XVI. THE ANTI-SCORBUTIC VALUE OF DRIED MILK.

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A CONSIDERABLE conflict of evidence exists as to the presence or absence in dried milk of the anti-scorbutic accessory food factor, or vitamin, commonly referred to as "water-soluble C." The experiments described in this paper were an attempt to clear up some of the discrepancies.

The animals used were guinea-pigs, and the methods those of previous investigators, except where it seemed to us necessary to make certain modifications by way of additional safeguards.

Care was taken as far as possible to eliminate abnormal animals before experiment. In every case the young animal weighing about 250 g. was put into a cage on a normal diet of green-stuff (cabbage or cauliflower-leaf) with oats and bran, and was only given a milk diet when it showed a normal and steady rate of growth, and had reached 300–350 g. Before the experimental diet was begun, all hay was removed from the cages, and sawdust alone used as bedding.

The normal diet of a guinea-pig contains no moisture other than that associated with the cabbage-leaves or other vegetable matter on which it lives, and we found that, in the absence of green-stuff (as in the case of certain animals that we put on to a purely scorbutic diet), only about 50 cc. of water were taken during the day. Whatever the nature of the food, it is obviously unreasonable to expect animals, whose constitution is only adapted to the taking of a small quantity of liquid, to take several times this quantity and still maintain perfect health. To avoid, therefore, undue dilution of the food the milk-powder was made into a thin cream with *three times its weight of hot water*. We were thus giving our reconstituted milk in a form twice as concentrated as that used by Barnes and Hume [1919], and other investigators.

In order to make the reconstituted milk as palatable as possible to the animals, it was made up freshly every morning and evening, about twice as much being given for the night as for the day. It was reconstituted with hot water and not with cold or boiling water, in order to postpone as far as possible the formation of a layer of solid fat on the surface, which seemed

to be resented by the animals, and also because they appeared to us actually to prefer it with the chill off. Fresh oats and bran were given every morning, but not until after the milk had been put in the cages, in order to prevent the animal satisfying its appetite on the oats and bran, and then feeling disinclination for the milk. Hand-feeding was only necessary in quite a few cases, for the first day or two of an animal's change of diet. The disturbing effect on a guinea-pig of so unusual a diet as milk is only accentuated by so unusual a method of nutrition as hand-feeding with a glass-tube. In all cases sufficient food was given to ensure that some was left over when the next allowance was supplied.

Four series of experiments were undertaken. In the first three, comparison was made of summer milk, winter milk, and winter milk neutralised with sodium bicarbonate before drying. All three were dried by the roller process, in which the milk is in contact with the source of heat for less than three seconds. The fourth variety was full-cream milk dried by the spray process. This was purchased direct from the makers and packed into air-tight tins immediately on arrival. We have no details as to the time for which such milk is heated during manufacture but the remarks made by Hart, Steenbock and Smith [1919], and others, would suggest that it is subject to prolonged heating.

The winter milk was dried by one of us (H. J.) in the first week of December, 1919. The weather was cold and wet, and no cattle were in the fields. The milk when received was perfectly fresh, having an acidity of 17.3° and was immediately dried, sifted, and placed in air-tight tins. The neutralised winter milk was dried at the same time. The acidity of the milk before drying was 26.5° and 11 ozs. of sodium bicarbonate were added to 38 gallons. The summer milk was part of a considerable quantity of milk dried at the same factory in late August and early September. Its acidity before drying was 19.9° , and the powder was stored in hermetically sealed tins, and transferred to smaller air-tight tins for experimental purposes. We give below the composition of the powders:

Table I.

	Winter milk	Summer milk	Neutralised winter milk	Spray process
Moisture	2.6	2.8	3.3	4.3
Ash	5.9	5.8	6.6	5.6
Fat	26.7	27.0	27.2	28.2
Lactose	37.3	36.3	35.4	35.6
Protein	27.0	27.8	26.5	25.6
	99.5	99.7	99.0	99.3

It will be observed that the general composition of these milk powders was almost identical.

Our experiments were begun in January 1920 in the case of the winter and the summer milk series. The experiments in the summer milk series

were completed by the end of May, while the experiments in the winter milk series were concluded at the beginning of July. The experiments in the neutralised milk series were begun in the middle of March and were concluded at the end of July.

It will thus be seen that the winter milk was from two to eight months old during the course of the experiments, while the summer milk was from four to ten months old. The neutralised milk was from three to seven months old; we have no information as to the age of the spray milk.

Fig. 1. Diet of oats, bran, and summer dried milk (roller process).

Curves 3, 6, 7 and 8. Death from disease; no scurvy in 7, 10, 21 (Curves 7, 6, 3); slight scurvy in 19 a (Curve 8).

Curve 5. This animal (16) hurt its foot at (a); its food consumption fell off, and it developed mild scurvy; from this it recovered completely, without any change of diet.

Curves 1 and 2. (b) indicates change over to full normal vegetable diet.

Fig. 2. Diet of oats, bran, and winter dried milk (roller process).

Curve 12. Death from disease; no signs of scurvy. Animals 2, 18, and 20 showed similar curves.

Curve 14. Attack of scurvy repelled without change of diet.

Curves 11 and 13. (b) indicates change over to full normal vegetable diet.

Fig. 3. Diet of oats, bran, and neutralised winter milk (roller process).

Curve 24. This animal died from disease, but post-mortem examination showed many of the characteristics of mild scurvy.

Fig. 4. Diet of oats, bran, and dried milk (spray process).

Curves 25, 26, and 27. (c) indicates change of diet to roller process milk. In the case of these three animals (38, 42, and 40) no cure was effected.

Curves for animals 35 and 41, which were cured in this manner, are shown separately (Fig. 5).

Fig. 5. Diet of oats, bran, and dried milk (spray process) followed by dried milk (roller process).

Curves 29, and 30. (c) indicates change from spray process to roller process (summer) dried milk; (b) indicates change over to full normal vegetable diet.

The following table and the curves in Figs. 1-5 record the results of feeding groups of animals upon the four varieties of dried milk.

Table II.

Series I. Summer milk.

Animal no.	Daily consumption in cc., averaged over 30-day period			Length of experiment (days)	Initial weight in g.	Highest weight in g.	Final weight in g.	Remarks
	1.	2.	3.					
5	88	145	121	103	308	572	567	No scurvy
6	122	157	124	108	339	692	685	No scurvy
7	90	119	—	82	302	543	420	Death from disease
10	84	108	123	96	330	564	465	Death from disease
15	97	124	136	115	365	633	612	No scurvy. Chloroformed
16	85	122	129	115	368	636	636	No scurvy. Chloroformed
17	85	120	—	77	302	437	332	Slight scurvy. Death from disease
19a	73	86	—	65	336	427	311	Slight scurvy. Death from disease
21	128	—	—	47	309	542	370	Death from disease

Series II. Winter milk.

1	88	90	160	98	277	530	530	In good health. Chloroformed. Signs of old scurvy
2	107	100	—	73	265	429	421	Died of disease. Slight scurvy in early life
4	117	140	—	73	259	645	645	No sign of scurvy
11	98	143	147	112	546	707	706	No sign of scurvy. Survived
12	88	96	—	71	300	471	308	Slight scurvy. Disease main cause of death
13	91	101	85	112	300	590	577	No scurvy. Chloroformed
18	72	105	—	65	340	392	309	No scurvy. Death from disease
19	117	156	153	129	342	700	683	No scurvy. Chloroformed
20	95	128	—	71	313	604	432	No scurvy. Death from disease

Series III. Neutralised winter milk.

22	117	147	165	137	310	732	714	Slight scurvy. Chloroformed
23	86	132	95	98	306	557	505	Mild scurvy. Chloroformed
24	89	107	96	93	306	539	340	Scurvy main cause of death
25	88	142	126	125	306	565	496	Mild scurvy. Chloroformed

Series IV. Spray process milk.

34	105	—	—	54	338	470	335	Acute scurvy. Moribund. Chloroformed
36	91	—	—	41	350	399	293	Acute scurvy. Moribund. Chloroformed
37	101	98	—	63	358	451	310	Acute scurvy. Moribund. Chloroformed
38	68	(97)	—	52	338	395	248	Severe scurvy
39	78	—	—	38	333	351	223	Acute scurvy. Moribund. Chloroformed
40	95	—	—	37	299	378	235	Died of acute scurvy ¹
42	92	—	—	35	308	380	244	Died of acute scurvy ¹
43	98	(69)	—	38	317	399	247	Acute scurvy. Chloroformed
44	80	(80)	—	48	332	367	241	Died of acute scurvy
45	70	—	—	29	337	328	233	Died of acute scurvy

¹ See below.

In the case of animals 40 to 45 spray process milk was given at a concentration of 1 in 3 in order to ascertain if the animals could be induced to take sufficient of the milk to avoid scurvy.

Average weight curves are given for each series, as well as the normal growth curve obtained by keeping nine animals on full vegetable diet, but under otherwise the same cage conditions as the animals fed upon milk. The curve thus secured shows, unlike that of Barnes and Hume [1919], a gradual decline in steepness, becoming flatter as the animals grew older. It will be noticed that it is a somewhat steeper curve than any of the others; this is only to be expected, for milk is, at best, an inferior food for an animal with a natural vegetarian diet. Nevertheless, the difference between this normal curve and those obtained for summer and winter dried milk is remarkably small (see Fig. 6).

Fig. 6. Average curves for four series of experiments on dried milk, and for a full normal vegetable diet.

It is not considered necessary to give full protocols of all the animals used, but certain of the animals have been of sufficient interest to merit individual comment; we give also individual curves for the majority of animals in each series.

Four animals, two on winter milk (experimental animals 5 and 6) and two on summer milk (experimental animals 11 and 13), after over 100 days on experimental diet had reached a weight that was practically constant, and, to ascertain whether they were full-grown or whether their lack of further growth was due to absence of a sufficient supply of growth-promoting factor, they were then given a normal diet of oats and bran and about 100 g. of cabbage-leaf per diem. No marked growth occurred in any case and the dotted parts of Curves 1, 2, 11 and 13 (Figs. 1 and 2) illustrate this feature.

Certain animals on spray process milk diet were, after they had become definitely scorbutic, placed on summer dried milk (dried by the roller process). In two cases (experimental animals 40 and 42), haemorrhage had already set in, and the animals after a brief rally died (see Table II, Series IV). In two cases, however, a complete cure was effected by the change over from spray process to roller process dried milk.

Table III.

Animal No.	Consumption in cc., average over 30-day periods				Length of experiment (days)	Initial weight in g.	Final weight in g.
35	80 ¹	112	142	170	136	330	611
41	103 ²	118	(121)	—	73	330	490

See also Fig. 5.

¹ Change over on 23rd day, weight 364 g.

² Change over on 29th day, weight 320 g.

In order to give a general comparison of the anti-scorbutic value of the four milks used, we summarise our results in the following table:

Table IV.

	Summer milk	Winter milk	Neut. milk	Spray milk
Under experiment	9	11	4	12
Developed scurvy	2	3	3	12
Survived, or died of causes other than scurvy	6	9	1	—

Two animals on summer milk which died from disease (probably *coccidium* or *pseudo-tuberculosis*) showed signs of scurvy. One consumed throughout considerably less than the average ration of milk (experimental animal 19 a), and the other showed a marked falling-off in its food intake during the three weeks preceding death (experimental animal 17).

Of the three animals on winter milk that showed signs of scurvy, one recovered without change of diet (experimental animal 1), one (experimental animal 12) was weakened by a fall and consequent loss of appetite, and one was recovering from an early attack of scurvy when it succumbed to disease (experimental animal 2). Our conclusion on this point must, therefore, be negative; we have, in fact, found no appreciable difference in anti-scorbutic value between winter and summer dried milk.

When we turn to the actual anti-scorbutic value of dried milk as compared with raw milk, we find considerable discrepancy between our results and those of Barnes and Hume [1919] and Chick, Hume and Skelton [1919]. We are greatly indebted to these workers for the invaluable suggestions contained in their pages, and, as will have been made clear above, have followed their methods very closely. We wish, in conclusion, to point out how we consider this discrepancy has arisen, and how it can be explained.

For purposes of comparison we have expressed the amount of dried milk taken in terms of equivalent cc. of ordinary milk. (Thus, when an animal took 60 cc. of our double-strength concentrated milk containing 15 g. of dried milk and 45 g. of water, we refer to it as having consumed 120 cc. of milk).

It should be observed, in the first place, that up to the publication of a paper by Hart, Steenbock and Ellis [1920] there was no record of any guinea-pig on experimental diet reaching a weight of 500 g.; in most cases, indeed, even 400 g. was not achieved. In the paper of the above mentioned workers a certain advance was made, and out of 54 animals, one reached a weight of over 600 g. and eleven more a weight of over 500 g.

None of our animals when weighing between 300 and 400 g. consumed more than 135 cc. of milk, and in some cases, the average daily consumption was lower than 100 cc. Barnes and Hume [1919, p. 308] state explicitly "that scurvy could be prevented and health maintained in those cases in which 150 cc. (of raw milk) were taken." On the other hand, recent workers [Hart, Steenbock and Ellis, 1920] claim to have obtained complete protection with 50 cc. of fresh summer milk. It seems possible that one source of discrepancy may be the failure to take into account, as well as the amount of food necessary for protection, the size of the animal protected from scurvy.

In very few cases did any of our animals consume appreciably more than 150 cc. of milk, and, even then, only when their weight was well over 500 g. Frequently, at a later period, the consumption actually dropped off; the animal was no longer growing rapidly, and food was mainly needed to maintain metabolism for an animal leading a sedentary life, and not to supply the matter or the energy for building new tissue. Even so, with this smaller intake, the animals still remained free from scurvy.

It would appear, therefore, that on the average growing guinea-pigs can find in milk reconstituted from dried milk as much protection as, according to Barnes and Hume, is afforded by raw milk. It is desirable, if possible, to gain some idea as to what is the minimum of such reconstituted milk required for protection. We have adopted the following method for arriving at this quantity. Ignoring those animals that showed signs of scurvy, since protection in their case was not complete, and eliminating, in the case of those that died from other causes, that part of their career where they were eating little and losing weight (to include those parts would, obviously, quite inaccurately lower the estimated minimum protective dose of milk), we have then divided the time of normal growth into 8-day periods, and for each 8-day period we have calculated the average weight of the animal (W) and the average consumption of milk in cc. (V). The expression $\frac{100V}{W}$ represents the average daily consumption per 100 g. body-weight. This has been calculated for each animal and the average for the two series where there was complete protection from scurvy is also taken. We give below a summary of the results:

Table V.

Animal No.		Period (days)	Minimum $\frac{100V}{W}$	Maximum $\frac{100V}{W}$	Average $\frac{100V}{W}$
1	Winter milk	4- 27	32	36	33
4	" "	4- 73	24	37	29
11	" "	1-104	17	28	23
13	" "	1- 96	19	27	22
18	" "	4- 51	20	35	23
19	" "	4- 67	24	33	29
20	" "	3- 54	20	31	26

Average for winter milk series $\frac{100V}{W} = 27$.

5	Summer milk	10-103	22	35	27
6	" "	3-106	19	31	25
7	" "	3- 88	22	32	27
10	" "	6- 85	21	26	23
15	" "	1- 96	20	34	26
16	" "	35- 96	21	30	25
17	" "	3- 70	19	32	26
19a	" "	4- 59	23	20	25
21	" "	1- 36	29	33	32

Average for summer milk series $\frac{100V}{W} = 26$.

Average for two series (16 animals) $\frac{100V}{W} = 26$.

It will be seen from these figures that the average daily intake of milk in cc. per 100 g. body-weight varies considerably. There is variation in the case of each animal for different 8-day periods in its history; there is variation between the general average of one animal and another. The averages, for the two series, are identical within the degree of accuracy possible for this kind of experiment. The average for the whole series indicates that the minimum protective dose of milk reconstituted from dried milk for a guinea-pig of 400 g. body-weight is about 110 cc.

It has previously been stated [Harden and Zilva, 1918] that alkali has a destructive effect upon vitamins and the results obtained with neutralised milk would indicate that there is some destruction of the anti-scorbutic vitamin by alkali in the quantities used in this experiment. The reduction of the anti-scorbutic value was, however, slight and in no case were symptoms of scurvy observed in less than 100 days; even then they only appeared when rather less than the average quantity of raw milk required for protection was taken.

In series IV, even when animals which took small quantities of milk are eliminated, it can only be concluded that the anti-scorbutic factor had been very considerably affected, even if not entirely destroyed. A description of the spray process, from which it would appear that the milk is held at high temperatures for a very considerable time, is given by Hart, Steenbock and Smith [1919]. It has been suggested by these workers and others [*e.g.* Delf, 1920] that heat is responsible for diminution in the anti-scorbutic value of certain cooked or manufactured foods. Recent work, however, suggests that

oxidation may be an important factor in destroying the anti-scorbutic vitamin [Zilva, 1920].

That winter milk should be found to have as high anti-scorbutic value as summer milk is contrary to the opinions generally held, and to certain other experiments, but enquiry has shown that the cattle were being partly fed upon swedes at the time when the winter milk was obtained, and it is probable that the anti-scorbutic value was due to this cause.

There is some evidence to show that the method of drying may have a considerable influence on the degree to which the anti-scorbutic vitamin in milk is affected. The milks with which we have experimented were subjected to the high temperature of drying for not more than $2\frac{1}{2}$ seconds and, when the milk left the rollers, it was still steaming. This clearly indicates that there was considerable moisture present when the milk ceased to be in contact with the hot surfaces, and its temperature cannot, therefore, have appreciably exceeded 100° at any time. The comparatively low temperature of drying, the short period of contact with the hot rollers, and the relatively short exposure while hot to oxidation (compared with the spray process), may all contribute to the results we have found.

We are aware that under unsuitable conditions of storage, especially exposure to the air, dried milk undergoes marked change, and it is possible that the accessory food factors present are affected. This is a purely theoretical conclusion based upon physical changes in dried milk, and we have not, at present, any experimental evidence to support this view. Other workers [Hess and Unger, 1919] have expressed similar opinions.

We have called attention earlier in this paper to the fact that milk, especially full-cream milk with its high percentage of fat, is hardly a suitable diet for guinea-pigs. The question, therefore, arises how far such experiments are applicable to infant feeding. It has been argued that pasture-fed cattle yield milk of higher anti-scorbutic value than do stall-fed animals. It might equally be argued that human milk would, for the same reason, be markedly deficient in anti-scorbutic value, as compared with cow's milk. Whether or not this is the case cannot be known until experiments have been made with human milk.

We can only conclude from our own work that the results give a considerable measure of support to the clinical opinion, widely held in places where dried milk has been used for infant feeding during many years, that perfectly fresh milk dried by the roller process and stored in air-tight vessels contains sufficient anti-scorbutic accessory food factor to prevent scurvy in infants fed solely on a milk diet [*e.g.* Coutts, 1918].

It should be recognised, however, that whilst such dried milk is apparently equal to raw milk in anti-scorbutic value neither is rich in anti-scorbutic vitamin and where infants are only taking small quantities of milk or are upon a diet which is mainly farinaceous, the addition of a little fruit-juice is not only desirable but essential.

SUMMARY.

Animals have been fed upon summer milk, winter milk, and neutralised milk dried by a roller process and upon milk dried by the spray process. In the case of the summer and winter milks the anti-scorbutic values were found to be about equal to one another and to those of the original raw milks. The neutralised milk had an anti-scorbutic value slightly less than that of the raw milk and the spray process dried milk was markedly deficient in anti-scorbutic value.

Of the summer and winter dried milks a daily dose of 26 cc. per 100 g. body-weight, of reconstituted milk was, on the average, sufficient to afford protection against scurvy in the guinea-pig.

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