BULLETIN 518 JANUARY, 1933

Studies on the Nutritive Value of Milk

II. The Effect of Pasteurization on Some of the Nutritive Properties of Milk

W. E. Krauss, J. H. Erb, and R. G. Washburn

OHIO
AGRICULTURAL EXPERIMENT STATION
Wooster, Ohio

CONTENTS

STUDIES ON THE NUTRITIVE VALUE OF MILK

II. THE EFFECT OF PASTEURIZATION ON SOME OF THE NUTRITIVE PROPERTIES OF MILK

W. E. KRAUSS, J. H. ERB[1], AND R. G. WASHBURN

INTRODUCTION

The great food value of raw cow's milk is universally accepted. In the past, aside from differences caused by inherent qualities of milk produced by different breeds of cows, there was no evidence to show that one quart of milk was different in food value from any other quart of milk, no matter where produced. It is now known that the ration fed to cows is of considerable importance in determining the nutritive value of milk. However, when the milk from a large number of herds of cows is combined, as is the case in ordinary market milk, breed and feed differences are minimized. Nevertheless, market milks that have been standardized to a certain fat percentage differ in that some may be raw and some pasteurized.

With the introduction of pasteurization it was natural to inquire into the effect of this heat treatment on the properties of milk. This has led to many experiments; however, most of the earlier ones were general in nature and not carefully controlled.

The literature dealing with the nutritive properties of raw and pasteurized milk is voluminous. Although all kinds of experimental animals, as well as many human subjects, have been used and various nutritional phases of the question have been touched upon, there is still considerable controversy over the question. This is due to the fact that many of the reports in the literature are contradictory, that some of the earlier work has since been revised or corrected, and that small differences having no practical significance have been over-emphasized.

The practice of pasteurizing milk has increased rapidly in recent years. This has been due largely to the efforts of public health officials and to the fact that experimental work had shown, up to 1929, that the only significant, adverse effect of pasteurization, from the nutritive standpoint, was partial destruction of

---

[1]Department of Dairy Technology, The Ohio State University, Columbus, Ohio.

NOTE: There are different methods of pasteurization, but, in general, the term as applied to milk means heating the milk to and holding it at a temperature of 142° to 145° F. for 30 minutes, followed by rapid cooling. The purpose of pasteurization is to destroy disease-producing bacteria. It has nothing to do with making dirty milk clean, as is often popularly believed.

vitamin C and slight destruction of vitamin B and that these deficiencies of pasteurized milk could be readily overcome by properly supplementing the diet. However, in 1931, a report (35) appeared which showed that raw milk from specially fed cows was greatly superior to ordinary commercially pasteurized milk. This report received wide publicity and has recreated popular interest in the whole question.

There are few generally available publications dealing with various phases of the pasteurization question. This, together with the renewed interest in the question and the fact that we have been working on the problem in the light of newer discoveries in nutrition, has prompted the writing of this bulletin. The purpose of the bulletin is two-fold: First, to summarize briefly the available evidence; second, to present new evidence based upon our own experiments. This has been done entirely from the standpoint of food value without reference to the public health problems involved.

REVIEW OF THE LITERATURE

Three standard references may be taken for authoritative summaries of the status of the question at the time each was published. In a bulletin called *Milk and Its Relation to Public Health*, published by the Hygienic Laboratory in Washington in 1909, M. J. Rosenau (31), an authority on the subject, summarized the evidence on pasteurization as follows:

"There is a prevalent impression that the pasteurization of milk improves that important article of diet. Heating does not render milk better in any way as a food. All it does is to destroy certain bacteria and some of their toxic products. It checks certain processes of fermentation and putrefaction, thus rendering the milk safer. On the other hand, the evidence seems clear that the pasteurization of milk at 60° C. for twenty minutes does not appreciably deteriorate its quality or lessen its food value...... ."

"Comparative observations upon infants under the same conditions show that they flourish quite as well upon heated milk as upon raw milk. Laboratory experiments as well as clinical observations coincide with the view that heated milk is quite as digestible as raw milk. In fact, it is now claimed to be more so. Metabolism experiments indicate that the utilization of calcium and iron in the body is more complete in children fed upon boiled cow's milk than in those fed upon raw cow's milk."

A second authoritative source of information considers the literature up to 1916—*Milk and Its Hygienic Relations*, by Dr. Janet

E. Lane-Claypon (24). The mass of evidence presented in this book is concerned mostly with high heat treatment, particularly boiling, from which Dr. Claypon draws the following general conclusion:

"Generally, it may be stated that no form of artificial feeding will produce results of as favorable a nature as are obtained by natural methods of feeding. Where artificial feeding must be employed, there is no evidence that milk loses any of its nutritive value by boiling. The work of numerous observers indicates that rather more satisfactory progress is made with boiled than with raw milk."

A third authoritative summary of the available information up to 1928 is found in the book *Fundamentals of Dairy Science*, by Associates of L. A. Rogers (1). The effect of pasteurization on the food value of milk is summarized as follows:

"A great deal of work has been done on the effect of heat on the chemical and nutritive properties of milk. Rupp, for instance, finds that pasteurization at a temperature somewhat above that usually employed has no significant effect on the solubility of the calcium and phosphorus compounds of milk, does not coagulate the lactalbumin, and causes the casein to coagulate only slightly more rapidly with rennin.

"Later Bell has worked on the solubility of the calcium and phosphorus compounds of milk, and has confirmed Rupp's results .... There is a very satisfactory agreement that pasteurization, as ordinarily practiced, has only a small effect on such physical and chemical properties of milk as have so far been studied.

"A large amount of work has been done on the nutritive properties of raw and heated milk with the general aim of solving the practical question whether it is advantageous to heat milk which is intended for the nourishment of human infants. In the course of this work a number of species of animals have been involved and some observations have been made on babies. In many of the experiments milk was not the sole article of food used, the points observed were the gain in weight, general health, and mortality of the animals studied...... ."

"Since 1916 attention has been largely focused on the vitamins contained in milk, and many investigators have worked on the question of the effects of heat on these substances...... . There is a satisfactory agreement that pasteurization has no deleterious effect on vitamins A and B, but has a decided tendency to destroy vitamin C.

"Work on the effects of heat on the nutritive properties of milk is still being actively carried out...... . Some of these recent results indicate that fresh or rapidly boiled milk is superior in its nutritive qualities to pasteurized milk and that this superiority can not be wholly explained as due to the tendency of extended heating to destroy the antiscorbutic vitamin in milk. But the results in question are so much in conflict with each other on certain important points that the interpretation of their practical significance must be postponed until more work has been done in the field.

"In considering the practical conclusions to be drawn from the work which has just been described, it must be remembered that it is not usually wise to use milk as the sole article of diet, even for very young animals. The specialists on infant feeding recommend that babies have a small amount of orange juice as a part of their diet from the first few days of life onward, even when they are nursed by their mothers, and the same recommendation applies with still more force to babies who do not receive their mother's milk. Most of the experiments where raw and heated milk have been fed along with other articles of diet have failed to show any superiority of the former, and general practical experience seems to be in harmony with this outcome of the experiments. There can be little question, therefore, that the dangers which are known to be avoided by pasteurization should receive more consideration than the rather questionable point of superiority of raw milk. Until infectious diseases are under much more complete control than at present, it will be wise for our larger communities to hold to and perhaps extend the practice of pasteurization."

In a recent bulletin of the U. S. Department of Agriculture (2) the following is found:

"From a chemical standpoint serious objections have been raised against pasteurized milk, on the ground that the heating produces changes which render the milk less digestible, particularly by infants. However, Rupp found that milk pasteurized at a temperature as high as 145° F. for 30 minutes does not undergo any appreciable chemical change; he found that soluble phosphates do not become insoluble and that the albumin does not coagulate, but that when higher temperatures are used chemical changes do occur...... . Further evidence that low-temperature pasteurization does not injure the digestibility and nutritive value of milk has been brought out by feeding experiments with babies. In experiments conducted by Weld a number of babies were fed raw milk and pasteurized milk, and there was only a slight difference in the average net daily gain in weight during the feeding period. The slight difference was in favor of pasteurized milk. Hess, however, has found that milk pasteurized for 30 minutes at 145° may cause, in infants, a mild form of scurvy, which yields readily to so simple a remedy as orange juice.

"The high temperature heating of earlier days must not be confused with the low-temperature pasteurization of the present day. Many of the objections which have been raised to pasteurization have been founded on the observation of milk heated to high temperatures. However, the fallacy of the objections to pasteurization has been shown, through scientific research in the last few years, and as a result the value of the process has been firmly established...... .

"...... the only serious effect of pasteurization on the vitamins is on the antiscorbutic vitamin C and the antineuritic vitamin B, and it is evident that the feeding of orange and tomato juice, or other foods rich in these vitamins, readily makes up for the deficiency of these vitamins in pasteurized milk."

The preceding reviews cover most of the available information up to the first part of 1932. However, certain articles not included in these reviews should be mentioned.

Probably the most extensive nutritional experiment ever attempted was one recently conducted in Scotland (25). In this investigation 20,000 school children, under careful supervision, were involved. One group of 10,000 children received daily over a period of 4 months a supplementary ration of three-fourths of a pint of milk. The remainder of the children served as controls and received no milk. Of the 10,000 who received milk, 5,000 received raw milk and 5,000 pasteurized milk. Bulked, Grade A, tuberculin-tested milk was used, one-half of the supply being pasteurized. A statistical study of the results obtained, based on gains in weight and height, was made by the authors and led to the following conclusion: "In so far as the conditions of this investigation are concerned the effects of raw and pasteurized milk on growth in weight and height are, so far as we can judge, equal." This experiment has been severely criticized by Fisher and Bartlett (13), who point out by statistical treatment that the response in height to raw milk was significantly greater than that to pasteurized milk. Their interpretation of the data led to the assertion that the pasteurized milk was only 66.0 per cent as effective as the raw milk in the case of boys and 91.1 per cent as effective in the case of girls in inducing increases in weight, and 50.0 per cent as effective in boys and 70.0 per cent in girls in bringing about height increases.

In September, 1932, there appeared a report by the United States Public Health Service on the growth-promoting property of heated and raw milk (14). The data for this report were accumulated by the survey method and involved over 3,700 children between the ages of 10 months and 6 years in 39 cities of 10 states. The term "heated milk" as used in this study included pasteurized milk, boiled milk, evaporated milk, and milk powder. The conclusion reached as a result of this study was that the growth-promoting capacity of heated milk, plus the supplementary diet received by the average American child of 10 months to 6 years, is not measurably less than the growth-promoting capacity of raw milk, plus the supplementary diet received by the average American child of 10 months to 6 years.

Willard and Blunt (42) showed that children receiving approximately four-fifths of a quart of commercially pasteurized milk daily, furnishing 90-95, 65-78, and 40-50 per cent of the total calcium, phosphorus, and nitrogen, respectively, had positive balances of these elements. On equivalent intakes of evaporated milk, the balances were slightly more favorable. These authors concluded that evaporated milk appeared to be a satisfactory source of calcium, phosphorus, and nitrogen and seemed slightly superior to the pasteurized milk.

Kramer, Latzke, and Shaw (22) obtained less favorable calcium balances in adults with pasteurized milk than with "fresh milk" and made the further observation that milk from cows kept in the barn for 5 months gave less favorable calcium balances than did "fresh milk" (herd milk from a college dairy).

Wallen-Lawrence and Koch (41), conducting test-tube digestion experiments, found that, when trypsin—one of the enzymes concerned in digestion—was added to pasteurized milk, digestion occurred more rapidly than when it was added to raw milk. The explanation offered for this is that in raw milk there is an anti-substance which hinders the action of trypsin and that when milk is heated this anti-substance is destroyed.

Hill (19) has shown that the softness of the curd of milk upon coagulation with pepsin is related to the digestibility of the milk. Pasteurization of milk at 143° F. for 30 minutes had very little effect upon the hardness of the curd formed by its coagulation with pepsin; however, boiled milk formed a curd which was only 31 per cent as hard as that obtained from the raw milk. This offers a possible explanation for the superiority shown by boiled milk in many experiments.

Brannon and Prucha (3) have for several years been comparing raw and pasteurized milk from the nutritive standpoint and have as yet been unable to demonstrate any difference between them.

The question of the effect of pasteurization on the vitamin content of milk is often raised. Five vitamins, A, B, C, D, and G, are of sufficient importance in the nutrition of infants and children to warrant consideration.

No clear-cut direct evidence is known that shows the effect of pasteurization on vitamin A. 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. Mann (26), reporting at the World's Dairy Congress in 1928, claimed, after 4 years' work with boys of school age, that the addition of milk pasteurized at 145° F. for 30 minutes to a diet poor in vitamins A, C, and D resulted in marked improvement, indicating that the vitamin value of the milk was undamaged by the heat treatment. According to S. Schmidt-Nielsen and Schmidt-Nielsen (32), when milk pasteurized at 63° C. (145° F.) was fed to mature rats, early death or diminished vitality resulted in the offspring. This was attributed to the destruction of vitamin A.

The vitamin which was formerly known as vitamin B has recently been found to be a complex of at least two factors. These are now designated as vitamin B (B_1) and vitamin G (B_2). The former prevents beri-beri or polyneuritis and, hence, is referred to as the antineuritic vitamin; the latter is concerned with the prevention of pellagra and is sometimes called the antipellagric vitamin.

Daniels, Giddings, and Jordan (8) found that there was a suggestion of destruction of the antineuritic vitamin of milk pasteurized by the open method but that milk pasteurized by the closed method gave no evidence of destruction of this vitamin. In this work the standard assay method was not used. The behavior of suckling rats nursed by mothers fed a basal diet deficient in the antineuritic vitamin, to which was added various kinds of heat-treated milks, was used as the criterion.

Mattick and Golding (27) reported some preliminary experiments which indicated that pasteurization destroys some of the dietetic value of milk, including partial destruction of vitamin B_1. These same workers found the raw milk to be considerably superior to sterilized milk in nutritive value.

No published work dealing with the effect of pasteurization on vitamin G is known, although commercially pasteurized milk obtained on the open market was found by Todhunter (38) to be a good source of this vitamin.

There is considerable evidence, based upon animal and clinical work, on the effect of pasteurization on vitamin C. Most of this work has shown that an appreciable destruction of vitamin C occurs when milk is heated to 142°-145° F. for 20 to 30 minutes. That this loss in vitamin-C potency must be offset in the feeding of infants has been aptly pointed out by Hess (18) as follows:

"Although pasteurized milk is to be recommended on account of the security which it affords against infection, we should realize that it is an incomplete food. Unless an antiscorbutic, such as orange juice, the juice of an orange peel, or potato water, is added, infants will develop scurvy on this diet."[2]

On the other hand, Mann (26) failed to observe scurvy in infants receiving pasteurized milk, nor did he find the addition of orange juice beneficial from a nutritive standpoint. The mass of evidence, however, favors the viewpoint expressed by Hess. It should be pointed out in this connection that the vitamin-C content of milk depends largely upon the feed of the cow and, consequently, is variable. Consumption of raw milk, therefore, does not necessarily insure an adequate intake of vitamin C.

The resistance of vitamin D to heat treatment has led to the belief that this vitamin would not be affected by pasteurization; very little direct evidence as to this exists, however. Daniels and Jordan (7) showed that superheating irradiated milk (autoclaved at 15 pounds pressure for one hour) did not affect the antirachitic properties of the milk. In this experiment growth alone was used as the criterion.

Within the past few years it has been repeatedly demonstrated that young animals, particularly rats, develop nutritional anemia when fed on raw milk exclusively (16, 23, 39). The reason for this was later found to be that milk is deficient in two elements necessary for blood formation—iron and copper. Many laboratories have since adopted the exclusive raw milk method of feeding as standard procedure for the development of nutritional anemia in rats.

This means that raw milk produced by cows fed in many different ways in various parts of the country has been used to produce anemia in rats. Furthermore, it has been definitely shown that the iron and copper content of milk cannot be increased through the feeding of iron and copper supplements to the cow (11, 12, 15).

In view of these recent findings, considerable interest was created by the appearance of the articles by Scott and Erf (34, 35), in which it was shown that rats fed exclusively on raw milk from specially fed cows did not develop nutritional anemia whereas rats

[2]Other vitamin-C supplements might have been added to this list.

fed commercially pasteurized milk did. Pasteurization was also found to affect the hematogenic and growth-promoting properties of the special milk. These first experiences were substantiated by further work (36) in which it was emphasized that "milk from properly fed cattle is apparently a complete food, and when taken exclusively brings about normal growth and a normal blood picture in the albino rat." Also, "Experiments seem to prove that the application of heat to milk (or other foods) causes a decrease in the nutritive value, as indicated by the growth curve, the red blood cell count and hemoglobin per cent of the albino rat."

Since in our own laboratory we were deeply involved in nutritional anemia studies and since preliminary experiments had shown no difference in the rate at which rats developed nutritional anemia on raw or pasteurized milk, the appearance of the work of Scott and Erf (35) stimulated us to a more detailed study of the problem. This led to studies on other phases of the problem upon which it was felt that more definite information was needed.

EXPERIMENTAL PROCEDURE

ANEMIA DEVELOPMENT

For 6 years in this laboratory a study of the nutritive properties of milk, particularly with respect to the effect of the cow's ration on these properties, has been made. One of the discoveries made in the course of this work was that when weanling rats were placed on an exclusive raw milk diet they soon died from nutritional anemia. The reason for this was later found to be that milk is deficient in iron and copper (17), two elements necessary for blood formation.

Early in the course of these experiments a diet of raw milk exclusively was compared with one of pasteurized milk exclusively. Only a few animals were used, and the only criteria taken for comparing the value of the two kinds of milk were rate of growth and external physical appearance. No difference between the two groups could be detected. Later, after it had been established that rats develop nutritional anemia on an exclusive milk diet, this trial was repeated, and, in addition to the above-mentioned criteria, hemoglobin determinations were made periodically, using a Dare hemoglobinometer. Again no difference could be detected between the two kinds of milk. Since these preliminary trials gave negative results, the matter was dropped. However, upon the appearance of the report by Scott and Erf (35) it was decided to repeat our earlier work on an extensive scale and exercise every precaution to make the only limiting factor the heat treatment of the milk.

Three Holstein cows from the Ohio Experiment Station herd were selected to furnish the milk for the experiments conducted at Wooster. These cows were normal individuals receiving the regular herd ration of alfalfa hay, corn silage or soaked beet pulp, and a grain mixture of corn, oats, bran, and linseed oilmeal. Milk was not obtained from the same three cows throughout the experiments. Whenever one of the original three cows became dry, another individual on the same ration was substituted until the original animal had been fresh a suitable length of time.

The cows were milked twice daily; on the days when collections were made (usually twice weekly), the milk was caught in enamel buckets. A small portion of the milk from each cow was transferred to a glass bottle at each milking on collection days. A sufficient quantity of milk to last 3 or 4 days was then transferred to two Erlenmeyer flasks. One of these was immediately placed in a cooler at about 40° to 45°F. The other was placed in a De Khotinsky constant temperature water bath and heated as rapidly as possible to 145° F. (62.5° C.), at which temperature it was held for 30 minutes. The milk was then cooled with running water as rapidly as possible and placed in the cooler with the raw milk sample. It required about 15 minutes to bring the temperature of the milk up to 145° F. (62.5° C.). During the heating process the flask was stoppered, and the temperature was read by means of a thermometer extending through the stopper and into the milk. The milk was stirred every few minutes, and at the completion of the heating process any material adhering to the sides of the flask was scraped down with a rubber policeman and incorporated with the milk. The milk heated as described above is referred to here as "laboratory pasteurized milk"; the unheated milk was the source of the "raw milk" used in the experiments carried out at Wooster.

The laboratory pasteurization procedure and the method of collecting the milk eliminated all possibility of metallic contamination. This was of importance in view of the part played by iron and copper in preventing anemia. However, under commercial conditions, milk comes in contact with metals of various kinds, depending upon the particular type of equipment used in the dairy plant. In order to get some information as to the effect of commercial production and handling, the work done at Wooster was duplicated at Columbus, using milk before and after pasteurization in the plant of the Department of Dairy Technology at the Ohio State University.

The milk used was produced by the purebred dairy herd (made up of the major dairy breeds) of the Ohio State University. As roughage these cows received ground alfalfa hay (western grown) of medium quality, except during the pasture season when a small allowance of coarse alfalfa hay was fed. Corn silage was fed at all times except during May and June. The cows were on good bluegrass pasture from May 15 to October 1. During the first trial the grain mixture consisted of cottonseed meal 140, linseed oilmeal 100, gluten feed 400, wheat bran 300, white hominy 340, standard middlings 200, ground oats 260, dried distillers' grains 200, finely ground limestone 20, steamed bone feeding meal 20, salt 20. During the second trial at Columbus yellow corn meal replaced the white hominy in this grain mixture. During the third trial the grain mixture consisted of standard wheat middlings 260, linseed oilmeal 240, cottonseed meal 160, wheat bran 500, ground oats 300, yellow corn meal 480, steamed bone feeding meal 20, finely ground limestone 20, salt 20.

Raw milk was received from the Ohio State University herd in well-tinned, 10-gallon cans. This was dumped into a tinned iron weigh tank and from there conducted to a tinned copper, horizontal coil vat. After the milk was dumped into the coil vat, it was agitated and the sample of raw milk for use in the rat feeding trials was taken. The milk was dipped out of the vat with an aluminum dipper and placed in glass quart bottles. The milk in the vat was then heated to about 100° F. and pumped through a cloth filter at this temperature. From the filter, the milk flowed into a 200-gallon spray pasteurizing vat. This vat was of tinned copper construction and contained no exposed copper. Pasteurization was accomplished by heating the milk to a temperature of 143 to 144° F. and holding it at that temperature for 30 minutes. The milk was then cooled over a tinned-copper surface cooler to a temperature of 40° F. This cooler had a small amount of exposed copper on the first coil. The milk then went into the bottling machine, from which quart samples were taken for the feeding trials.

Bottles of raw and pasteurized milk, taken as previously described, were placed in a cooler at a temperature of 42° F. until used. In feeding, the sample was poured directly from the bottle into a glass feeding dish which was placed on a scale. The amount of milk desired was then weighed out.

It should be noted that the milk was not standardized for fat content. The pasteurized milk contained the same percentage of fat as the original raw milk, approximately 4 per cent. Samples were taken about three times a week, the raw and pasteurized samples coming from the same batch of milk.

Young albino rats from regular stock females out of our own rat colony were used throughout the experiments. The mothers received our regular stock diet, which included whole milk *ad libitum*. The young had access to the mother's diet until weaned at 24 days of age, at which time hemoglobin determinations and erythrocyte counts were made. They were then transferred to individual glass

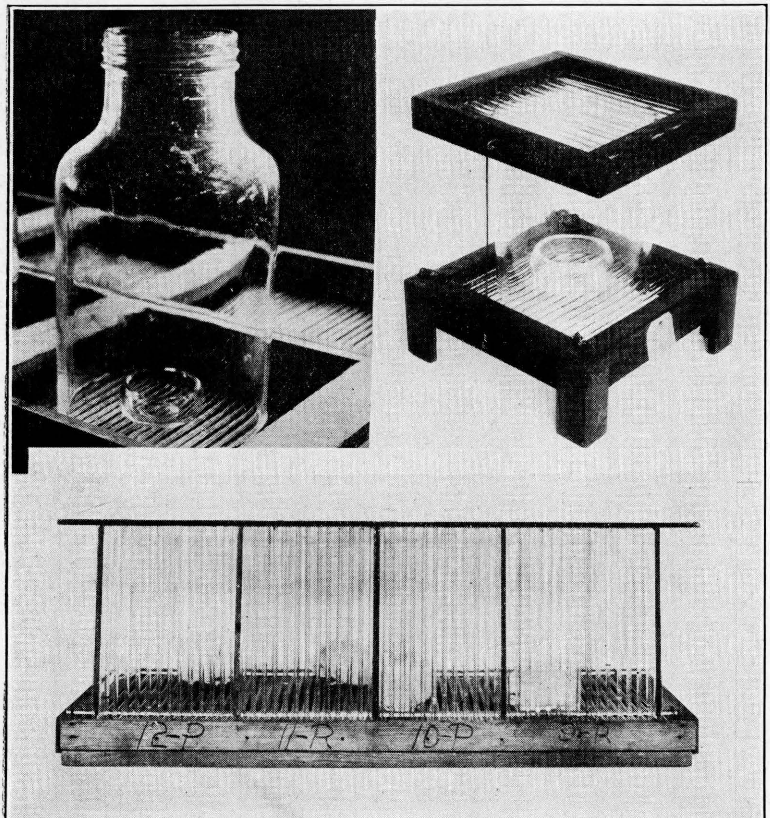

Fig. 1.—Three types of glass cages used in the anemia experiments. The two upper cages were used at Wooster; the lower one was used at Columbus

cages (Fig. 1) and placed on either raw milk or pasteurized milk exclusively. The paired feeding method of Mitchell and Beadles (27) was used. One animal of each pair received raw milk; the other received pasteurized milk. In pairing the animals, the hemoglobin value and red cell count were taken into consideration, along with sex and weight. The milk was measured out daily into glass dishes with a pipette, the amount fed to each pair depending upon the amount consumed on the previous day by the poorer eater of the pair. Unconsumed milk was measured by pouring it into a graduate.

Hemoglobin determinations and red cell counts were made weekly, and the animals were weighed at the same time. The hemoglobin determinations were made with a Bausch and Lomb hemoglobinometer (improved Newcomer model). The red cells were counted in a Levy-Hausser counting chamber with improved Neubauer ruling, Hayen's solution being used as the diluting fluid. Blood samples were obtained by clipping the end of the tail with a safety-razor blade. The wound thus made was cauterized with a hot iron.

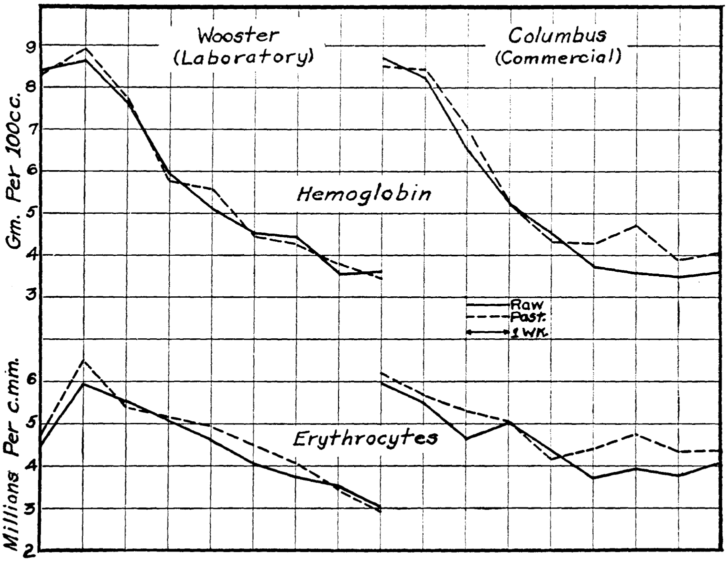

Fig. 2.—Curves showing the rate of anemia development on raw and pasteurized milk. 16 pairs of rats were used at Wooster and 16 pairs at Columbus

Complete data were obtained on 16 pairs at Wooster, using laboratory pasteurized milk, and 16 pairs at Columbus, using commercially pasteurized milk. The results obtained are presented graphically in Figure 2. Each point on the curves represents the average value of all the animals in each group.

It will be noted first of all that, based on hemoglobin values and red cell counts, the rats receiving raw milk developed nutritional anemia within 8 weeks. Those receiving pasteurized milk developed nutritional anemia at about the same rate. The red cell counts and hemoglobin values of the rats receiving commercially pasteurized milk were slightly higher than those of the raw-milk rats at the end of the 8-week period. These differences, although quite small, indicate what might be expected under commercial conditions where the milk may have an opportunity to become contaminated through metallic contact.

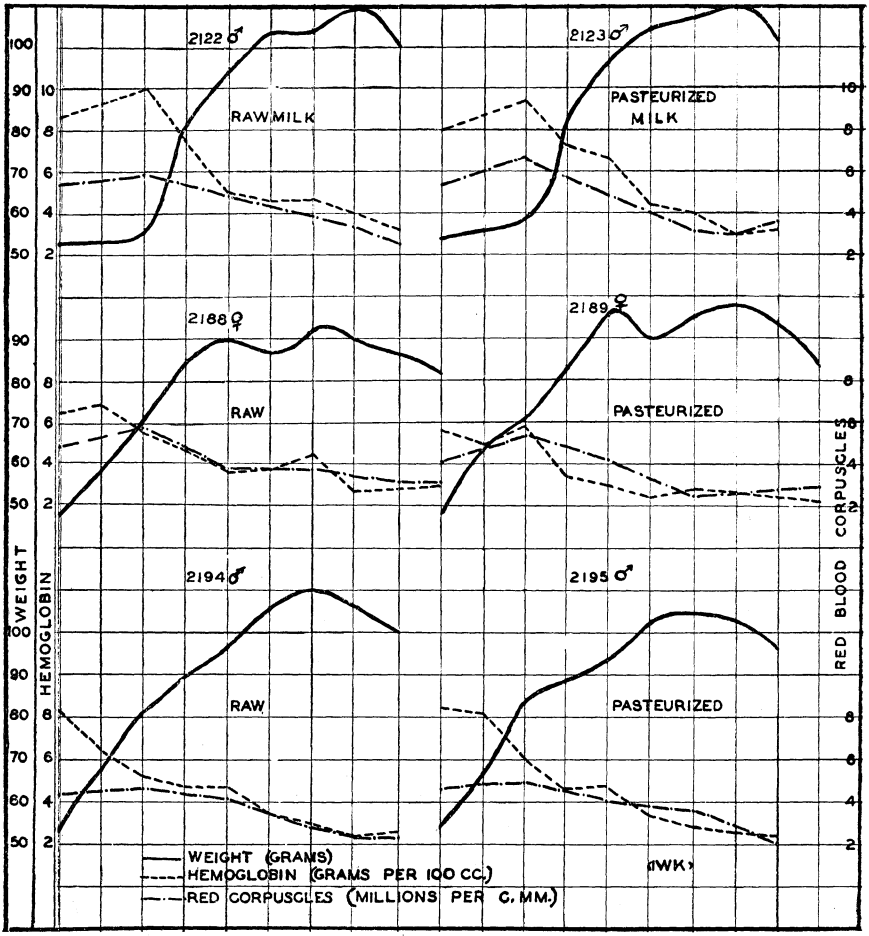

Fig. 3.—Details of the behavior of three typical pairs on raw and pasteurized milk

In Figure 3 the curves for the individuals of three typical pairs are given. Some idea of the physical condition of the animals after 8 weeks is given in Figure 4. It was pointed out by Scott and Erf (34) that "those upon the special and certified milks showed a sleek, smooth, coat, were in good flesh, their eyes were clean, their dispositions good and they seemed to enjoy being petted; while those receiving the pasteurized milk showed a roughened coat, they were dull, listless, and huddled together in the cage, their eyes lacked luster and they became quite irritable and showed a tendency to bite upon being handled." We have never observed any difference in the physical appearance or disposition of the animals on either kind of milk. In our experiments all the animals were handled with bare hands without trouble, even when the blood samples were drawn.

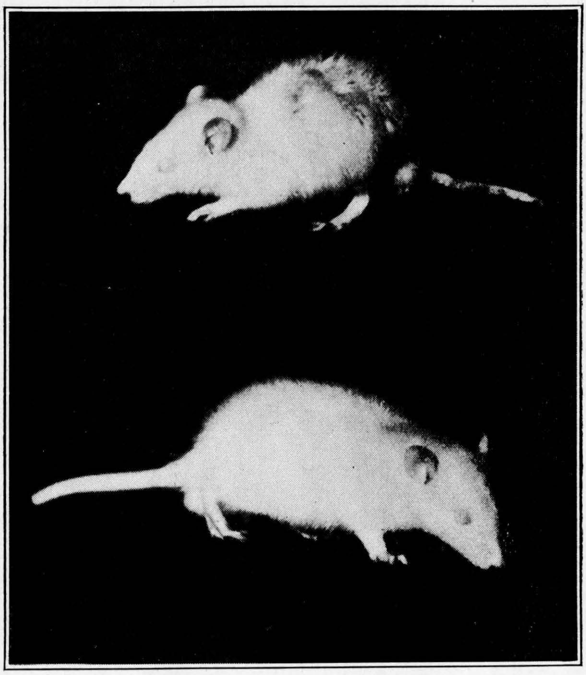

Fig. 4.—Litter mates after 8 weeks on (above) raw milk and (below) pasteurized milk. Note lack of color in the eyes. Both rats have severe nutritional anemia.

Because there has been some controversy over the effect of pasteurization on the mineral constituents of milk, nine samples of raw milk and nine of the same milk after laboratory pasteurization were analyzed for iron and copper, with the following results:

	Mg. Iron[3] per liter	Mg. Copper per liter
Raw Milk	2.03	0.19
Pasteurized Milk	2.05	0.18

[3]Subsequent analyses by the method of Stugart (37) gave lower absolute values for iron.

Iron was determined by the method of Elvehjem and Hart (9) and copper by the Elvehjem and Lindow modification of the Biazzo method (10).

Although these analyses do not show whether or not the forms in which the iron and copper are present in milk are altered by pasteurization, they do show that there is no loss of these elements.

GROWTH

In order to compare the total growth-promoting power of raw milk and pasteurized milk, the exclusive milk method of feeding was employed. To overcome the factor of nutritional anemia, 0.5 mg. of iron as ferric chloride and 0.15 mg. of copper as copper sulfate were added daily to the milk fed each rat. The paired feeding method was again used. Hemoglobin determinations were

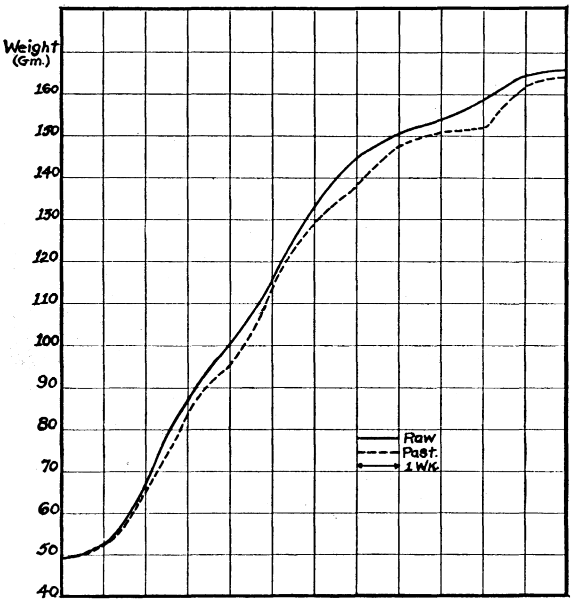

Fig. 5.—Rate of growth on raw and pasteurized milk when the factor of anemia was eliminated by adding iron and copper to the milk. The curves are composites of 12 pairs.

made on each animal at the beginning of the experiment and monthly thereafter, as well as at the close of the 12-week feeding period. The rats were housed in individual, hardware cloth cages and were weighed once a week. The pasteurized milk used in this experiment was "laboratory pasteurized", as previously described.

The results of this trial are shown in Figure 5. It is apparent that the composite curve for the rats receiving pasteurized milk is slightly but significantly below that for the rats receiving raw milk throughout the entire feeding period; from this it might be concluded that the pasteurized milk was not as effective as the raw milk in promoting growth. However, when each pair is considered separately and statistical methods for paired feeding (5) are applied to the total gain in weight, it is found that the difference is not significant. The important fact to be pointed out about this experiment is that the composite growth curve of the rats on pasteurized milk did not assume a downward trend at any time and was actually very close to the curve for the rats on raw milk. That the factor of anemia had been eliminated was shown by the hemoglobin values. These remained at a normal level throughout.

The growth curves shown in Figure 5 are below the normal curve of growth for rats on a suitable mixed diet. This difference is probably due to an inadequate intake of manganese, as pointed out by Kemmerer, Elvehjem, Hart, and Fargo (21).

CALCIFICATION

The term "calcification" as used here designates storage of minerals in the body. Calcium and phosphorus are the chief elements concerned in bone formation, although these and numerous other mineral elements are stored in the entire body. It was felt that ash determinations of the femurs of rats on raw or pasteurized milk would give a fair measure of the ability of the animals to use calcium and phosphorus from the two kinds of milk. Ash determinations of the entire body would give the end result of mineral storage, and calcium and phosphorus determinations on the entire body would give added information as to the utilization of these two elements.

Consequently, when animals in the anemia experiments died or were removed from the trial, their femurs were removed and freed from all adhering tissues. These bones were then dried in a steam oven for 24 hours, after which they were crushed, wrapped in filter paper, and extracted in a Soxhlet extractor with 95% alcohol for 36 hours and with ethyl ether for 12 hours. After driving off any remaining ether, the bones were placed in a constant temperature oven at 100° C. for 24 hours. They were then cooled in a desiccator and weighed, after which they were ashed in a muffle furnace. The resulting ash was weighed and the per cent of ash calculated on a moisture-free, fat-free basis. The femurs of five pairs used in the growth experiments were likewise treated.

TABLE 1.—Calcifying Properties of Raw and Pasteurized Milk (Anemia Experiments)

Number of pairs	Ash in femurs (per cent)	
	Raw milk	Pasteurized milk
17	56.40	56.32*
17	58.77	59.20†

*Laboratory pasteurized.
†Commercially pasteurized.

The bodies of the remaining eight pairs used in the growth experiment were dried and ashed (after removing the alimentary tract contents); calcium and phosphorus determinations were made on a solution of the ash. The method of McCrudden (28) was used for calcium and the usual official methods for phosphorus. The results of these calcification studies are given in Tables 1 and 2.

TABLE 2.—Calcifying Properties of Raw and Pasteurized Milk (Growth Experiment)

Entire body (Alimentary tract contents removed)

	Fresh weight	Dry weight	Weight of ash	Ash		Calcium		Phosphorus	
				Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis
	Gm.	*Gm.*	*Gm.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Raw milk, 8 pairs	141.0	49.96	5.93	4.23	11.97	1.29	3.65	0.796	2.25
Pasteurized milk, 8 pairs	132.0	46.80	5.54	4.22	12.10	1.29	3.71	0.792	2.27

It is obvious that in these experiments the rats utilized the minerals of pasteurized milk just as well as those of raw milk. It should be emphasized that these experiments measure the end results of mineral metabolism over a considerable period of time and differ from ordinary metabolism studies which measure the mineral balance over short periods.

VITAMINS

In the experiments just reported the exclusive milk method of feeding was used. Under such a system of feeding the amount of milk consumed may be great enough to overcome some deficiency, particularly a vitamin deficiency, which would become apparent only on a limited intake of milk. To get information as to the critical levels necessary to furnish adequate amounts of the various vitamins, raw and pasteurized milk were assayed for vitamins A, B, G, and D. The samples of milk were obtained and treated as previously described. Pasteurization was carried out in the laboratory.

VITAMIN A

Regular stock females with young (six to a litter) were deprived of the stock diet when the young were 14 days of age and were placed on a diet deficient in vitamin A.[4] The young were weaned at 24 days of age and continued on the A-free diet until growth ceased. They were then placed in individual hardware cloth cages with raised bottoms and fed, in addition to the basal A-free diet, various amounts of either raw or pasteurized milk. The rats were weighed weekly and observations were made as to the incidence and severity of ophthalmia and respiratory trouble.

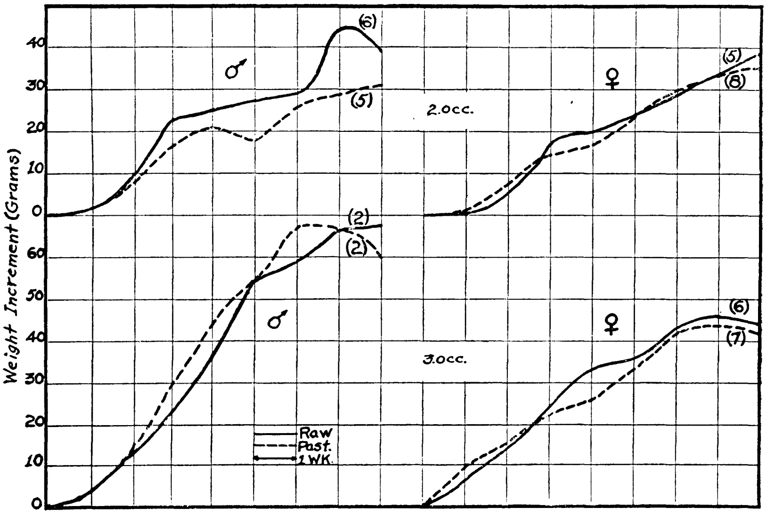

Fig. 6.—Effect of pasteurization on vitamin A. No measurable destruction of vitamin A occurred. The number of animals is indicated in parentheses

Three levels of milk were fed—1.0 cc., 2.0 cc., and 3.0 cc. daily. The 1.0 cc. level of either kind of milk was too low and most of the animals did not survive the 8-week supplementary feeding period. A group of control rats likewise died within a short time. The data for the 2.0 and 3.0 cc. levels only, therefore, are shown in Figure 6. Loss of three females on the 2.0 cc. level of raw milk changed the sex distribution and necessitated charting the sexes separately (Fig. 6).

[4]Argentine casein (purified), 18.0; Dextrin, 58.0; Crisco, 10.0; Salts 185, 4.0; Agar-agar, 2.0; Yeast, 6.0; Irradiated yeast, 2.0.

The rather uneven character of the curves for males on the 2.0 cc. level was due to the fact that one animal in each group was in a critical condition most of the time, the one on raw milk finally dying at the end of the sixth week. This raised the average weight of the raw-milk group considerably during the next week and accounts for the apparent difference between the two curves. As shown by the curves for females at this level, 2.0 cc. of either milk produced about the same response. This was further substantiated by examination of the records with respect to ophthalmia and respiratory infection. On the 3.0 cc. level the same response was obtained with both kinds of milk.

Careful consideration of the growth response, the incidence of ophthalmia, and respiratory trouble leads to the conclusion that no destruction of vitamin A resulted from heating milk at 145° F. for 30 minutes in a closed container.

VITAMIN B

Weanling rats, weighing from 45 to 50 grams, were placed in hardware cloth cages with raised bottoms and fed a diet deficient in vitamin B.[5] As a source of vitamin G, 0.5 gram of autoclaved yeast per rat was fed daily. When the animals ceased to grow (in about 2 weeks), they were transferred to individual cages and fed various quantities of either raw or pasteurized milk.

Three levels of milk were fed—5.0 cc., 7.5 cc., and 10.0 cc. per rat daily. The 5.0 and 10.0 cc. levels were fed first in order to establish an upper and lower limit. The 5.0 cc. level was too low; whereas at the 10.0 cc. level the rats on raw milk gained more than the amount required by the Sherman system to equal one unit. Consequently, the 7.5 cc. level was chosen as the one likely to represent one unit. This proved to be the case insofar as the raw milk was concerned, but this level represented less than one unit of pasteurized milk (Fig. 7). Likewise, the 10.0 cc. level of pasteurized milk fell short of being equivalent to one unit of vitamin B. From the growth increment differences on raw and pasteurized milk fed at different levels as the sole source of vitamin B, it is estimated that the vitamin-B potency of the raw milk was reduced at least one-fourth by the process of pasteurizing employed. That some destruction of this factor occurred is in agreement with the preliminary experimental results obtained by Mattick and Golding (27).

[5]Argentine casein, 18.0; Agar-agar, 2.0; Salts 185, 4.0; Cod-liver oil, 2.0; Crisco, 10.0; and Starch, 64.0.

It will be observed that at the 10.0 cc. level the response to pasteurized milk was less than at the 7.5 cc. level. This is difficult to explain, since the response of rats on 10.0 cc. of raw milk was greater than that of rats on 7.5 cc., as would logically be expected.

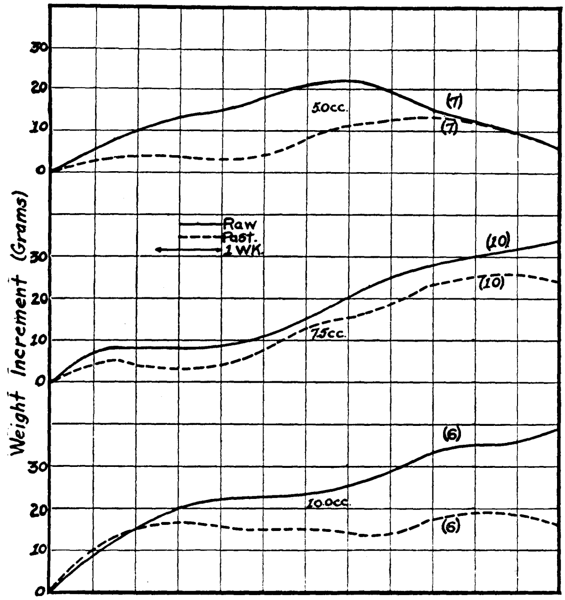

Fig. 7.—Some destruction of vitamin B (B_1) occurred when milk was pasteurized in a closed container, as shown here. The number of animals is indicated in parentheses.

The curves for the 5.0 cc. level were not of much significance as most of the rats represented by these curves developed polyneuritis at an early stage, thus interfering with the growth response. On the 7.5 cc. level two rats on raw milk developed mild polyneuritis toward the end of the trial; whereas three rats on pasteurized milk developed polyneuritis early, which became severe as the trial drew to a close. On the 10.0 cc. level none of the rats on raw milk developed polyneuritis, but three on pasteurized milk were severely afflicted.

VITAMIN G

The same technique and the same basal diet used in the vitamin-B experiments were used in studying vitamin G, except that as a source of vitamin B each rat was fed daily 1.0 cc. of an alcoholic extract of rice polishings, this amount being equal to 1.0 gram of the original polishings.

Three levels of milk were fed—3.0 cc., 5.0 cc., and 7.5 cc. daily. The 3.0 cc. level was too low to allow regular, continuous growth, and the curves shown for this level are, therefore, without much significance. At the other two levels the curves for raw and

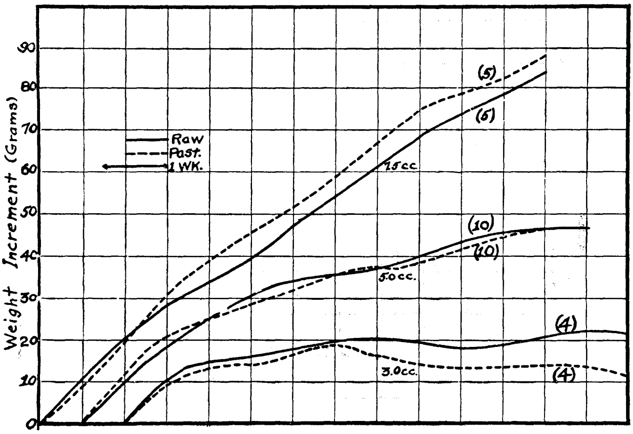

Fig. 8.—These curves show that pasteurization did not affect vitamin G. The number of animals is indicated in parentheses

pasteurized milk are practically identical (Fig. 8). The heat treatment did not destroy any of the vitamin G originally present in the raw milk. This is to be expected, since it is known that vitamin G resists autoclaving at hydrogen-ion concentrations within the range in which the pH of milk normally falls.

VITAMIN D

The vitamin-D content of foods may be determined either curatively or prophylactically. Both methods were used to determine whether pasteurization destroyed any of the vitamin D in milk.

Weanling rats were placed on the Steenbock and Black rickets-producing diet[6] for 21 days. At the end of that time they were fed, in addition to the basal diet, measured amounts of butterfat or cream, both of which were obtained from milk before and after pasteurization. Butterfat and cream were used on the assumption that practically all the vitamin D in milk would be found in the fat (6).

The butterfats were prepared by filtering melted butters which had been churned from two samples of cream, one of which came from raw milk and the other from pasteurized milk out of the same

[6]Yellow corn, 76; Wheat gluten, 20; Calcium carbonate, 3; Salt, 1.

batch. The cream samples fed were obtained by separating milk in the usual way, after which half the cream was pasteurized in a closed glass vessel (heated to 145° F. for 30 minutes). The raw and pasteurized cream were then stored in a refrigerator at 40° F., small quantities being removed each day for feeding.

TABLE 3.—Effect of Pasteurization on the Vitamin-D Content of Milk (Butterfat and Cream)

Curative

Sample	Levels fed		Number of rats		Line test	
	Raw	Pasteurized	Raw	Pasteurized	Raw	Pasteurized
Butterfat	600 mg. 800 mg.	600 mg. 800 mg.	7 7	8 7	1.1 + 1.4 +	0.8 + 1.3 +
Cream	2.0 cc. 3.0 cc. 4.0 cc. 5.0 cc.	2.0 cc. 3.0 cc. 4.0 cc. 5.0 cc.	5 10 6 7	5 9 7 6	0.80+ 1.35+ 2.25+ 2.14+	0.60+ 1.39+ 2.29+ 2.33+
Cream*	0.07 cc. 0.08 cc. 0.09 cc.	0.07 cc. 0.08 cc. 0.09 cc.	11 11 6	11 11 6	0.8 + 1.4 + 1.9 +	1.1 + 1.7 + 1.7 +

*Cream from milk produced by cows fed irradiated yeast. Milk was pasteurized in a commercial plant at 143° F. for 30 minutes. Raw and pasteurized samples came from the same batch.

Two levels of butterfat were fed, 600 mg. and 800 mg. per rat daily for 10 days. At the end of that time the rats were killed and their radii and ulnae removed. These were immersed in 10% formalin for at least 4 hours, after which they were cut longitudinally with a safety-razor blade, immersed in 3% silver nitrate, and exposed to a bright light until the calcified areas turned black. (This procedure constitutes what is known as the "line test".) The degree of healing was expressed in plus and minus signs, the average values for which are given in Table 3 in the column headed "Line test". The greater the amount of vitamin D in the sample, the greater is the plus value. At the 600 mg. level there is some indication that the raw milk contained more vitamin D than the pasteurized milk. However, this level of fat furnished too little vitamin D to allow much significance to be attached to the line-test values given. At the 800 mg. level about the same degree of healing was obtained on both kinds of fat.

Four levels of cream were used in assaying for vitamin D curatively. From the line-test values given in Table 3, it will be seen that pasteurization of the cream did not destroy any of the vitamin D originally present.

To obtain additional evidence as to the effect of pasteurization on the vitamin-D content of milk, arrangements were made with a commercial dairy to obtain samples of cream from the milk of cows fed irradiated yeast. Milk produced by cows thus fed is rich in vitamin D, and it was felt that any destructive effect of pasteurization would be more readily detected in samples rich in the antirachitic factor.

A sample of raw milk was taken after the batch had been placed in the vat previous to pasteurization. The remaining milk was then heated to 143° F. and held at that temperature for 30 minutes, after which the pasteurized milk sample was taken. The two samples of milk thus obtained were then separated and the resulting cream samples were brought to Wooster. Distilled water was used to adjust the fat percentage of each cream sample to 34 per cent. The cream from each source was then assayed by the curative technique, using daily levels of 0.07 cc., 0.08 cc., and 0.09 cc.

The results of this assay are given in Table 3, from which it is evident that the commercial process of pasteurization used in this experiment did not destroy any of the vitamin D in the original milk.

TABLE 4.—Effect of Pasteurization on the Vitamin-D Content of Milk (Butterfat)

Prophylactic

Sample	Levels fed		Number of rats		Calcification		Bone ash	
	Raw	Pasteurized	Raw	Pasteurized	Raw	Pasteurized	Raw	Pasteurized
	Mg.	*Mg.*					*Pct.*	*Pct.*
Butterfat	400	400	6	6	1.9+	1.8+	34.92	33.71
	600	600	5	5	2.6+	2.7+	39.69	40.57
Basal diet only			4		0.0		26.28	
Basal diet plus 2 per cent cod-liver oil			5		3.8		46.16	

The results of the curative assay of the butterfat were substantiated by a prophylactic trial. In this procedure rats were fed the Steenbock and Black rickets-producing diet over a period of 5 weeks. In addition, each rat received daily either 400 mg. or 600 mg. of butterfat from raw or pasteurized milk. At the end of 5 weeks the rats were killed and their femurs removed. Ash determinations were made on the femurs according to the procedure described under "Calcification". The ash values thus obtained are given in Table 4. The greater the percentage of ash, the greater was the amount of vitamin D in the sample. It is obvious from these figures and from those under "calcification" (which were obtained as described for the curative technique) that there was no difference in the vitamin-D content of the two butterfats. This agrees with the work of Daniels and Jordan (7), who subjected milk to even more severe heat treatment.

CURD TENSION

Much interest has been aroused through the work of Hill (19, 20) in the curd tension of milk. Hill reported that pasteurization had very little effect in reducing the curd tension of milk. Since in the experiments just reported it was necessary to pasteurize milk frequently, advantage was taken of this opportunity to determine, by use of the Hill technique, the curd tension of the milk before and after pasteurization. The curd tension of 24 samples of milk was determined by the Hill method before and after pasteurization. As compared with the curd before pasteurization, the curd of the milk after pasteurization was softer in 16 trials, harder in seven trials, and the same in one trial. The average curd test of the raw milk was 45.2 grams; the average test after pasteurization was 43.2 grams. The difference between these tests is small but it appeared significant when the statistical method suggested by Crampton for paired data was applied (5). This effect might be emphasized in milk having a higher curd tension than that used in our work. That such a possibility exists is shown in some data given by Hill (19). Two samples of raw milk having curd tensions of 131 and 188 grams gave readings of 82 and 134 grams, respectively, after heating to 143° to 144° F. for 30 minutes. Six samples having curd tensions ranging from 17 to 47 grams did not show such marked decreases after pasteurization. One sample showed no change and one increased slightly in curd tension after the heat treatment.

DISCUSSION

What practical significance may be attached to the results herein reported? The only adverse effect of pasteurization found was a partial destruction of vitamin B (B_1). This, together with other work showing that vitamin C is also adversely affected, narrows the question down, so far as our present knowledge goes, to a consideration of the effect of a diminution in the amount of vitamins B and C in milk upon the adequacy of the dietary in these two factors.

Bottle-fed infants constitute the group most apt to be affected, inasmuch as cow's milk comprises their sole or chief food. Undoubtedly, infants receiving pasteurized milk should receive some vitamin-C supplement, such as orange juice or tomato juice. However, there is no assurance that raw milk will furnish an adequate amount of vitamin C, since the amount present in milk depends upon the ration of the cow. Furthermore, when milk is boiled before feeding to infants (a common modern practice), some destruction of vitamin C occurs (33). The use of vitamin-C supplements is rapidly becoming a universal practice, even beyond the infant age, inasmuch as the virtues of vitamin C are now known to extend beyond its antiscorbutic effect.

Milk cannot be considered a good source of vitamin B, and any processing of milk that would cause some destruction of this factor might necessitate the use of vitamin-B-containing supplements in infant feeding. Many commercial infant foods now contain such supplements. Fortunately, cereals are relatively rich in vitamin B, as are green vegetables and egg yolk, all of which are fed to infants at a relatively early age.

It would seem, therefore, that the reduced amounts of vitamins B and C in pasteurized milk can be readily compensated for by proper dietary control.

The differences between the results obtained by Scott and Erf (34, 35, 36) and those reported herein require some explanation. In the first paper by these authors, the raw milk used came from specially fed cows and the pasteurized milk from a local grocery, except in one minor experiment involving a few animals. This fallacy was pointed out elsewhere (4). However, in later work (36) this objection was overcome by pasteurizing part of the special raw milk supply. The difference between the raw milk and the same milk after pasteurization was not nearly so great as that obtained when milk from different sources was used. Apparently, from the work of Scott and Erf, the cow's ration is the factor which makes the difference between the food value of raw and pasteurized milk, implying that the factor imparted to the milk by their system of feeding is destroyed by mild heat treatment. Furthermore, this factor is associated with blood formation. Rats fed raw milk from specially fed cows did not develop nutritional anemia; those fed the same milk after pasteurization did. To explain this satisfactorily it must be shown that the special system of feeding employed increased the iron and copper content of milk or that some unknown heat-labile factor concerned with blood formation was imparted to the milk. It has been conclusively demonstrated that iron and copper are the two elements concerned in the prevention of nutritional anemia on an exclusive milk diet (17, 23, 40) and that the amounts of these elements in milk can not be increased by feeding (11, 12, 29). It has also been shown in the data presented in this bulletin that no loss of copper or iron occurs when milk is pasteurized. Furthermore, under conditions of commercial pasteurization there is likelihood of the milk becoming contaminated with these elements. This is well illustrated by the experience of Rose and Vahlteich (30) while studying the effect of various foods on hemoglobin regeneration. They state: "At first pasteurized milk from a large city market was used but the production of anemia was slow and irregular, hence to avoid contamination with copper, certified city milk was substituted".

In the experiments reported by Scott and Erf exclusive milk diets were used throughout. It is difficult to see how, unless one or more of the vitamins were completely destroyed by pasteurization, such a great difference in growth would be obtained when such large quantities of milk were consumed. Even 25 or 30 cc. of milk daily will furnish sufficient vitamin D, the vitamin in which milk is most deficient. The suggestion of Scott and Erf that vitamin A may be associated with the results they obtained would seem to have no scientific basis, since as little as 2.0 cc. of milk from winter-fed cows will cure ophthalmia and restore growth in rats receiving a vitamin-A-free diet.

One of the conclusions drawn by Scott and Erf (36) was that "the milk from properly fed cattle is apparently a complete food, and when taken exclusively brings about normal growth and a normal blood picture in the albino rat." The cows used to furnish the milk for our experiments, although kept under winter feeding conditions during the time some of our trials were run, were fed as well as our present knowledge of dairy cattle feeding would recommend and better than the average dairy herds producing market milk. On the other hand, the cows furnishing the special milk used by Scott and Erf were fed materials not now generally recommended by feeding authorities. It is felt, therefore, that the milk used in our experiments was representative of the milk ordinarily finding an outlet through market milk channels.

SUMMARY

The rate of nutritional anemia development in albino rats fed on raw, laboratory pasteurized, or commercially pasteurized milk exclusively was compared. Based on hemoglobin determinations and red cell counts, nutritional anemia developed on each kind of milk and at about the same rate.

Copper and iron determinations showed that no loss of these elements occurred when milk was heated to 145° F. (62.5° C.) for 30 minutes in a closed vessel.

When the factor of anemia was eliminated by daily additions of copper and iron to the milk fed, no significant difference was found in the total growth of rats fed raw or pasteurized milk exclusively over a 12-week period.

No difference was found in the ash content of the femurs and entire bodies or in the calcium and phosphorus content of the entire bodies of rats fed either raw or pasteurized milk exclusively.

Using standard methods for determining vitamins A, B, G, and D, it was found that pasteurization destroyed at least 25 per cent of the vitamin B in the original raw milk.

Twenty-four samples of milk were tested for hardness of curd, using the Hill technique. After pasteurization, 16 were softer and seven were harder than before pasteurization. One sample showed no difference.

CONCLUSIONS

1. Albino rats develop nutritional anemia when fed exclusively on raw milk from cows fed as well as present knowledge of dairy cattle feeding would recommend.

2. When this milk is heated to 145° F. (62.5° C.) in a closed vessel and is held at that temperature for 30 minutes or when it is pasteurized under commercial conditions (held at 143-144° F. for 30 minutes) and then given as the sole food of albino rats, the rate at which nutritional anemia develops is not increased over that obtaining when the original raw milk is fed.

3. No loss of iron or copper occurs when milk is pasteurized.

4. When the exclusive milk method of feeding is used (fortifying with copper and iron to eliminate the anemia factor), no significant difference is apparent in the total nutritive effect of raw or pasteurized milk.

5. The calcium and phosphorus in pasteurized milk are as readily available as in raw milk.

6. Heating milk to 145° F. (62.5° C.) for 30 minutes in a closed glass vessel does not affect vitamins A, G, and D but does destroy at least 25 per cent of the vitamin B originally present.

7. The curd tension of milk is slightly reduced by pasteurization.

8. The nutritive deficiencies of pasteurized milk [vitamins B (B_1) and C] can be readily overcome by proper dietary control, and the continued use of pasteurized milk offers no serious problem from the food standpoint.

REFERENCES CITED

1. Associates of L. A. Rogers. 1928. Fundamentals of dairy science. The Chemical Catalog Company. pp. 469-471.
2. Ayers, S. H., R. P. Hotis, and C. J. Babcock. 1932. The present status of the pasteurization of milk. U. S. D. A. Revised Bull. 342.
3. Brannon, J. M. and M. J. Prucha. 1930-1931. Ill. Agr. Exp. Sta. An. Rept., p. 133.
4. Brown, E. G. 1931. Report of committee on milk. Conference of state and provisional health authorities of North America. Public Health Reports **46**: 1455-1460.
5. Crampton, E. W. 1930. Statistical analysis of comparative feeding trial data. Sci. Agr. **11**: 281-285.
6. Crawford, M. E. F., J. Golding, E. O. V. Perry, and S. S. Zilva. 1930. The fat-soluble vitamins of milk. Biochem. Jour. **24**: 682-691.
7. Daniels, A. L. and D. Jordan. 1929. Influence of superheating on antirachitic properties of irradiated foods. Proc. Soc. Expt. Biol. and Med. **26**: 453-455.
8. Daniels, A. L., M. L. Giddings, and D. Jordan. 1929. The effect of heat on the antineuritic vitamin of milk. Jour. Nutrition **1**: 455-466.
9. Elvehjem, C. A. and E. B. Hart. 1926. Iron in nutrition. II. Quantitative methods for the determinations of iron in biological materials. Jour. Biol. Chem. **67**: 43-51.
10. Elvehjem, C. A. and C. W. Lindow. 1929. The determination of copper in biological materials. Jour. Biol. Chem. **81**: 435-443.
11. Elvehjem, C. A., R. C. Herrin, and E. B. Hart. 1927. Iron in nutrition. III. The effects of diet on the iron content of milk. Jour. Biol. Chem. **71**: 255-262.
12. Elvehjem, C. A., H. Steenbock, and E. B. Hart. 1929. The effect of diet on the copper content of milk. Jour. Biol. Chem. **83**: 27-34.
13. Fisher, R. A. and S. Bartlett. 1931. Pasteurised and raw milk. Nature **127**: 591-592.
14. Frank, L. C., F. A. Clark, W. H. Haskell, M. M. Miller, F. J. Moss, and R. C. Thomas. 1932. Do children who drink raw milk thrive better than children who drink heated milk? Public Health Reports **47**: 1951-1960.
15. Hamilton, T. S., H. H. Mitchell, and W. B. Nevens. 1929. Ill. Agr. Exp. Sta. An. Rept., 1928-1929. p. 120.
16. Hart, E. B., H. Steenbock, C. A. Elvehjem, and J. Waddell. 1925. Iron in nutrition. I. Nutritional anemia on whole milk diets and the utilization of inorganic iron in hemoglobin building. Jour. Biol. Chem. **65**: 67-80.
17. Hart, E. B., H. Steenbock, J. Waddell, and C. A. Elvehjem. 1928. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. Jour. Biol. Chem. **77**: 797-812.
18. Hess, A. F. 1916. Infantile scurvy. III. Its influence on growth (length and weight). Amer. Jour. Dis. Children **12**: 152-165.
19. Hill, R. L. 1928. The physical curd character of milk and its relationship to the digestibility and food value of milk for infants. Utah Agr. Exp. Sta. Bull. 207.
20. Hill, R. L. 1931. Soft-curd milk. Utah Agr. Exp. Sta. Bull. 227.
21. Kemmerer, A. R., C. A. Elvehjem, E. B. Hart, and J. M. Fargo. 1932. The nutritive value and efficiency of mineralized milk. Amer. Jour. Physiol. **102**: 319-324.

22. Kramer, Martha M., E. Latzke, and M. M. Shaw. 1928. A comparison of raw, pasteurized, evaporated, and dried milks as sources of calcium and phosphorus for the human subject. Jour. Biol. Chem. **79**: 283-295.

23. Krauss, W. E. 1931. Studies on the nutritive value of milk. I. The deficiencies of an exclusive milk diet and how to overcome them. Ohio Agr. Exp. Sta. Bull. 477.

24. Lane-Claypon, Janet E. 1916. Milk and its hygienic relations. Longmans, Green and Co. pp. 175-205.

25. Leighton, G. and P. L. McKinlay. 1930. Milk consumption and the growth of school children. His Majesty's Stationery Office.

26. Mann, H. C. C. 1928. The safety factor of pasteurised milk. Proc. World's Dairy Congress, 127-133.

27. Mattick, E. C. V. and J. Golding. Relative value of raw and heated milk in nutrition. Lancet **220**: 662-667.

28. McCrudden, F. H. 1911-1912. The determination of calcium in the presence of magnesium and phosphate: the determination of calcium in urine. Jour. Biol. Chem. **10**: 187-199.

29. Mitchell, H. H. and J. R. Beadles. 1930. The paired-feeding method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins. Jour. Nutrition **2**: 225-243.

30. Rose, M. S. and E. M. Vahlteich. 1932. Factors in food influencing hemoglobin regeneration. I. Whole wheat flour, white flour, prepared bran, and oatmeal. Jour. Biol. Chem. **96**: 593-608.

31. Rosenau, M. J. 1909. Milk and its relation to the public health. Treasury Department Hygienic Laboratory Bull. 56, 637-680.

32. Schmidt-Nielsen, S. and Schmidt-Nielsen. 1929. Kgl. Norske Videnskab. Selsk. Forhandl. (Trondhjem) **1**: 126-128. Abstracted in Biol. Absts. **4**: 94 (1930).

33. Schwartze, E. W., F. J. Murphy, and R. M. Hann. 1930. Studies on the destruction of vitamin C in the boiling of milk. Jour. Nutrition **2**: 325-352.

34. Scott, E. and L. A. Erf. 1930. The influence of different grades of milk in the production of anemia in the albino rat. Proc. Amer. Assoc. Medical Milk Comm., Inc., and Certified Milk Producers' Assoc. of America, Inc., 78-87.

35. Scott, E. and L. A. Erf. 1931. Natural milk proves best. Jersey Bull. **50**: 210-211; 224-226; 237.

36. Scott, E. and L. A. Erf. 1931. Effects of cattle feeding and pasteurization on food value of milk. Proc. Internat. Assoc. Dairy and Milk Inspectors. pp. 18.

37. Stugart, R. 1931. Determination of iron in milk and other biological materials. Ind. and Eng. Chem., Anal. Ed., **3**: 390-393.

38. Todhunter, E. N. 1932. A comparison of the vitamin G values of pasteurized milk, evaporated milk and eggs. Jour. Amer. Dietetic Assoc. **8**: 42-46.

39. Waddell, J., H. Steenbock, C. A. Elvehjem, and E. B. Hart. 1928. Iron in nutrition. V. The availability of the rat for studies in anemia. Jour. Biol. Chem. **77**: 769-775.

40. Waddell, J., H. Steenbock, and E. B. Hart. 1929. Iron in nutrition. X. The specificity of copper as a supplement to iron in the cure of nutritional anemia. Jour. Biol. Chem. **84**: 115-130.

41. Wallen-Lawrence, Z. and F. C. Koch. 1930. The relative digestibility of unsweetened evaporated milk, boiled milk and raw milk by trypsin in vitro. Amer. Jour. Dis. Children **39**: 18-33.

42. Willard, A. C. and K. Blunt. 1927. A comparison of evaporated with pasteurized milk as a source of calcium, phosphorus, and nitrogen. Jour. Biol. Chem. **75**: 251-262.