XCIV. THE INFLUENCE OF DIET AND SUN- LIGHT UPON THE GROWTH-PROMOTING AND ANTI-RACHITIC PROPERTIES OF THE MILK AFFORDED BY A COW.

BY ETHEL MARJORIE LUCE (*Beit Memorial Research Fellow*).

From the Department of Experimental Pathology, Lister Institute.

(*Received May 1st, 1924.*)

CONTENTS.

	PAGE
I. Introduction	716
II. General method of experiment	718
III. Technique employed for evaluation of the milk	719
1. Growth-promoting value	719
2. Anti-rachitic value	721
IV. Influence of diet and light upon the growth-promoting value of the milk	723
V. Influence of diet and light upon the anti-rachitic properties of the milk	724
(a) Post-mortem evidence and histology	724
(b) Calcium content of the bones of rats	726
(c) Experiments with McCollum's 3143 diet	728
The calf	731
Summary of Section V	733
VI. General discussion of results	734
VII. Conclusions	735

I. INTRODUCTION.

THE effect of diet on the growth-promoting properties of cow's milk has been studied by several investigators. Dutcher and Kennedy [1921, 1922] investigated the influence of the diet of the cow on the quality of the milk as regards its content of vitamins A and B. They found that the vitamin content of the milk depended on the feeding of the animal, that both vitamins A and B could be supplied to a stall-fed cow by a proper combination of grain and leafy foods, and also that a vitamin-rich milk can only be obtained by feeding a cow on pasture, if the pasturage is fresh and green.

Drummond, Coward and Watson [1921] have shown that the growth-promoting value of butter varies with the season, and this is attributed by them to the difference in diet of cows during summer and winter. They have recently communicated the results of further experiments, in which they reduced the vitamin A content of milk by feeding the cow on a diet deficient

in this vitamin, and conversely increased it by the addition of cod-liver oil to the dietary. On the other hand, Hume [1923] was unsuccessful in improving the growth-promoting value of the milk of a stall-fed cow, although it was fed upon abundant green food for a period of three months. Following on her discovery that animals whose growth had become arrested upon a diet deficient in vitamin A, recommenced to grow at a normal rate, if irradiated with ultraviolet light for a few minutes daily, she explored the possibility that the milk of a cow in sunlight might show an enhanced value over that of an animal on the same diet in a dark stall. A cow was kept out of doors for 12 hours daily during the height of summer, but no significant improvement in the growth-promoting value of the milk was detected after two months. The diet was deficient in vitamin A and consisted of cereals, straw and roots.

In a recent paper [1924] Reyher gives clinical evidence that the milk from cows on fresh green fodder has a higher growth-promoting power than that from cows on dry fodder. He fed infants with milk from cows on green and dry fodder alternately, and found that their weight was arrested with surprising constancy when they received milk from cows on dry fodder.

I am unaware of any observations on the influence of diet upon the anti-rachitic properties of cow's milk, although, from analogy, it has been assumed that these would also be modified by the food supplied. This view is supported by the fact that it is impossible to produce rickets in litters of young rats for a considerable time after weaning, if the mother is well supplied with cod-liver oil during pregnancy and lactation, and conversely rickets is more easily induced, and in a severer form, in young rats when the mother has been subject to diet deficiencies during pregnancy and lactation [Korenchevsky, 1921, 1922, 1923].

The effect on the anti-rachitic value of the milk of exposing the cow to more or less sunlight has not, I believe, been investigated. After the recent discoveries of the influence of light in the prevention and cure of rickets in children and in animals, it would not be surprising should the anti-rachitic value of milk respond to variations in the radiant energy impinging upon the cow. The average anti-rachitic properties of cow's milk cannot, however, be very great, for many children reared upon it suffer from rickets. Indeed, whilst nearly every observer admits that cod-liver oil has a specific effect in the prevention and cure of rickets, there is divergence of opinion as to whether cow's milk possesses any anti-rachitic properties. Mellanby [1919, 1921] working with dogs, and Korenchevsky [1921, 1922] working with rats, however, agree that the withdrawal of milk fat from certain dietaries and its substitution by a vegetable oil determines the occurrence of rickets in these animals. Paton and Watson [1921], however, do not agree that milk fat can prevent rickets in puppies.

The experience of Hess and Unger [1920] is that children develop rickets in spite of the consumption of large amounts of cow's milk. The possibility

of the milk being of unequal value at different times of the year was not lost sight of by these workers, and in a later paper [1921] on the interpretation of the seasonal variation of rickets, they record observations on two groups of children, amongst those under their care in an institution, which were fed upon dried milks prepared from stall-fed and pasture-fed cows, respectively. Two or three out of each group developed mild rickets. Hess and Unger are evidently among those who consider milk to be devoid of anti-rachitic properties, for they formulate their conclusions thus: "the milk of cows supplied with green fodder does not acquire anti-rachitic properties." Findlay [1922] supports the view of Hess and Unger, because in his clinic many children on a diet poor in milk fat present no evidence of rickets, and some of the most severe examples of this disease occur in children fed on a diet rich in fat. The same conclusion was reached by Galbraith [1923] as a result of a large series of clinical observations.

During the clinical observations of Chick, Dalyell, Mackay and others [1923] on rickets in Vienna, children admitted with active rickets during winter and spring, were fed upon milk from stall-fed cows receiving different diets, including a diet of fresh green fodder. No healing was demonstrated in the bone lesions, in absence of other specific treatment.

The pathology of rickets in animals and in children is so essentially similar, that the discordance between clinical and experimental results demands explanation.

There are other factors which are now known to influence the development of rickets besides the presence or absence of a fat-soluble, anti-rachitic factor in diet. These are notably the degree of exposure to light and the absorption of Ca and P. It is evident that milk, unlike cod-liver oil, does not contain any superabundance of this anti-rachitic principle, and may, therefore, not have been the controlling influence in particular cases. This interpretation of clinical observations is consistent with experience in the laboratory. It is emphasised by it. Young rats receiving a ration of milk may become rachitic or not, according to the amounts of phosphorus in their diet, and the amount of direct sunlight to which they are exposed.

II. GENERAL METHOD OF EXPERIMENT.

The following experiment was started in July, 1922, and continued until December, 1923. A fawn-coloured Jersey cow, which had previously been for three months on pasture, and was, at the beginning of the experiment, three months pregnant with her seventh calf, was kept during this time under carefully controlled conditions at the Serum Department of the Lister Institute in Herts. I am much indebted to Dr MacConkey, for kindly undertaking to arrange for the feeding and management of the cow, and for the collection and pasteurisation of the milk samples.

The observations may be divided into five periods.

INFLUENCE OF DIET AND LIGHT ON VITAMINS OF MILK 719

Period I (July 5th–November 8th, 1922). *The cow was kept on a diet deficient in fat-soluble vitamins, but was out of doors in the sunshine.* She was kept out all day in a ploughed enclosure, and received daily: white maize meal, 3½ lbs., gluten meal, 3 lbs.; oats and barley (crushed), 3 lbs.; mangolds, 50 lbs.; barley straw *ad lib*.

Period II (November 9th, 1922–May 24th, 1923). *Dry fodder in the dark.* The same diet was continued as in period I, but the cow was placed in a well-ventilated, but completely dark stall. The door of the stall was divided into an upper and lower half; the upper half was replaced by a louvre for purposes of ventilation, and remained fixed. The lower half was opened a short distance each day for purposes of cleaning and milking; for the remainder of the time it was tightly closed. No direct light entered the stall, and the conditions of darkness, therefore, were as complete as was compatible with the care and well-being of the animal.

Period III (May 25th–July 31st, 1923). *Green fodder in the dark.* During this time the conditions of darkness described in period II were continued, but the diet of the cow was changed to one of freshly cut meadow grass and clover, given in equal parts, of which she ate 120–130 lbs. per day.

Period IV (August 1st–October 22nd, 1923). *Pasture feeding.* She was turned out into the fields all day, and kept at night in a light shed, built in the pasturê. The weather, especially during August, was bright and sunny, and the pasture fresh and green.

Period V (October 23rd–December 19th, 1923). The conditions described in period I were repeated.

The milk was tested at monthly intervals on rats. Equal samples of the whole morning's milking were taken, with sterile precautions, over a period of seven days. These were pooled, and CO₂ was bubbled through for five minutes, followed by pasteurisation for 20 minutes at 70° C. in an atmosphere of CO₂, to prevent oxidation of the fat-soluble vitamins. The milk was kept thereafter in the refrigerating chamber at about 0°. By this method it was found that the samples remained fresh for 8–10 weeks or even longer.

III. TECHNIQUE EMPLOYED FOR THE EVALUATION OF THE MILK.

1. *Determination of the growth-promoting value.*

Rats which had ceased to grow, from deprivation of vitamin A, were used for these tests. The rats were bred in the Institute from animals fed on a diet of fresh cow's milk, white bread, oats and bran, carrots or cabbage, with, occasionally, cooked meat. The same breeding diet was used throughout the experiment and the litters showed a fairly uniform response to the vitamin A deficiency in their diet.

The young rats were kept in a well-lighted and well-ventilated room at a temperature of 60–65° F. The windows faced N.N.E., and were kept shut;

the animals were not exposed to direct sunlight. For a preliminary period of 3-4 weeks the rats received a modification of the basal diet recommended by Zilva and Miura [1921] which consisted of the following ingredients: egg white (cooked), 20 %; starch, 50 %; salt mixture, 5 %; marmite, 5 %; lemon juice, 5 %; cotton-seed oil, 15 %; water 90 cc.

The salt mixture used was as follows:

Sodium chloride (NaCl)	51.9
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) ...	164.0
Sodium acid phosphate ($\text{NaH}_2\text{PO}_4 + 2\text{H}_2\text{O}$)	104.1
Potassium phosphate (K_2HPO_4)	286.2
Calcium phosphate [$\text{CaH}_4(\text{PO}_4)_2 + \text{H}_2\text{O}$] ...	162.0
Calcium lactate [$(\text{C}_3\text{H}_5\text{O}_3)_2\text{Ca} + 5\text{H}_2\text{O}$] ...	390.0
Ferric citrate... ..	35.4

The basal diet contained 0.28 % Ca and 0.6 % P in the fresh diet. This has been shown by Korenchevsky [1922] to be sufficient to promote normal calcification in rats receiving sufficient fat-soluble vitamins in their diet.

When growth had ceased for 10-14 days, the diet was supplemented with daily amounts of 3, 5, 7, 10, 15 and sometimes 20 cc. of the milk, in order to find the dose necessary to restore a normal rate of growth. Three to four rats were used for each dose. Twenty or more rats were used in determining the value of each sample, and individuals from different litters were distributed as evenly as possible over the experiment, with controls receiving no milk. Milk feeding was continued for 35 days, and the standard of normal growth adopted was an average of 10-12 g. increase in weight per week during the experimental period. The mean between the dose that secured this growth and the one below it, was considered to contain one vitamin unit.

Use of egg white as the sole source of nitrogen in these experiments.

Boas [1924] has shewn that when cooked egg white is used as the sole source of protein, a loss of hair and declining weight occur in quickly growing rats, after 4-6 weeks.

For the first three months of my experiment the basal diet appeared to be perfectly satisfactory, the milk-fed animals had normal coats, whilst those of the controls shewed nothing more than the staring condition associated with vitamin A deficiency. There was no definite baldness.

In later trials the condition of baldness described by Boas occurred in some, but by no means all, of the rats during the preliminary period of feeding on the basal diet. This condition persisted in some of the controls, but was cured in the milk fed animals. The small amounts of caseinogen and lactalbumin given with the milk apparently supplied the protein deficiency. An analysis of bone made by rats of the same family with and without this coat condition, shewed that it did not affect the deposition of calcium in the bones. It was therefore decided to continue the use of egg white throughout

the experiment, as it was of the utmost importance to employ a rigid technique for the biological tests. The only variable factors in this experiment are the diet and light conditions of the cow, and the results are only to be interpreted by comparison with one another.

2. *Investigation of the anti-rachitic value of the milk.*

Whilst the titration of the growth-promoting power of the milk presents little difficulty, and the results are sufficiently accurate to permit of graphic representation, the anti-rachitic value cannot be determined with the same quantitative accuracy. It is the experience of all experimenters with rats, that when a group of animals is submitted to the same conditions, post-mortem signs of rickets do not occur consistently in each member of the group, unless the animal is, at the same time, starved of phosphorus, as is the case when McCollum's basal diet No. 3143 is employed.

With the basal diet described above, which contains an adequate supply of Ca and P, only a proportion of the control rats manifest post-mortem signs of rickets such as enlarged costochondral junctions, diminished rigidity of the skeleton, deformity of the chest and spontaneous fractures of the ribs. Taking all the control animals observed over 18 months, the proportion was 50 % by post-mortem, 60 % by histological diagnosis. When rickets ensued, consequently, it did not fall equally on all animals of a group. Sometimes all, but more often only 1, 2 or 3 of the 4 receiving a particular dose of milk would display obvious signs of rickets, whereas the others appeared to have more or less normal skeletons.

In view of this variation in the incidence of rickets, the post-mortem observations from period II onwards were supplemented in three ways. *Firstly*, a histological examination of the rib-junctions was made to confirm post-mortem observations. Two ribs from each animal, fixed in normal saline solution containing 10 % formalin for 48 hours, were partially decalcified in Müller's fluid, embedded in paraffin, sectioned and stained with haematoxylin and eosin. *Secondly*, the degree of calcification which had taken place in the five weeks' period of observation under the influence of the different rations of the various milk samples was determined, and compared with the deposition of calcium salts in the control animals which had received the basal diet only. The calcium content of the bones of both hind legs (femur, tibia and fibula) was estimated by Aron's method [Aron and Sebaauer, 1910]. *Thirdly*, a determination was made of the minimal daily dose of a particular sample of milk required to prevent any histological manifestation of rickets in young rats fed upon McCollum's rickets-producing diet No. 3143¹. Rats aged three to four weeks, when fed upon this diet without the addition of cod-liver oil, or other animal fat, invariably develop severe rickets. Using this method, it was possible to assign to each sample the possession of a

¹ Wheat 33 %, maize 33 %, gelatin 15 %, wheat gluten 15 %, NaCl 1 %, CaCO₃ 3 %.

Fig. 1. Vitamin A (growth-promoting) value of milk expressed as units per 100 cc. Samples I-XV
Total Vitamin output of the cow in units per day

INFLUENCE OF DIET AND LIGHT ON VITAMINS OF MILK 723

certain number of "anti-rachitic units," that is, the method was roughly quantitative. Young rats weighing 47-55 g. were fed on McCollum's 3143 diet supplemented with different rations of the various milks for 28 days and compared with control animals receiving no milk.

The employment of McCollum's diet is a very severe test for the anti-rachitic factor. Its content of phosphorus is inadequate, and this is a severe handicap to the process of calcification. On the other hand the estimation of the amount of calcium in the bones is a more sensitive indication of the disturbance of calcification and can be employed with a basal diet in which the amount of salts is adequate.

IV. INFLUENCE OF DIET AND LIGHT UPON THE GROWTH-PROMOTING VALUE OF THE MILK.

In order to obtain comparable values for the growth-promoting value of the various samples of milk an arbitrary standard was fixed. This standard was the dose that restored a growth of 10 to 12 g. on an average per week in rats whose weight had become stationary on a diet deficient in fat-soluble vitamins.

This fixing of such an arbitrary end-point is open to criticism. The most one can say about its accuracy is that one particular dose was sufficient, whilst the one below was insufficient, to restore a certain rate of growth. The end-point, in reality, lies between the two, and is more accurately expressed in a graph showing the mean between two limits. This method of representation has been adopted in Fig. 1 and the two limits are indicated by the vertical lines. Tests were made with 15 samples of milk, taken at intervals over the five periods described at the beginning of the paper; the results showed that the limits from which the end-point was calculated varied according to the spacing of the doses, as shown in the following table:

Date	Period	Sample	Growth-promoting, end-point between	Growth units in 100 cc.
July 1922	I	I	2- 3 cc.	50-33
Aug.	"	II	3- 4	33-25
Sept.	"	III	4- 5	25-20
Oct.	"	IV	7- 8	14-12
Nov.	II	V	7-10	14-10
Dec.	"	VI	7 (-10)	14 (-10)
Jan. 1923	"	VII	5-10	20-10
Feb.	"	VIII	5-10	20-10
April	"	IX	10-15	10- 6
May	"	X	7-10	14-10
July	III	XI	5-10	20-10
July	"	XII	3- 5	33-20
Sept.	IV	XIII	2- 3	33-50
Oct.	"	XIV	2- 3	33-50
Dec.	V	XV	6- 8	18-12

The continuous line in Fig. 1 represents the number of vitamin units per 100 cc. in the samples tested; the broken line shows the total output in vitamin units per day reckoned from the total milk excretion of the cow.

From February–April, 1923, the cow was suckling her calf, so that during this period, as indicated in the graph, the total milk excretion was not measured.

It is evident that the growth-promoting power of the milk is controlled by the *diet of the cow*. In period I when conditions of illumination were unchanged, a drop from 41 to 13 units per 100 cc. was obtained by changing from a diet of pasture to one of dry food deficient in fat-soluble vitamins; and a rise in period III from 12 to 26 units by the substitution of fresh green food for dry fodder.

It is also evident that the effect of diet is *not immediate*. It took three months for the growth-promoting power of the milk to fall from 41 to 13 units per 100 cc. in period I. At the beginning of the experiment the animal was in good condition. She had previously been out at pasture for three months, and during this time had received in addition a daily ration of hay and oil cake. A preliminary test showed her to be giving milk of high vitamin A value (41 units per 100 cc.), and she had, in all probability, a considerable store of vitamin A in her tissues. It is, therefore, not surprising that it should take time to deplete this store. It is also possible that *three months was too short a time to produce the maximum effect*, and that the further small drop obtained during period II might have occurred, had the cow been kept out of doors, on the same diet, a couple of months longer.

The calf was born on January 27th, 1923. The last ante-partum sample of milk tested (sample VI) was of low vitamin A value¹, the first two post-partum samples (VII and VIII) showed a slightly improved value. Whilst this may be only a variation, within normal limits, of the growth-promoting power of the milk, there is also the possibility of there being an ante-partum storage, and subsequent post-partum liberation of the growth factor, for the needs of the calf.

The observations afford *no evidence as to whether the degree of illumination of the cow influences the growth-value of the milk*.

(a) The small diminution from 13 to 8 vitamin A units per 100 cc. which occurred in darkness following three months on dry fodder out of doors and (b) the striking improvement from 26 to 41 units per 100 cc. when pasture feeding was substituted for grass feeding in the dark, both suggest a beneficial influence of light. Both, however, might have happened had the periods been extended during which illumination was kept constant.

V. THE INFLUENCE OF DIET AND LIGHT UPON THE ANTI-RACHITIC PROPERTIES OF THE MILK.

(a) *Evidence from post-mortem observations and histological diagnosis on rats receiving the basal diet supplemented by different doses of the milks.*

At the beginning of the experiment in period I, when the cow was fed on cereals and oats and straw, and kept out of doors, the post-mortem appear-

¹ The scale of doses used for testing this sample did not go above 10 cc., which was inadequate as indicated by dotted line in graph.

ance of the rats receiving milk was not sufficiently striking to warrant a histological examination being undertaken. Three out of twelve control animals showed post-mortem evidence of rickets, but none of the rats receiving 2 cc. of milk or upwards showed macroscopic evidence of rickets.

A striking change was observed in the post-mortem appearances of the rats receiving milk from sample VI (period II, Protocol 4). Out of 15 animals in this group, receiving doses of milk ranging from 3–10 cc., 11 showed post-mortem, and 15 histological, evidence of rickets—2 out of 3 control animals in this group were also rachitic, the other showed an extreme degree of osteoporosis. It is obvious, therefore, that a daily dose of 10 cc. of this milk was not protective.

As a result of this observation it was resolved in future to supplement post-mortem observations in all animals by histological examination of costochondral junctions and determinations of the calcium content of the bones.

Six samples of milk were tested during period II, whilst the cow was on dry fodder but kept in complete darkness, and during this time the rats receiving both 3 and 5 cc. doses of milk showed an even higher percentage of rickets than the controls of this period, and many receiving up to 10 and 15 cc. were also rachitic.

During the following two months, period III, when the cow was still in the dark but fed on fresh grass and clover, the rats receiving 3 cc. doses still showed a higher percentage of rickets than the controls of this period, whilst the percentage occurrence of rickets in those receiving 5 cc. doses was slightly lower. Two out of six rats receiving 10 cc. of milk during this period showed evidence of slight rickets histologically.

The milk obtained after six weeks of pasture feeding (period IV) showed a marked change. Out of 40 rats receiving doses varying from 1 to 15 cc. daily, only one showed evidence of rickets at post-mortem examination. This was one of eight animals which received a 1 cc. dose of milk and proved on histological examination to show evidence of osteoporosis and not of rickets. Four out of eight control animals in this series showed histological evidence of rickets.

On reverting to the diet of dry fodder for two months (period V), the cow being still out of doors, the milk retained its high anti-rachitic value. None of the rats receiving 3 cc. doses and upwards showed any signs of rickets, while two out of the four control animals showed histological evidence of rickets.

An analysis of the incidence of rickets among the rats receiving the milk corresponding to the different periods of the cow's history tells the following story:

(a) When the cow was kept out of doors in the light, whether on dry fodder or pasture, none of the rats receiving 3 cc. doses and upwards showed histological evidence of rickets, which occurred in 50 % of the control animals.

(b) The highest incidence of rickets in milk-fed rats occurred with milk obtained during the period of dry feeding in the dark. Animals receiving 3 and 5 cc. doses of this milk daily showed an even higher percentage of rickets than the control animals. Doses of 15 cc. and upwards were usually needed to prevent rickets.

(c) The incidence of rickets was still high in animals receiving the milk obtained during the period of grass feeding in the dark¹.

(b) *Evidence from the calcium content of the bones of the above animals.*

The calcium determinations tell nearly the same story as the post-mortem and histological observations. The results of these are displayed in Fig. 2, which shows the percentage of calcium in the bones of rats used for titrations of milk samples V–XV plotted against the daily quantity of milk fed.

During the period of dry feeding in the dark (period II) 3 cc. of milk daily caused no improvement in calcification as compared with that of the controls; there is some, but no very great improvement with 5 cc. (Fig. 2 and Protocols *A* and *B*). Sample VI, already referred to as being low in its growth-promoting and anti-rachitic power, is seen to possess the lowest calcium depositing power of the samples belonging to period II (Fig. 2 and Protocol *A*). 10 cc. of this milk gave about the same calcification as 5 cc. of the previous and two later samples. This sample, as already stated, was the last one tested before the birth of the calf. This sample of milk, then, besides being of low anti-rachitic and growth-promoting value, showed also the lowest calcium depositing value of any milk used throughout the experiment.

With green feeding in the dark (period III, Protocol *C*) there is some improvement in the calcifying power of the milk obtained; most obvious in the case of the low doses of sample XII, a milk taken after two months of this feeding. This was not accompanied by a decrease in the incidence of rickets as shown by histological examination, a fact possibly connected with the deprivation of light, suffered by the cow during this period.

The best calcification, however, was obtained with the rats receiving milks of period IV, when the cow was pasture fed. Of the two samples tested, the first (sample XIII) caused better calcification with a 5 cc. dose (20.4 % Ca) than had been obtained with a 20 cc. dose of any previous sample. For the testing of this sample, however (Protocol *D*), only two families of rats were used, and only two rats on each dose and in this case a higher average percentage calcification was obtained in the control animals than was the case in the antecedent groups. The animals used for this test were possibly at a higher level as regards their bodily store of fat-soluble vitamins, than those used in previous tests, as is seen by the increased growth and calcification in the controls. This observation was made from July to September, during the time when laboratory light conditions were at a maximum. The animals were

¹ The histological picture in nearly all the milk-fed animals showed some degree of osteoporosis.

never exposed to direct sunlight, the windows being kept closed. In spite of this fact, however, the control rats showed no histological evidence of rickets, only a moderate degree of osteoporosis. The second sample (Protocol *D* 1) of pasture milk, tested a month later, showed a similar high calcium-depositing value and in this case the control animals were comparable to those of periods II and III, inasmuch as they grew badly, had badly calcified bones, and all showed post-mortem and histological evidence of rickets. *All the animals receiving milk, from 1 cc. and upwards, in this period, when the cow was on pasture, showed an improved calcium content of bone as compared with the controls. The contrast was well-marked with the low doses.*

Fig. 2. Average percentage of calcium (dry weight) in bones, femur and fibula of rats used for titration of milk samples V-XV (see Protocols *A* to *E*). Time of experiment 35 days. 3-4 rats were used for titration of each dose.

The calcium content of the bones of the rats used to investigate the milk of period V (dry feeding in the light, Fig. 2 and Protocol *E*) shows a drop in the case of the animals receiving low doses compared with those receiving the milk from the cow when on pasture.

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

Samples X, XII, XIV and XV, taken at the end of periods II, III, IV and V respectively, were used to determine the anti-rachitic power of the milks. Each of these samples was titrated over a family of eight or nine rats receiving doses of 2, 5, 7, 10, 15 and 20 cc., with two controls receiving no milk. As far as possible animals of the same sex and initial weight received the same dose in each of the four samples. The animals were fed for 28 days on McCollum's 3143 diet, with the addition of doses of the various milks. In every case a histological examination of the rib-junctions, and calcium determination of the leg bones (femur, tibia and fibula) were made.

The protective dose of milk was taken to be the one received by rats giving an approximately normal histological picture.

All the *control animals* in the four groups showed gross and histological evidence of severe rickets. The *macroscopic findings* were marked enlargement of the costochondral junctions, angulation and spontaneous fractures of the ribs, enlarged radio-ulnar epiphyses, with marked diminished rigidity and deformity of the chests. *Histologically* there was very imperfect calcification, excess of osteoid tissues, both cortical and peri-trabecular, angulation of ribs, increase in width and disorganisation of the zone of hypertrophic cartilage cells, and disorganisation of the zone of provisional calcification. The milk-fed animals showed evidence of rickets that varied with the quantity and quality of the different milks.

The results are seen from Tables I-IV and are summarised in Table V and illustrated by Figs. 3-7 (Plates II and III).

1. *At the end of period II* (dry feeding in the dark) (Table I and Fig. 7) 15 cc. of sample X was barely protective. Rat 599 receiving 15 cc. of this milk showed a histological picture of slight rickets. Rat 600 receiving 15-20 cc. showed an approximately normal picture.

2. *At the end of period III* (green feeding in the dark) (Table II and Fig. 6) a normal histological picture was obtained in rats that had received 20 cc. daily, but all the other milk-fed animals showed a histological picture of severe or pronounced rickets.

3. *At the end of period IV* (pasture feeding) (Table III and Figs. 3, 4 and 5) there was a marked improvement in the histological picture obtained with rats that had received 2 cc. daily, and a normal picture with those that had received 5 cc. The same result was obtained with a second family of rats tested with low doses only (1-5 cc.).

4. *At the end of period V* (dry feeding in the light) (Table IV) the quantity of milk required to produce a normal histological picture in rats on McCollum's diet had increased to 10 cc. The test was made with two litters.

Calcium-content of the bones of these animals. In every group the animals showed an increasing deposition of calcium with increasing doses of milk.

Fig. 3. Rib-junction, rat 796; 28 days on basal diet without milk addition. Severe rickets, rib-junction bent at an angle, structure disorganised: wide zone of hypertrophic cartilage (*HC*); excess of osteoid tissue (*O*); zone of proliferating cartilage (*PC*).

Fig. 4. Rib-junction, rat 797; 28 days on basal diet with addition of 2 cc. daily of milk sample XIV (period IV, pasture-feeding). Slight rickets. Zone of hypertrophic cartilage (*HC*) still wide, but reduced in width in comparison with rat 796: much less osteoid tissue (*O*).

Fig. 5. Rib-junction, rat 801; 28 days on basal diet with addition of 5 cc. daily of milk sample XIV (period IV. pasture feeding). Structure nearly normal, well-marked zone of provisional calcification (ZPC); and well-ossified bony trabeculae (TR).

Fig. 6. Rib-junction, rat 724; 28 days on basal diet with addition of 5 cc. daily of milk sample XII (period III fresh green food in a dark stall). Pronounced rickets. Wide zone of hypertrophic cartilage (HC) with very deficient calcification in zone of provisional calcification (ZPC); excess of osteoid tissue (O).

Fig. 7. Rib-junction, rat 596; 28 days on basal diet with addition of 5 cc. daily of milk sample X (period II, dry-fodder in a dark stall). Pronounced rickets. Structure much disorganised, wide zone of hypertrophic cartilage (HC), much osteoid tissue (O); zone of provisional calcification remains almost uncalcified.

Figs. 3-7. Basal diet=McCullum's rickets-producing 3143 diet.

ERRATUM

Table, bottom of p. 729, 1st column, *should read*

Sample		Sample
X	} <i>instead of</i> {	XIV
XII		XVI
XIV		XVIII
XV		XIX

INFLUENCE OF DIET AND LIGHT ON VITAMINS OF MILK 729

ANTI-RACHITIC POWER OF THE MILKS. RATS ON McCOLLUM'S 3143 DIET.
MILK FEEDING 28 DAYS.

Table I. *Period II. (Dry fodder, dark.) Sample X.*

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
593	♂	A	No milk	49	93	+++	63.2	4.2	11.4	Severe rickets
594	♀	"	"	53	83	+++	63.1	3.8	10.2	"
Av. no milk:							63.1	4.0	10.8	
cc.										
595	♂	"	2	54	95	++	57.0	6.4	14.7	Pronounced rickets
596	♀	"	5	49	90	++	55.6	6.9	15.6	"
597	♂	"	7	52	120	++	55.0	7.5	16.8	"
598	♀	"	10	47	98	++	Estimation lost			"
599	♂	"	15	48	142	+	51.3	8.8	18.9	Slight rickets
600	♀	"	*15-20	48	110	?	48.8	9.9	19.5	Nearly normal

Table II. *Period III. (Green fodder, dark.) Sample XII.*

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
721	♂	B	No milk	47	67	+++	62.4	4.3	11.3	Severe rickets
722	♀	"	"	53	82	+++	61.0	4.1	10.5	"
Av. no Milk:							61.7	4.2	10.9	
cc.										
723	♂	"	2	52	106	+++	61.6	4.3	11.3	"
724	♀	"	5	45	84	+++	56.4	6.6	15.1	Pronounced rickets
725	♂	"	7	48	102	+++	59.4	5.8	14.3	"
726	♀	"	6-10	37	68	+++	60.8	4.7	12.1	"
727	♂	"	12-15	47	110	++	59.1	5.8	14.2	"
728	♂	"	*20	55	138	?	59.1	8.6	17.8	Nearly normal

* Protective dose.

There was no definite correspondence, however, between the proportion of calcium in the bones and the histological picture.

The possible influence of increased phosphorus given with the milks. The possibility that the curative effect of feeding the various milks is partly due to the ingestion of phosphorus, apart from the presence of the organic anti-rachitic factor, has not been lost sight of. An analysis of the four samples of milk used in these experiments, kindly made by Miss Boas, gave the following results:

Sample	% P	% Ca
XIV	0.1039	0.123
XVI	0.0803	0.116
XVIII	0.0901	0.118
XIX	0.0913	0.1323

The ratio of Ca to P in McCollum's 3143 diet is 4 : 1. The extra Ca and P given in a daily addition of 2 cc. of milk alters it to 3.8 : 1, and addition of 5 cc. of milk to 3.5 : 1. With the higher doses 10, 15 and 20 cc. of milk

Table III. *Period IV. Pasture (July to October). Sample XIV.*

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
795	♀	C	No milk	50	70	+++	61.1	5.4	14.0	Severe rickets
796	♂	"	"	52	61	+++	62.7	4.5	12.1	"
Av. no milk:							61.9	4.9	13.1	
cc.										
797	♂	"	2	50	83	+	57.2	7.1	16.9	Slight to moderate rickets, healing
801	♀	"	*5	49	90	?	49.3	8.8	17.5	Nearly normal
799	♂	"	7	51	92	?	53.4	8.4	18.1	"
800	♀	"	10	47	107	?	50.9	9.2	19.0	Slight osteoporosis
802	♀	"	20	48	107	—	51.4	9.4	19.5	Nearly normal

Second family. Low doses only:

811	♂	D	No milk	49	69	+++	58.7	5.8	14.2	Severe rickets
812	♀	"	"	53	82	+++	58.7	5.5	13.4	"
Av. no milk:							58.7	5.6	13.8	
cc.										
818	♀	"	1	47	72	+++	55.8	6.3	14.4	"
819	♀	"	1	45	65	++	53.5	7.2	15.5	Pronounced rickets
Av. 1 cc.:							54.6	6.7	14.9	
817	♂	"	2	48	86	?	53.9	7.1	15.6	Slight rickets
815	♀	"	3	48	83	?	52.9	7.5	16.1	Nearly normal
816	♀	"	3	46	83	?	54.5	7.1	15.7	Slight rickets
Av. 3 cc.:							53.7	7.3	15.9	
813	♀	"	*5	49	93	?	55.4	7.1	15.9	Nearly normal

Table IV. *Period V. (Dry fodder, light.) October to December. Sample XV.*

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
914	♂	E	No milk	53	85	++	59.9	5.7	14.2	Pronounced rickets
915	♀	"	"	51	75	+++	62.1	4.7	12.4	Severe rickets
Av. no milk:							61.0	5.2	13.3	
cc.										
916	♂	"	2	52	85	++	59.2	5.8	14.2	"
917	♀	"	5	50	107	+	56.0	7.2	16.3	"
918	♀	"	7	52	104	+	55.6	7.2	16.3	Pronounced rickets
919	♀	"	*10	51	110	?	50.6	9.2	18.7	Nearly normal

* Protective dose.

Table V. *Rickets-preventing value of milk samples.*

No. of sample	Description	Protective dose against rickets for rats on McCollum's 3143 diet, between
X	After 6 months on dry fodder given in dark stall	15 cc. — and 20 cc. +
XII	After 2 months on fresh green fodder in dark stall	12 to 15 cc. — and 20 cc. +
XIV	After 3 months of pasture-feeding (July–October)	2 cc. — and 5 cc. +
XV	After 2 months on dry fodder given out of doors (Oct.–Dec.)	7 cc. — and 10 cc. +

the change is greater and the ratio becomes 2.5-2.7 : 1, and it is possible that this proportional increase in the amount of P may contribute to the preventive effect of certain high doses of milk.

The results of these experiments, however, have been interpreted after comparison of the effect of doses of similar size, the only variable factor being the sample of milk tested. They are important in so far as they show a positive anti-rachitic effect of *small doses (1-5 cc. daily) of milk from the cow on pasture feeding*. With these doses the added phosphorus is in all probability negligible, and the result obtained indicates a high anti-rachitic potency of the milk.

The results obtained with the higher doses cannot be interpreted to indicate only anti-rachitic potency, as the extra P given with the milk probably contributes to the result.

The Calf.

The calf (♀) was born on January 27th, 1923, during the period when the cow was in the dark and fed on cereals, roots and straw. She weighed 70 lbs. at birth and was apparently normal and healthy. She was kept with the mother in the dark and fed exclusively on the mother's milk. Three months later she was killed. By this time she was well grown and weighed 300 lbs.

Post-mortem examination showed nutrition good, subcutaneous and body fat plentiful: bones of skull and limbs hard: no enlargement of costochondral junctions: viscera normal.

A *calcium analysis* was made of the 6th rib, a length equal to 2 cm. cut at equal distances from the costo-vertebral margin on both sides, and gave the following result.

	Analysis of rib		
	% H ₂ O	% Ca wet	% Ca dry
6th rib—right side	28.7	15.8	22.2
„ —left side	29.0	15.8	22.2

A *histological examination* of the costochondral junction showed no excess of osteoid tissue, no increase in width of the zone of hypertrophic cartilage cells, or disorganisation of this area, a normal area of provisional calcification, and bone trabeculae regularly arranged and well calcified. Very slight osteoporosis.

Results from observations on the calf. The milk used for feeding the calf was that of period II in which the growth-promoting and anti-rachitic value, as tested on rats, had reached a minimum. In spite of this she grew normally and showed no signs indicative of rickets.

The mother, during the first three months of pregnancy, had been on pasture, receiving in addition a daily ration of hay and oil cake. Previously to this she was known to have had several healthy calves, and to be a "heavy milker." She must, therefore, have been in excellent condition at the beginning of the experiment, with a maximum storage of growth and anti-rachitic factors.

The calf, then, lived for the first three months of intra-uterine life under optimal conditions of nutrition, the mother receiving abundance of light, and a diet rich in the fat-soluble vitamins. A deprivation of fat-soluble vitamins for six months and of light for two months did not apparently affect the calf, which was probably, *in utero*, drawing upon the mother's reserve of growth and anti-rachitic factors during this time. In the lactation period of three months that followed, the milk proved adequate for the needs of the animal.

Rickets is not a common disease amongst calves, but there is some evidence in support of the view that the onset of the disease may take place after three months of age if the period of lactation is prolonged.

McCandlish [1923], investigating milk as an exclusive diet for calves, found that it would not support life after the first three or four months. Two bull calves fed exclusively on milk, did well for the first 13 weeks, but after that declined and died within six months. In these animals there was post-mortem evidence of softening of the bones, and spontaneous fractures of the ribs. In a later paper he investigated the effect of the addition of mixed grain and alfalfa hay to the milk ration. A calf fed on the diet of milk and mixed grain ultimately developed "stiff and swollen leg joints with knees bowed outward and forward." The photograph of this animal gives an almost typical picture of rickets, although, as this animal was subsequently cured by the addition of alfalfa hay it did not come to post-mortem examination.

It is possible that had the milk feeding been continued longer with unchanged feeding and management of the mother a condition resembling rickets might have been induced in the calf, born during the present experiment. The reason why rickets is not a disease of common occurrence amongst calves may be that at three months of age the diet of a calf is normally changed from one of milk to one of grain, hay, pasture, roots, etc. Milk is of low anti-rachitic value as compared with green fodder and hay, and the reinforcement of the calf's diet with food of high anti-rachitic value may serve to tide the animal over a period of susceptibility.

A sample of colostrum milk, taken 18 hours after calving, was titrated for 35 days with a single family of six male rats. The table shows *a definite increase in the growth-promoting power of low doses of colostrum, compared with that of the preceding milk, sample VI, taken during period II* (compare Protocol A).

With the exception of rat 428, which showed a slightly better calcification than the control animal of this series, this increased growth was not accompanied with increased calcification in rats receiving doses up to 7 cc. The incidence of rickets in this group of rats was as high as in those receiving previous samples of the milk of this period when the cow was in the dark on dry feeding.

Colostrum milk. 18 hours after calving.

Rat	Sex	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
						H ₂ O	Ca wet	Ca dry	
422	♂	No dose	53	74	+	43.4	8.0	15.5	Osteoporosis + mod. rickets
428	♂	3 cc.	58	119	-	46.3	8.9	16.6	Osteoporosis
426	♂	5	49	101	+	46.1	8.3	15.3	Osteoporosis + mod. rickets
427	♂	7	52	104	+	46.4	7.9	14.8	" "
423	♂	10	54	122	-	45.4	9.2	17.1	Osteoporosis
425	♂	10	57	129	-	46.4	9.4	17.5	Osteoporosis + slight rickets
Av. 10 cc.						45.9	9.3	17.3	

Variations in the amount of sunshine during periods I, IV and V.

To control the diet of a cow is a simple matter, but light conditions vary with the time of year and cannot be controlled or standardised. Records of the amount of sunshine (in hours per day) taken at Rothamsted Meteorological Station, Herts., show that in period I, July–November, 1922, there was a decreasing amount of sunshine ranging from an average of 4.8 hours per day in July to 1.9 hours per day in November. The maximum amount of sunshine recorded in this year was 8.2 hours per day in May and June, before the beginning of the experiment. The amount of sunshine per day impinging on the cow during period I, which averaged 4.2 hours per day from July to October, was decreasing all the time and never approached the maximum for the year.

In period IV the records show an average of 7.3 hours of sunshine per day, which was *the maximum for the year*.

In period V the records compare with those of period I, that is to say, the light was decreasing and averaged 3.4 hours per day in November and 1.4 hours per day in December.

These observations show that whereas the intensity of sunshine during the two periods of dry fodder in the light (periods I and V) is roughly comparable, neither can be compared with that prevailing during the period of pasture feeding (period IV). It is possible that before light can be effective in raising the anti-rachitic value of milk, it must reach a certain level of intensity. Further experiments of dry feeding *during maximum conditions of sunshine* are being attempted, in order to elucidate this point.

Summary of Section V.

The milk, at the beginning of the experiment, when the cow had been out at pasture, gave evidence of anti-rachitic properties in doses of 2 cc. and upwards. A fall in the anti-rachitic value was not recognisable until the cow had been fed on a diet deficient in fat-soluble vitamins for $4\frac{1}{2}$ months, by which time the growth-promoting (vitamin A) value of the milk had reached a minimum.

On changing the diet to fresh grass the cow being kept still in the dark, the anti-rachitic value of the milk improved slightly, but this improvement was not great enough to be detected with McCollum's 3143 rickets-producing diet.

On letting the cow out to grass, the milk regained its original value, *and this improvement was also evident by the severe test with McCollum's 3143 rickets-producing diet*. Two months after dry food had been again substituted for pasture, the cow remaining out of doors, the milk still contained anti-rachitic properties to a considerable degree.

VI. GENERAL DISCUSSION OF RESULTS.

There are three possible theories in explanation of the results obtained in these experiments:

1. *That the growth-promoting factor in milk is controlled by the diet of the cow, whilst its anti-rachitic value depends upon the amount of light impinging on the animal.* This theory gains some support from the fact that during both periods I and V, in which the cow was fed on dry fodder out of doors, the growth value of the milk decreased almost to a minimum, whilst the anti-rachitic value remained fairly high, in spite of the fact that the intensity of light was on the decline. Conversely, during periods II and III, when the cow was kept in the dark stall on dry fodder, and fresh green food, respectively, the percentage incidence of rickets in the milk-fed animals was greater than during the three periods when she was out of doors. This low anti-rachitic value appeared to hold, notwithstanding the increased growth-promoting value observed in the colostrum milk yielded during period II and in the milks tested after the cow's diet had been changed to fresh green food in period III.

2. *That both growth-promoting and anti-rachitic factors in milk are influenced only by the diet of the cow.* A fall in the anti-rachitic value of the milk occurred after a $4\frac{1}{2}$ months' deficiency of fat-soluble vitamins in the diet of the cow. The growth-promoting value was reduced almost to a minimum after three months' deficient feeding. Furthermore, both factors in the milk were at their maximum and minimum values together. It is possible that both are controlled by diet, but that it takes longer to drain a cow of the anti-rachitic than of the growth-promoting principle.

If this were so it should be possible to reduce both factors to a minimum by dry feeding out of doors, and raise them to a maximum by green feeding in the dark. The periods employed in these observations were not long enough to permit of this conclusion.

3. *That the diet of the cow has the main effect on both factors, whilst light controls to some extent the anti-rachitic value of the milk.* It is evident that diet affects both factors, but how far the anti-rachitic value of milk is dependent on the amount of sunlight impinging on the cow is not apparent, though there are indications that it has a part, possibly a subsidiary one, to play. As long as the cow remained in the light, even light of a fairly low intensity (period I, July–November, and period V, October–December), the anti-rachitic value of the milk remained at a higher level than that reached when the animal was in the dark. Also no definite fall was observed in the anti-rachitic value of the milk until the cow had been, for some weeks, in the dark. During the comparatively short period in which she received fresh

grass fodder in the dark the anti-rachitic value of the milk rose slightly, as judged by the calcium content of the bones of the animals fed upon it and might have risen more had this period been prolonged.

A maximum level of anti-rachitic potency, however, was not reached until the cow was placed under conditions of maximal light and optimal diet, and it is conceivable that, in the absence of sunlight, it would be impossible to raise the anti-rachitic value of the milk above a certain level.

In the absence of results from further experiments it is impossible to do more than draw broad conclusions. In the above experiments the variations in anti-rachitic value did not follow precisely those in (vitamin A) growth-promoting factor, although both were at their maximum and minimum together. Both are evidently influenced by diet. The discrepancy between them could be interpreted to mean, either that the anti-rachitic value of milk is influenced by the illumination of the cow more than the vitamin A value, or that it takes longer to exhaust the milk of the former active principle.

VII. CONCLUSIONS.

1. Milk from the same cow has been shown to vary within wide limits, as regards its anti-rachitic and growth-promoting power.

2. The diet of the cow appeared to be the main factor in determining the growth-promoting value of the milk. When the cow received a diet of fresh green grass the milk possessed a higher growth-promoting value than when she received dry fodder deficient in fat-soluble vitamins. This result was obtained irrespective of whether the cow was kept out of doors in sunlight or in a dark stall. The evidence as to whether light does, or does not, contribute, is not conclusive from these experiments.

3. The anti-rachitic value depends on the diet of the cow, and possibly also on the degree of illumination to which she is exposed. *Milk from a pasture fed cow has a definite and high anti-rachitic value*; the same animal when stall fed in the dark, yielded a milk much inferior in anti-rachitic properties.

4. A possible contributory cause of the seasonal incidence of rickets in children, may be the use of milks whose anti-rachitic value is higher in summer than in winter.

In conclusion, my thanks are due to Dr MacConkey and his staff at the Serum Department of the Lister Institute, Elstree, Herts., for their kind co-operation in carrying out the management of the cow, and much tedious work connected with this investigation. Without their co-operation the experiment could not have been undertaken. I also acknowledge with sincere pleasure my gratitude to Dr C. J. Martin for much valuable support and criticism during the course of the experiment, to Dr V. Korenchevsky and Dr H. Chick for technical advice, and to Miss M. Boas for kindly undertaking chemical analyses of the milks used. I wish also to thank Miss M. Rickards for assistance with section cutting, and G. Favell, for help in feeding and care of the animals.

PROTOCOLS A-E. SHOWING RESULTS OF TYPICAL ANALYSES OF MILKS FROM PERIODS I-V.

Protocol A. Period II. Sample VI. Dry fodder—dark.

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
415	♀	1	No milk	72	70	++	52.7	8.0	13.3	Osteoporosis + pronounced rickets
400	♀	2	"	68	70	?	53.0	8.5	14.0	Extreme osteoporosis
395	♀	3	"	53	62	++	48.1	7.0	14.0	Osteoporosis + moderate rickets
Av. no milk:							50.1	7.7	13.7	
cc.										
401	♂	2	3	65	70	++	48.2	6.9	13.5	Osteoporosis + slight rickets
402	♀	2	3	56	68	++	52.7	6.0	12.7	Pronounced rickets
408	♂	1	3	56	93	++	53.7	6.6	14.3	Osteoporosis + slight rickets
Av. 3 cc.:							51.5	6.5	13.5	
396	♀	3	5	52	69	+	41.4	7.4	14.7	Slight rickets
406	♀	2	5	74	80	+	46.7	6.8	12.9	"
409	♀	1	5	74	93	+	49.3	7.2	14.1	"
411	♂	1	5	66	106	+	49.0	7.7	15.2	Osteoporosis + moderate rickets
Av. 5 cc.:							46.6	7.3	14.2	
397	♀	3	7	53	85	-	42.9	7.8	13.7	Moderate rickets
399	♀	3	7	63	95	+	44.9	8.3	14.9	Osteoporosis + slight rickets
412	♂	1	7	65	100	+	52.0	6.8	14.3	" "
Av. 7 cc.:							46.6	7.6	14.3	
398	♂	3	10	61	100	+	48.7	6.9	13.6	" "
405	♀	2	10	58	88	+	48.1	7.9	15.4	Slight rickets
403	♀	2	10	71	94	-	45.5	6.9	13.5	Osteoporosis + slight rickets
414	♀	1	10	54	105	-	49.6	8.2	16.4	" "
Av. 10 cc.:							47.4	7.6	14.7	

Last ante-partum sample tested. Showing high incidence of rickets and low % calcium in bones.

Protocol B. End of Period II. Sample IX. Dry fodder—dark.

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
553	♂	1	No milk	55	113	+	48.6	7.6	15.1	Osteoporosis + pronounced rickets
536	♂	2	"	57	117	+	51.2	7.1	14.6	Osteoporosis + moderate rickets
573	♀	3	"	67	65	+	49.9	7.3	14.3	Osteoporosis + slight rickets
Av. no milk:							49.9	7.3	14.6	
cc.										
537	♀	2	3	92	113	+	45.0	9.3	16.9	Osteoporosis + pronounced rickets
554	♀	1	3	73	88	+	45.5	8.5	15.6	Osteoporosis + slight rickets
561	♂	1	3	73	95	+	44.1	7.9	14.2	" "
572	♂	3	3	67	120	-	Estimation lost			" "
Av. 3 cc.:							44.8	8.5	15.5	
562	♀	1	5	60	91	-	45.4	8.4	15.5	Osteoporosis
563	♀	1	5	90	125	-	40.7	10.1	16.9	Osteoporosis + slight rickets
Av. 5 cc.:							43.0	9.2	16.2	
541	♀	2	10	103	133	-	44.1	9.9	17.8	Osteoporosis
551	♀	1	10	87	127	+	47.5	8.5	16.2	Osteoporosis + slight rickets
555	♂	1	10	78	128	+	47.5	9.1	17.4	" "
574	♂	3	10	63	117	+	46.9	8.8	16.7	Osteoporosis + pronounced rickets
Av. 10 cc.:							46.5	9.0	17.0	
543	♀	2	15	89	133	-	44.0	10.6	19.0	Slight osteoporosis
552	♀	1	15	95	138	-	41.1	10.8	18.3	Osteoporosis + slight rickets
560	♂	1	15	87	142	-	47.9	9.4	18.2	" "
Av. 15 cc.:							44.3	10.2	18.5	
564	♀	1	20	85	144	-	41.1	10.1	18.0	Slight osteoporosis
565	♀	2	20	85	158	-	40.8	10.1	17.1	" "
550	♂	1	20	94	167	-	46.8	9.5	17.9	Osteoporosis
Av. 20 cc.:							40.9	10.1	17.5	

Showing high incidence of rickets.

Protocol C. Period III. Sample XI. Fresh green fodder—dark.

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
601	♂	1	No milk	57	65	+	48.1	7.0	13.5	Osteoporosis + slight rickets
655	♀	2	"	53	57	-	48.7	6.7	13.2	"
654	♂	3	"	74	71	? +	48.7	6.7	13.2	Marked osteoporosis
Av. no milk:							48.5	6.8	13.3	
cc.										
602	♀	1	1	72	85	+	46.0	6.6	12.4	Osteoporosis + slight rickets
610	♀	1	1	57	70	+	45.5	8.0	14.8	"
664	♀	2	1	50	68	-	43.4	7.9	14.1	Osteoporosis
Av. 1 cc.:							44.9	7.7	13.7	
603	♀	1	3	80	105	-	54.3	7.0	15.4	Osteoporosis + slight rickets
611	♂	1	3	72	108	+	43.3	8.4	14.9	"
663	♂	3	3	57	85	-	42.8	8.8	15.4	"
659	♂	2	3	62	81	-	43.8	7.7	13.7	Osteoporosis
Av. 3 cc.:							46.0	7.9	14.8	
653	♀	2	5	68	122	-	45.8	8.9	16.5	Osteoporosis + slight rickets
605	♀	1	5	70	104	-	45.3	8.4	15.4	Slight osteoporosis
658	♂	3	5	55	102	-	44.2	9.4	17.0	"
608	♂	1	5	82	132	-	47.0	7.9	15.0	Osteoporosis + slight rickets
Av. 5 cc.:							45.3	8.6	15.9	
606	♀	1	10	53	113	-	44.8	9.0	16.3	"
612	♀	1	10	81	145	-	45.4	8.0	15.1	"
661	♂	2	10	64	137	-	42.4	9.1	15.9	Osteoporosis
660	♂	2	10	60	115	-	44.5	9.8	17.7	"
Av. 10 cc.:							44.3	8.9	16.2	
607	♀	1	15	76	124	-	42.5	9.7	17.0	"
657	♀	3	15	56	113	-	43.5	10.4	18.5	Nearly normal
Av. 15 cc.:							43.0	10.0	17.7	
609	♀	1	20	72	133	-	41.1	11.4	19.4	"
656	♂	3	20	58	138	-	42.3	10.4	17.9	Osteoporosis
Av. 20 cc.:							41.7	10.7	18.6	

Slightly improved % calcification with low doses as compared with Protocol B. High incidence of rickets.

Protocol D. Period IV. Sample XIII. Pasture.

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
744	♂	1	No milk	82	106	-	39.7	10.6	17.5	Osteoporosis
737	♀	1	"	66	87	-	40.2	9.6	16.1	Slight osteoporosis
747	♀	2	"	52	73	? +	47.3	8.1	15.5	Osteoporosis
Av. no milk:							42.4	9.4	16.3	
cc.										
739	♂	1	1	88	130	-	43.1	10.4	18.3	Normal
743	♀	2	1	76	90	-	35.7	12.3	19.1	Osteoporosis
Av. 1 cc.:							39.4	11.3	18.7	
738	♀	1	2	60	103	-	38.9	11.1	18.2	Slight osteoporosis
748	♀	2	2	69	115	-	43.6	11.3	20.1	Normal
Av. 2 cc.:							41.5	11.2	19.1	
740	♀	1	3	70	127	-	39.9	11.9	19.9	"
749	♀	2	3	53	107	-	48.5	9.5	18.6	"
Av. 3 cc.:							44.2	10.7	19.2	
741	♀	1	5	66	103	-	Estimation lost			"
750	♀	2	5	60	112	-	43.9	11.5	20.5	"
754	♀	2	5	60	111	-	45.0	11.2	20.3	"
Av. 5 cc.:							44.4	11.3	20.4	
742	♀	1	10	73	116	-	40.1	12.3	20.6	"
746	♀	2	10	57	97	-	45.0	11.6	21.0	"
Av. 10 cc.:							42.5	11.9	20.8	
752	♀	2	15	67	132	-	41.8	12.4	21.2	"
753	♀	2	15	63	120	-	41.7	12.2	21.0	"
Av. 15 cc.:							41.7	12.3	21.1	
745	♀	1	20	71	115	-	37.0	13.0	20.6	"
751	♂	2	20	75	167	-	43.5	12.1	21.4	"
Av. 20 cc.:							40.2	12.5	21.0	

No rickets in control of milk-fed animals. High calcification with 5 cc. doses and over.

Protocol D 1. Period IV. Sample XIV. Pasture.

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
767	♂	1	No dose	63	62	++ 16 ribs spontaneous fractures slightly enlarged, cch. junctions	50.0	7.0	14.6	Pronounced rickets
759	♀	2	"	78	60		58.1	6.6	15.7	Kept for demonstration
774	♀	3	"	58	77	+	42.6	7.6	13.4	Slight rickets
			cc.			Av. no milk:	50.2	7.0	14.6	
760	♀	1	1	64	80	? +	44.1	8.7	15.6	Osteoporosis
765	♀	1	1	74	83	-	43.3	7.8	13.9	"
763	♀	2	1	70	107	No fractures, cch. junctions normal	46.0	9.0	16.7	Kept for demonstration
770	♀	3	1	68	86	-	46.1	9.2	17.2	Slight osteoporosis
						Av. 1 cc.:	44.9	8.7	15.8	
761	♂	1	2	80	117	-	43.6	9.0	16.1	Normal
766	♀	3	2	78	109	-	42.5	8.2	14.3	"
757	♀	2	2	73	113	No fractures, cch. junctions normal	43.6	10.5	18.7	Kept for demonstration
758	♀	2	2	64	103	-	44.2	9.5	17.1	Normal
						Av. 2 cc.:	43.5	9.3	16.5	
771	♀	3	3	83	121	-	47.0	8.5	16.1	Osteoporosis
769	♀	1	3	78	127	-	47.5	9.3	17.7	Slight osteoporosis — nearly normal
764	♀	1	3	89	144	-	46.3	9.4	17.6	"
755	♀	2	3	92	136	-	44.7	10.2	18.5	Normal
						Av. 3 cc.:	46.4	9.3	17.5	
756	♀	1	5	74	136	-	44.6	10.5	19.0	"
780	♂	3	5	64	115	-	40.8	10.8	18.3	"
778	♀	3	5	78	145	-	44.7	9.2	16.7	"
779	♀	3	5	67	113	-	40.9	10.9	18.4	"
						Av. 5 cc.:	42.7	10.3	18.1	

No rickets in milk-fed animals. Improved calcification with low doses.

Protocol E. Period V. Sample XV. Dry fodder in the light.

Rat	Sex	Family	Dose	Initial weight	Final weight	Post-mortem evidence of rickets	In bones %			Histological diagnosis
							H ₂ O	Ca wet	Ca dry	
840	♂	1	No milk	92	106	—	48.4	8.0	15.6	Osteoporosis + slight rickets
863	♀	2	"	79	108	—	48.3	7.9	15.3	"
826	♀	3	"	55	—	—	Died and eaten			Osteoporosis
855	♀	4	"	72	87	—	46.2	9.1	16.9	"
						Av. no milk:	44.3	8.3	15.9	
820	♂	3	cc. 1	76	106	+	50.1	8.0	16.1	Osteoporosis + slight rickets
835	♀	1	1	72	69	—	44.2	7.6	13.8	Osteoporosis
864	♀	2	1	72	81	—	44.3	8.7	15.6	"
						Av. 1 cc.:	46.8	8.1	15.2	
822	♀	3	2	82	119	—	49.8	8.7	17.5	Osteoporosis + slight rickets
839	♂	1	2	64	88	—	45.4	8.0	14.7	"
						Av. 2 cc.:	47.6	8.3	16.1	
825	♀	3	3	50	77	—	50.2	7.4	14.9	Osteoporosis
828	♀	3	3	79	88	—	43.4	9.7	17.2	"
866	♂	2	3	78	100	—	43.3	9.7	17.2	"
						Av. 3 cc.:	45.6	8.9	16.4	
832	♀	3	5	69	103	—	46.3	8.3	15.6	Slight osteoporosis
845	♀	1	5	73	124	—	46.5	9.8	18.4	Normal
861	♀	2	5	65	105	—	44.9	9.3	17.0	Slight osteoporosis
867	♀	4	5	76	117	—	44.6	9.8	17.8	"
						Av. 5 cc.:	45.5	9.3	17.2	
853	♀	4	8	63	107	—	45.1	10.2	18.5	Normal
851	♀	4	8	69	110	—	42.7	11.7	20.5	"
868	♂	2	8	62	126	—	46.8	9.0	16.9	"
						Av. 8 cc.:	44.8	10.3	18.6	
858	♀	2	10	69	124	—	42.7	10.8	18.9	"
869	♀	2	10	56	111	—	47.0	9.6	18.1	"
870	♂	2	10	76	147	—	47.8	8.6	16.5	Slight osteoporosis
						Av. 10 cc.:	45.8	9.6	17.8	

Low % calcification with low doses as compared with pasture milk (Protocol D).

REFERENCES.

- Aron and Sebauer (1910). Abderhalden's Handb. der biochem. Arbeitsmethoden, **1**, 413.
- Boas (1924). *Biochem. J.* **18**, 422.
- Chick, Dalyell, et al. (1923). *Med. Res. Council Spec. Rep. Series*, No. 77.
- Drummond, Coward and Watson (1921). *Biochem. J.* **15**, 540.
- Dutcher and Kennedy (1921). *J. Ind. Eng. Chem.* **13**, No. 12.
- Findlay (1922). *Lancet*, i, 825.
- Galbraith (1923). *Quart. J. Med.* **64**, 321.
- Hess and Unger (1920). *J. Amer. Med. Ass.* **74**, 217.
- (1921). *Amer. J. Dis. Child.* **22**, 186.
- Hume (1923). *Med. Res. Council Spec. Rep. Series*, No. 77.
- Kennedy and Dutcher (1922). *J. Biol. Chem.* **50**, 339.
- Korenchevsky (1921). *Brit. Med. J.* Oct. 8.
- (1922). *Med. Res. Council Spec. Rep. Series*, No. 71.
- (1923). *J. Path. Bact.* **26**, 389.
- McCandlish (1923). *J. Dairy Science*, **1**, 4.
- Mellanby (1919). *Lancet*, i, 407.
- (1920). *Lancet*, i, 604.
- (1921). *Med. Res. Council Spec. Rep. Series*, No. 61.
- Paton and Watson (1921). *Brit. J. Exp. Path.* **2**, 75.
- Reyher (1924). *Klin. Wochensch.* Feb. 5th.
- Zilva and Miura (1921). *Biochem. J.* **15**, 654.