STUDIES OF EXPERIMENTAL SCURVY.

EFFECT OF HEAT ON THE ANTISCORBUTIC PROPERTIES OF SOME MILK PRODUCTS.*

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The recent work of Chick and Hume, Cohen and Mendel, Hess and Unger, and Givens and Cohen gives full support to the earlier views of Hoist and Frölich that scurvy is the result of a deficiency of some nutritive factor in the diet. If we understand by the term vitamine the chemically unknown substances of food origin essential for the normal performance of the function of animal life there is perfect propriety in applying to the above mentioned factor the name antiscorbutic vitamine. As polyneuritis is caused by a lack of the water-soluble vitamine (anti-neuritic vitamine) and xerophthalmia by a lack of the fat-soluble vitamine (antixerophthalmic vitamine), scurvy is caused by a lack of the antiscorbutic vitamine.

Opposed to this view of the etiology of scurvy was the theory of McCollum and Pitz which postulated that scurvy is related to

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intestinal putrefaction and retention of feces. As pointed out by Chick and Hume, the conclusions of the aforementioned investigators were based on data obtained from experiments where a vitiating factor was constantly operative, in that all the experimental animals were allowed milk *ad libitum*. As milk contains the antiscorbutic vitamin, the incidence of scurvy would consequently be dependent not only on the animal's idiosyncrasy in liking or disliking milk, but also on any change in experimental conditions which tended to modify the fluid intake. Chick and Hume were able to secure some protection against experimental scurvy in guinea pigs when 50 to 100 cc. of milk were given daily per individual, and when 100 to 150 cc. were given scurvy was entirely prevented. In all cases the milk allowance was superimposed on a basal ration of oats and bran.

This work on the rôle of milk we have repeated and confirmed, entirely preventing the development of scurvy with a diet of rolled oats, hay, and 100 cc. of whole milk per individual per day, and delaying the appearance of scurvy in guinea pigs for 18 weeks by a daily allowance per individual of 30 cc. of whole milk and a diet of rolled oats and dried hay. It is altogether probable, therefore, that the development of the theory of the intestinal origin of scurvy became connected with variations in the milk consumed by animals receiving different treatments.

The fact that Hess\(^7\) was able to cure scurvy in guinea pigs by intravenous injection of orange juice gives incontrovertible evidence of the existence of an antiscorbutic vitamin. So conclusive has become the evidence for the existence in our foodstuffs of this third class of unknowns that we accept this point of view as now fully established.

The point of view that there does exist a relatively unstable antiscorbutic vitamin in our foods offers a satisfactory explanation of the prevalence of scurvy among infants fed milk of which the origin and heat treatment may have been variable. It opens for study the question of the variation in antiscorbutic vitamin content of milks produced under various conditions. The fact, first demonstrated by Holst and Frölich and further developed by Hess and by Givens and his associates, that mere

drying of plant tissue destroys considerable quantities of this vitamin, possibly places the feeding of cows for special purposes, as for the production of a milk rich in the antiscorbutic factor, in a new light and calls for investigation. Further, with the recognition of a third class of vitamins, in addition to the fat-soluble (antixerophthalmic) and water-soluble (antineuritic) types, as essential to the life of certain species as man, monkey, and guinea pigs, comes the question of its relation to the life of other species of animals. Does the guinea pig need an abundance of this vitamin and therefore fail on a diet of corn-meal, peas, and dried alfalfa flour, — low in the antiscorbutic factor— while the rat, cow, or pig needs less or none of it, and therefore succeeds on such a ration? These are questions of great interest and fundamental to a complete understanding of all the factors involved in animal nutrition. A recent paper by Harden and Zilva involves an investigation of this question with special reference to the rat.

The data presented in this paper involve a repetition of some of the earlier experiments conducted in this laboratory and, in addition, demonstrate that commercial condensed milks, commercial milk powders, and milks sterilized for 10 minutes at 120°C. have suffered the destruction of their antiscorbutic vitamin as determined experimentally with the guinea pig.

An excellent description of the symptomatology of guinea pig scurvy is given by Cohen and Mendel. Our experience in following the course of this disease coincides very well with their observations. There occurs tenderness of the joints which provokes crying when touched; a progressive swelling of the affected joints, which, if not cured, become permanently hardened into exostoses; and a slowly increased inability to use the hind legs, which finally develops into an apparent paralysis of those parts. At this latter stage the animal lies on its side or back a considerable part of the time, taking the “face ache” position; the lower molars become loose, being easily picked out with a forceps, and in some cases there is an appearance of hemorrhage at the base of the lower incisors. At autopsy the junctions of the ribs and cartilages are enlarged, the bones are easily breakable, and conditions of hemorrhage or congestion are usually noticeable.

Harden, A., and Zilva, S. S., Biochem. J., 1918, xii, 408.
The diet chosen as one invariably producing scurvy in the guinea pig was rolled oats and dried hay—a mixture of June grass and timothy hay (see Chart 1). Fresh milk in the diet was avoided unless its consumption was quantitatively controlled because of its power, in an oat-hay diet, to prevent scurvy if consumed in large enough quantities. In the absence of hay and with a diet of rolled oats and fresh whole milk, involving an average daily consumption of 47 cc. of milk per individual, the guinea pig will develop scurvy (see Chart 2). In the presence of the hay a somewhat less amount of milk (30 cc.), consumed daily per individual, will prevent or at least greatly delay the onset of scurvy (see Chart 3). These results can be interpreted in two ways: either the hay still contains some of the antiscorbutic vitamine, which in addition to that in the milk and grain is sufficient to prevent the development of the disease, or the hay, as a source of roughage, or of mineral matter as pointed out by Pitz, improves the conditions for normal nutrition to a degree sufficient for a smaller amount of the antiscorbutic factor to suffice. With a daily allowance of 30 cc. of fresh whole milk per individual, together with a ration of rolled oats and hay—the latter always fed separately and ad libitum—there was a delayed development of scurvy, but absolute protection was not secured (see Chart 3). One animal, after giving birth to young, gradually developed the disease, while the other three animals showed symptoms of enlarged joints only after being on the ration 20 weeks.

Where 100 cc. of whole raw milk were allowed daily per individual and an average daily consumption per individual of 84 cc. was actually recorded with a basal ration of rolled oats and hay, absolute protection from scurvy was secured (see Chart 4). Even at the present writing and after a period of observation of 26 weeks, the animals appear in perfect condition. This work confirms the work of Chick and her associates on the quantitative relation of raw milk consumption to the development of a scorbutic condition in the guinea pig.

With either rolled oats and hay or rolled oats and milk we have been unable to prevent the development of scurvy by the administration of mineral oil. Lot 5, Chart 5, receiving a diet of

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Pitz, W., *J. Biol. Chem.*, 1918, xxxiii, 471.
rolled oats and hay was given 1 cc. of mineral oil per individual every other day after the first symptoms of scurvy appeared. They all died of the disease. Lot 6, Chart 6, on a diet of rolled oats and milk *ad libitum* (but an average daily consumption of 47 cc. per individual per day) developed scurvy from which they died at the end of 6 to 8 weeks. They had received 1 cc. of mineral oil per individual on alternate days starting from the time of the inauguration of the experiment. Even with a diet of rolled oats and hay, but with 1 cc. of mineral oil per individual administered daily from the beginning of the experiment, the development of scurvy could not be prevented (see Chart 7). There is but one conclusion possible from these data, namely, that mineral oil, acting as a laxative, cannot *per se* prevent the development of scurvy.

Phenolphthalein administration was also ineffective in preventing the development of scurvy on a diet of rolled oats and hay (see Chart 8). To this group 2 mg. of phenolphthalein per individual were administered on alternate days by gelatin capsule. Whether the administration of the phenolphthalein was begun at the initiation of the experiment (see Chart 9) or after the onset of scurvy symptoms, the final results were the same. All the animals died of scurvy. The more mature animals were as sensitive to the development of scurvy as those less mature and the disease terminated their lives in 5 weeks.

Pitz had observed that lactose could prevent the development of scurvy in guinea pigs receiving a diet of rolled oats, a salt mixture, and fresh milk *ad libitum*. The fact that again milk was accessible to the animals for *ad libitum* consumption makes the conclusion questionable. The lactose may have increased the milk consumption or possibly may have carried some of the antiscorbutic vitamine, as the product was not especially purified in respect to its vitamine content. The results secured with Lot 10, Chart 10, where unheated lactose (Merck) was used with a diet of rolled oats and hay and with Lot 11, Chart 11, where the same quality and quantity of lactose were used, but after having been heated to 120°C. for 4 hours, are conclusive evidence that lactose in itself is not an antiscorbutic. All the animals in both groups died of scurvy in 4 to 5 weeks. They ate the rations well for 2 weeks as indicated by their increments in live weight.
Of special interest to us was the effect on the guinea pig of certain rations known by long experience to be satisfactory for cattle and swine. Rations consisting of intimate mixtures of rolled oats 84 parts, dried corn-stover 15 parts, and common salt 1 part (see Chart 12); or dried alfalfa hay 25 parts and rolled oats 75 parts (see Chart 13); or dried corn-stover 49 parts, rolled oats 50 parts, and common salt 1 part (see Chart 14); or corn-meal 35.7 parts, gluten feed 14.3 parts, dried corn-stover 49 parts, and common salt 1 part (see Chart 15); all produced scurvy in the guinea pig in from 4 to 5 weeks. Such rations gave no apparent nutritional disturbances over long continued use with cattle or swine. For example, we have reared cattle to maturity and obtained very successful reproduction on a ration of corn-meal 35.7 parts, gluten feed 14.3 parts, dried corn-stover 49 parts, and common salt 1 part. It is true, as far as we are aware, that scurvy has not been reported in cattle or swine, but it is difficult to conceive why any mammal should be exempt from the needs of this class of vitamins, while the guinea pig, monkey, and man are extremely sensitive to its absence or a low supply. Probably it is wholly a matter of different quantitative demands and therefore the rations mentioned may have supplied a sufficient quantity of this nutritive factor for the normal performance of such species as cattle, swine, or rats.

Antiscorbutic Properties of Sterilized Milk.

Fröhlich¹⁰ and later Chick, Hume, and Skelton¹¹ have called attention to the poverty of heated milks in the antiscorbutic vitamin. Hess and Fish¹² and later Hess¹³ reported a mild outbreak of infantile scurvy caused by the use, for several months, of a diet of cow's milk previously pasteurized at 63°C. for 30 minutes. The scurbutic symptoms disappeared on the restoration of the previously used antiscorbutic, orange juice, or on the substitution of raw milk for the heated milk.

¹⁰ Fröhlich, T., Z. Hyg. u. Infektionskrankh., 1912, lxxii, 155.
We have not as yet done any work with pasteurized milk, but have investigated, with respect to their antiscorbutic properties, milk sterilized at 120°C. for 10 minutes, two brands of unsweetened condensed milk, and one commercial brand of skimmed milk powder.

Milk sterilized at 120° for 10 minutes has lost its scorbutic protective powers for the guinea pig receiving a diet of rolled oats and dried hay (see Chart 16). A daily allowance per individual of 100 cc. of milk was made, but not all of this was consumed. The average daily consumption per individual, had it been raw milk, would have adequately protected them against scurvy. As it was, two of the animals died of scurvy at the end of 7 and 9 weeks respectively, after having consumed daily 47 cc. of milk per individual. The other two animals showed severe symptoms of the disease at the end of 4 weeks and had begun to lose weight. Their average daily milk consumption per individual had been 43 cc. At this point 1 cc. of orange juice per individual was administered daily by pipette with rapid recovery and gain in weight, demonstrating the adequacy of the diet in respect to all other factors but the antiscorbutic vitamine. The average daily milk consumption of these animals was much less during the orange juice-free period, but, including the orange juice-free period, the average milk consumption was 79 cc. per day per individual for the entire period of observation.

Antiscorbutic Properties of Unsweetened Condensed Milk.

The brands14 of unsweetened condensed milk with which we have worked have also lost their antiscorbutic properties. The heat treatment given milks in the milk condensing industry varies considerably during different seasons of the year and also in different localities. A large condensing concern in reference to this matter of heat treatment states as follows:

14 "The general statement can be made that the pre-heating temperatures vary from 82.5°C. to 88.5°C. and the time from one minute to twenty minutes. The condensing temperature runs from 55°C. to 70°C.; the time

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depending on the amount of milk condensed. The sterilizing temperatures run from 107°C to 115.5°C, the time varying from twenty minutes to fifty minutes."

This is a more severe treatment than the sterilization of milk at 120°C for 10 minutes. To test its antiscorbutic properties the condensed milk was diluted with an equal volume of water, making its content in solids equivalent to normal milk. A daily allowance to each animal of 100 cc. of this diluted milk was made, together with the usual ration of rolled oats and hay. The average daily milk consumption, had it been raw milk, would have amply protected against such an early attack of scurvy (see Chart 17). Two of the animals died from scurvy at the end of 3 and 5 weeks respectively. Their average daily consumption of milk per individual was 42 cc. The other two animals developed severe symptoms of the disease at the end of 5 weeks, at which time 1 cc. of orange juice per individual was administered by pipette daily. Rapid recovery followed, with pronounced increase in weight. The average daily consumption of milk per individual was 50 cc. for the entire period. While 30 cc. of whole raw milk on a rolled oats-hay diet offered protection against scurvy for 15 to 18 weeks, yet with these products, over 40 cc. per individual per day offered no protection whatever.

Antiscorbutic Properties of Milk Powder.

Judging from our limited data on milk powders accumulated up to the present time these products have likewise lost their antiscorbutic properties. Probably most of the milk powders on the market today are made by the spray process and here again there is no rigid uniformity among manufacturers as to time and heat treatments in the various stages for the process. One manufacturer informs us that in his plant the skimmed milk is heated for 20 minutes at 94°C and then condensed in a vacuum from about five to one volume. The milk is next cooled rapidly and held in storage for the powder mill. The condensed milk is then run through a Jensen pasteurizer and heated to 60°C just ahead of the hydraulic pump which forces the milk through the spray. The powder room is held at a temperature ranging from 70–77°C. The powder lies in this room during the day’s run and is taken
out in the evening. Another manufacturer first condenses the milk and then pre-heats it to 49°C. and with the aid of a pump developing a pressure of 2,000 pounds per square inch, forces the spray into the powder room. A continuous current of hot air at an approximate temperature of 149°C. is forced into this room at a rate of 14,000 cubic feet per minute, which, when it comes in contact with the milk spray, immediately loses its high temperature of 140°C. and falls to 82°C. Gradually the temperature of 82°C. is reduced through the various channels of operation to a temperature of 55–65°C.

It is clear that the milk powders on the market have been exposed to heat treatments of varying degrees and probably in most cases of sufficient severity to destroy the antiscorbutic vitamine or at least greatly reduce its quantity. More data, however, are required to establish this point fully. In the light of our experience the report of the United States Public Health Service15 on British experience in the use of milk powder in infant feeding is confusing. This report indicates a general success with milk powders in infant feeding, although it advises that "The occasional use of fruit juice is desirable."

In the records shown in Charts 18, 19, and 20 but a single brand16 of milk powder was used. In our first experiments, Chart 18, 10 parts of the milk powder were mixed with 90 parts of rolled oats and the mixture was fed with the dried hay; the latter, as in all other experiments, was allowed in a separate container. Normal guinea pigs of 200 to 250 gm. in weight will consume daily 15 to 18 gm. of the oat-milk powder mixture. This amount of food, containing 10 per cent of milk powder, would mean the consumption of an equivalent in raw milk of but 15 to 18 cc. per day. All the animals developed scurvy and died in 4 to 5 weeks. The allowance of milk powder was insufficient to test adequately the problem in hand.

In our next experiment with the same powder (Chart 19) the milk powder constituted 25 per cent of the oat-milk powder mixture. With a similar daily consumption per individual of 15 to 18 gm. of the oat-milk powder mixture an equivalent of 40 to 45

16 This brand was a Merrill Soule powder, concerning which no record of heat treatment could be secured.
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cc. of raw milk would have been consumed. This quantity of raw milk would have been adequate for at least a long delay in the development of scurvy symptoms. Yet all these animals died with typical scurvy in 5 to 6 weeks.

In Lot 20, Chart 20, the proportion of skimmed milk powder was further increased and made 50 per cent of the oat-milk powder ration. With guinea pigs of 250 to 275 gm. in weight and a daily consumption of 15 to 18 gm. of the mixture per individual, there would be a daily consumption of milk powder equivalent to 75 to 90 cc. of raw skimmed milk. Yet on this liberal allowance, which as raw milk would have permanently protected against scurvy, all the animals succumbed to the disease in 5 to 15 weeks.

The lesson is obvious. Either the results with guinea pigs on experimental scurvy should not be translated to infantile scurvy, or we should follow the wiser course of using some antiscorbutic in conjunction with the exclusive use in infant feeding of such heated milk products as described in this paper.

SUMMARY.

1. On a diet of rolled oats and hay the prevention of scurvy by the use of raw milk will depend upon the amount of raw milk allowed. This confirms the results of Chick and her associates.

2. Such laxatives as mineral oil or phenolphthalein or the sugar, lactose, are not in themselves preventives of scurvy.

3. Milk sterilized at 120°C, for 10 minutes, commercial unsweetened condensed milk, and the commercial milk powder examined had lost their antiscorbutic properties when used in quantities equivalent to an amount of raw milk which would prevent scurvy in guinea pigs on a diet of rolled oats and dried hay.
CHART 1. Lot 1 received a diet of rolled oats and dried hay (a mixture of timothy and June grass). These materials were fed separately, the hay being put in the cage uncut. The hay was eaten with a relish. This ration provided all the factors of nutrition, except a good protein mixture and the antiscorbutic factor. Guinea pigs invariably died of scurvy on such a ration.

S = Scurvy.
+ = Died.

CHART 2. All the animals in Lot 2 died of scurvy. Without the hay and with nothing but rolled oats and milk ad libitum (actual consumption of milk shown in chart) they were unable to survive. The quantity of whole milk consumed was undoubtedly too small to act as an efficient scorbutic protector.
CHART 3. This lot received rolled oats, dried hay, and a small allowance per individual of fresh whole milk. By a daily consumption of 30 cc. of fresh whole milk per individual, there was great delay in the onset of scurvy. The hay probably contributed some of the necessary anti-ascorbatic, in addition to roughage, salts, and fat-soluble vitamin, and through this improvement in the ration over a rolled oats-milk diet made possible an effective protection against scurvy with a small supply of milk. Animals 13, 15, and 16 showed enlargement of the joints at the end of 20 weeks, indicating a mild condition of scurvy.

CHART 4. Complete protection against scurvy on a rolled oats-dried hay diet when the fresh whole milk consumed daily per individual equaled 60 cc. or more.
On a diet of rolled oats and dried hay mineral oil did not protect the guinea pig against the course of scurvy after it had developed. The theory that scurvy is primarily related to intestinal disorders is untenable.

The behavior of this lot is further evidence of the non-protection against scurvy by the use of a laxative such as mineral oil. A diet of rolled oats and whole milk (with an average daily consumption per individual of 42 to 56 cc.) produced scurvy even with an administration on alternate days of 1 cc. of mineral oil per individual. The milk addition improved the ration for growth. Where cures or preventions of scurvy by the use of a laxative have been accomplished on a diet of rolled oats and milk ad libitum, such as reported by McCollum and Pitz, it is probable that a larger consumption of milk occurred than was here observed.
CHART 7. Administration of the mineral oil from the time the animals were placed on the diet of oats and hay was equally ineffective in protection against scurvy (cf. Chart 5).

CHART 8. Phenolphthalein was ineffective as an antiscorbutic with guinea pigs receiving a diet of oats and dried hay. Administration of the phenolphthalein was begun after the early symptoms of scurvy appeared.
CHART 9. Administration of phenolphthalein on alternate days from the beginning of the experiment failed to protect guinea pigs, receiving a diet of rolled oats and dried hay, against the development of scurvy.

CHART 10. This Chart illustrates the record of guinea pigs receiving a diet of rolled oats, dried hay, and unheated lactose. They all died with scurvy. On a diet of rolled oats, salt mixture, and milk, Pitz had succeeded in dispelling the symptoms of scurvy by the use of lactose. The probable explanation of that result lies in a larger milk consumption by the animals after lactose administration. This would then mean that recovery was really due to an increased intake of the antiscorbutic vitamine.
CHART 11. Lactose heated for 4 hours at 120°C., to destroy any antiscorbutic factor that it might contain, differed in no way from unheated lactose in its effects on the development of scurvy on a diet of rolled oats and dried hay.

CHART 12. This Chart illustrates how a diet, satisfactory for one species, will fail with another. Cattle or swine could be reared at a slow rate of growth and maintained on a ration of rolled oats 84 parts, corn-stover 15 parts, and common salt 1 part, but this dried material is too poor in the antiscorbutic to prevent scurvy in the guinea pig. This may mean only that cattle and swine demand less of the antiscorbutic factor than do guinea pigs.
Another illustration of the failure of a dried roughage, such as alfalfa, to protect against scurvy in the guinea pig. A ration of rolled oats and dried alfalfa would be adequate for swine, cattle, or rats, as far as maintenance is concerned, although it would not allow normal growth in swine or rats; but with the guinea pig it leads to scurvy. This is interpreted as a difference in the antiscorbutic demands of the species and as an indication that the rolled oats and dried alfalfa have lost much of their antiscorbutic vitamin in drying or aging.

Even a larger proportion of dried corn-stover in the diet (49 per cent as contrasted with 15 per cent, see Chart 12) will not protect the guinea pig against scurvy. The roughages, such as alfalfa or corn-stover, that were used in these experiments were the ordinary dried material stored in the University barn from the crop of 1918.
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Chart 15. A ration which has grown and maintained cattle for 4 years as the sole diet, but which produced scurvy in the guinea pig. Either cattle are not susceptible to the disease or their demands for the antiscorbutic vitamin are relatively less.

Chart 16. Milk sterilized at 120°C for 10 minutes has lost or suffered a great reduction in its antiscorbutic content. On a diet of rolled oats, hay (dried), and 30 cc. of fresh milk daily per individual the development of scurvy can be greatly delayed (see Chart 3). Yet, with a similar diet and a consumption of 46 and 47 cc. of sterilized milk scurvy terminated the life of Animals 99 and 100 in 7 to 9 weeks. The curves of Animals 101 and 102 illustrate the recovery from scurvy brought about by a diet of rolled oats, hay, and sterilized milk, but with the daily administration of 1 cc. of orange juice per individual. After the administration of the orange juice and recovery the average milk consumption per day was considerably increased.
CHART 17. The brands of unsweetened condensed milk used in these experiments had also lost their antiscorbutic properties. The consumption of this milk, diluted so as to be equivalent in concentration to normal fresh milk, was 36 to 52 cc. per individual per day. All developed scurvy in 3 weeks and the disease was allowed to run its course in the cases of Animals 95 and 96. To the other two animals 1 cc. of orange juice was given daily by pipette with complete recovery, except for the permanent enlargement of the joints.

CHART 18. The brand of commercial milk powder examined possessed no protective qualities against scurvy. On a diet of rolled oats, dried hay, and a daily allowance of milk powder, equivalent to 15 to 18 cc. of skim milk per individual, scurvy terminated the life of the animals in 5 weeks.
CHART 19. Another failure on milk powder. When the allowance of milk powder was increased to an amount equivalent to 40 to 45 cc. of raw milk per individual per day, with a diet of rolled oats and hay, scurvy rapidly developed and all of this group died of the disease in 5 to 6 weeks.

CHART 20. A still more liberal allowance of milk powder than was made with the lots whose curves are shown in Charts 18 and 19, failed to prevent the development of scurvy. The milk powder allowed per individual per day was equivalent to 75 to 90 cc. of fresh skim milk. Three of the animals rapidly succumbed to the disease, lasting but 5 to 7 weeks. Animal 56 developed scurvy as early as the other three in the lot, but in spite of the progress of the disease it started to regain in weight after the first period of 6 weeks, but finally died of scurvy at the end of 15 weeks.
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