CXIII. A PRELIMINARY STUDY OF FACTORS INFLUENCING CALCIFICATION PROCESSES IN THE RABBIT.

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EARLY in 1918 E. Mellanby published a preliminary report of his experimental work on rickets in puppies, emphasising the importance of a vitamin similar to vitamin A in the development of the disease. This calcifying vitamin has recently been called vitamin D. Since 1920 much work has appeared on the subject, the majority of the additional workers using rats as their experimental animals. Arising out of E. Mellanby's work one of us (M. M.) has been for the past eight years investigating the factors influencing the development of teeth and jaws in puppies and other animals with the object of attempting to discover the main causes of the bad teeth of civilised nations. It was shown that foods contain two factors potently affecting the formation of the teeth and their reaction to harmful stimuli. One of these is the calcifying vitamin and the other, acting in the opposite way, some factor present in cereals [M. Mellanby, 1918, 1920, 1923, 2].

This work has also been extended in two ways to children.

(1) A large number of deciduous teeth of children have been examined and it was found

(a) that they were badly calcified, to the extent of over 80 %, not 3 % as usually stated [M. Mellanby, 1923, 1];

(b) that there is a direct relationship between structure and caries, in general the worse the structure the more the caries [1923, 1];

(c) that, when teeth are well calcified and yet carious, their resistance after eruption must have been poor as evidenced by the badly formed or deficient secondary dentine; and, *vice versa*, when teeth are badly formed and yet non-carious, the secondary dentine is abundant and well calcified, indicating good resistance. Inferring from the results of the experimental production of secondary dentine in puppies, it seemed probable that, at the time when the noxious influences affected the children's teeth, bad or good secondary dentine, respectively, was formed owing to the diet at the time being on the one hand defective in calcifying powers and on the other potently calcifying [1923, 3].

(2) On the basis of the above results an investigation was undertaken by M. Mellanby, Pattison and Proud [1924] to test the effect of diet on caries in

erupted teeth of children. The children were divided into three groups, the diets of which varied in their calcifying powers as estimated by the animal experiments.

Group A had a potently calcifying diet,

„ B had a much less potently calcifying diet,

„ C had an intermediate diet as regards calcifying properties.

It was found that the spread of caries was nearly three times greater in group B than in group A, and intermediate in group C. From all these facts it may be inferred

(a) that well and badly calcified teeth can be produced at will by altering in the diet the relative amounts of calcifying vitamin, found in milk, egg-yolk, cod-liver oil, etc., and anti-calcifying substances found chiefly in cereals,

(b) that the better calcified the teeth the less liable are they to be attacked by caries, and

(c) that after eruption a calcifying diet tends to increase the resistance of the teeth, whatever their structure, to the onset and spread of caries.

To put all these inferences to a final test it is essential that experimental caries should be capable of production at will in animals, for it is difficult by any other means than the animal experimental method to control all the factors except the one to be tested.

For a long time experiments have been carried out by one of us (M. M.) principally on dogs with little or no success as far as experimental caries *in vivo* is concerned. McCollum, Simmonds, Kinrey and Grieves [1922] in America claim to have produced "caries-like" lesions in the teeth of rats, but as these animals are very small, and as so far we have not succeeded in reproducing the results obtained by the American workers, we sought for another experimental animal. We had heard privately from Sir Frank Colyer that rabbits sometimes suffer from caries under domestication and we therefore determined to make use of these animals in spite of the fact that their molars are of the persistently growing type.

Up to the present time we have not seen caries in any of the experimental animals, but we have now obtained diets which will allow the animals to live for periods up to one year and yet which might be expected on the basis of the work above mentioned to lead ultimately to caries, for the teeth and bones of animals fed on these diets are poorly calcified. A drawback to the rabbit, which applies also to the rat, is that it is impossible to examine the molars during life so that the condition of these teeth can only be determined *post mortem*. In the meantime it was thought worth while to publish the results up to date as they may prove useful to other investigators working on the calcification processes in animals and especially on the subject of rickets and the anti-rachitic effect of foods and ultra-violet radiations.

Using the puppy experiments and the investigations on children as a basis, the first object was to find a diet deficient in the calcifying vitamin and yet so closely resembling the usual diet of these animals when under domestication

that they would eat it readily. A mixture consisting of four parts of oats to one part of bran, plus 1.5 % CaCO_3 and 6 cc. decitrated orange or lemon juice, proved to be a suitable basal diet; animals on this diet usually grow fairly well and develop rickets (Plate III, 1 B) and badly calcified teeth (Plate V, 3 B). Papers have recently appeared by Goldblatt and Moritz [1925], who have produced rickets in rabbits by feeding them on diets built up on the lines found to give rickets in rats; the diets used by these workers, however, were somewhat artificial and the duration of the experiments short. The feeding methods described below appear to exclude these two drawbacks, for the foods are more natural to the animals and consequently they live for periods up to at least one year. Other workers [Nelson and Lamb, 1920] have shown that rabbits fed on a diet deficient in fat-soluble vitamin A, for periods of 60 days or more, develop xerophthalmia. Some degree of xerophthalmia has been noticed in only four cases on the above-mentioned basal diet. Probably this slight incidence may be explained by the fact that our diets were not composed of purified foodstuffs and therefore even the basal diet was not free from fat-soluble vitamin A. Nelson and Lamb do not state whether they observed any symptoms of rickets in their animals. A summary of the results now to be described was given before the Physiological Society on Feb. 20th, 1926 [M. Mellanby and Killick, 1926].

Experimental Methods.

Litters of rabbits were always used in comparative experiments. The special diet was usually started when the animals were about eight weeks old and weighed from 350-650 g., according to the breed. Each experimental animal was kept in a separate cage in a moderately well lighted room. The cages were of galvanised iron wire, so that they could easily be sterilised. The animals got very little exercise, except in two experiments in which the effect of exercise was tested, as the cages were small. The room temperature, particularly for young rabbits, was found to be of some importance, the most suitable appearing to be about 55° F. When the temperature fell much below 50° F., the young animals frequently developed diarrhoea and died. As an additional safeguard against cold, the litters were kept in wooden hutches until they were required for experiment, and, in the early days of the experimental period, they were put together at night. Daily notes were made of any special features seen. The animals were weighed twice a week; radiographs of each experimental series were taken when the first clinical signs of rickets or scurvy appeared in any member of the series, and in some cases, at intervals throughout the experiment.

Diagnosis of the state of bones and teeth was made by:

- (1) clinical signs during life;
- (2) radiographs of left foreleg during life and of jaws at death;
- (3) *post mortem* findings;

(4) histological structure (decalcified and undecalcified sections of rib, radius, ulna and teeth);

(5) estimation of Ca in the femur shaft.

In this publication the majority of illustrations and other data given are those of the bones because differences are more easily seen. The changes in teeth and jaws (Plate V, 3 and 4) run parallel.

Feeding. In the earlier experiments the animals were allowed to eat as much dry oats and bran as they would, and only the variable in the diet was regulated in amount. The results obtained in this way were of course not strictly comparable, as the degree of rickets in animals fed on a vitamin-deficient diet depends partly on the rate of growth [E. Mellanby, 1920]. When, therefore, a suitable basal diet for producing badly calcified teeth and bones had been found, all the food given was, as far as possible, either weighed or measured for each animal. Special ingredients of the diet, such as lemon juice, any oils or fats, yeasts, etc., were measured and mixed with the oats and bran. The usual custom in the later experiments was to give water, equal in weight to the dry part of the diet. The water balance was found to be of importance to the health of the animals.

All the animals of one series were given the same quantity of food and water; any leavings were recorded, so that the amount could be regulated, if so desired, by the animal of the litter eating the least food. In most cases, any vegetable to be eaten was given as an extra in addition to the amount of other food. In several cases, however, those animals which had vegetable *ad lib.* were given a smaller amount of oats and bran than the others, the amount being regulated so that the rate of growth in all members of the series was as nearly the same as possible.

The experiments were complicated at first by several cases of scurvy, three animals showing definite and extensive haemorrhages at the *post mortem* examination when fed on oats, bran and a small quantity of grass. Two of them had winter grass, the third had heated and oxygenated grass. A fourth, fed on oats, bran and 20 g. cabbage, showed definite haemorrhages at *post mortem*. Symptoms of scurvy were sometimes noted clinically and also by radiographs taken during life; the diagnosis was also confirmed histologically after death. Other animals fed on an intentionally scorbutic diet showed varying results. Two developed definite scurvy; the majority showed some indications of the disease, while a few developed no such symptoms.

Rabbits appear to be on the border-line between those animals, such as guinea-pigs, which develop scurvy readily when fed on a diet deficient in antiscorbutic vitamin, and those much less sensitive to a lack of it, such as the rat. In order, therefore, to study rickets and allied teeth conditions, uncomplicated by possible scorbutic changes, some antiscorbutic substance had to be included in the basal diet. White turnip proved to be very successful as an antiscorbutic, improving the appetite and general health of the rabbits, and showing a complete lack of calcifying power, but, owing to the difficulty

in obtaining a continuous supply of this vegetable, it was thought more suitable to use lemon juice, decitrated by Zilva's method, as a standard.

The mixture of four parts oats to one part bran with 6 cc. of decitrated lemon juice was first adopted as the basal diet. Rabbits fed on this diet did not eat much after the first few weeks. They soon ceased to grow, then lost weight and died. The period of life varied from $2\frac{1}{2}$ to 11 weeks. All the animals showed some signs of rickets, although in the majority of cases it was very slight (see Plate III, 2 A), the actual degree varying with the rate of growth and the length of time the animal was on the diet.

With the object of improving the diet with respect to the Ca : P ratio, 1.5 % CaCO_3 was added to the oats and bran mixture. The Ca : P ratio, calculated from figures given by Sherman [1924], was thus altered from 0.08 : 0.55 to 0.68 : 0.55, a ratio very much closer to the optimal for good calcification as given by McCollum for rats. The effects of this addition were very striking; the animals ate better, grew better and lived much longer, but developed badly calcified bones and teeth. Plate III, 2, shows the radiographs of the forelegs of two rabbits of the same litter, A and B, one without (A) and one with CaCO_3 (B). From the weight curves (Fig. 1) and radiographs (Plate III, 2) it will be seen that the animal receiving CaCO_3 ate more and grew better and had worse rickets than the one without this salt.

Table I. *The effect of adding CaCO_3 .*

Age at beginning of experiment: 9 weeks.
 Weight at beginning of experiment: 430–500 g.
 Time of year at beginning of experiment: December.
 General diet: Rolled oats 4 } *ad lib.*
                   Bran 1
                   Lemon juice.

No. of exp.	Variable factor	Time on diet weeks	Gain in weight g.	Calcium		X-ray	Histology
				% wet weight	Amount g.		
65	No CaCO_3	$6\frac{1}{2}$	130	11.4	0.097	Slight R.	Slight R.
67	No CaCO_3	11	200	9.6	0.101	"	"
68	1.5 % CaCO_3	17	420	13.1	0.130	Bad R.	Rickets
69	1.5 % CaCO_3	$11\frac{1}{2}$	360	10.9	0.150	"	"
70	1.5 % CaCO_3	17	450	13.24	0.117	"	Bad R.

It will be seen from Table I that, although the bones of the two animals with no CaCO_3 showed signs of only slight rickets when examined macroscopically or microscopically, yet their low Ca content indicated that the defect in calcification was there, but was not manifest because of the failure of the animals to grow well. This suggested that with a deficiency of calcifying vitamin and excess of oats, the degree of rickets which developed depended mainly upon the rate of growth of the animal, and that the effect of the added CaCO_3 in causing bad rickets was largely a growth effect [E. Mellanby, 1918, 1919].

To test this hypothesis, two animals were fed on oats, bran, 1.5 % CaCO_3 and lemon juice. Rabbit 168 was given as much of the mixture as it could

it the one unexplained exception to the general result. Thus it appears that if a healthy, normally growing young rabbit is given this basal diet it eats moderately well and develops rickets within 7 weeks, and sometimes as early as 10 days according to its rate of growth and probably also to its antenatal history.

Fig. 2. Weight curves showing the effect of doubling the basal diet. The rickets developed by A, 168 (2x g. diet) was much greater than that developed by B, 167 (x g. diet).

The results with regard to bone changes were fairly constant, and it has also been found that animals on this rickets-producing basal diet develop poorly calcified alveolar bone and badly formed dentine containing many interglobular spaces (photomicrograph and radiograph, Plate V, 3 B and 4 B).

THE CALCIUM AND PHOSPHORUS CONTENT OF THE RICKETS-PRODUCING DIET.

Whereas it is true that the normal calcification of bones and teeth can only be produced when the diet contains a minimum of calcium and phosphorus, it is equally certain that these necessary minima depend upon other factors, the more important of which are:

- (1) the amount of calcifying vitamin eaten,
- (2) the rate of growth,
- (3) the amount of exposure of the animal or certain food constituents to ultra-violet radiations,
- (4) in some (*e.g.* dog) and possibly all animals the amount of some anti-calcifying substance in cereals.

It will be seen later that the calcifying vitamin prevents rickets and allows the production of normally developed teeth and jaws in rabbits as in puppies; and it may be said with some certainty that, if there is abundance of anti-rachitic vitamin in the food, the question of calcium-phosphorus ratio and balance is negligible in questions of the aetiology of rickets and defective tooth calcification, and that even the absolute amounts of calcium and phosphorus in the diet, so far as ordinary foods are concerned, are of but small importance. The calcium and phosphorus retaining powers possessed by the calcifying vitamin of cod-liver oil are so great as to make it almost impossible

to produce abnormality of calcification of bones and teeth by ordinary foods in the presence of this oil. The question we have to deal with now, however, is the importance of these elements when there is a deficiency of calcifying vitamin in the food eaten.

The effect of the rate of growth on calcification has been seen above to be one of great importance. If x g. of a certain diet produce a small amount of rickets, $2x$ g. of the same food, containing twice the calcium and phosphorus intake, make the rachitic condition worse when all other factors are equal. It is very probable that the increase in rickets is due to the greater rate of growth. Therefore, even if there were no factors influencing the development of rickets other than growth, it is obviously impossible to assign great importance to the calcium-phosphorus ratio of the diet in the aetiology of this disease, for this ratio is the same whether $2x$ or x g. are eaten.

So much importance has, however, been attached to the questions of the calcium-phosphorus intake in relation to rickets, especially by American investigators, that this aspect of the problem deserves further consideration in the light of the experimental work on rabbits. It is undoubted that, when the diet is deficient in calcifying vitamin, the amount of calcium and phosphorus ingested bears some relation to the ultimate calcification of bones and teeth. The improvement in bone and teeth formation resulting from the addition of calcium carbonate to a diet deficient in calcifying vitamin, and especially when butter formed a constituent of the mixture eaten, has been described [E. Mellanby, 1921]. Again, Elliott, Crichton and Orr [1922] found that rickets in pigs could be prevented by adding a salt mixture containing calcium phosphate, calcium carbonate, magnesium sulphate, potassium carbonate, ferric chloride, sodium chloride and sodium hydroxide to a diet of oatmeal, rice and skimmed milk.

The following table, slightly modified from that of Goldblatt [1924] represents the calcium and phosphorus contents and ratios in some of the standard diets used by the respective workers for producing defective bone formation in rats and that described above for rabbits:

Table III.

	Diet	Animal	Effect on animal	Calcium %	Phosphorus %	Ca : P ratio
Sherman and Pappenheimer [1921]	84	Rat	Rickets	0.553	0.087	1 : 0.15
McCollum, Simmonds, Shipley and Park [1922]	3143	"	"	1.231	0.301	1 : 0.25
McCollum, Simmonds, Becker and Shipley [1922]	3407	"	"	1.202	0.217	1 : 0.18
Korenchevsky [1921]	- A - P	"	Osteoporosis	0.256	0.22	1 : 0.86
Goldblatt [1924]	- A - P + CaCO ₃	"	Rickets	1.08	0.22	1 : 0.20
Chick and Roscoe [1926]	F.R.	"	Osteoporosis	0.27	0.37	1 : 1.37
M. Mellanby and Killick [1926]	—	Rabbit	Rickets	0.67	0.550	1 : 0.81

The optimum amounts of calcium and phosphorus for normal bone formation in rats has been described by McCollum as 0.75 % and 0.59 % respectively,

or a ratio of 1 : 0.67. One of the main features, therefore, about the diets mentioned in Table III and used in the rat-feeding experiments for producing rickets would seem to be the low phosphorus intake and especially the small amount of phosphorus as compared with the calcium. A ratio nearer that given as optimal for calcification in rats is seen in Korenchevsky's — A — P diet [1921]. This diet does not regularly produce definite rachitic changes but results in osteoporosis. Again, the Chick and Roscoe diet [1926] has a Ca-P ratio of 1 : 1.37 and produces osteoporosis and not rickets. Goldblatt [1924] suggested that this lack of regularity in rickets production was due to the ratio between the calcium and phosphorus intake, for, when calcium carbonate was added to the — A — P diet of Korenchevsky so as to change the Ca : P ratio from 1 : 0.86 to 1 : 0.20, rickets was readily produced. At first sight it might appear that the 1.5 % CaCO_3 in the standard diet for producing poor calcification in rabbits is comparable to the addition of CaCO_3 by Goldblatt to Korenchevsky's diet. This, however, does not appear to be the case, for the Ca : P ratio in our diet is 1 : 0.81; this resembles the ratio 1 : 0.86 of the original — A — P diet of Korenchevsky which does not regularly produce rickets in rats. In other words, the ratio of calcium to phosphorus which has been found to be effective in producing rickets in rabbits is nearer the optimum said to prevent rickets in rats (*i.e.* 0.75 % Ca, 0.5 % P, ratio 1 : 0.67).

If the Ca : P ratio of the basal rickets-producing diet for rabbits be varied by adding less calcium carbonate than 1.5 %, then the conditions do not appear to be as good for producing growth. When, for instance, 0.75 % CaCO_3 is added to the diet, the growth of rabbits is less and the bones, although osteoporotic, are less rachitic. The addition of 3 % CaCO_3 instead of 1.5 % also apparently brings about a slower rate of growth, but nevertheless the bony changes are usually now of a severe rachitic nature.

The point might be raised that, although the optimum Ca : P ratio for producing rickets in rabbits under these conditions is such as tends to prevent the disease in rats, yet the absolute amount of these elements eaten may be low. This, however, does not appear to be the case, for the percentages of Ca and P in the diets which are well eaten and allow good growth in the rabbits are 0.67 % and 0.55 % respectively. These figures are high compared with the figures 0.256 % and 0.22 % of Korenchevsky's — A — P diet, and are roughly comparable with those said by McCollum to be required for normal calcification in rats.

If 1.55 % $\text{Ca}_3(\text{PO}_4)_2$ be added to the oats and bran mixture instead of 1.5 % CaCO_3 the calcium content of the diet remains constant, but the additional phosphorus changes the Ca : P figures from 0.67 : 0.550 or 1 : 0.81 to 0.67 : 0.86, or 1 : 1.28. In this case the animal grows as well as when the diet contains CaCO_3 , but the calcification appears much improved (Table IV).

Acid-base ratio of the ash.

The importance of the acid-base ratio of the ash of the diet in the production of rickets has been considered by various workers, including McCollum, Simmonds, Shipley and Park [1922], Zucker, Johnson and Barnett [1923] and E. Mellanby [1925], but no general agreement on this subject has been obtained. Some experiments were therefore made on rabbits to test the acid-base hypothesis by substituting sodium carbonate and sodium phosphate respectively for the calcium carbonate. Under the experimental conditions, however, only poor growth resulted, and, whether sodium carbonate or sodium phosphate (Na_2HPO_4) was added, an intense and thick band of calcification was produced at the growing epiphyseal ends of the bones (see Plate III, 3; Exp. 340, Table IV). The condition is apparently the opposite to that of rickets, calcification being more powerful than growth. In some ways this condition simulates the changes seen in achondroplasia.

Further work on the Ca : P ratio and other mineral constituents and their relation to calcification is necessary before any conclusions can be reached. At present, however, it can be said that the optimum Ca : P ratio for bad calcification in rabbits is a figure approximating to that which is said to be best for antagonising the development of this disease in rats. It also happens that the growth of young rabbits is good under the experimental conditions when the diet contains the said quantities of calcium and phosphorus and that this is probably a determining factor.

Table IV. *The effect of adding various salts to the diet.*

Age at beginning of experiment: about 7 weeks.

Weight at beginning of experiment: 240-380 g.

Time of year at beginning of experiment: May.

General diet: Crushed oats 4 } ad lib.

Bran 1
6 cc. neutral lemon juice.

No. of exp.	Variable factor	Time on diet up to radiographs weeks	Gain in weight g.	X-rays
337	1.5 % CaCO_3 + unsaponifiable substances from 1 cc. cod-liver oil	10	380	Normal
339	5.37 % Na_2HPO_4 , $12\text{H}_2\text{O}$	10	40	Dense band calc.
340	1.6 % Na_2CO_3	10	60	Very dense band calc. (Plate III, 3)
342	1.5 % CaCO_3	10	340	Rickets
343	1.55 % $\text{Ca}_3(\text{PO}_4)_2$	10	340	Very slight rickets

It will be seen from the above table that with Na_2CO_3 (340) and Na_2HPO_4 (339) the animals scarcely ate any food and put on very little weight. In both cases there was a dense band of calcification at the top of the diaphysis (see Plate III, 3). The other three animals put on weight at an equal rate: 342, with CaCO_3 developed rickets, 343 with $\text{Ca}_3(\text{PO}_4)_2$ had only very slight rickets, whilst 337 with CaCO_3 and the unsaponifiable substances from 1 cc. of cod-liver oil was normal in every way.

THE CALCIFYING ACTION OF SOME FOODS IN THE CASE OF RABBITS.

1. *Foods rich in calcifying vitamin.*

(a) *Cod-liver oil.* Having found a diet which resulted in poor calcification, the next problem was to see what additions to the diet would produce normal calcification of teeth and bones. It proved at first difficult to adapt the basal diet so as to give normal calcification, together with good growth and general health. The common method now used in other animals of adding to the basal diet some fat rich in fat-soluble vitamin is open to objection in the case of rabbits, since these animals do not digest fat easily. When 2 cc. *per diem* of fat is included in their diet their general health is impaired after some weeks. For instance, rabbits given the basal diet plus 2 cc. cod-liver oil *per diem* grew at first at about the same rate as control animals on the basal diet only, but showed no symptoms of rickets (see Plate III, 4). After a period of 7–8 weeks, however, the animals having cod-liver oil suffered a loss of appetite, their weight went down rapidly and, if not killed, they died (Fig. 3). These animals showed no constant lesion of the intestinal tract, although a larger proportion on this diet than on any other were found to have gastric ulcers.

Table V. *The effect of adding cod-liver oil to the basal diet.*

Age at beginning of experiment: 8½ weeks.
 Weight at beginning of experiment: 380–480 g.
 Time of year at beginning of experiment: May.
 General diet: Oats 4 | 1.5 % CaCO₃, ad lib.
                   Bran 1

No. of exp.	Variable factor	Time on diet weeks	Gain in weight g.	Calcium in femur shaft		X-ray		Histology	
				% wet weight	Amount g.	Bones	Teeth	Bones	Teeth
127	Turnip, 20 g.	7½	280	12.9	0.091	Bad rickets	Calc. imperfect	Bad rickets	Calc. poor
130	Turnip, 20 g. Cod-liver oil, 2 cc.	7½	315	—	0.161	Normal	Calc. good	Calc. good	Normal
131	Orange juice, 6 cc.	7½	180	12.1	0.093	Rickets	Calc. imperfect	Rickets	Calc. poor
132	Orange juice, 6 cc. Cod-liver oil, 2 cc.	7½	165	13.4	0.116	Normal	Calc. good	Calc. good	Normal

Table VI. *The effect of cod-liver oil with less CaCO₃.*

Age at beginning of experiment: 8 weeks.
 Weight at beginning of experiment: 600–620 g.
 Time of year at beginning of experiment: February.
 General diet: Oats 4 | 0.75 % CaCO₃, ad lib.
                   Bran 1
                   6 cc. neutral lemon juice.

No. of exp.	Variable factor	Time on diet weeks	Gain in weight g.	Calcium in femur shaft		X-ray	Histology
				% wet weight	Amount g.		
83	—	13	240	10.7	0.144	Some rickets	Calcification abnormal
84	Cod-liver oil, 2 cc.	8½	270	13.1	0.197	No rickets	Calcification good

Fig. 3. Weight curves of 4 rabbits.

A received turnip in addition to basal diet (127).

B received turnip + 2 cc. cod-liver oil and basal diet (130).

C received orange juice and basal diet (131).

D received orange juice, cod-liver oil and basal diet (132) (B and D).

Note loss of weight in both animals receiving cod-liver oil after 6 weeks.

The method of administering the cod-liver oil did not influence these results, as it was varied in different experiments, as follows: (1) by mouth separately, (2) mixed with a small quantity of oats and bran, (3) mixed with the whole bulk of the food, in each case with the same result. 1 cc. of cod-liver oil *per diem* in general acts in a similar way to 2 cc., but when only 0.5 cc. of the fat is given the animals live longer; the appetite of the animals, however, is not good and after a period of about 2 months they stop growing and finally die at the end of about 4 months. When the unsaponifiable fraction from 1 cc. of cod-liver oil is used as the source of the calcifying vitamin, growth is slightly better and the general health is certainly improved. Four animals on this diet are still alive and going up slowly in weight; one has been on the diet for 6 months. In all cases in which cod-liver oil or the unsaponifiable substance of cod-liver oil has been added to the basal diet the calcification is very good.

When 20 g. or more of green cabbage are added to the basal diet as well as small quantities of cod-liver oil, conditions are much improved; growth, general health and calcification all seem excellent whether 0.5 cc. of cod-liver oil is given as such (Plate IV, 2 D) or whether the unsaponifiable substance from 1 cc. of the oil is given (Plate IV, 2 c). Animals have been kept alive and are still alive on these diets showing constant increase in weight for over 6 months (Fig. 4) and the calcification of their bones and teeth is very good.

Table VII. *The effect of adding cod-liver oil or its unsaponifiable fraction together with cabbage to the basal diet.*

Age at beginning of experiment: 7½ weeks.
 Weight at beginning of experiment: 450–530 g.
 Time of year at beginning of experiment: February.
 General diet: Crushed oats 4 } 1.5 % CaCO₃.
                   Bran 1   }
                   6 cc. neutral lemon juice.

No. of exp.	Variable factor	Time on diet up to radiograph weeks	Gain in weight in 21 weeks g.	X-ray	Notes	
291	Savoy cabbage up to 45 g. Mixture up to 70 g.	21	775	Very bad rickets	Alive after 6 mths.	
292	As 291 plus unsaponifiable substance 1 cc. cod-liver oil	21	1100	Normal	do.	do.
294	As 291 plus 0.5 cc. cod-liver oil	21	1050	Normal	do.	do.

Fig. 4. The weight curves of 290, 291, 292, 294. Radiographs are shown in Plate IV, 2. Note the parallel rate of increase in weight.

(b) *Egg-yolk.* The addition of egg-yolk to the basal diet had similar results to those following the addition of cod-liver oil. When one-quarter of an egg-yolk a day was given at the beginning of the experiment and increased to half a yolk *per diem*, the calcification of bones and teeth was good (Plate III, 5). 138 (Plate III, 5 A), on the basal diet only, developed fairly bad rickets and poorly calcified teeth, whilst 137 (Plate III, 5 B), with additional egg-yolk, appeared to have well calcified teeth and bones. After 8 or 9 weeks, however, the same effect was observed as with the cod-liver oil; the animals having egg-yolk lost weight and died if allowed to remain on this diet (Fig. 5).

Table VIII. *The effect of egg-yolk.*

Age at beginning of experiment: about 10 weeks.
Weight at beginning of experiment: 850-900 g.
Time of year at beginning of experiment: May.
General diet: Oats 4 } 1.5 % CaCO₃, 30 g. turnip.
                           Bran 1 }

No. of exp.	Variable factor	Time on diet weeks	Gain in weight g.	Calcium in femur shaft		X-ray	Histology	
				% wet weight	Amount g.		Bones	Teeth
137	$\frac{1}{2}$ egg-yolk	11	430	17.3	0.294	Normal	Normal	Calc. good
138	—	11	465	16.1	0.216	Bad rickets	Bad rickets	Calc. bad

Fig. 5. Weight curves of 137 and 138 (see Plate III, 5). Note rapid loss of weight of rabbit on egg-yolk after 60 days of diet.

In one series of experiments, when cabbage was added to the basal diet as well as egg-yolk, the animal grew better and, when killed at the end of 8½ weeks, was still putting on weight and seemed in excellent health. Photomicrographs of ribs of 250 and 243 (Plate III, 6) show that on the basal diet and cabbage bad rickets resulted (Plate III, 6 B), whereas when egg-yolk was added as well as cabbage calcification was good.

Table IX. *The effect of egg-yolk added to the basal diet and cabbage.*

Age at beginning of experiment: 9½ weeks.
Weight at beginning of experiment: 770-820 g.
Time of year at beginning of experiment: December.
General diet: Crushed oats 4 } 1.5 % CaCO₃.
                           Bran 1 }
                           2 g. yeast, 6 cc. neut. lemon juice, 20 g. cabbage.

No. of exp.	Variable factor	Time on diet weeks	Gain in weight g.	Calcium in femur shaft		X-ray		Histology	
				% wet weight	Amount g.	Bones	Teeth	Bones	Teeth
243	$\frac{1}{2}$ egg-yolk	8	820	15.3	0.362	Normal	Normal	Normal	Normal
250	—	8	550	13.6	0.224	Rickets	Poor calc.	Rickets	Poor calc.

2. *Vegetable foods.*

Many observations have been made by different workers on the anti-rachitic action of green vegetables in the case of rats and the general consensus of opinion is that their calcifying vitamin content is small and their vitamin A content fairly high. Most of the experiments have been carried out on spinach. Thus McClendon and Shuck [1923] found that 0.1 to 0.5 g. of dried spinach cured ophthalmia but did not protect against rickets in rats, even when it formed 75 % of the diet. Zucker and Barnett [1923] also found that an alcoholic and ether extract of spinach did not protect against rickets in rats but promoted their growth when the diet was otherwise devoid of vitamin A. Similar results were obtained with spinach by Goldblatt and Zilva [1923]. Quite recently the subject has been studied afresh by Chick and Roscoe [1926], who point out that the deficiency of phosphorus in the diets of previous workers on rickets in rats detracted from the reliability of their results. In order to overcome this difficulty Chick and Roscoe raised the phosphorus content of the basal diet to 0.37 %, leaving the calcium at 0.27 %. These investigators found that spinach grown in the open in winter, spring or autumn possessed no demonstrable anti-rachitic properties but that when grown in midsummer it had appreciable anti-rachitic value. Boas [1926] also concluded that winter spinach contained a negligible amount of vitamin D but abundance of A.

E. Mellanby [1925] in his experiments on dogs found that green cabbage had a definite but not powerful anti-rachitic action. When oatmeal was the rickets-producing element of the diet the effect of cabbage was very small, but when white flour was the cereal eaten the anti-rachitic action of the cabbage was more evident. In his experiments and possibly in other experiments on carnivorous animals, one possible source of error in determining the anti-rachitic action of green and other vegetables is the difficulty such animals have in digesting them completely. Often much of the vegetable is passed, apparently undigested and unchanged, in the faeces. Experiments to determine the anti-rachitic properties of grass and vegetables made on rabbits have the merit that these particular kinds of food are normal to the animal and more easily digested.

Since most of the experiments with vegetable foods were carried out in series of animals in litters, so that the effect of one vegetable could be compared with another, the details of some experiments will be given in groups after the main effects of these substances have been briefly described.

(A) *Green foods.*

(i) *Grass.* The addition of grass to the basal rickets-producing diet was followed by varying effects on calcification, the variations depending partly on the amount of grass added and partly on the season of the year during which the grass was grown. This addition always improved the appetite and general health of the animals.

The following are some of the main effects:

(a) When grass grown in summer was eaten *ad libitum* in addition to oats 4 parts, bran 1, with and without CaCO_3 , the animals grew well and calcification was good (Plate III, 7 A).

(b) Summer grass to the amount of 50 g. daily sometimes induced normal calcification (Exp. 124, Table XV), but was often accompanied by the development of either slight rickets or a characteristic enlargement of the shafts of radius and ulna (Plate IV, 5 E and Table XIV). This enlargement was associated with a bending of the bones and an eversion of the front paws, and microscopically the bones were abnormal.

(c) Summer grass added to the basal diet when limited to 30 g. daily resulted in rickets.

(d) Winter-grown grass up to 50 g. daily brought about the development of some rickets but, what was more striking, a condition of scurvy.

(e) When the winter grass was increased to 75 g., the long bones of the rabbit were often enlarged and bent (Plate III, 7 B).

It would appear from these results that even small quantities of grass added to the basal diet have some anti-rachitic action, which becomes prominent when fairly large quantities are eaten; also that the potency of this anti-rachitic effect is greater in summer-grown than in winter-grown grass. It is of interest to note that the same difference appears to hold in the anti-scorbutic effect of grass grown at different times of the year, the summer variety being more strongly antiscorbutic than the winter.

(ii) *Cabbage*. If the amount of the basal diet be kept constant, it will usually be found that the addition of cabbage up to about 50 g. makes the rachitic condition worse. This increase in rickets is accompanied by a distinct improvement in the animals' health and in more rapid growth (Fig. 1). From the point of view under which this investigation was started, the effect of adding cabbage to the basal diet is of great interest because it is now possible to keep rabbits for long periods in good health under conditions which allow defective bones and teeth to be formed. Rabbits are still alive which have had the basal diet together with 30–50 g. of cabbage daily for periods up to one year. Such a state of affairs on the basis of the work mentioned in the beginning of this paper might appear to be suitable for the production of dental caries.

The addition of 25 g. of green cabbage leaf daily to 40 g. of the basal diet will result, in the course of a few weeks, in a severer form of rickets and in more defectively calcified teeth than in the animal eating the basal diet only. Doubling the cabbage intake to 50 g. may make the rachitic condition even more severe (Plate IV, 1 A and B). Fifty rabbits receiving, in addition to their basal diet, amounts of cabbage varying from 20 to 50 g. daily, some with and some without lemon juice, developed rickets, in most cases of a severe nature. Plate IV, 1 A and B, represent radiographs of two animals (363 and 362) after $6\frac{1}{2}$ weeks of diet, receiving respectively 30 and 15 g. of cabbage daily. It will

be seen that 363 (30 g. cabbage) has worse rickets than 362 (15 g. cabbage). There was but little difference in the gain in weight of these animals during the 6½ weeks of the diet, 363 gaining 330 g. and 362, 320 g. When the unsaponifiable fraction of 1 cc. of cod-liver oil was eaten in addition to the basal diet and the cabbage, the bones were normally calcified.

Table X. *The effect of adding cabbage to the basal diet.*

Age at beginning of experiment: 6 weeks.
Weight at beginning of experiment: 300–350 g.
Time of year at beginning of experiment: May.
General diet: Rolled oats 4 } 1.5 % CaCO_3 .
 Bran 1
 6 cc. neutral lemon juice.

No. or exp.	Variable factor	Time on diet up to radiograph weeks	Gain in weight g.	X-rays
362	Green cabbage leaf, 15 g.	6½	320	Rickets (Plate IV, 1 B)
363	Green cabbage leaf, 30 g.	6½	330	Bad rickets (Plate IV, 1 A)
360	Green cabbage leaf, 30 g. Unsaponifiable subs. from 1 cc. cod-liver oil	6½	345	Normal

If the amount of oats and bran plus CaCO_3 in the basal diet be reduced to 25 g. and the cabbage increased to the eating capacity of the rabbit (from 40 to 400 g. according to the size), the calcification processes are greatly improved and the bones and teeth are nearly normal in structure (Plate IV, 2 A, and Fig. 4).

Table XI. *The effect of giving cabbage ad lib.*

Age at beginning of experiment: 7½ weeks.
Weight at beginning of experiment: 500 g. each.
Time of year at beginning of experiment: February.
General diet: Crushed oats 4 } 1.5 % CaCO_3 .
 Bran 1
 6 cc. neutral lemon juice.

No. of exp.	Variable factor	Time on diet up to radiograph weeks	Gain in weight g.	X-rays
290	Savoy cabbage <i>ad lib.</i> up to 400 g. Oats and bran mixt., average amt. 25 g.	21	875	Very slight rickets (Plate IV, 2 A)
291	Savoy cabbage up to 45 g. Oats and bran mixt. up to 70 g.	21	775	Very bad rickets (Plate IV, 2 B)

Boiling cabbage in water in an open vessel for 8 hours appears to make both the growth and the calcification somewhat worse than with the corresponding amount of uncooked cabbage (Fig. 6 and Plate IV, 3).

No differences in the calcifying powers of cabbage during winter and summer months have been detected nor between the green leaf and the white parts.

These experimental results indicate that the calcifying properties of green cabbage are small compared with the anti-calcifying properties of the basal diet. When the intake is low, *i.e.* below 50 g. daily, it may actually make the

rachitic changes in the bones worse in spite of the improvement in the general health of the rabbits which results.

Table XII. *The effect of boiling cabbage.*

Age at beginning of experiment: 9½ weeks.
Weight at beginning of experiment: 480–510 g.
Time of year at beginning of experiment: September.
General diet: Rolled oats 4 } 1.5 % CaCO₃.
                  Bran 1
                  6 cc. neutral lemon juice.

No. of exp.	Variable factor	Time on diet months	Gain in weight g.	X-ray	
				10 weeks	10 months
202	Cabbage uncooked, 20–40 g.	10	1200	Rickets (Plate IV, 3 A)	Rickets healing
204	Cabbage cooked in open vessel 8 hours, 20–40 g.	10	750	Worse rickets (Plate IV, 3 B)	Rickets healing very irregularly

Fig. 6. Weight curves of 202 and 204.

(iii) *Dandelion leaves* were tested with the basal diet in one series during the summer months and resulted in fairly well calcified bones and teeth (Plate IV, 5).

B. Root vegetables.

(i) *Turnip*. Animals fed on white turnip up to 100 g. daily in addition to a diet of oats 4 parts, bran 1 part and 1.5 % CaCO₃ grew well and developed bad rickets (Plate IV, 4). Growth on white turnip, oats 4, bran 1, without CaCO₃, was not nearly so good but rickets developed nevertheless. White turnip seemed to have no anti-rachitic powers but improved the rate of growth and general health of the animals.

(ii) *Swede turnip* gave results depending partly on the quantity eaten and partly on some undetermined factor. Animals given 50 g. swede turnip with the usual basal diet grew well and often showed fairly well calcified, although not normal, bones and teeth (Plate IV, 5 c, and Table XIV).

Animals given 10, 20 and 30 g. swede turnip in addition to the basal diet grew somewhat less rapidly than those with 50 g., but developed rickets.

(iii) *Carrot*. The addition of carrot to the basal diet was also followed by variable results. In eight out of ten experiments calcification was fairly good (Plate IV, 5 d and Table XIV), whilst in two, fed at the same time of the year and with the same amount of carrot, calcification was poor (Table XIII).

Table XIII. *The relative effect of carrot and cabbage.*

Age at beginning of experiment: 10 weeks.
Weight at beginning of experiment: about 800 g.
Time of year at beginning of experiment: May.
General diet: Oats 4 } 1.5 % CaCO_3 .
                   Bran 1 }
                   6 cc. neutral lemon juice.

No. of exp.	Variable factor	Time on diet weeks	Gain in weight g.	Calcium % wet weight	X-ray	Histology
118	Carrot to 50 g.	6	735	14.6	Rickets	Rickets
120	Cabbage to 50 g.	6	705	14.0	Rickets	Rickets

(iv) *Potato* has been tested in one case only. The animal was given up to 50 g. potato with the basal diet: it grew fairly well and developed bad rickets (Table XV).

Table XIV. *The relative effect of different vegetables.*

Age at beginning of experiment: 8½ weeks.
Weight at beginning of experiment: 400–600 g.
Time of year at beginning of experiment: July.
General diet: Oats 4 } 1.5 % CaCO_3 , *ad lib.*
                   Bran 1 }

No. of exp.	Variable factor	Time on diet up to radio-graphs weeks	Gain in weight g.	Calcium in femur shaft		X-ray		Histology
				% wet weight	Amount g.	Radius and ulna at death	Jaws and teeth	
169	Dandelion leaves, 20–50 g.	12½	900	16.2	0.346	Nearly normal (more rickets in earlier X-ray) (Plate IV, 5 A)	Nearly normal (Plate V, 4 A)	Nearly normal
170	Cabbage, 20–50 g.	12½	580	14.0	0.136	Bad rickets (Plate IV, 5 B)	Calcification defective (Plate V, 4 B)	Bad rickets
172	Swede turnip, 20–50 g.	12½	550	15.7	0.212	Nearly normal (slight rickets in earlier X-ray) (Plate IV, 5 c)	Nearly normal, but not as well calcified as 169	Calcification fairly good but not normal
173	Carrot, 20–50 g.	12½	770	17.5	0.253	Nearly normal (Plate IV, 5 d)	Nearly normal	Nearly normal
174	Grass, 20–50 g.	12½	900	16.0	0.244	Enlarged ulna (earlier X-ray slight rickets) (Plate IV, 5 E)	Nearly normal	Abnormal

Table XV. *The relative effect of different vegetables.**Age at beginning of experiment: about 8 weeks.**Weight at beginning of experiment: 450–550 g.**Time of year at beginning of experiment: May.**General diet: Oats $\frac{4}{1}$ Bran 1 } 1.5 % CaCO_3 , ad lib.*

No. of exp.	Variable factor	Time on diet up to radiographs weeks	Gain in weight g.	Calcium in femur shaft, % wet weight	X-ray		Histology of costo-chondral junctions
					Radius and ulna at death	Jaws and teeth	
122	Potato, 20–50 g.	20	380	15.0	Bad rickets	Poorly calcified	Bad rickets
123	Swede turnip, 20–50 g.	20 $\frac{1}{2}$	860	16.2	Nearly normal	Well calcified	Abnormal—some osteoporosis
124	Grass, 20–50 g.	17	1070	17.2	Nearly normal	Well calcified	Some osteoporosis

DISCUSSION OF THE EFFECT OF VEGETABLES.

It is surprising that the calcifying influence of the vegetables tested is so small, more especially in view of the great improvement in the general health of the animals which follows their addition to the basal diet. Experiments with puppies suggested that those dietetic conditions which promote poor calcification of bones and teeth lead fairly rapidly, *i.e.* after 4 to 6 months, to impaired health, loss of appetite and even death. Experience with rabbits, however, has shown that, for at least a year, when the diet contains cabbage in addition to the basal substances, they may remain in good health in spite of very deformed limbs and defectively calcified teeth. Cabbage, and probably other vegetables, therefore, provide some essential dietetic factors which are absent from or deficient in the basal diets. One of these substances may be vitamin A, but this alone would probably not account for the beneficial effect on the general health. It is clear from the experiments in which normal calcification of bones and teeth resulted when calcifying vitamin was added, either in the form of the unsaponifiable fraction of cod-liver oil or as egg-yolk, that this particular vitamin is only present in small quantities in the vegetables tested.

Of the green foods, grass is apparently more potently anti-rachitic than cabbage, especially when summer-grown. It has also been observed that even the anti-scorbutic action of grass is greater in the summer time. In order to produce perfectly formed bones the intake of green cabbage leaf must be large, and that of the basal substances (*i.e.* oats, bran and CaCO_3) small.

White turnip seems to be devoid of anti-rachitic vitamin, but swede turnip is better in this respect, nearly normal calcification of bones being obtained on several occasions when 50 g. were eaten daily. Carrot, on the whole, is more anti-rachitic than other root vegetables but the variable results which were obtained, both with carrot and swede turnip, remain unexplained. The action of the carrot in different series of experiments does not seem to bear any relationship to the season of the year.

EFFECT OF IRRADIATING THE ANIMALS AND THE FOOD.

(a) *Animals.* The result of exposing rabbits to a mercury vapour lamp was similar to that obtained by other workers who have irradiated children, rats and dogs. For instance, rabbits 233 and 234, of the same litter, were fed on the basal diet alone and one of them (233) was exposed three times a week to the mercury vapour lamp. The irradiated animal showed normal calcification at the end of 10 weeks, the control developed rickets.

Table XVI. *The effect of ultra-violet radiation on the animal.*

Age at beginning of experiment: 8 weeks.
Weight at beginning of experiment: 450–500 g.
Time of year at beginning of experiment: November.
General diet: Crushed oats 4 } 1.5 % CaCO_3 .
                   Bran 1   }
                   6 cc. neutral lemon juice.

No. of exp.	Variable factor	Time on diet weeks	Gain in weight g.	X-ray
233	Animal irradiated 3 times a week	10	470	Normal
234	—	10	400	Rickets

Similar results were obtained with animals fed on the basal diet and green cabbage (Plate V, 1 B, Table XVII, Exp. 282).

(b) *Foods.* Irradiating the foodstuff also greatly assists calcification and may result in normal or nearly normal bones and teeth. In series 280–286 (see Table XVII), the animals all had the basal diet together with 20–45 g. of cabbage. They grew well (Fig. 7), but the calcification is seen to be nearly normal not only in 282 (Plate V, 1 B), when the animal was irradiated, but also in 280 (Plate V, 1 A) when the basal diet was irradiated, and in 286 (Plate V, 1 D), when the cabbage only was irradiated.

Table XVII. *The effect of irradiating the food.*

Age at beginning of experiment: $7\frac{1}{2}$ weeks.
Weight at beginning of experiment: 310–450 g.
Time of year at beginning of experiment: February.
General diet: Crushed oats 4 } 1.5 % CaCO_3 .
                   Bran 1   }
                   6 cc. neutral lemon juice, 20–45 g. cabbage.

No. of exp.	Variable factor	Time on diet weeks	X-ray
280	Oats, bran and CaCO_3 irradiated	24	Nearly normal
282	Animal irradiated	24	Nearly normal (slightly worse at 10 weeks)
283	—	24	Rickets
286	Cabbage irradiated	24	Nearly normal

See also photomicrographs of the costo-chondral junctions of three rabbits of the same family, 227, 229 and 230 (Plate V, 2), of which 227 had the basal diet only, 229 had the basal diet and irradiated cabbage and 230 received

additional cabbage but without irradiation. It will be seen that 227 (Plate V, 2 A) has rickets, 229 (Plate V, 2 B) is nearly normal, whilst 230 (Plate V, 2 c) has even greater disorganisation of the growing cartilage than 227.

Fig. 7. Weight curves of animals 280, 282, 283, 286, whose radiographs are shown in Plate V, 1. Note the parallel rate of increase in weight.

SUMMARY.

(1) Diets are described which, while allowing good growth and good general health in rabbits, produce very defective calcification of teeth and bones. It is hoped by feeding on these diets to produce ultimately dental caries.

(2) The degree of abnormal calcification is related to the growth of the animal. If x g. of a diet produces rickets, $2x$ g. of the same diet will produce worse rickets.

(3) The worst calcification is obtained in rabbits under these experimental conditions when the calcium and phosphorus content of the diet approaches figures which have been described by workers on rickets in rats as likely to prevent the disease.

(4) Calcification in rabbits responds immediately, in the same way as in other animals studied, to an increase in calcifying vitamin in the diet and to exposure either of the animal or of food to ultra-violet radiations.

(5) Examination of some vegetable foods indicates that they are for the most part deficient in calcifying vitamin. Grass, especially summer-grown grass, contains more than cabbage. Of the root vegetables, carrot and swede turnip contain more than white turnip.

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EXPLANATION OF PLATES.

Plate III, 1. Photographs of (A) normal and (B) rachitic rabbits.

Plate III, 2. Radiographs of forelegs showing the rickets-producing effect of adding 1.5 % CaCO_3 to a diet of oats, bran and lemon juice. (A) no CaCO_3 (67), (B) CaCO_3 (69).

Plate III, 3. Radiograph of rabbit (340) in which Na_2CO_3 replaced CaCO_3 in the basal diet. Note the thick band of ossification at epiphysis.

Plate III, 4. Radiograph showing improved calcification on adding cod-liver oil to basal diet.

A. Basal diet + cod-liver oil—good calcification (130).

B. Basal diet only—poor calcification (127).

Plate III, 5. Radiographs showing effect of adding egg-yolk to basal diet.

A (138) received basal diet only—bad rickets.

B (137) received basal diet + egg-yolk—normal calcification.

Plate III, 6. Photomicrographs of costo-chondral junctions showing defective calcification produced by diet which included cabbage, and normal ossification produced by the addition of egg-yolk.

A (243) basal diet + cabbage + egg-yolk—good calcification.

B (250) basal diet + cabbage—very poor calcification and much osteoid tissue.

Plate III, 7. Radiographs showing the effect of grass on calcification.

A (41) summer grass *ad lib.* added to basal diet—good bone formation.

B (61) winter grass (75 g.) added to basal diet. Note great thickening of radius, an effect often produced by grass.

Plate IV, 1. Radiographs showing that rickets may be made worse by adding more cabbage to basal diet.

A (363) basal diet + 30 g. cabbage daily—bad rickets.

B (362) basal diet + 15 g. cabbage daily—moderate rickets.

The rate of growth of these animals was similar.

Plate IV, 2. Radiographs showing the relative effects of cabbage *ad lib.* and cod-liver oil on rickets in rabbits.

A (290) basal diet (25 g.) + savoy cabbage *ad lib.*

B (291) basal diet (70 g.) + 45 g. savoy cabbage.

C (292) basal diet (70 g.) + 45 g. savoy cabbage + unsaponifiable substance from 1 cc. cod-liver oil.

D (294) basal diet (70 g.) + 45 g. savoy cabbage + 0.5 cc. cod-liver oil.

Note that savoy cabbage *ad lib.* (A) has not produced quite the perfect calcification of C and D. B has very bad rickets.

Plate IV, 3. Radiographs showing that boiled cabbage produces worse rickets than uncooked cabbage.

A (202) basal diet + uncooked cabbage—moderate rickets.

B (204) basal diet + cooked cabbage—bad rickets.

Plate IV, 4. Radiograph showing that white turnip up to 100 g. daily added to basal diet allows very bad calcification.

Plate IV, 5. Radiographs showing relative effect on calcification of different vegetables added to basal diet.

A (169) basal diet + dandelion 50 g.—fairly good calcification.

B (170) „ + cabbage 50 g.—very bad calcification.

C (172) „ + swede turnip 50 g.—fairly good calcification.

D (173) „ + carrot 50 g.—fairly good calcification.

E (174) „ + summer grass 50 g.—thickened bones but little rickets.

Plate V, 1. Radiographs showing effect on calcification of irradiating animal and food with a mercury vapour lamp. All animals on same diet.

A (280) basal diet irradiated—fairly good calcification.

B (282) animal irradiated—fairly good calcification.

C (283) no irradiation—bad calcification.

D (286) cabbage of diet irradiated—good calcification.

Plate V, 2. Photomicrographs of costo-chondral junctions showing effect of cabbage and irradiated cabbage on calcification.

A (227) basal diet only—disordered growth of cartilage.

B (229) basal diet + cabbage (irradiated)—nearly normal growth.

C (230) basal diet + cabbage—disordered growth.

Plate V, 3. Photomicrographs of ground sections of molar teeth of two rabbits showing perfect and imperfect calcification of the dentine.

A. Dentine thick and well calcified.

B. Dentine thin and imperfectly calcified showing interglobular spaces.

Plate V, 4. Radiographs of half lower jaws of two rabbits showing well calcified and poorly calcified bones.

A (169) basal diet + 45 g. dandelion leaf—nearly normal calcification.

B (170) basal diet + 45 g. cabbage leaf—poorly calcified bone easily seen round incisors and growing portion of molars.

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