris, L. C.
Determining riboflavin in dried milk powders. Indus. Engin. Chem. Analyt. Ed. 11:535-540. 1939.

(41) Supplee, G. C., Ansbacher, S., Flannigan, G. E., and Hanford, Z. M.
The fluorometric estimation of lactoflavin. Jour. Dairy Sci. 19:215-220. 1936.

(42) Supplee, G. C., Flannigan, G. E., Hanford, Z. M., and Ansbacher, S.
Lactoflavin, a possible contaminant of vitamin-free diets. Jour. Biol. Chem. 113:787-792. 1936.

(43) Whitnah, C. H., Kunerth, B. L., and Kramer, M. M.
A rapid method for the determination of lactoflavin in milk. Amer. Chem. Soc. Jour. 59:1153. 1937.

(44) Van Duyne, Frances Oliva.
Riboflavin in tissues. Dissertation. New York. Columbia University, 16 p. 1940.