CLXVIII. FURTHER OBSERVATIONS ON THE INFLUENCE OF SUNLIGHT UPON THE GROWTH-PROMOTING AND ANTI-RACHITIC PROPERTIES OF COW'S MILK.

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IN a recent communication [Luce 1924], it was shown that during summer, when the cow was pasture-fed, the anti-rachitic value of the cow's milk was increased above that of the milk afforded by the same animal when stall-fed and kept in the dark. The growth-promoting value of the milk was also shown to vary within wide limits which corresponded to variations in the animal's diet. Upon a diet of fresh green grass she yielded a milk superior in growth-promoting quality to that obtained when her diet was a dry fodder, deficient in fat-soluble vitamins. An attempt was also made to determine the effect on the anti-rachitic and growth-promoting value of the milk of exposing the cow to varying degrees of illumination, whilst keeping her upon a constant diet, but the results obtained up to the time of publication were not conclusive.

Since then the experiments have been continued by keeping the animal for eight months on the diet deficient in fat-soluble vitamins and comparing the value of the milk given respectively when in a stall under winter conditions and when exposed to sunlight of summer intensity.

The cow was of Jersey breed and was kept under carefully controlled conditions at the Serum Farm of the Lister Institute at Elstree, Herts., for eighteen months previous to, and during, the present observations. My acknowledgements are due to Dr MacConkey and his staff, who, during the whole period, kindly undertook the care, management and feeding of the cow as well as the collection and pasteurisation of the various milk samples.

Method of Experiment.

The present experiment was in effect a continuation of the last period (Period V), described in the original paper [1924, pp. 718-23]. The same cow was used, and the same technique employed for evaluating the samples of milk. Three samples were tested between October 23rd, 1923, and July 2nd, 1924. During this time the diet of the cow was unaltered, and was deficient in fat-soluble vitamins. It consisted of a daily ration of white maize meal $3\frac{3}{4}$ lbs.,

gluten meal 3 lbs., oats and barley (crushed) 3 lbs., mangolds 50 lbs., barley straw *ad lib.* The following samples of milk were tested:

1. Sample XV, representing the pooled milk afforded from December 12th to 19th, 1923, after the cow had received the above diet for two months and been kept out of doors in a ploughed enclosure by day and in her stall by night. The results obtained with this milk were given in the first communication and are resumed here in Table I and Fig. 1 (*a*) for comparison.

2. Sample XVI, pooled milk afforded from April 9th to 16th, 1924. Owing to the weather it was found necessary to keep the cow in stall from December 20th, 1923, to April 16th, 1924. The stall, however, was kept as light as possible by removing the upper half of the door by day.

3. Sample XVII, pooled milk afforded from June 25th to July 2nd, 1924. On April 17th, 1924, the cow was again let out into the ploughed enclosure where she remained exposed to all the available sunlight till July 2nd, 1924.

This last interval included several periods of very fine weather: April 17th to 21st (average sunshine 10 hours daily), and June 14th to end of the period, July 2nd (average sunshine 9 hours daily). During the whole division (77 days) the average number of hours of sunshine daily was 6.3. The incidence of sunlight upon the cow was therefore in marked contrast to that of the periods preceding the taking of samples XV and XVI, where the average number of hours' sunshine daily was about three, and, moreover, the animal was for the most part kept indoors.

*Variations in the intensity of sunlight received by
the cow during the three divisions of Period V.*

The intensity of sunlight during the three divisions of Period V, at the end of which samples XV, XVI and XVII respectively were tested, can be roughly gauged by a study of the Meteorological Records taken at Rothamsted Experimental Station, Herts. These records show that during the first division, October 23rd, 1923, to December 19th, 1923 (sample XV), the light was of a gradually decreasing intensity: 5.6 hours per day are recorded during the last week in October, 1923, an average of 3.4 hours per day during November and 1.3 hours during December. During the second division, it increased from an average of 1.9 hours per day during January and February, 1924, to an average of 5 hours per day during March and April, but during this time the cow, as already stated, was not out in the open. During the third division the incidence of sunshine was 6.1 hours daily in May and 6.6 hours per day in June. During this time the cow was kept out of doors.

The following table gives the total number of hours of sunshine recorded in each of the three divisions of Period V.

Table I.

No.	Period V	Cow	Sample tested at end of period	No. of days	Total hours of sunshine	Average sunshine per day (hours)
1	Oct. 23-Dec. 19, 1923	In ploughed enclosure	XV	58	167	2.9
2	Dec. 20-April 16, 1924	In stall	XVI	118	374	3.2
3	April 17-July 2, 1924	In ploughed enclosure	XVII	76	482	6.3

THE GROWTH-PROMOTING VALUE OF THE MILK.

The criterion used for estimating the growth-promoting value of the milk was the same as that adopted previously, viz. the reciprocal of the daily dose necessary to induce young rats, which had ceased to grow on the basal "—A" diet, to resume growth in body weight to the extent of 10–12 g. per week, the period of observation being 35 days. With the three samples of milk tested (XV, XVI, XVII) this dose was found to be between 5 and 8 cc. daily. The milk therefore contained 20–12 growth units per 100 cc. and no difference in value could be detected between the three samples, see Protocols F–H.

It is evident therefore that the amount of sunlight received by the cow during the latter part of the time was ineffective in raising the growth value of the milk. This value, however, did not fall to the lowest level (10–6 units per 100 cc.), obtained during Period II (October, 1922–July, 1923) when the cow was fed on the same diet but kept in a dark stall.

These results confirm those obtained by Hume [1923] who failed to raise the growth-promoting value of the milk by exposing a cow, fed on cereals, roots and straw, to summer sunshine.

ANTI-RACHITIC PROPERTIES OF THE MILK.

(a) *Evidence from the determination of calcium-content in the bones of rats used in the titration of samples XV, XVI, XVII. Period V.*

The results obtained by estimating the percentage of calcium (on dry weight) in bones of rats (femur, tibia, and fibula), are displayed in Fig. 1 (a) (and Protocols F–H). The increase in calcium content, as compared with

Fig. 1. Average calcium content (percentage reckoned on dry weight of bone) in bones of rats receiving various daily supplementary doses of milk, compared with that in control animals on basal (—A) diet alone.

(a) Milk samples XV–XVII, Period V: cow on dry fodder and exposed to light.

(b) Milk samples V–X, Period II: cow on dry fodder but in a darkened stall (Fig. taken from Luce, 1924, Fig. 2, p. 727).

[illegible]

Protocol H. Period V. Sample XVII. Dry Fodder; Light +.

Rat	Sex	Family	Dose	Initial wt	Final wt	Post-mortem evidence of rickets	In bones % Ca (dry)	Histological diagnosis
974	♀	1	No milk	73	76	—	15.2	Osteoporosis
975	♀	1	"	73	52	Died	16.9	"
978	♀	2	"	72	57	"	14.9	"
981	♀	2	"	82	57	—	15.8	"
988	♀	3	"	66	53	—	13.7	"
989	♀	3	"	61	55	Died	15.9	"
Av. control 15.4								
973	♀	1	3 cc.	64	83	—	15.3	Osteoporosis
980	♀	2	3 "	72	88	—	16.7	"
983	♀	2	3 "	67	—	Died	—	—
987	♀	3	3 "	48	79	—	17.0	Slight osteoporosis
Av. 3 cc. 16.3								
971	♀	1	5 cc.	70	119	—	15.8	Osteoporosis
982	♀	2	5 "	73	103	—	17.7	Slight osteoporosis
984	♀	2	5 "	53	87	—	17.6	"
990	♀	3	5 "	63	117	—	18.0	"
Av. 5 cc. 17.3								
970	♀	1	8 cc.	82	128	—	16.6	Nearly normal
977	♀	2	8 "	74	110	—	19.8	"
985	♀	3	8 "	62	105	—	18.9	"
Av. 8 cc. 18.4								

control animals, in bones of rats receiving 3–5 cc. doses of milk daily of samples XV–XVII is seen from Fig. 1 (a) to be slightly greater than that obtained with rats receiving the same supplements of milk from Period II (samples V, VI, VII, VIII, IX and X, Fig. 1 (b)) when the cow was fed on the same diet, but kept in a specially darkened stall [1924, p. 727].

In order to interpret correctly the results obtained, it is necessary to consider not only the effect of sunlight on the cow, but the effect of varying conditions of light in the laboratory on the rats used for titrating the milk, and of any possible variation there may have been in the reserves of the anti-rachitic factor possessed by these animals.

It was sometimes noticed that control animals, receiving no milk, appeared to grow better and to show a higher calcium content of bone in summer when the illumination of the rooms was at a maximum [1924, p. 726] than in winter. In both seasons the light passed through window glass. Allowance has been made for this possible source of error. In Fig. 2 is shown the *average percentage increase in calcification above that of the controls*, in animals receiving 3, 5 and 10 cc. doses daily of the milk of Period II (samples V, VI, VII, VIII, IX and X) compared with that of Period V (samples XV, XVI and XVII). Each point in the lowest curve (Period II) of this figure represents the average percentage increase over controls in the case of 24 rats, and summarises the results of tests of six samples with four rats on each dose: 3 cc., 5 cc., 10 cc. respectively. In the middle curve (Period V) each average corresponds to

results obtained with twelve rats, as three samples of milk only were tested in this period.

From Figs. 1 and 2 it would appear that the milk from a cow exposed to light possesses a *slightly but definitely higher calcium-depositing power* than that from the same animal on the same diet, but shut up in a dark stall.

In Fig. 2 a curve showing results obtained during Period IV when the

Fig. 2. Mean percentage increase over control animals in bone-calcification of rats receiving:

Curve II. 3, 5 and 10 cc. daily of milk taken in Period II. Dry Fodder; Dark (milk samples V, VI, VII, VIII, IX, X).

Curve V. 3, 5 and 10 cc. daily of milk taken in Period V. Dry Fodder; Light (milk samples XV, XVI, XVII).

Curve IV. 3, 5 and 10 cc. daily of milk taken during Period IV. Pasture-feeding (milk samples XIII, XIV).

cow was pasture-fed, is included for purposes of comparison. Its significance is discussed later.

(b) *Evidence from post mortem and histological observations of rats used in titration of samples XV, XVI, XVII.*

In the group of rats used for the titration of sample XV, rickets occurred in two out of four control animals, but in none of the three animals receiving 3 cc. of milk; the rats receiving higher doses showed by histological examination of the costo-chondral junction either a normal picture or one of slight osteoporosis (Protocol F).

With the rats used in the titration of sample XVI the results obtained were similar. A histological picture of rickets was obtained in three out of six control animals, but the milk-fed animals receiving doses of 3 cc. and upwards showed no evidence of rickets (Protocol G).

The six control animals used in the titration of sample XVII gave a histological picture of osteoporosis only, and none of the rats receiving 3 cc. of milk and upwards showed evidence of rickets (Protocol H).

Table II. *Incidence of rickets in the rats used in titrating milk samples taken during Period II (Dry Fodder; Dark) and Period V (Dry Fodder; Light) respectively.*

Period	Controls		Milk 3 cc.		Milk 5 cc.	
	Total	No. showing rickets	Total	No. showing rickets	Total	No. showing rickets
II	18	11	16	15	15	14
V	16	5	11	0	12	0

Table II shows that the incidence of rickets in the control animals receiving no milk is twice as great in Period II as in Period V. The rats receiving 3 and 5 cc. of the milk of Period II showed a *higher percentage incidence of rickets than the controls.*

In the rats used for testing milk of Period V, rickets occurred in none of the animals receiving 3 cc. milk, so that the difference between the two periods in the incidence of histological rickets in the milk-fed animals is very marked, *being much greater during Period II when the cow was kept in the dark.*

(c) *Evidence from experiments with rats using McCollum's rickets-producing diet No. 3143.*

Three families of rats, 22 in all, were used for testing the rickets-preventing value of sample XVII, taken at the *end* of Period V. As is described in the original paper, rats were fed on McCollum's No. 3143 rickets-producing diet for a period of 28 days with milk supplements of 3, 5, 8 and 10 cc. daily, whilst two rats from each litter received no milk and acted as controls. Results of this experiment are given in Table III. The protective dose with this milk was found to be 10 cc., the same result as that obtained with sample XV at the *beginning* of Period V (see previous paper, Table V, p. 730).

Table III. *Anti-rachitic value of milk at end of Period V. Sample XVII (Dry Fodder; Light). Rats on McCollum's 3143 diet for 28 days with milk supplements.*

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones % Ca (dry)	Histological diagnosis
999	♂	1	No milk	48	81	+++	9.0	Severe rickets
1000	♂	1	"	42	62	+++	9.9	"
1007	♀	2	"	50	93	++	12.1	"
1008	♀	2	"	47	73	+++	11.6	"
1019	♀	3	"	52	85	++	12.5	Pronounced rickets
1020	♀	3	"	47	80	+++	10.6	Severe rickets
Av. control 10.9								
1001	♂	1	3 cc.	45	100	+++	11.9	Severe rickets
1009	♀	2	3 "	46	92	+	15.9	Mod. rickets
1010	♀	2	3 "	51	108	+	15.1	"
1021	♂	3	3 "	47	87	++	13.4	Pronounced rickets
Av. 3 cc. 14.0								
1002	♀	1	5 cc.	48	109	++	13.2	Pronounced rickets
1003	♀	1	5 "	46	90	+	15.0	"
1011	♀	2	5 "	15	116	+	14.5	"
1022	♀	3	5 "	46	93	++	13.5	"
Av. 5 cc. 14.1								
1023	♂	3	8 cc.	44	113	?+	16.0	Slight rickets
1004	♀	1	8 "	46	111	?+	17.0	"
1006	♀	1	8 "	44	89	?-	17.7	"
1013	♀	2	8 "	47	126	?-	17.3	"
Av. 8 cc. 17.0								
1012	♀	2	10 cc.*	45	108	-	18.6	Nearly normal
1005	♀	1	10 "	45	93	-	18.0	"
1024	♀	3	10 "	45	125	-	18.5	"
1014	♀	2	10 "	50	131	-	18.5	"
Av. 10 cc. 18.4								

* Protective dose.

It is evident from this that exposure of the cow to direct sunlight for an average of 6.4 hours daily for 77 days failed to raise the anti-rachitic value of milk to a degree that could be detected by this severe test.

At the same time the anti-rachitic value of this milk did not fall to the level of that tested at the end of Period II (Dry Fodder; Dark) and Period III (Green Fodder; Dark) when 15-20 cc. was found to be the dose necessary for protection against rickets.

DISCUSSION OF RESULTS.

The growth-promoting value of milk appears to depend principally, if not entirely, on the diet of the cow. After feeding her for a period of three months on a diet deficient in fat-soluble vitamins, the growth value of her milk fell to a low level and remained at this level for five months longer, in spite of the

fact that during part of this time she was exposed to sunshine of summer intensity.

A slightly lower level of growth-promoting value was reached in a previous experiment, when, on the same diet, the animal was shut up in a dark stall. It is possible that this difference may be within the limits of experimental error, and the conclusion reached, in confirmation of the previous work and of the results of other investigators, is that *variations in the growth-promoting power of the milk depend upon the presence or absence of fat-soluble vitamins in the diet of the cow.*

The anti-rachitic value of the milk. There is some evidence from these experiments that *exposure of the cow to sunlight plays a part in raising the anti-rachitic value of her milk, although this would appear to be a subsidiary one.* The following are the findings in support of this theory:

1. During Period II (dry fodder in the dark), the incidence of rickets in rats receiving 3 and 5 cc. doses of the six samples of milk tested was definite and high, and although this was correlated with a high incidence of rickets in the control animals, this indicated milk of low anti-rachitic potency, because the milk-fed animals on these doses showed a higher percentage of rickets than the controls.

2. During Period V (dry fodder in the light), when the cow was out of doors or exposed to light of low intensity, the anti-rachitic value of her milk was at a higher level than that tested during Period II. 3 cc. of these milk samples were protective.

3. The histological evidence given above is supported by results obtained from the calcium determinations of the bones of the same rats. With milk from Period II, the deposition of calcium in the bones was at a slightly, but consistently, lower level than that obtained with similar doses of milk from Period V (see Figs. 1 and 2).

The anti-rachitic potency of the milk during the period of pasture-feeding (Period IV) was, however, much greater than that yielded in Period V. The lift in the curve obtained with 3 and 5 cc. of milk [see Luce, 1924, fig. 2, p. 727], the level of the percentage increase in calcification over controls (Fig. 2, Curve IV) and, moreover, the high protective value of low doses of pasture milk shown in rats on McCollum's No. 3143 diet [Luce, 1924], give striking evidence of this.

The diet of the cow therefore appears to be the main factor in determining the anti-rachitic value of her milk, for with a diet deficient in fat-soluble vitamins it has been found impossible, by the action of sunlight alone, to raise materially the anti-rachitic value of her milk.

Hume [1923] has shown that there is an interaction between vitamin A and light, the latter causing an increased growth in rats maintained upon a diet in which this vitamin is lacking, but concludes that this interaction does not involve a photosynthesis of the vitamin but a mobilisation or economy of a store already existing in the animal's body.

Steenbock and Nelson [1923] suggest that light is without effect on *vitamin A* whilst potent to liberate a reserve of the *anti-rachitic organic factor*, and that the increased growth obtained in Hume's experiments under the influence of irradiation, is due rather to the action of light upon an *anti-rachitic* than a *growth-promoting* factor (vitamin A). In support of this theory, the results of the present experiment indicate that insolation of the cow has some effect in maintaining a certain level of anti-rachitic potency in milk, and little or no effect upon its power to promote growth. The converse has also been shown, for when the cow was fed upon fresh green food in the absence of light, an increased growth-value of the milk was observed, without a corresponding improvement in its anti-rachitic value [see Luce, 1924, p. 724]. It appears from this that *two factors are present in milk, that both are influenced by diet, and the anti-rachitic factor also, to some extent, by light.*

CONCLUSIONS.

In the original paper, of which this is an extension, it was concluded that the diet of a cow was the principal factor determining both the growth-promoting value and the anti-rachitic value of her milk. The experiment did not permit any conclusion to be drawn as to whether the light impinging upon the cow also contributed to a minor degree.

These further observations show that sunlight, of summer intensity in this country, has no significant effect in raising the growth-promoting value of cow's milk, when the diet of the cow is deficient in fat-soluble vitamins. The degree of exposure to sunlight appears, however, to have a small effect in determining the anti-rachitic value of the milk given.

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