LXXXVII. A STUDY OF THE COMBINED ACTION OF RAW COW'S MILK AND ORANGE-JUICE AS ANTISCORBUTIC SUBSTANCES.

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INTRODUCTORY.

THE experiments to be described were devised to determine whether raw cow's milk can replace orange-juice quantitatively as an antiscorbutic substance. If this proved to be the case it was proposed to utilise the results to estimate the antiscorbutic value of human milk.

It has been shown by Chick, Hume and Skelton [1918, 1] that raw cow's milk has a low antiscorbutic value. In the case of guinea-pigs, when less than 50 cc. were consumed daily, the animals showed no protection from scurvy. If 50–85 cc. were taken weight was better maintained. When 100–150 cc. formed the daily ration the animals grew normally and showed no symptoms of scurvy.

When such large quantities of milk have to be administered daily, the conditions of the experiment become difficult, as unless the animal co-operates heartily, it is impossible to obtain a daily consumption of more than about 80 cc.

It follows that if human milk has a comparable antiscorbutic potency, its direct quantitative estimation would not be easy to carry out.

Orange-juice, on the other hand, is very rich in antiscorbutic substance. Davey [1921] has carried out careful experiments to determine within narrow limits what is the minimum ration which will prevent scurvy. It was found that the animals receiving only 0.5 cc. of orange-juice developed scurvy. Of eleven animals receiving 1.5 cc., six were protected, three died of intercurrent disease, while only two succumbed to scurvy. The minimum ration which will allow of normal life is therefore 1.5 cc., though a certain proportion of animals employed will not show complete protection when receiving this amount.

If raw cow's milk and orange-juice could replace one another in direct proportion to their respective antiscorbutic values, by using both these substances at one time as the source of the antiscorbutic factor, much smaller quantities of milk would have to be administered. The conditions of the experiment would be facilitated, and by carrying out parallel experiments with human and cow's milk, it would be possible to obtain comparable values for cow's milk and human milk in terms of orange-juice.

The ration of cow's milk to be employed had first to be decided on. It was thought most convenient to administer about one-quarter of the minimum milk ration, *i.e.* about 35 cc. daily. It will be shown in the subsequent discussion that this ration has an adequate amount of the fat-soluble vitamin and will permit of quite satisfactory growth, though it is less than the amount necessary for optimum development.

The orange-juice ration was likewise divided into quarters, *i.e.* 0.5 cc., 1.0 cc., 1.5 cc., 2.0 cc., etc., and combinations of 35 cc. of cow's milk with varying amounts of orange-juice were tested on a series of animals.

TECHNIQUE.

Young guinea-pigs of about 250–350 g. were employed. The basal ration consisted of oats and bran moistened and rubbed together, and water *ad lib.* Measured amounts were presented daily and the amount actually consumed was carefully noted. The animals were divided into three groups.

A. The first group received 40 g. of cabbage in addition to the basal ration and thus served as normal controls.

B. The animals in the second group received 35 cc. of milk which had been heated to 120° for one hour in the autoclave. In addition, varying amounts of orange-juice from 0.5 cc.–2.5 cc. were given (see Table I).

C. The third group received 35 cc. of raw cow's milk and varying amounts of orange-juice (see Table II).

Table I.

Animal	Addition of orange-juice to basal diet + 35 cc. autoclaved milk cc.	Duration of expt. (days)	Remarks	Post-mortem
B 1	0.5	72	Left hind limb raised off ground and tender.	Haemorrhages. Widening of costochondral junctions. Left knee joint ankylosed.
B 2	1	68	Ankylosis right hind limb.	Haemorrhages. Costal junctions blurred. Lymph glands congested.
B 3	1.5	68	—	Normal.
B 4	2	54	—	Normal.
B 5	2.5	52	—	Normal.

Table II.

Animal	Addition of orange-juice to basal diet + 35 cc. raw milk cc.	Length of expt. (days)	Remarks
C 1	0.5	76	No symptoms of scurvy. Nil abnormal post-mortem.
C 2	1	60	
C 3	1.5	54	
C 4	2	56	
C 5	2.5	50	

The results obtained are summarised in the accompanying tables and diagram (Tables I and II and Fig. 1).

Fig. 1. Weight curves of C 1-5 (raw milk 35 cc. and orange-juice 0.5 cc.-2.5 cc.) and B 3 (autoclaved milk 35 cc. + orange-juice 1.5 cc.).

DISCUSSION.

It was thus found that of the animals fed on autoclaved milk only those receiving 0.5 cc. and 1.0 cc. orange-juice (B 1 and B 2) developed scurvy. B 3, which received 1.5 cc. daily, showed no abnormal signs. B 4 and B 5, receiving 2.0 cc. and 2.5 cc. of orange-juice daily, developed quite normally and no suggestive signs were found on post-mortem examination. These results are in agreement with those of Davey [1921] already quoted.

None of the guinea-pigs in the series C receiving raw milk showed any signs of scurvy during life, or any characteristic changes post-mortem. It thus appears that 0.5 cc. of orange-juice when combined with 35 cc. of raw milk was sufficient to prevent the onset of the disease.

A second confirmatory set of experiments was then performed. The basal ration consisted of oats and bran and water *ad lib*. 0.5 cc. of juice was administered to all the animals. In addition, small quantities of raw cow's milk, varying from 15 cc.-40 cc. were given. In the case of animals receiving less than 40 cc. of raw cow's milk, their total milk ration was made up to 40 cc. with autoclaved milk.

Fig. 2. Weight curves of D 1, 4, 5, 6, 8, 9. For details see discussion in text.

The results obtained are summarised in the accompanying table and diagram (Table III and Fig. 2).

Table III.

Animal	Addition of milk to basal ration + orange-juice 0.5 cc.	Length of expt. (days)	Remarks	Post-mortem
D 1	Raw milk 0 cc. Autoclaved milk 40 cc.	46	Face-ache position very definite	Emaciated. Definite haemorrhages into muscles. Costo-chondral junctions thickened. Lungs and intestines normal (scurvy present)
D 2	Raw milk 0 cc. Autoclaved milk 40 cc.	39	Tender limbs, looking ill (died)	Emaciated. Pneumonia. Haemorrhages. Thickened and congested costo-chondral junctions (scurvy present)
D 3	Raw milk 15 cc. Autoclaved milk 25 cc.	46	Lies crouched up. Looks ill	Emaciated. Intense congestion of costo-chondral junctions. Gastro-enteritis and Pneumonia (scurvy present)
D 4	Raw milk 15 cc. Autoclaved milk 25 cc.	86	Fit	Costo-chondral junctions normal. Intestines normal (no scurvy)
D 5	Raw milk 25 cc. Autoclaved milk 15 cc.	66	Limping slightly. Thin	Well-marked haemorrhages. Intestine healthy. Slight pneumonia. Costo-chondral junctions obscure. Two or three ribs matted together. Spontaneous fracture of rib and excessive callus formation (scurvy present)
D 6	Raw milk 25 cc. Autoclaved milk 15 cc.	81	Normal	Normal (no scurvy)
D 7	Died suddenly while being hand-fed			
D 8	Raw milk 35 cc. Autoclaved milk 5 cc.	93	Condition good. Appears healthy	Intestine and lungs healthy. Slight haemorrhages. Most of rib junctions on both sides show marked deformity from chronic overgrowth. Appearance resembles "rickety rosary" (? scurvy present)
D 9	Raw milk 35 cc. Autoclaved milk 5 cc.	81	No symptoms of scurvy. Not looking well	Lungs and intestine healthy. Nil abnormal found (no scurvy)
D 10	Raw milk 40 cc. Autoclaved milk 0 cc.	60	Normal	Nil abnormal found (no scurvy)

D 1 and D 2, which received no raw milk at all, developed definite symptoms of scurvy.

In the case of the remaining animals, D 3-D 10, owing to the general vagueness of the signs shown during life, a definite diagnosis could only be arrived at after a careful consideration of the weight charts taken in conjunction with the findings after death. Great stress was laid on the presence or absence of haemorrhages, but due attention was also paid to the bony changes. Histological bone lesions were not taken as evidence of scurvy in the absence of haemorrhages, as Tozer [Delf and Tozer, 1918] has shown that such lesions frequently occur in animals on non-scorbutic diets, and are probably due to deficiency of vitamin A.

Since the early days of the work on scurvy, it has been known that the weight curves did not vary solely with the antiscorbutic substances present in the diet. Chick, Hume and Skelton [1918] found it necessary to administer 60 cc. of autoclaved milk daily to obtain normal growth curves in the case of guinea-pigs which were receiving an adequate ration of the antiscorbutic substance. Hume [1921] has recently made an extensive investigation into the power of various natural foodstuffs to promote growth in guinea-pigs. In the case of cow's milk, only a limited number of experiments were performed. Guinea-pigs receiving 60 cc. of raw cow's milk daily showed an average gain of 46.9 % in weight, which closely approximates to the increase in the controls of 55.8 %. Two animals were also placed on 20 cc. of raw milk daily. At the end of 25 days one showed a slight loss and the other a slight increase in weight. After 30 days both were put on 40 cc. daily and subsequently showed a slow recovery.

In the case of autoclaved milk, 60 cc. daily resulted in an increase of 44.8 %.

On analysis of the results obtained in the first series of experiments (Table IV), it is found that the average increase in weight obtained at the end of 45 days was 33.5 %. This may be compared with the results of Hume and shows that 35 cc. of cow's milk daily contains appreciably less than the optimum amount of vitamin A. Quite good growth, however, occurs on such a diet.

Table IV.

Animal	Original weight	After 45 days	% increase
C 1	299	320	7.5
C 2	398	475	19.6
C 3	380	285	33.7
C 4	390	340	14.7
C 5	445	326	36.5
B 3	420	238	76.5
B 4	380	258	47.3
B 5	380	230	65.4

Average: 33.5 %

It is now possible to discuss the history of each of the animals receiving raw milk. From examination of the details given in Table III, it will be seen that D 3 and D 5 alone (receiving 15 cc. and 25 cc. of raw milk respectively) developed definite signs of scurvy. D 4, D 6, D 9 and D 10 all grew well, putting on from 50-100 % in weight, and no signs of scurvy could be detected.

D 8 maintained very steady progress for 65 days, putting on over 100 % in weight. During the last month of the experiment he fell off a little. He was killed on the 93rd day when the condition was quite good. As stated in the protocols, a definite diagnosis of scurvy could not be made.

Taking the above facts into consideration, the experimental findings may be summarised thus:

Definite scurvy	D 3 (15 cc. raw milk)
	D 5 (25 cc. „)
Doubtful scurvy	D 8 (35 cc. „)
No scurvy	D 4 (15 cc. „)
	D 6 (25 cc. „)
	D 9 (35 cc. „)
	D 10 (40 cc. „)

It is, therefore, possible to conclude that, when such small quantities of raw cow's milk as 15–35 cc. are added to a basal ration already containing 0.5 cc. of orange-juice, protection from scurvy may be obtained. This figure is considerably less than what one would expect if raw cow's milk and orange-juice interchanged in direct proportion to their respective antiscorbutic values. On a quantitative basis the ration of antiscorbutic substance administered amounted to half or less than half the minimum requirements of the animals. It thus appears that when a mixture of antiscorbutic substances are administered, the combination is somewhat more potent than when proportionate amounts of the same substances are administered separately.

It must be remembered, however, that different samples of cow's milk may show considerable variation in their antiscorbutic content. No direct determination was made of the minimum dose of the milk employed, the figure obtained by Chick, Hume and Skelton [1918] being adopted. Any such determination is liable to a probable error of 25–50 %. In view of these facts the results obtained need not necessarily involve any very marked difference between the combined and separate actions of cow's milk and orange-juice.

The opportunity was taken during the course of the present research to investigate the condition of the lymphoid tissue in scurvy. Cramer, Drew and Mottram [1921, 1] have shown that in rats fed on diets deficient in vitamins A and B, the only constant and characteristic change found is marked hypoplasia of the lymphoid tissues. The thymus almost entirely disappears, and the spleen, lymphatic glands and lymph nodules in the intestine show definite atrophy.

These changes were not found to be present to any degree in guinea-pig scurvy. The thymus showed little change. The spleen was so variable in weight that no definite conclusions could be drawn. The lymphatic glands were smaller than on the control animals and frequently showed congestion.

On histological examination the lymph paths were normal. The endothelial cells, however, frequently showed pigment granules. These were probably granules of haemoglobin which had been absorbed from areas where blood had been extravasated.

In a communication which appeared while this paper was passing through the press, Cramer and his co-workers [1921, 2] announce that the atrophy of the lymphoid tissues which they have described can occur when vitamin B alone is withheld from the diet, and that it is associated with a diminution of the lymphocytes of the circulating blood, the polymorpho-nuclear leucocytes being unaffected. The absence of vitamin A alone does not produce any of these changes.

Bedson [1921] has recently described the condition of the blood in scurvy. He finds no constant or striking changes in the total or differential leucocyte count.

It would, therefore, appear that in guinea-pig scurvy (as in vitamin A deficiency) no changes are found in the lymphoid tissues analogous to those which occur when vitamin B is withheld.

SUMMARY.

1. Experiments on guinea-pigs are described to determine whether raw cow's milk and orange-juice replace one another in direct proportion to their respective antiscorbutic values.

2. The question is discussed whether the minimum dose which can afford protection from scurvy is reduced when both the above substances are administered simultaneously.

3. The condition of the lymphoid tissues in scurvy is described.

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