

## XI. THE ANTI-SCORBUTIC VALUE OF COW'S MILK<sup>1</sup>.

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### INTRODUCTION.

THE experiments described in the following paper form a small group out of a much larger scheme. The main scheme was planned in order to obtain a comparison between the anti-scorbutic values of a number of foodstuffs, in response to war-time requirements, both civil and military, within the British Empire. Some of the preliminary results have already been published [Chick and Hume 1917; Chick, Hume and Skelton 1918] owing to urgent practical need for their application on active service and at home, and it was intended that a detailed report of the whole work should appear later, when the investigation was complete.

We believe, however, that any contribution to the study of the nutritional value of milk may be of immediate practical value in connection with the problems of infant feeding, particularly at the present time. The following experiments are by no means complete or exhaustive, but, though few in number, they form a complete and concordant series. Work of this character involves great labour and the experiments with milk have had temporarily to be abandoned to permit time and attention to be devoted to other, equally important foodstuffs. It has therefore seemed wisest not to delay publication of the results already obtained.

It has been usual to regard fresh cow's milk as a substance possessing valuable anti-scorbutic properties and capable of preventing scurvy, if present in a diet in even moderate amount. This view is held by many authorities on infant feeding. There does not, however, appear to be any sound basis for such an opinion and though direct evidence upon this point is

<sup>1</sup> A short summary of this paper appeared in the *Lancet*, Jan. 5th, 1918

scanty, what there is, is opposed to such a view. Curran [1847] in his description of the scurvy epidemic in Dublin in 1847, cites more than 80 cases among Union inmates, all of whom had received one pint of milk daily for at least six months before falling ill. The diet in the Unions, however, was deficient in fresh meat and vegetables, and potatoes had been scarce, owing to the potato famine.

More recent evidence upon this point is derived from careful experience of infant feeding. The observations of Hess and Fish [1914], Hess [1916], Miller [1917] and others also suggest that cow's milk is a food-stuff comparatively poor in anti-scurvy substances. They indicate further that many methods of preparation of food for the use of infants, such as heating, drying, etc., tend to diminish the small amount of these valuable constituents originally present, with a distinct resultant risk of causing slight and incipient infantile scurvy. The results of our experimental work are in accord with the observations of these workers, which deserve wide publication and will be referred to in detail later, see page 150.

#### *Experimental.*

From the close analogy in etiology, symptoms, method of cure and post-mortem appearances in case of death, guinea-pig scurvy has generally been accepted as the equivalent of human scurvy by those having experience of both conditions. The guinea-pig has been selected for the study of experimental scurvy on grounds of general convenience and because this animal is particularly susceptible to the disease. If all fresh vegetable food is removed from a young, growing guinea-pig on a diet of grain and water, scurvy symptoms will appear in about 20 days and death will occur in about a month (see Table I and Chart 1). The anti-scorbutic value of various foodstuffs can be estimated by adding these in varying amounts to the scurvy diet of grain and water.

Guinea-pigs were used by Holst and Frölich [1912] in their classic work on experimental scurvy and by Frölich [1912] who specially investigated cow's milk. He found that these animals could be protected from scurvy by an exclusive diet of fresh milk and that raw milk was a better diet than heated milk. It is difficult to interpret his experiments fully, as no indication is given of the quantities consumed.

Funk [1913] states that 50 cc. of fresh unboiled milk daily, in addition to oats, prevented loss of weight and onset of scurvy in guinea-pigs, a result

which we have not succeeded in confirming. His experiments are not, as far as we are aware, set out anywhere in detail.

Jackson and Moore [1916] found that of guinea-pigs fed on pasteurised milk *ad libitum*, 5 remained well out of 32; on raw milk, out of 9, all died. McCollum and Pitz [1917] fed guinea-pigs on oats and fresh cow's milk *ad libitum*, and found that some survived and some died. In both these researches no figures are given for the amounts of milk actually consumed by the animals. The present work is therefore the first in which a special effort has been made to include the quantitative factor.

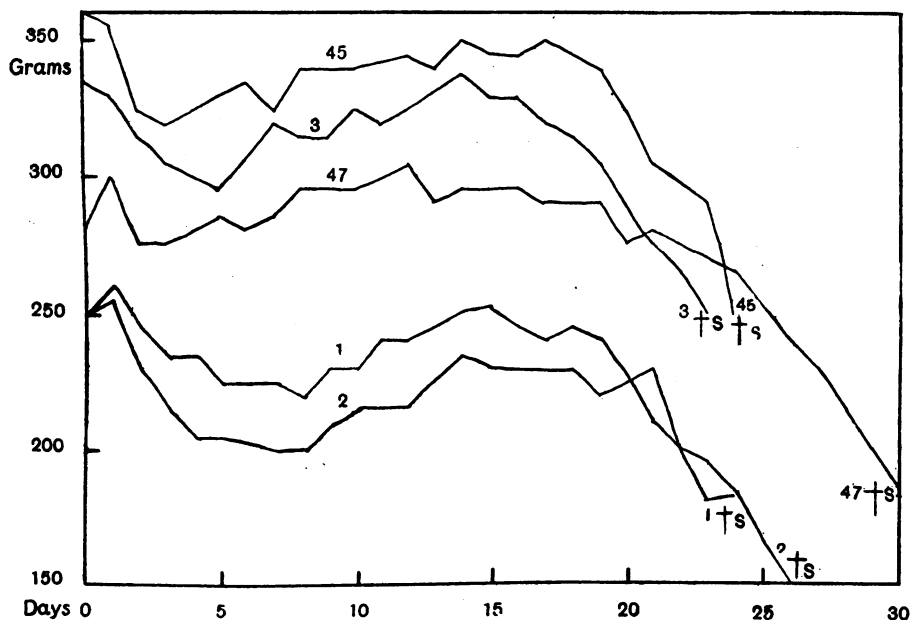


Chart 1. Weight curve of young guinea-pigs upon a diet of oats and bran (*ad lib.*) and water, see Table I. †s signifies death from scurvy.

The general course of events in an unprotected guinea-pig, when grain and water alone are given, is seen on reference to Chart 1 and Table I. The details of 5 young guinea-pigs fed upon oats, bran and water *ad libitum* show a considerable degree of consistency. The initial weight, 250–360 grams, is maintained, but barely exceeded, for a period of 15 to 18 days (see Chart 1), after which it gradually falls till at death the loss is 25–30% of the original. Definite scurvy symptoms were noted after periods of 17–22 days; and death from scurvy occurred between the 23rd and 30th day.

TABLE I. *Development of Scurvy in Young Guinea-pigs upon a diet of Grain and Water.*

No. of experiment	No. of animal	Diet	Weight of animal, g.				Length of experiment, days	Day on which symptoms of scurvy were first noted	Result	
			At beginning	Highest reached	At end	Change in weight, %				
1	1	{ Oats, wheaten bran, water, <i>ad lib.</i> * }	250.	260	182	-37	24	uncertain	{ Death from scurvy, }	24th day
2	2	do.	250	250	150	-40	26	22	do.	26th "
3	3	"	335	338	250	-25	23	17	"	23rd "
4	45	"	360	360	250	-31	24	21	"	24th "
5	47	"	280	300	185	-34	30	17	"	30th "

\* In health the animals consumed about 30-50 g. oats and bran daily; when sickening with scurvy the amount decreased to 10-20 g. or less.

The use of a basal diet of oats, bran and water is open to the criticism that it is deficient in another accessory food-factor besides the anti-scorbutic one, *i.e.* the "Fat Soluble A" of McCollum and others, and that therefore any experiments performed with it will be subject to two limiting factors instead of one. We have therefore made it our general custom in studying experimental scurvy to add to the diet of oats and bran, 60 cc. a day of milk autoclaved at 120° C. for an hour: of this from 30 to 60 cc. were usually consumed. We have not found that this addition delays the onset of scurvy symptoms but it does alter the character of the weight curve. If Chart 1 (oats, bran and water) be compared with Chart 2 (oats, bran and autoclaved milk), it is seen that while on the first diet, weight is maintained till the first onset of scurvy symptoms, on the second diet there is a steady gain in weight for about the same period.

This effect of milk in maintaining growth after it has been deprived of its anti-scorbutic vitamine is interesting in view of the results obtained by Hopkins [1912] and later by McCollum and Davis [1913] and by Osborne and Mendel [1913] in their experimental work on growth. The accessory growth factor, termed "Fat Soluble A," of which one important source is butter fat, would appear to be operating in our autoclaved milk, and to be distinct from the anti-scorbutic factor.

Further experiments are in progress in order to test whether this explanation is correct or whether the influence of the milk ration in promoting growth is, in this case, due simply to its content of fat, protein and salts.

These American investigators, working with young rats, have arrived at the conclusion that, in addition to adequate provision of protein, carbohydrate, fat and salts, for nutritive purposes two accessory factors are required, if satisfactory growth is to be maintained. The first is termed "Fat Soluble A" [McCollum and Kennedy 1916], and is to be found among other sources in butter fat and egg fat [McCollum and Davis 1913], in beef fat [Osborne and Mendel, 1915], in codliver-oil [Osborne and Mendel, 1914], and in some green leaves [McCollum, Simmonds and Pitz, 1918]. The second "Water Soluble B" [McCollum and Kennedy, 1916], of which rich deposits are found in the germ of cereals [McCollum and Davis, 1915] and in yeast [Funk and Macallum, 1915], is considered [McCollum and Kennedy, 1916] to be identical with the accessory

factor (anti-beri-beri or anti-neuritic vitamine) protecting from avian polyneuritis and human beri-beri. McCollum and his co-workers do not admit that there is an additional factor protecting from scurvy. It is however possible that had their work been carried out with another experimental animal they might have reached another conclusion. It is well known that the rat is insusceptible to scurvy, and it may well be that it requires only a small amount of the anti-scorbutic factor and that sufficient traces may remain in the substances of animal and plant origin which supply the two "growth" factors.

Our work leaves us in no doubt that, in the case of guinea-pigs and man, an accessory anti-scorbutic factor is required to maintain health. Although in many cases growth will cease in young animals if this anti-scorbutic factor is omitted from the diet, it does not appear likely from a study of their respective distributions that this anti-scurvy vitamine is identical with either of the two growth factors described by the American workers. In our own experiments, the anti-beri-beri vitamine (probably identical with the growth factor "Water Soluble B") is present in abundance in the ample ration provided of whole oats and wheaten bran. The second growth factor, "Fat Soluble A," may be operating in our milk ration (even when strongly heated) and is distinct from the anti-scurvy accessory factor.

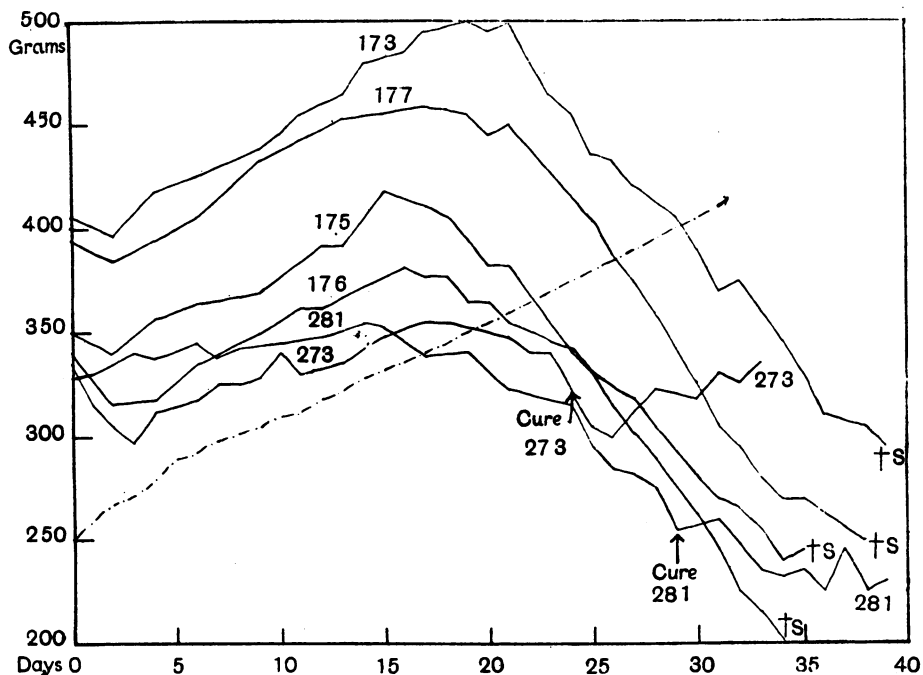


Chart 2. Weight curve of young guinea-pigs upon a diet of oats and bran (*ad lib.*) and autoclaved (1 hour at 120° C.) cow's milk up to 60 cc. daily, see Table II, Expts 1-6.

· · · · · signifies normal weight curve upon a diet of oats and bran and fresh cabbage *ad lib.*

†s signifies death from scurvy.

↑ Cure signifies cure begun with fruit juice.

It is necessary to give a short description of what is meant in the Tables by "scurvy symptoms." The first symptom to be observed is soreness of joints and limbs, more especially of shoulders and knees, so that the animal squeaks when pressure is applied to these places. Some animals squeak when

TABLE II. Influence of addition of (a) strongly heated (120° C. for one hour) milk and (b) dried milk, upon Scurvy in Guinea-pigs upon diet of Oats and Bran compared with (c) other anti-scorbutic materials.

No. of experiment	Diet		Average daily ration of milk, cc.		Weight of animal, g.			Length of experiment, days	Day on which symptoms of scurvy were first noted	Result
	Average daily ration of oats and bran, <i>ad lib.</i>	Average daily ration of oats and bran 1st 21 days Later	At beginning	Highest reached	At end	Change in weight, %				
1	60	55	405	500	298	-26	40	21	Death from scurvy, 40th day	
2	45	20	347	418	200	-43	34	21	" " 34th "	
3	41	40	340	382	245	-28	35	21	" " 35th "	
4	47	32	395	458	240	-39	37	18	" " 37th "	
5	45	34	339	355	320	-6	24	15	In condition of acute scurvy, 24th day, cure with lemon juice started	
6	"	42	330	356	255	-23	29	not noticed	"Cure" with fresh lime and fresh orange juice started 29th day	
7	"	"	346	373	237	-32	34	28	Death from scurvy, 34th day	
8	"	"	350	395	350	0	23	21	"Cure" with preserved lemon juice started, 23rd day	
9	<i>ad lib.</i> + 2.5 g. fresh cabbage	ca. 60	320 increasing to 572		572	+79	95	—	Good health	
10	<i>ad lib.</i> + 5 g. fresh cabbage	ca. 60	299		565	+89	92	—	" "	
11	<i>ad lib.</i> + 3 cc. fresh orange juice	ca. 60	365		598	+64	93	—	" "	
12	<i>ad lib.</i> + 10 cc. fresh raw beef juice	0	350	350	240	-31	23	15	Death from scurvy, 23rd day	

\* Reckoned as the equivalent of ordinary milk.

handled under any circumstances, and when in perfect health; but if they are examined regularly from the beginning of the experiment, it is possible to distinguish those which are feeling pain. The presence of painful members is also shown by the assumption of what we have called the "scurvy position," which seems to indicate haemorrhage and consequent discomfort in the muscles of the limbs. The animal rests on its side, and the painful leg is held off the ground and may be seen twitching. A second attitude, which we have called the "face-ache position," is also indicative of scurvy in young guinea-pigs; the animal lies curled up with the side of its face pressed on the floor of the cage. This is a frequent attitude in adult guinea-pigs when in normal health, but we have never seen a young animal adopt it except when ill with scurvy. It seems to indicate haemorrhage of the jaw, with soreness and looseness of the teeth. The state of the molar teeth and of the whole gums cannot be inspected during life, and it is only possible to judge of the condition by the greater or less capacity for eating, and by the assumption of the "face-ache position."

*Post-mortem appearances.* The macroscopic signs of scurvy in guinea-pigs consist mainly of haemorrhage and bone lesions and have been well described by Holst and Frölich [1907, 1912]. It may be convenient to recapitulate them here.

The haemorrhage may occur in almost any situation; for example, in the subcutaneous connective tissue, especially in the region of the axilla or groin, and over the ribs, or among the muscles, more frequently of the upper and lower limbs, and less often in the intercostal muscles. In chronic cases of long standing, the whole musculature appears darker in colour than in the normal animal. Haemorrhages in the walls of the viscera are also frequent; in these cases blood is often passed with the faeces during life and death occurs suddenly.

The changes in the bony tissues are characteristic, and are conveniently observed in the knee joints. In a severe case these are very much swollen, and on dissection the tibial epiphysis (or more rarely the femoral) is found to be disorganised and often broken across; the condyles can best be described as papery, the shafts of the long bones are brittle and can be easily broken with forceps, and the marrow is much darker than the normal. Periosteal haemorrhages, which form so marked a feature of infantile scurvy, are seen in comparatively few cases. The jaws and teeth are usually profoundly affected, the teeth become brittle and loose, and the jaw is easily fractured on pressure.

Typical changes take place in the costo-chondral junctions. They become swollen, and exhibit a transverse yellow bar and are frequently surrounded with haemorrhages in the adjoining muscles. These appearances correspond with the complete disorganisation of the bone-cartilage junction, and with disappearance of the cartilaginous trabeculae and of the rows of cartilaginous growing cells. In many cases the bone is found to be completely fractured and there is great proliferation of connective tissue across the junction.

*Experiments with fresh Cow's Milk.*

## GROUP I.

The experiments with fresh milk fall into two groups.

The first group of experiments consists of those in which not more than 30 to 50 cc. of fresh milk were presented to each animal daily, together with an *ad libitum* (measured) ration of oats and bran. The larger quantity of milk was given under the impression that we should protect our animals from scurvy by such a ration [Funk, 1913]. This, however, proved not to be the case, and in every instance acute scurvy was the result.

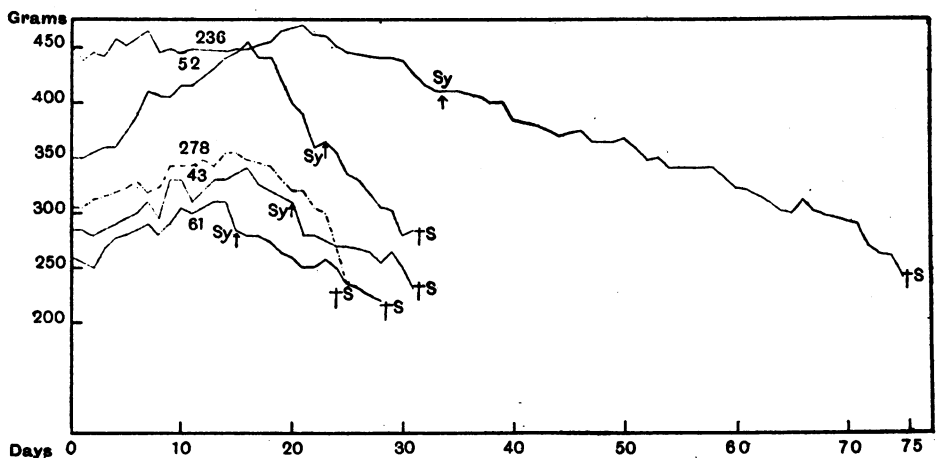


Chart 3. Weight curve of young guinea-pigs upon a diet of oats and bran (*ad lib.*) and fresh cow's milk 30-50 cc. daily, see Table III. In case of No. 236, the daily ration of 50 cc. was maintained continuously by hand-feeding.

Sy † signifies scurvy symptoms noted.

†s signifies death from scurvy.

--- signifies 1 cc. liquid paraffin given every second day.

The details concerning the 8 animals in this group are set out in Table III and Chart 3, and present a very uniform result. All (with the exception of No. 236, described later) developed scurvy symptoms in 15 to 28 days, an average time not very much longer than when the milk was omitted (see Table I). In cases where no treatment was essayed, the symptoms became progressively severer, great loss of weight occurred, and the animals became moribund about 10 days later. They were usually killed by chloroform at this stage (Nos. 1, 2, 4, 7, Table III).

In the case of other animals in this group fresh fruit juices were given after definite scurvy symptoms had been observed, with the result that successful cures were obtained. The animals, after a short interval, began to



TABLE III. *Influence of Daily Ration of 30-50 cc. fresh Cow's Milk upon Scurvy in Guinea-pigs. The diet consisted otherwise of Oats and Bran ad libitum, the amount of which consumed varied between 20 and 40 g. daily.*

No. of experi- ment animal	Daily ration of milk offered, cc.	Weight of animal, g.			Length of experiment, days	Day on which symptoms of scurvy were first noted	Result	Post-mortem examination result
		At beginning	Highest reached	At end				
1	30	285	340	215	31	20	Very ill, killed 31st day	Scurvy
2	30	290	295	205	29	not noticed	Moribund, killed 29th day	"
3	50	320	375	260	31	20 slight	"Cure" 5 cc. fresh orange juice daily started 31st day. Killed 77th day in fair health. Weight=420 g.	Traces of old scurvy
4	50	350	450	285	31	28	Very ill, intestinal haemorrhage. Killed 31st day	Scurvy
5	50	355	410	290	31	20	"Cure" 5-10 cc. fresh orange juice daily started 31st day. Splendid condition 90th day. Weight=470 g. Used for breeding	
6	50	280	385	340	17	18	"Cure" 10 cc. fresh orange juice daily, 18th day. Killed 63rd day, in fair health. Weight=450 g.	Traces of old scurvy
7	50	200	310	220	30	15	Death from scurvy, 30th day	Scurvy
8	60 + 1 cc. liquid paraffin each second day	305	355	237	25	not noticed	" " 25th "	"
9	"	318	335	295	24	15	"Cure" attempted with lime juice beginning 24th day	Traces of old scurvy

put on weight, the teeth, if loose, began to tighten up, and very slowly the soreness of the joints began to improve. The animals were killed after varying periods of time, and, although in good health, traces of the old scurvy were demonstrable even when some months had elapsed since the date of acute symptoms. These traces consisted in survival of the bone changes in acute scurvy, such as swellings at costo-chondral junctions, and malformation of leg bones with bony outgrowths due to partial or complete separation of the tibia at the upper epiphysis and subsequent joining up again of the fractured bone in the wrong position. Disturbance in the position of the teeth was also sometimes noted.

No animal in this group failed to develop scurvy. The ration of milk offered (*Expts.* 1, 2, 30 cc.; *Expts.* 3 to 9, 50 to 60 cc.), was usually consumed as long as the animal remained well; with the onset of scurvy symptoms the amount of milk taken decreased until the end of the experiment, 10 to 15 days later. The supply of anti-scurvy accessory substance is here so deficient that individual idiosyncrasy and power of resistance play an insignificant part. In our experience no guinea-pig can remain in health over a long period upon a diet of oats and bran, to which as little as 30 to 50 cc. of fresh milk daily is added.

#### GROUP II.

In the second group of experiments, the amounts of fresh milk consumed were greater, viz.—from 50 cc. upwards.

This group (see Table IV, Charts 3 (No. 236) and 4) contains few animals, but the nature of the work made this unavoidable. Throughout the scurvy investigation in the Institute the rule has been that the animals are not left to the care of the laboratory servants, but are handled and weighed by the worker in charge, who also measures the amount actually consumed of each constituent of the diet. We have found that this is the only way in which a really satisfactory knowledge of the working of the experiment can be gained, although the amount of the daily routine is greatly multiplied. In addition to these precautions, it was often necessary, with the animals in the second group, to reinforce with hand-feeding, in order that the standard of milk consumption should be kept up. It is not, however, possible to give more than about 50 cc. of milk by hand during the day and, unless the animal also cooperates heartily in the experiment during the night, the total daily consumption does not rise above 70–80 cc.

The ration of oats and bran was cut down to a minimum, 10 g. in order

TABLE IV. Influence of Daily Ration of 50-130 cc. fresh Cow's Milk upon Guinea-pig Scurvy.

No. of experiment animal	No. of Average daily ration of milk consumed, cc.	*Ration of oats and bran, g.	Weight of animal, g.		Change in weight, %	Length of experiment, days	Day on which symptoms were first noted	Result	Post-mortem examination results	
			At beginning	At highest reached						
1	236	50†	451	469	240	-47	75	33	Very ill scurvy. Killed 75th day	Scurvy
2	232	65	467	507	382	-18	39	28	Accidentally killed 39th day	"
3	234	1st period, 14 days	275	340	268	-2	83	21	Moribund, killed 83rd day	"
		2nd " 32 "								
		3rd " 34 "								
4	242	1st period, 44 days	453	490	300	-34	91	45	Death from scurvy, 91st day	"
		2nd " 21 "								
		3rd " 24 "								
5	243	85	480	gradually in. 555	creasing to 586	+16	24+	never	Good health, birth of 2 young on 24th day	No scurvy
6	235	1st period, 22 days	260	"	586	+125	113+	never	Good health	"
		2nd " 31 "								
		3rd " 30 "								
		4th " 30 "								

\* In health the animals fed *ad lib.* consumed 30-50 g. daily; when sickening with scurvy the amount decreased to 10-20 g. or less.  
 † This animal did not voluntarily take as much as 50 cc. daily, the amount not consumed was fed by hand, so that the daily consumption was regularly 50 cc.

that a sufficient volume of milk could be accommodated; we also found that a moderately high temperature of the laboratory was helpful in increasing thirst. In all the experiments the milk was given twice daily. At first it was ordinary dairy milk, but much trouble was encountered through souring during the night, after which most animals refused to touch it. For a short time boracic acid (0.5 g. per litre) was added to the night milk to preserve it, but this did not improve the animals' appetite. For the latter part of the time, in the experiments of this group, very pure, specially delivered, country milk was used. No further trouble was experienced with souring.

The usual procedure was to offer 50 cc. of milk in the morning which had to be consumed during the day, with the help of some hand-feeding, if necessary. At night, a measured amount, graded to the particular appetite of the animal in question, was offered, and the residue was carefully measured next morning.

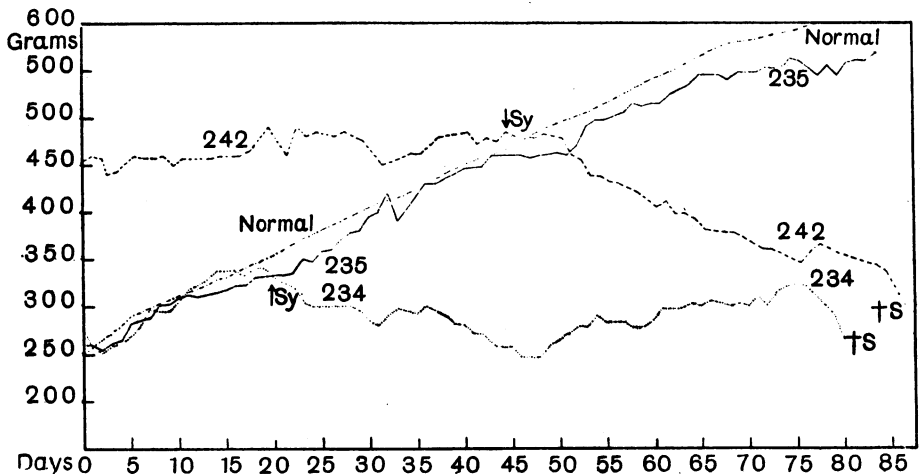


Chart 4. Weight curves of young guinea-pigs upon a diet of oats, bran and fresh cow's milk 50-130 cc. daily, see Table IV: No. 234, 60-80 cc., No. 242, ca 80 cc., No. 235, 80-130 cc.

— · — · — signifies normal weight curve upon a diet of oats, bran and fresh cabbage *ad lib.*

†S signifies death from scurvy.

↓Sy signifies scurvy symptoms observed.

The details concerning animals in Group II are set forth in Table IV, and the weight charts of three typical cases are shown in Chart 4; the animals themselves, however, deserve individual notice.

No. 236 (initial weight 450 g.), offers an interesting contrast with the animals of Group I, and its weight chart is set out in Chart 3, for purposes of comparison with the animals of that group. This animal consumed exactly 50 cc.

of milk daily throughout the experiment. Incipient symptoms of scurvy appeared about the 33rd day, and a gradual decline of weight set in, but the animal did not become acutely ill until the 75th day. This is in marked contrast with the other animals in Group I, and is attributable to the fact that the daily ration of 50 cc. fresh milk did not fall off when the animal became ill, but was fed daily to it by hand.

The weight curve of No. 236 we regard as characteristic of partial protection against scurvy. The anti-scorbutic accessory factor is present in the diet but in insufficient quantity and the life of the animal is prolonged, though it ultimately succumbs with symptoms of scurvy of long standing.

No. 232 (initial weight 467 g.), consumed an average of 65 cc. milk, and did well for a time. About the 22nd day it showed symptoms of scurvy, and its weight fell rather fast. At this period even with hand feeding it was occasionally receiving less than 50 cc. of milk daily. About the 30th day it became very thirsty and consumed 70 to 80 cc. daily and the weight improved. Unfortunately the animal became enfeebled with an attack of diarrhoea, its daily consumption of milk reached 100 to 110 cc., but in its eagerness it unfortunately fell into its milk pot and was drowned.

No. 234 (initial weight 275 g.). The average daily consumption of milk varied from 68 cc. for the first two weeks, to 57 cc. for the next four weeks, and 73 cc. for the last five weeks. The weight of the animal was well maintained throughout the 83 days of the experiment, but symptoms of scurvy were apparent from the 21st day.

No. 242 (initial weight 453 g.). The average daily ration of milk was at first greater than in case of 234, viz., about 86 cc.; later it fell below it. On the whole, however, the result corresponded very nearly with that of No. 234 though the animal was much larger to begin with. Scurvy symptoms were developed at the end of the third week and death supervened after 91 days.

No. 243 (initial weight 480 g.) did well, consuming an average of 85 cc. and increased in weight until the 25th day, when two living young were born. It immediately fell ill and died of a general septicaemia a few days later. No symptoms of scurvy were observed in life or at the post-mortem examination.

No. 235 (initial weight 260 g.) was started at the same time as, and in a manner in every way similar to, No. 234. From the first it co-operated well in the experiment and took its milk with enjoyment. It needed very little hand feeding. For three weeks it consumed an average of 84 cc. and put on weight steadily; later its daily average increased to about 120 cc., while on some days it consumed as much as 200 cc. It survived for more than 16

weeks; it more than doubled its weight, and during this period showed no symptom of scurvy.

The results of experiments in Groups I and II taken together suggest that it is the *quantity* of fresh milk consumed which is the important factor in influencing the survival of guinea-pigs upon a diet of oats, bran and fresh milk. If these results be compared with those summarised in Table I, where milk was omitted and where the guinea-pigs subsisted on oats, bran and water only, or with Table II where oats and bran and autoclaved milk were given, the following conclusions are reached with regard to the anti-scorbutic value of fresh milk:

1. When less than 50 cc. are taken daily, the animals showed no protection from scurvy.

2. When exactly 50 cc. are taken (No. 236) some degree of protection was apparent, life was prolonged, but the weight declined from a comparatively early date.

3. When 50–85 cc. are taken, increased protection from scurvy was noticed, and weight was better maintained (Nos. 232, 234, 242, 243).

4. In the one instance where 100–150 cc. was the daily ration (No. 235) the animal grew in a manner closely approximating to the normal (see Chart 4) and showed no symptoms of scurvy for 16 weeks.

Our interpretation of these results leads us to a concept of fresh cow's milk as a substance containing the anti-scorbutic accessory substance, but in small amount. It is therefore necessary that a large daily ration should be consumed, in fact that the diet should be composed entirely or almost entirely of milk, if scurvy is to be prevented by its agency alone. Such a concept is consistent with the rôle played in nature by mammalian milk, which is destined to form a complete food for a definite, but limited, period for the young of the species.

The low anti-scorbutic value of fresh milk is realised from Table II (c), where results obtained with more potent substances are given for purposes of comparison. It will be seen that scurvy in guinea-pigs can be effectively prevented by a daily ration of 2.5 to 5 g. fresh cabbage leaves, or 3 cc. fresh orange juice, whereas upwards of 100 cc. fresh cow's milk is required.

Our experimental work with fresh milk thus falls into line with other work pointing to scurvy as a "deficiency disease," caused by absence from, or deficiency in, the diet of an accessory food factor.

A similar set of experiments (already alluded to, p. 133), have recently

been published by McCollum and Pitz [1917] and results resembling our own were obtained. The interpretation placed upon them by these authors was, however, entirely different from our own and led them to reject altogether the "vitamine hypothesis" of guinea-pig scurvy. They arrive at the conclusion that this disease is not due to any deficiency in the diet, but that it is the result of chronic constipation, caused by the physical structure of a "scurvy diet."

They are led to this conclusion by the fact that a certain diet, *i.e.*, rolled oats, casein, salts and butter fat (the last-named supplying the accessory growth factor, "Fat Soluble A") sufficient to maintain the rat in perfect health, is insufficient to maintain the guinea-pig. The latter goes down hill and dies, but whether from starvation and general inanition, or whether from the symptoms described by Holst and Frölich [1907, 1912] and other workers as scorbutic, *i.e.* haemorrhages, fragility of the bones and loosening of the teeth, together with specific histological changes in bone and bone marrow, is not stated. A further set of experiments with guinea-pigs is described, in which the diet consisted of rolled oats and fresh milk. In this case a certain proportion (two out of four) died of scurvy in 5-7 weeks; the remaining two animals survived, and it is argued that if some survive the diet must be chemically adequate for all. No allowance is made for individual idiosyncrasy or for possible differences in the amounts consumed.

McCollum and Pitz consider that the metabolism of any two mammals, *e.g.*, the rat and the guinea-pig, must be similar, so that a diet which is chemically sufficient for the one must be chemically sufficient for the other, and if the one animal fails on it while the other survives, the failure must be due to some other cause than the deficiency in diet. They repudiate the idea that the guinea-pigs die from a "deficiency" scurvy and they attribute the deaths which occur on these diets to chronic constipation. They consider that the guinea-pig is peculiar in needing foods which will produce bulky easily eliminable faeces, and oats and milk they do not regard as belonging to such a category. They find support for this view in the fact that post-mortem examinations of guinea-pigs dead of scurvy generally show an empty stomach and a gorged caecum; further, they consider that scurvy in guinea-pigs can be both prevented and cured by the administration of laxatives, without any other alteration of the diet.

The results of experimental work carried out in this Institute [Chick and Hume, 1917] in confirmation of that of Holst and Frölich [1907, 1912, 1913], Fürst [1912], etc., in Christiania, have furnished abundant data in support

of the explanation of guinea-pig scurvy as a deficiency disease, analogous as regards etiology, symptoms and methods of cure with the condition called scurvy in man. And, furthermore, the results which we have obtained by feeding guinea-pigs on common articles of human diet, including milk, correspond in a striking manner with current experience and old records of human scurvy.

We believe that the experiments of McCollum and Pitz will bear an entirely different interpretation from that which they have put upon them, an interpretation which falls into line with the "vitamine" hypothesis and requires no assumption of constipation as the causative agent. This interpretation we believe to be as follows: those guinea-pigs (2 out of 4) in their experiment which survived on a diet of unlimited fresh cow's milk and oats were those which consumed a large quantity of milk and very little oats, while those which developed scurvy were those that consumed much less milk. It is clear that an *ad libitum* ration of fresh milk may mean anything and as the quantities consumed were not measured, the above explanation could not emerge.

There remains the case of those animals developing scurvy on a diet of fresh milk and oats, whose condition improved after regular administration of a laxative [*e.g.* McCollum and Pitz, 1917, Nos. 2 and 4, Chart 7, p. 249]. A fall in weight coinciding with scurvy symptoms at about the 5th week, was checked and the animals did well subsequently. It will be found that weight charts, closely resembling these, are to be found where no curative doses were given [*e.g.*, McCollum and Pitz, 1917, Chart 3, p. 245, Chart 4, p. 246]. We have noticed that a distaste for solid food and greatly increased appetite for milk frequently accompanies an attack of scurvy. The improvement in weight following a fall, which was noticed in cases where a laxative was administered (and recorded also, without comment, in some cases where none was given) may possibly have corresponded to an increased consumption of the fresh milk. In our own experiments the length of life and welfare of the animals were found to be nicely graded to the amount of milk taken, and had McCollum and Pitz recorded the amount of milk actually consumed by their animals, we believe that their work would have yielded the same result.

As regards the constipation hypothesis of McCollum and Pitz, we have not observed that this condition is a constant feature of guinea-pig scurvy, indeed we should have said that diarrhoea with intestinal, duodenal and sometimes gastric haemorrhage, entered more often into the clinical picture. In some



cases it is true, the failure to eat resulting from loosened teeth, does lead to a post-mortem picture showing an empty stomach and small intestine, and a stagnant caecum. No doubt cases of constipation occur in this way, but we have not found them the regular concomitant of scurvy symptoms. On the other hand many facts have emerged from the work in this Institute which are quite incompatible with the view that scurvy in guinea-pigs is caused by a chronic state of constipation.

Dr E. Marion Delf<sup>1</sup> has found that guinea-pigs can be protected from scurvy by 5 g. of peas, germinated for 48 hours [cf. Fürst 1912] when added to a diet of grain and milk (autoclaved at 120° for an hour). A very much larger ration, about 30 g., is needed to do this when the peas are merely soaked in water for 24 hours. It is hard to believe that the process of germination, which has proceeded for so short a time, can so alter the physical properties of a diet that its power of preventing or curing constipation should have increased sixfold.

McCollum and Pitz refer to Holst and Frölich's [1912] experiment with dried greens and suggest that the loss of power to prevent scurvy, which cabbage suffers on drying, is due to the failure of the greens to take up water in the intestine and to produce the bulky food needed to prevent constipation. We have repeated Holst and Frölich's work with cabbage, dried and kept for three months. Before being fed to guinea-pigs, the greens were soaked and became fully re-imbibed, yet an amount equal to more than 20 times the minimal ration of fresh cabbage was found incapable of preventing scurvy.

The changes in the food stuffs which are described in these two cases are not mere changes in physical condition but appear much more likely to be subtle chemical changes involving the appearance or disappearance of an, at present unisolated, chemical complex, such as a "vitamine" or accessory food factor is conceived to be.

It appeared worth while, however, to put the matter to an experimental test. Guinea-pig No. 278 (Chart 3 and Table III) received oats, bran and 50 cc. fresh milk daily; in addition 1 cc. liquid paraffin was administered every second day. This animal behaved like those which received a similar ration of fresh milk without any laxative. A fall in weight began on the 15th day, scurvy symptoms occurred on the 27th day.

In a second experiment guinea-pigs 269 and 270 were fed on a scurvy diet of oats, bran and autoclaved milk; 1 cc. liquid paraffin was given every second day, beginning from the 23rd day, the date at or about which scurvy

<sup>1</sup> Unpublished experiments.

symptoms were noticed. A few days later a steady fall in weight set in and death from scurvy took place on the 39th and 50th days respectively. The weight charts of these animals are set out in Chart 5, and their histories should be compared with those dealt with in Table II, where the animals received a similar diet without addition of any paraffin. The third curve in

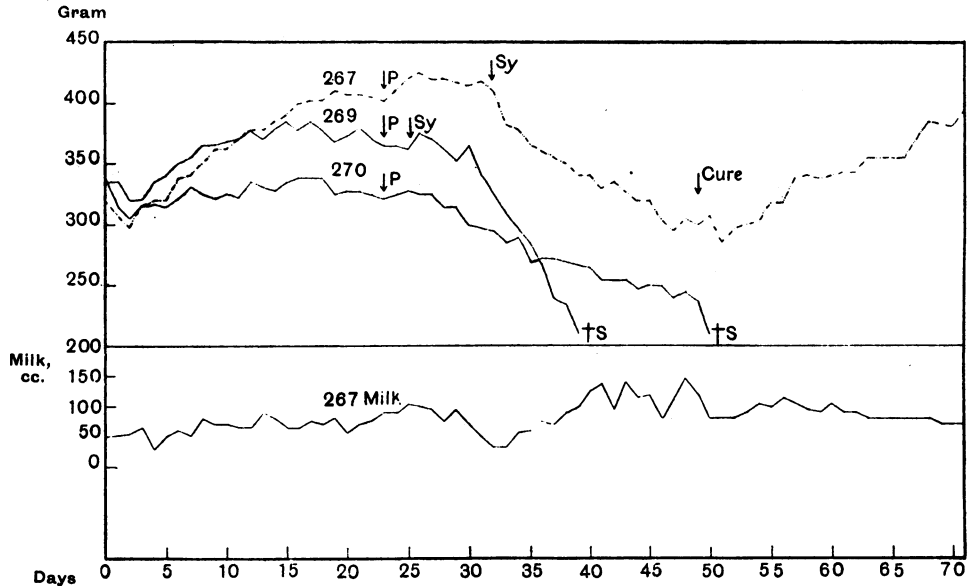


Chart 5. Weight curves of young guinea-pigs upon a diet of oats, bran and autoclaved milk.

↓ P signifies that 1 cc. liquid paraffin was given every second day subsequently.

↓ Sy signifies scurvy symptoms noted.

↓ cure signifies cure with orange juice started, paraffin discontinued.

†S signifies death from scurvy

Lower curve shows daily consumption of autoclaved milk in case of No. 267. It is interesting to note in this case that although the consumption of autoclaved milk increased from the 33rd day, the weight curve continued to fall steadily until after the orange juice was administered. Increased consumption of milk autoclaved at 120° C. for an hour had therefore no beneficial effect in scurvy.

Chart 5 is concerned with guinea-pig No. 267, which developed scurvy on a diet of oats, bran and autoclaved milk, and was first treated with paraffin (as Nos. 269 and 270), but also received no benefit; later this treatment was changed for a daily dose of orange juice and immediate recovery from scurvy began.

Our experiments upon this point, as far as they go, do not, therefore, confirm the conclusion of McCollum and Pitz.

APPLICATION OF THE EXPERIMENTAL RESULTS TO THE QUESTION  
OF INFANT FEEDING.

The foregoing results indicate that anti-scorbutic substances are present in fresh cow's milk, but in small amount. It is therefore clear that when the milk is destined for the use of infants, any method of preparation which tends to diminish this small amount must be regarded with suspicion. Among these processes are included the heating and the drying of milk.

*Use of Heated Milk.*

It has become the almost universal custom to subject milk to preliminary heating before feeding to infants, in order to prevent transmission of bacterial disease.

Our own experiments with heated milk have only dealt with very severe heating indeed (at about 120° C. for one hour) and the ration taken has as a rule been less than 60 cc., an amount which is insufficient to protect against scurvy even when the milk is fresh. In the few cases, however, in which we have seen much larger amounts consumed, there was no sign that any anti-scorbutic substance was present which could assist in the process either of protection or of cure.

Frölich [1912] found that a diet of oats and milk, heated to 70° C. for 30 minutes or to 98° C. for 10 minutes, produced scurvy in guinea-pigs more frequently than did raw milk, but as he did not measure the quantities consumed, it is not possible to draw quantitative deductions from his work.

Practical experience of infant feeding also affords proof that damage is done when the heating takes place at temperatures lower than boiling point. Neumann [1902] states that in 1901-1902 he encountered 21 cases of infantile scurvy in his private practice, whereas previously he had rarely seen the disease. Of these 21 cases, 20 had for several months received milk from the same dairy, a dairy in which since 1901 it had been the custom to pasteurise the milk at 90-95° C. before delivery. In all cases the milk had been heated a second time after delivery in a Soxhlet or other apparatus, at or near 100° C., for 10-15 minutes. A similar experience is related by Heubner [1903]. This Berlin physician noted only 23 cases of infantile scurvy in Berlin between the years 1894 to 1900 inclusive. In 1901-1902 he met with 59 cases, of which 31 had been fed on milk previously heated in various ways and 11 had received condensed milk or patent food. He, in common with Neumann, attributed the marked increase in infantile scurvy at this period to the introduction of pasteurisation or boiling of milk before feeding to infants.

Perhaps the best instance in the literature is that studied in great detail by Hess and Fish [1914] and Hess [1916] in which 23 cases of mild infantile scurvy were shown to be caused by a diet for several months of cow's milk previously pasteurised at 145° F. (63° C.) for 30 minutes. This outbreak occurred at the Hebrew Infant Asylum, New York, where it had long been the custom to use pasteurised milk for the infants and in addition to give a regular ration of fresh orange juice. After the American Medical Milk Commissions had published the conclusion [1912] that for purposes of infant feeding, heated milk might be considered as the equivalent of raw milk, the use of orange juice was discontinued in the Infant Asylum. The result was that an outbreak of mild scurvy took place about two to four months later. The babies were not acutely ill, and the symptoms were mild in comparison with the clinical picture of severe infantile scurvy. The patients were fretful, showed marked pallor, loss of appetite and some degree of anaemia; they also ceased to gain weight or to grow in length. In a few cases there were distinct haemorrhages in the mouth and elsewhere and in others a thin blue line of induration could be detected round the margin of the teeth. The infants were all more than six months old, and it would have been reasonable to attribute the illness to teething troubles. The scorbutic nature of the disorder was, however, proved by the fact that all symptoms cleared up speedily after restoring the extra anti-scorbutic to the diet or substituting raw milk for the heated milk.

Miller [1917] has described a series of similar cases from his private practice. The illness was mild, and other diagnoses were suggested, but successful cures following on treatment with anti-scorbutic material proved the trouble to be incipient infantile scurvy.

#### *Use of Dried Milk.*

Dried milk we believe to be largely, if not entirely, deprived of anti-scurvy vitamine. Whether this happens during the process of drying or in the long keeping which follows drying we are unable to say. We have, so far, discovered no conditions under which the anti-scorbutic vitamine can be preserved intact apart from living tissues for any considerable time at room temperature. It is slowly destroyed by some process at present unknown, and in dried milk there is no exception to the rule.

The history of two guinea-pigs (Nos. 163 and 162) are given in Table II, section (b). These animals consumed daily an average amount of dried milk equivalent to 60 cc. and 76 cc. milk respectively and there was no

significant protection from scurvy. The loss of anti-scorbutic principle originally contained in the milk is seen by comparing the results of these two experiments with Experiments 2, 3 and 4 in Table III, where similar amounts of fresh raw milk were consumed.

If infants are nourished on dried milk or on "artificial" milk (prepared from dried skim milk with addition of vegetable fats) nutritive disorders are to be apprehended if the diet is maintained for several months, and if no extra anti-scorbutic is added. Expert medical opinion is divided upon this point, as upon the question of the adequacy of heated milk. Naish [1914], for example, believes that "the risk of scurvy upon such a diet is non-existent," and adds that he is not in the "habit of ordering any orange juice or other anti-scorbutic." Pritchard [1914, p. 304], on the other hand, states that "when dried milks or other preserved foods are employed, it is well to give some fresh fruit juice or other anti-scorbutic."

It must be remembered that scurvy is a disease which develops after a long period of imperfect nutrition, extending usually for several months. The symptoms will vary in severity according to the susceptibility of the individual and to the degree of deficiency in the diet. Every gradation may therefore be expected from almost imperceptible changes in mild cases to the well recognized severe symptoms of acute infantile scurvy. This point merits the consideration of those authorities who deny the need for extra anti-scorbutics in artificial feeding of infants on the ground that infantile scurvy is a very rare disease, in comparison with the great frequency of artificial feeding without extra anti-scorbutic. It is possible, however, that more careful observation may discover that in its milder manifestation, infantile scurvy is not so uncommon an ailment as has been suspected.

It is essential, where infants are concerned, that the very best conditions should prevail and it is the only prudent course, in the present uncertain state of our knowledge, to supply additional anti-scorbutic substance when anything other than fresh cow's milk is given. Of these, orange juice is easily the most suitable, but when the conditions are such as to put it out of the reach of the poor, raw swede juice may be substituted. We have found that when freshly squeezed, it is a valuable anti-scorbutic, though not as potent as orange juice; a larger dose should therefore be given.

Research at this Institute is now in progress to determine the exact relative values against scurvy of these and other foodstuffs, and also if possible to discover other anti-scorbutic materials which may usefully be employed in infant feeding.

## SUMMARY.

(1) A set of nutritional experiments with young guinea-pigs is described in which the diet consisted of oats, wheat bran and fresh milk. The special feature of the work is the measurement of the amounts actually consumed.

(2) The basal scurvy-producing diet for control purposes consisted of oats, bran and water, or oats, bran, and 60 cc. milk autoclaved at 120° C. for an hour. With the former diet no growth was produced and it seemed possible that it lacked both the anti-scorbutic factor and the "Fat Soluble A" growth factor of McCollum and his co-workers. With the addition of the autoclaved milk growth took place until the onset of scurvy symptoms; it was concluded therefore that this diet was in every way a sufficient one, save only that it lacked the anti-scorbutic factor.

(3) If the daily consumption of fresh milk was less than 50 cc. the guinea-pig developed scurvy in a period not different from that in the control experiments.

(4) If the daily ration varied from 50-100 cc., a greater or less protection from scurvy was observed, varying proportionately with the amount consumed.

(5) If 100-150 cc. was taken daily, satisfactory growth and development occurred and no symptoms of scurvy were observed. This practically amounts to a complete milk diet.

(6) The above facts accord well with the vitamine deficiency hypothesis of the etiology of guinea-pig scurvy. Milk is evidently a food poor in the anti-scurvy accessory factor and a ration large in comparison with that of other anti-scorbutic materials is necessary to afford satisfactory protection from scurvy.

(7) In our opinion these experiments offer a reasonable explanation of the anomalous results obtained by other observers, when guinea-pigs were fed on diets consisting of grain and fresh milk, and when no measurements were made of the amount of milk actually consumed. Irregularity was noticed in the onset of scurvy symptoms and the observers were led to seek an explanation of the ill-health in causes other than a deficiency in diet. Jackson and Moore [1916] and Jackson and Moody [1916] have arrived at the conclusion that guinea-pig scurvy is an infection of bacterial origin; McCollum and Pitz [1917] think that it is due to chronic constipation, caused by the physical properties of the oats and milk diet.

(8) We have obtained no evidence that chronic constipation is a constant concomitant of guinea-pig scurvy. Instances are given, where certain

modifications in diet, to which no extra laxative effect can be attributed, have cured or prevented guinea-pig scurvy. Experiments are also described in which the administration of a laxative alone has failed to cure or prevent scurvy. The results, on the one hand, afford additional confirmation to the theory that guinea-pig scurvy is due to the deficiency in the diet of a specific accessory substance and on the other hand to the theory that it is analogous in etiology and method of cure with human scurvy.

(9) Application of the results is made to infant feeding with cow's milk and attention is called to the risk of tampering with a substance which even in the fresh condition is but feebly anti-scorbutic. In order to give the child optimum conditions, it is strongly urged that whenever milk is heated in any way, or dried, an additional source of anti-scorbutic vitamins should be provided. Clinical experience and animal experiments point to orange juice as the most suitable anti-scorbutic to employ, but if, owing to war or other conditions, it is unobtainable, raw swede juice in rather larger doses may be substituted.

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