CLXIV. THE INFLUENCE OF THE COW'S DIET ON THE FAT-SOLUBLE VITAMINS OF WINTER MILK.

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IN the early stages of research on vitamins Funk [1913] advanced the suggestion that the secretion of these factors in the milk was controlled by their presence in the food of the lactating animals. Subsequent investigations have proved this conjecture to be true. McCollum, Simmonds and Pitz [1916], working with rats, found that the development of the young was greatly influenced by the presence or absence of vitamin A or B in the diet of the nursing mothers. Hart, Steenbock and Ellis [1920], Dutcher and co-workers [1920], and Hess, Hunger and Supplee [1920] demonstrated that the antiscorbutic potency of cow's milk depended on the antiscorbutic character of the diet of the cow, whilst Hughes, Fitch and Cave [1921], and Kennedy and Dutcher [1922] were responsible for proving that the vitamin A or B content of the milk was similarly influenced by the diet of the animals. The last of these investigators introduced vitamin A into the cows' ration in the form of alfalfa hay and corn silage. We [Drummond *et al.*, 1923, 1924] were able to confirm the observations of previous workers in this field, showing that by restricting the vitamin A content of the diet of the stall-fed cow a corresponding diminution of the vitamin takes place in the butter-fat. This fall in the vitamin A potency was shown by us to be rectified by the introduction of cod-liver oil into the deficient winter ration of the cows.

Apart from the direct evidence that the vitamin content of the milk of the cow is influenced by the diet of the animal, there is also some indirect evidence available supporting this view. In this connection one might quote the observations of Barnes and Hume [1919] on the seasonal variations in the antiscorbutic properties of cow's milk and those of Drummond, Coward and Watson [1921] on the variation in the amount of vitamin A in butter. Although a few investigators were unable to confirm this influence of diet on the vitamin content of the milk, the weight of evidence obtained from so many independent and careful investigations leaves no doubt as to its reality.

All the experiments referred to above were carried out at a time when the

separate identity of the antirachitic vitamin (vitamin D) and of vitamin A was not fully appreciated and consequently the "Vitamin A" measure of the cows' diet and that of the milk obtained from these animals as determined by these earlier researches give us no idea of the relative proportions of the two fat-soluble principles present in either.

The discovery of McCollum, Simmonds, Shipley and Park [1922, 1, 2, 3] that the two "fat-soluble" vitamins were not identical was soon corroborated by various workers and it became apparent that certain green vegetables did not contain much of the antirachitic vitamin [McClendon and Schuck, 1923; Zucker, 1923; Goldblatt and Zilva, 1923; Chick and Roscoe, 1926; Boas, 1926]. The experiments of Goldblatt and Zilva made the low calcifying potency of spinach as compared with cod-liver oil particularly plain. They found that whilst a daily dose of 0.1–0.25 g. of spinach promoted growth in rats on a diet deficient in the fat-soluble vitamin, a dose of 3 g. did not have the slightest effect in promoting calcium deposition, although 2.2–4.5 mg. of cod-liver oil was found sufficient in this respect. If, therefore, other green fodder behaved similarly to spinach, the milk from winter cows fed on a basal ration supplemented with green vegetables would be poor in the antirachitic vitamin in spite of its growth-promoting properties. On the other hand, the milk from the cows which received an addition of cod-liver oil with their winter ration might yield a milk of enhanced antirachitic potency. Recent work [Luce, 1924; Chick and Roscoe, 1926] has shown that the presence of green fodder in the ration of the cow increases the vitamin A content of the milk, whereas it has only a slight effect on the antirachitic factor.

In attempting to test this hypothesis experimentally we were faced with some technical difficulties. In our first effort in the winter of 1924–25 we employed the old growth method of Zilva and Miura [1921] for the estimation of vitamin A. In view of important work on this subject by Steenbock and his collaborators [1923, 1924] growth cannot be accepted as a reliable criterion for the assay of vitamin A unless other vitamins are available in adequate amounts. These workers have shown that each fat-soluble vitamin is able to promote growth for as long a time as the other is present as a reserve in the animal, and consequently the interpretation of the results obtained in our first experiments was not warranted, although there were definite indications that our surmise as regards the influence of the green fodder and cod-liver oil of the cows' ration on the milk was justified. The work was therefore repeated in the winter 1925–26. In this effort we were greatly aided by the experiences of the preceding year. Above all, we had the advantage of utilising for our tests a standardised breed of young rats which are now specially bred at the Lister Institute for use in the estimation of the fat-soluble vitamins [Smith and Chick, 1926]. Sources of error such as the variation in the reserves of these two vitamins in the experimental animals were thus eliminated. The importance of this has been pointed out by Chick [1926] in her critical study on the biological methods for the estimation of the fat-

soluble vitamins. Vitamin A was in the present investigation estimated by a method based on Steenbock's principles, namely, by administering the butters to rats which were declining in weight on a diet free from fat-soluble vitamins but containing irradiated hardened cotton-seed oil as a source of vitamin D.

This final set of experiments produced definite evidence that cows receiving a supplement of green vegetables (kale) with their stall diet yield a butter rich in vitamin A but poor in the antirachitic vitamin, whilst cows receiving a supplement of cod-liver oil produce a milk rich in both fat-soluble vitamins. In spite of their superior vitamin A content the antirachitic value of the butters from the cows receiving kale was found to be of the same magnitude as that of the butters from the cows receiving the basal diet without supplement which was a diet altogether free from fat-soluble vitamins. The significance of this fact from the hygienic point of view will be discussed later.

EXPERIMENTAL.

Six dairy shorthorn cows from the herd of the National Institute for Research in Dairying at Reading were employed in the 1925-26 experiment. The animals were divided into three groups but the milk of each individual cow was kept separate. The groups were arranged as follows:

Table I.

		Last calved	No. of calves	Weight 19. i. 26		Weight 13. iv. 26	
Basal diet	Lily II	9. x. 25	1	8 cwts.	97 lbs.	9 cwts.	55 lbs.
	Welcome	7. x. 25	3	10	105	11	52
Basal diet and kale	Bertha	5. xi. 25	3	9	111	9	104
	Firefly II	24. ix. 25	2	10	32	10	43
Basal diet and cod-liver oil	Rosemary	6. xi. 25	8	10	92	10	54
	Doll II	1. xi. 25	2	9	64	9	79

The cows were stalled at the end of November. From the first week in December their diet consisted of mangolds, oat straw and meal mixture. The experiment started on Feb. 4th. The cows on the basal rations, Lily and Welcome, received oat straw, roots, and meal mixture¹. Bertha and Firefly received kale instead of roots, whilst Rosemary and Doll received the basal ration of oat straw, roots and meal mixture supplemented by a daily dose of cod-liver oil². The cod-liver oil dose was started at 2 oz., increased after 8 days to 4 oz., after another 19 days to 6 oz., and the final dose of 8 oz., which was administered after a further interval of 21 days, was continued to the end of the experiment in the middle of April. The oil was given with the ration and was always consumed quantitatively. The consumption of food by the cows

¹ The meal mixture was composed of two parts of extracted palm kernel meal, two parts of bean meal, one part of decorticated extracted ground nut meal, one part of crushed barley, one part of crushed wheat and of a little steamed bone flour and calcium carbonate.

² The cod-liver oil, as tested by the technique to be described later, was found to contain vitamins A and D.

per day towards the end of the experiment represented approximately the average consumption and was as follows:

Table II.

Food	Lily II	Firefly II	Welcome	Doll II	Bertha	Rosemary
Straw	10 lbs.	7 lbs.	10 lbs.	8 lbs.	7 lbs.	10 lbs.
Roots	80	—	80	60	—	80
Kale	—	40	—	—	40	—
Cod-liver oil	—	—	—	8 ozs.	—	8 ozs.
Meal mixture	10	11	14	15 lbs.	14	14 lbs.

The cows were kept in well-ventilated stalls where the access of light was very poor and exposure to direct sunlight was excluded. The animals were milked twice a day, at about 7 a.m. and 4 p.m., and their walk from their stalls to the milking sheds was their only exercise. Water was laid on for each cow.

About the middle of April the butters were prepared from the respective milks for testing purposes. All the milk from four consecutive milkings on two days was employed for the butter of each cow. After separating the milk, the cream was ripened by a special starter prepared in separated milk. When the ripening was complete the butter was churned in a 2-minute churn, washed in the usual way with water and made up. Precautions were of course taken to prevent the butter of one cow from coming into contact with that of another in the churning process. The butters from the cows on basal diet were white and those from the "cod-liver oil" cows were equally colourless and did not possess a "fishy" flavour or any other taint. The butters from the kale-fed cows, especially from Firefly, possessed the usual yellow colour. The vitamin determinations which were carried out at the Lister Institute began early in May. During the period which elapsed between the preparation and the termination of the testing, the end of June, the butters were kept in the dark in cold store.

There was a very marked drop (about 30 %) in the percentage of fat of the milk from the cows receiving cod-liver oil when the dose was increased to 8 oz. The percentage of the fat in the milk from the cows fed on kale on the other hand remained the same as in that obtained from the control cows. This phenomenon was previously observed by us [Drummond *et al.*, 1924, Channon *et al.*, 1924] as well as by Wagner and Wimberger [1925]. The reduction in the *actual* amount of fat produced by the "cod-liver oil cows" per day was not, however, as great as it might appear from the percentage figures. This is seen from Table III in which the yield of milk-fat per day (an average of three successive days) at the commencement and at the end of the experiment from each cow is given.

The figures show that there was a diminution in the daily yield of milk-fat in all the cows due to the natural fall in the quantity of milk as the lactation period advances. The "cod-liver oil cows," however, showed a decidedly lower production of milk-fat per day. If the average fall in yield of fat by the other cows be taken into account the extra diminution amounted to about 15 %.

Table III.

		Average daily milk yield	Average daily fat yield	Drop in fat yield	Mean drop in fat yield	
Diet and cow	Dates	lbs.	lbs.	%	%	
Basal diet + cod-liver oil						
Doll II	Feb. 1st, 2nd, 3rd	28	1.299	32.9	29	
	Apr. 10th, 11th, 12th	26	0.872			
Rosemary	Feb. 1st, 2nd, 3rd	27	1.131	25.1		
	Apr. 10th, 11th, 12th	29	0.847			
Basal diet						
Lily II	Feb. 1st, 2nd, 3rd	17	0.699	8.4	14	
	Apr. 13th, 14th, 15th	15	0.640			
Welcome	Feb. 1st, 2nd, 3rd	25	1.001	17.9		
	Apr. 12th, 13th, 14th	20	0.822			
Basal diet + kale						
Firefly II	Feb. 1st, 2nd, 3rd	20	0.906	17.7		
	Apr. 13th, 14th, 15th	17	0.746			
Bertha	Feb. 1st, 2nd, 3rd	22	1.180	15.4		
	Apr. 10th, 11th, 12th	21	0.998			

The influence of cod-liver oil on the milk-fat yield of the cow will form the subject of another communication.

Vitamin A was estimated by a method which has been recently used at the Lister Institute with success [see Chick and Roscoe, 1926] similar to that described by Steenbock, Nelson and Black [1924]. Young rats about 4 weeks old and weighing about 35 g. were placed on the diet deficient in fat-soluble vitamins used by Zilva and Miura [1921] and made up as follows:

Heated caseinogen	...	...	20 g.
Starch	...	...	50 g.
Hardened cotton-seed or sesame oil	...	...	15 g.
Marmite	...	...	5 g.
*Salt mixture	...	...	5 g.
Decitrated lemon juice	...	...	5 g.
Water	...	...	50 g.

* No. 185, MacCollum, Simmonds and Pitz [1917].

When the rats on the above diet ceased to grow in weight after 3-4 weeks, the hardened cotton-seed oil was replaced by a similar oil previously irradiated for 30 minutes at a distance of 33 cm. from a quartz mercury lamp. Growth was then restored for another 3-4 weeks when the second decline in weight due to the vitamin A deficiency set in. The butters were then administered. Doses of 0.2 g. and 0.6 g. from each butter were given daily, care being taken to ensure the total consumption of the doses. As a negative control a group of rats were left on the basal ration containing the irradiated oil only. Another group of rats receiving cod-liver oil served as a positive control. The degree of growth was taken as a criterion for the vitamin A assessment.

The antirachitic potency of the butters was arrived at by feeding them to rats subsisting on McCollum's 3143 rickets-producing diet low in phosphorus. Rats about a month old were placed on this diet supplemented by the butter doses. In this case daily doses of 0.2 g. and 0.6 g. of each butter were also used. The group of rats serving as a negative control received only the basal diet,

whilst some of the animals in the positive control group received one drop, others three drops of cod-liver oil. After 28 days the animals were killed and the femur, tibia, and fibula were dissected and carefully cleaned of all adhering flesh and weighed. The dry weight was obtained after drying the bones for 24 hours at 110°. The dry bones were then wrapped up in finely meshed silk material, broken up and extracted with boiling alcohol for 8 hours and subsequently with ether for 16 hours, after which they were removed to weighing bottles, dried again at 110° for 2 hours and weighed. The ash content of the bone was obtained by incineration to constant weight.

The main difference in composition of the bones between rats receiving a sufficient dose of cod-liver oil as an addition to the above diet and those maintained on the experimental diet alone lies in the water content and in the ash content of the bone. Chick, Korenchevsky and Roscoe [1926] suggest a convenient standard of comparison, namely, the ratio A/R of the ash in the bone to the organic material other than substances extracted by hot alcohol and ether. We found this standard to be a reliable index of the degree of calcification and very convenient for comparative purposes.

Vitamin A. Three rats were utilised for each dose of butter. In this experiment about 50 rats of approximately the same age and reared under the same conditions had to be collected. Consequently, it was difficult to prevent the inclusion of a few animals which did not come up to the high standard of the physical condition of the majority of the group. Any suspicious animals in this respect were placed in the group of the positive control, the animals of which group received cod-liver oil daily. Some of the delicate animals died in the early stages of the experiments. The remainder, as will be seen from Table IV, in spite of their condition, responded well to the addition of vitamin A in the form of cod-liver oil to the otherwise complete diet. Negative controls which received no vitamin A lost in weight and eventually succumbed as a result of this vitamin deficiency. The rats receiving the butters from the cows on the basal diet (Lily and Welcome) showed hardly any growth on the 0.2 g. dose and some growth on the higher dose of 0.6 g. The butter from the cow Welcome was decidedly worse than that from her fellow on the same diet. When we turn to the group of rats fed on the butters from the cows which received kale in addition to the basal diet (Bertha and Firefly) we find a decidedly higher growth-promoting activity in this butter as compared with that from the cows on the basal ration. This activity is still more marked in the butters from the cows whose ration was supplemented by cod-liver oil. These results are represented graphically in Figs. 1 and 2, in which the average increase in weight is plotted against time. This experiment leaves little doubt that the addition of kale or cod-liver oil to the basal ration of cows improves the vitamin A content of the butter.

Vitamin D. Detailed results of these estimations are given in Tables IV and V, a summary of the averages of these figures in Table V, whilst Figs. 1 and 2 give a graphic representation of the average values of the A/R ratio

Table IV.

Rat	Initial weight g.	Final weight g.	Weight of bone g.	% of water	% of fat	% of organic residue	% of ash	Ratio A/R
A. Negative controls								
658 ♀	34	51	0.7835	66.79	2.49	23.74	6.98	0.294
669 ♀	40	63	0.8892	66.44	1.96	23.61	7.98	0.338
678 ♀	48	65	0.9742	64.98	4.95	21.89	8.17	0.373
689 ♂	51	83	1.1235	64.57	3.51	22.62	9.30	0.411
698 ♀	44	65	0.9720	64.07	5.12	22.10	8.71	0.394
706 ♀	46	59	0.8990	64.07	3.77	22.39	9.77	0.436
712 ♀	42	69	0.9340	62.15	4.39	22.49	10.97	0.488
724 ♂	48	63	0.9878	66.18	3.86	22.63	7.33	0.323
B. Positive controls								
One drop cod-liver oil								
668 ♀	39	47	0.7939	57.74	4.95	21.67	15.64	0.722
677 ♀	43	56	0.8305	56.68	5.74	21.39	16.19	0.757
688 ♀	36	42	0.7195	—	—	21.64	13.27	0.613
697 ♂	41	62	0.9985	57.93	6.39	21.58	14.10	0.653
705 ♂	37	54	0.8070	59.23	4.97	23.20	12.60	0.543
Three drops cod-liver oil								
657 ♀	32	44	0.7190	60.15	5.01	20.93	13.91	0.664
711 ♀	37	48	0.7561	55.14	5.99	21.93	16.94	0.773
723 ♂	40	53	0.8021	—	—	21.76	16.76	0.770
C. Basal ration (Lily)								
0.2 g. butter								
670 ♀	45	59	0.8859	64.45	3.99	23.37	8.19	0.352
649 ♀	36	49	0.7839	67.17	2.85	22.97	7.01	0.306
663 ♂	46	74	1.0993	66.86	3.87	22.33	6.94	0.311
679 ♀	42	66	0.9570	60.08	8.64	23.31	7.97	0.342
690 ♂	43	60	0.9605	63.61	5.49	21.96	8.94	0.408
713 ♂	44	66	0.9366	63.91	3.01	22.82	10.26	0.450
0.6 g. butter								
654 ♂	35	40	0.7782	66.19	4.70	20.88	8.09	0.388
685 ♀	41	60	0.8833	63.84	3.44	22.53	10.19	0.452
694 ♀	42	56	0.8680	60.83	6.54	21.29	11.34	0.532
717 ♀	41	58	0.8790	58.68	4.31	21.82	15.19	0.665
D. Basal ration (Welcome)								
0.2 g. butter								
650 ♂	39	63	0.9126	67.29	2.28	23.31	7.12	0.305
664 ♀	42	63	0.9524	65.07	2.46	23.49	8.98	0.382
671 ♀	46	70	1.0017	65.21	5.18	21.83	7.78	0.357
680 ♂	44	67	1.0821	65.75	2.85	22.91	8.49	0.370
699 ♀	37	47	0.7155	61.58	5.71	22.59	10.12	0.448
707 ♂	42	61	0.9058	60.38	4.45	22.75	12.42	0.543
0.6 g. butter								
660 ♀	42	56	0.8610	63.94	4.86	21.71	9.49	0.436
674 ♂	45	60	0.9105	59.21	6.59	21.91	12.29	0.643
702 ♀	36	45	0.7440	60.40	7.20	21.75	10.65	0.489
718 ♀	37	47	0.7661	61.74	2.86	23.23	12.17	0.523
E. Kale (Bertha)								
0.2 g. butter								
651 ♀	37	52	0.8222	66.78	3.61	22.61	7.00	0.310
665 ♂	43	60	0.9856	66.82	4.69	23.59	4.90	0.208
681 ♀	42	58	0.9320	63.63	3.63	23.35	9.39	0.402
691 ♀	44	55	0.9465	57.95	8.11	23.20	10.74	0.463
708 ♀	42	56	0.8650	59.77	7.32	22.66	10.25	0.452
714 ♂	43	57	0.8794	62.54	5.93	23.34	9.19	0.391
0.6 g. butter								
655 ♂	34	41	0.7866	66.10	4.53	21.26	8.11	0.381
686 ♀	39	59	0.8465	63.22	3.53	22.67	10.58	0.467
695 ♀	42	53	0.8255	57.12	8.72	21.58	12.58	0.582
719 ♀	38	58	0.8670	60.55	5.13	21.57	12.75	0.591

Table IV (*cont.*).

Rat	Initial weight g.	Final weight g.	Weight of bone g.	% of water	% of fat	% of organic residue	% of ash	Ratio A/R
F. Kale (Firefly II)								
0.2 g. butter								
652 ♀	35	54	0.7990	66.64	3.89	21.96	6.89	0.312
666 ♀	44	57	0.8861	65.28	3.04	23.76	7.92	0.333
672 ♂	50	66	0.9970	63.65	6.28	22.19	7.88	0.355
682 ♂	45	63	1.0265	63.84	3.50	22.83	9.83	0.431
700 ♀	37	45	0.7890	63.13	4.03	23.39	9.45	0.404
715 ♂	41	59	0.8940	62.03	4.86	21.87	11.24	0.511
0.6 g. butter								
661 ♀	42	58	0.8935	65.78	4.63	21.13	8.46	0.401
675 ♀	44	47	0.9003	61.03	7.17	21.25	10.55	0.497
703 ♂	36	47	0.7860	62.21	5.35	21.44	11.00	0.514
720 ♂	40	47	0.7741	53.76	10.67	21.84	13.73	0.639
G. Cod-liver oil (Rosemary)								
0.2 g. butter								
653 ♀	34	55	0.8432	67.06	2.95	22.46	7.53	0.335
667 ♂	42	69	1.0238	66.90	4.22	20.88	8.00	0.384
683 ♀	41	59	0.9530	63.55	3.93	22.00	10.52	0.465
692 ♀	43	65	0.8730	60.57	6.83	21.94	10.66	0.486
709 ♀	37	47	0.7695	59.71	12.13	18.23	9.93	0.541
716 ♂	40	59	0.8720	62.21	4.24	21.62	11.93	0.639
0.6 g. butter								
656 ♂	36	63	0.9273	62.89	4.84	20.30	11.97	0.590
687 ♀	37	43	0.8120	59.17	4.47	21.64	14.72	0.680
696 ♀	42	55	0.8835	57.55	7.91	21.43	13.11	0.612
721 ♂	40	57	0.8619	56.66	6.97	21.40	14.97	0.699
H. Cod-liver oil (Doll II)								
0.2 g. butter								
659 ♀	44	68	0.9336	64.53	4.69	21.19	9.59	0.452
673 ♀	47	68	0.9480	63.05	5.78	21.23	9.94	0.469
684 ♂	45	59	0.9855	63.12	3.93	22.07	10.88	0.473
693 ♀	43	54	0.8690	60.64	6.59	22.47	10.30	0.459
701 ♂	43	56	0.9150	62.18	3.71	22.36	11.75	0.526
710 ♂	41	67	0.9430	60.77	6.20	21.95	11.08	0.505
0.6 g. butter								
662 ♀	42	64	0.9453	64.05	4.12	21.13	10.70	0.507
676 ♂	44	54	0.8657	56.67	7.73	19.94	15.66	0.785
704 ♀	34	47	0.7560	60.85	5.79	19.97	13.39	0.669
722 ♀	36	43	0.7338	54.75	7.56	21.34	16.35	0.767

obtained with rats fed on the butters from the different cows. It is seen that the rats receiving 0.2 g. of butter from the basal cows and from the kale-fed cows behaved very much like the negative control rats receiving no butter supplement in their diet. On the other hand, the same dose of butter from the "cod-liver oil cows" showed a definite antirachitic potency (Fig. 1). When the doses of the butters given to the test rats were trebled (Fig. 2) the butter from the basal and kale-fed cows showed an antirachitic potency of the same magnitude. This potency was, however, decidedly lower than that shown by the butters from the "cod-liver oil cows." The latter butters were almost as protective in this dose as the one drop of cod-liver oil given to some of the animals of the positive control rats. As there was only a small difference in the behaviour of the two sets of animals in the positive control group, one may conclude that the maximum standard of calcification possible to demonstrate

Table V.

Diet and cow	Dose of butter g.	% H ₂ O	% fat	% organic residue	% ash	Ratio A/R
Basal						
Lily	0.2	64.35	64.28	4.64	22.79	0.368
Welcome	„	64.21	3.82	4.23	22.73	0.401
Lily	0.6	62.77	61.55	4.75	21.63	0.509
Welcome	„	61.32	5.38	5.07	22.15	0.523
Kale						
Bertha	0.2	62.92	63.51	5.55	23.13	0.371
Firefly II	„	64.10	4.27	4.91	22.67	0.391
Bertha	0.6	61.75	61.23	5.48	21.77	0.505
Firefly II	„	60.70	6.96	6.22	21.43	0.513
Cod-liver oil						
Rosemary	0.2	63.33	62.86	5.71	21.19	0.475
Doll II	„	62.38	5.15	5.43	21.53	0.481
Rosemary	0.6	59.07	59.08	6.05	21.19	0.645
Doll II	„	59.08	6.30	6.18	21.10	0.632
Negative controls	—	64.89	3.76	22.68	8.65	0.382
Positive controls	1 drop	57.89	5.51	21.90	14.36	0.658
	3 drops	57.65	5.50	21.54	15.87	0.735

by this method has been almost reached by the administration of one drop of cod-liver oil.

In comparing the results of the vitamin D tests of the butters with those of the vitamin A tests it becomes evident that whilst the addition of kale to the basal ration raises the vitamin A content of the milk it does not contribute to the enhancement of its antirachitic potency. In the case of the "cod-liver oil cows" there is a marked augmentation in the potency of both vitamins.

In our experiments the water content was higher and the ash of the bones lower than in those of Chick, Korenchevsky and Roscoe [1926]. This is most probably due to the fact that the batch of rats employed by us had lower initial reserves of the antirachitic vitamin. Our results, however, fully confirm the observation of these workers. There is a decided higher water and lower ash content in the bones of the negative control animals as compared with the positive control group. The corresponding differences in fat content and the "organic residue" are also similar. It will be seen that the value of the ratio of ash to "organic residue," as suggested by the above workers, serves as a very convenient index of the antirachitic protection effected by substances incorporated in the basal diet.

The comparative superiority of the calcium-depositing properties of butters from cows receiving cod-liver oil over those of butters from kale-fed cows also emerges from the results we obtained in our 1924-25 experiments. The test rats were fed on a basal diet complete in every respect except the fat-soluble vitamins, and growth was observed by the method of Zilva and Miura and the mineral ash content of the bones of the test rats estimated after a period of 7 weeks on the experimental diet to which small daily rations of the butter were added. The butters were tested on three different occasions during the period in which the cows were on the experimental diets. In the third test the

Fig. 1.

Fig. 2.

Figs. 1 and 2. The curves show the average increase in weight of the test rats. The preliminary period is omitted. Individual deaths in the groups are marked by a cross.

results were most marked, since by then the reserves of the antirachitic vitamin of the cows were run out and consequently the influence of the antirachitic character of the diet on the milk was most pronounced. In Table VI only the averages of the percentages of ash in the fat-extracted bone from the rats on the various butters in the third test are given. Three doses of butter were used in this investigation, namely, 0.2 g., 0.4 g., 0.8 g. The last dose, however, was found to be too high and had a disturbing effect on the rats, for which reason we adopted 0.2 g. and 0.6 g. as test doses in the 1925-26 investigation.

From Table VI it is seen that whilst the percentages of the ash content of the bones from the rats receiving the butters from the basal diet cows and from

Table VI.

Cow and diet				% of ash in dry extracted bone	
				0.2 g. butter	0.4 g. butter
Basal diet					
Bertha	...	...	...	52.27	52.52
Carlet	...	...	...	52.77	
Basal diet and kale					
Firefly II	...	...	...	52.99	52.58
Welcome	...	...	...	53.25	
Basal diet and cod-liver oil					
Doll II	...	...	...	55.94	56.08
Winsome	...	...	...	56.54	
Negative controls				51.90 (only basal diet)	—
Positive controls				59.16 (3 drops of cod-liver oil)	—

the kale-fed cows are the same and almost of the same magnitude as the negative controls, the percentage of the ash in the group of rats on the "cod-liver oil butter" is definitely higher. There is no differentiation between the behaviour of the animals on the 0.2 g. dose and those on the 0.4 g. dose. This we can only explain by the fact that the less vigorous rats of the available supply were used purposely in testing the higher dose. The results of this experiment, however, when taken in conjunction with those of the 1925-26 investigation indicate an improvement in the antirachitic potency of the "cod-liver oil butters" as compared with the butters from the kale-fed cows and with those from the cows on the basal diet.

CONCLUSION.

Taking the A/R ratio in the bones of our test animals as the basis of comparison for the antirachitic potency of the different butters it is seen that the antirachitic value of the milk varies with the increase in the A/R ratio above the figures obtained with the negative controls. By comparing these variables it is found that the antirachitic potency of the "cod-liver oil butters" is twice as high as that of the butters from the kale-fed cows and from the cows fed on the basal ration. An increase of this magnitude can only be explained by

a passage of the antirachitic vitamin of the cod-liver oil into the milk. One must of course consider the possibility of an increase in the vitamin content of the butter owing to the decreased yield of butter-fat brought about by the administration of the cod-liver oil. Such an increase, however, could, as we have seen, only be of the order of 15 %. Experiments with smaller doses of cod-liver oil will throw further light on this aspect of the question.

The passage of the antirachitic vitamin from the diet into the milk of the lactating animal cannot be looked upon as an unexpected result when one considers the analogous behaviour of the other vitamins in this respect. The work of McCollum, Simmonds and Pitz [1916] on rats, mentioned in the introductory part of this communication, Miss Hume's investigations at Vienna on human milks [1923], Korenchevsky's [1921, 1923, 1925] contributions on the influence of the presence of the antirachitic vitamin in the diet of the pregnant and lactating rat on the progeny, all pointed to the passage of the antirachitic principle from the diet into the milk of the mother.

By observing the effect upon children suffering from florid rickets Wagner and Wimberger [1925] studied clinically the relative antirachitic potency of milks from cows receiving cod-liver oil in their ration and from cows subsisting on a basal diet. Parallel laboratory tests were carried out on the milks used by Wagner and Wimberger by Dr E. M. Luce at the Lister Institute with the object of ascertaining the relative vitamin A and vitamin D activity of the samples of milk. In view of the fact that the growth-promoting test was employed in the estimation of vitamin A these results may not be correctly interpreted, but the antirachitic tests, in spite of the small number of rats employed, and the rather doubtful basal diet of the cows (clover, brewer's grains, clover hay and bran) show that the "cod-liver oil" milks were definitely superior to the control milks. Thus for test rats on a dose of 5 cc. of milk the percentage of calcium in dry bone (not extracted) was 16.5 for the "cod-liver oil milk rats" as compared with 14.1 for the "control milk rats." This differentiation was rather less marked with the higher dose of 10 cc., probably because of the appreciable antirachitic potency of their control milk. The cure of florid rickets was presumably too severe a test and influenced by the negative results which they obtained, Wagner and Wimberger, to our mind, discount the suggestive nature of the results on rats when they say "Im Zusammenhang mit den Versuchen am menschlichen Säugling aber darf man auch dieser geringen Überlegenheit der 'Lebertranmilch' keinen zu grossen Wert beilegen."

Our results are not without practical significance when we consider that infants born during the winter and brought up on cow's milk would depend almost entirely, unless cod-liver oil were given, for their supply of the antirachitic vitamin on the milk, during the early months of their existence. The antirachitic value of the average winter milk would not be of a much higher order than that of the milk from our basal- or kale-fed cows. The antirachitic potency of such milk, it appears from our results, can be doubled by administering cod-liver oil to the cows during the winter months. Several points have

still to be cleared up. It is of importance to ascertain whether lower doses of cod-liver oil, such as do not depress the yield of milk fat, will enhance the anti-rachitic potency of the milk to the same extent as the higher doses. Also whether the administration of cod-liver oil to the cows during the summer months could raise the antirachitic activity of their milk in summer also, and lastly to obtain clinical confirmation of the above laboratory observations. The latter task is fraught with many difficulties, but should not be insuperable in spite of the negative results so far obtained. The clinical confirmation of the laboratory work in connection with the other vitamins encourages one to think that a parallelism also exists in this case.

SUMMARY.

(1) The inclusion of kale in the winter ration of the cow raises the vitamin A content but not the vitamin D content of the milk.

(2) The inclusion of cod-liver oil in the winter ration of the cow raises the vitamin A and the vitamin D content of the milk.

(3) Previous observation that the administration of high doses of cod-liver oil to cows reduces the percentage of fat in their milk is confirmed.

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