XXIII. THE ANTI-RACHITIC VALUE OF WINTER SPINACH¹.

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(Received January 4th, 1926.)

1. THE INFLUENCE OF FRESH GREEN WINTER SPINACH UPON THE RETENTION OF CALCIUM AND PHOSPHORUS BY YOUNG RATS.

AN account of previous observations upon the content of the anti-rachitic factor (vitamin D) in fresh green leaves is given in the preceding paper [Chick and Roscoe, 1926] and therefore only those investigations will be referred to here in which the Ca and P retention of animals fed upon green food was studied.

Hart, Steenbock and Hoppert [1921], in a study of the Ca metabolism of a goat, found that the retention of this substance was increased by the addition of fresh green oats to an otherwise dry diet; raw cabbage did not however seem to have any such effect. Hart, Steenbock, Hoppert and Humphrey [1922] working on milking cows converted a negative Ca balance into a positive one by adding dry alfalfa hay to the diet, whilst the substitution of this by fresh green alfalfa further increased the storage of Ca. In two cases out of three the storage of P was also increased. Hart, Steenbock, Hoppert, Bethke and Humphrey [1922] changed negative Ca and P balances in milking cows into positive ones by replacing the dry timothy hay in the diet by green alfalfa hay. Gaessler and McCandish [1923] found positive Ca balances in cows fed upon a diet with a low fat content but containing large amounts of fresh alfalfa hay. When this was replaced by alfalfa hay which was 1½ years old a negative balance appeared.

The results of these investigations agree in demonstrating the influence of green plants in raising the Ca retention of herbivorous animals fed upon them, and in showing that the fresh green plants are superior in this respect to the dried material.

The present paper records an investigation of the effect of the fresh green leaves of winter spinach upon the Ca and P metabolism of the young rat. For this purpose the retention of Ca and P was compared in groups of young rats fed in the following three different ways:

¹ The first experiment in this paper formed part of a thesis approved for the degree of Doctor of Philosophy in the University of London.

- (1) upon a basal control diet deprived of fat-soluble vitamins,
- (2) upon the same diet supplemented by a daily ration of fresh green spinach leaves, and
- (3) upon the basal diet supplemented by a daily ration of cod-liver oil.

The metabolic experiments were of two types. In the first type a litter of rats was divided into three groups, each of which received one of the above diets, the retention of Ca and P in each group being compared with that in the others. In the second type of experiment the same rats received first the basal diet alone for some weeks, and subsequently the basal diet supplemented by spinach.

Experimental Methods.

The method for estimating Ca and P retention already described [Boas, 1924] was employed. Certain refinements were introduced, the most important being the separate collection of faeces, urine and uneaten food. The glass separators designed by Gross and Connell [1923] were used and proved satisfactory.

Two different basal diets were used.

Basal Diet 1 had the following composition:

Inactivated caseinogen	120 g.
Wheaten starch	342 „
Yeast extract	36 „
Hardened sesame oil	90 „
Salt mixture [see Boas, 1924]	10.5 „
Ferric citrate	0.5 „
Orange juice	36 c.c.
Distilled water	280 „

Sufficient CaCO_3 and K_2HPO_4 were added to produce a diet with a final Ca content of 0.54–0.57 % and a P content of 0.54–0.60 % reckoned on the wet weight, 0.95 % Ca and 0.96 % P reckoned on the dry weight.

Basal Diet 2 had the following composition:

Inactivated caseinogen	100 g.
Wheaten starch	250 „
Yeast extract	25 „
Hardened cotton seed oil	75 „
Ferric citrate	0.4 „
Calcium carbonate	3.0 „
Potassium monohydrogen phosphate	3.8 „
Salt mixture [see Boas, 1924]	9.0 „
Lemon juice	25 cc.
Distilled water	300 „

In this case the salts containing Ca and P were mixed with the dry constituents of the diet. Two samples of each batch of the diet after the water was added were taken for the determination of the content of Ca and P.

This averaged 0.24 % Ca and 0.26 % P reckoned on the wet weight, 0.44 % Ca and 0.48 % P reckoned on the dry weight of the diet. This diet had therefore a considerably lower Ca and P content than the first basal diet.

A normal mixed diet for a rat, consisting of milk, brown bread and cabbage, contains about 0.23 % Ca and 0.32 % P reckoned on the dry weight, and it was therefore thought that the first basal diet contained an amount of these substances far in excess of the normal requirement though it was similar to the diets used by other workers [see Goldblatt, 1923, p. 301].

The leaves of prickly seeded winter spinach (*Spinacea oleracea*) were used. After being carefully washed and dried the stalks and large ribs were removed. All leaves which were at all discoloured were rejected. At the beginning of each week the weekly ration was weighed out to the nearest gram into a stoppered bottle and placed in the refrigerating room, a representative sample being taken at the same time for the estimation of Ca and P content. Each rat received its ration separately, but if not consumed within reasonable time the residue was mixed with the basal ration. The successive batches of spinach had a Ca content ranging from 0.12 % to 0.21 % and a P content of from 0.0 % to 0.13 % of the fresh leaves. The water content averaged 90 %.

Experiments and Results.

Three metabolism experiments each lasting for 8 to 11 weeks were carried out on young rats.

Exp. 1 (February to May, 1924). Six female rats from one litter were placed in couples in three metabolism cages when they were 22 days old and had an average body weight of 44 g. In the first group (Group A) the rats received basal diet 1. In the second group (Group C) each rat received 3 drops of cod-liver oil daily in addition to the basal diet, whilst the rats comprising the third group (Group S) were given a daily ration of fresh spinach starting at 1 g. per rat, and increasing to 5 g. before the end of the first fortnight. The ration was kept at this level during the rest of the experiment. The first fortnight was used as a preliminary period during which no determinations were made, and followed by three periods, each consisting of two weeks, during which the excreta were collected and the retention of Ca and P was estimated.

Results of Exp. 1. Fig. 1 shows the mean curve of increase in body weight per rat of the three groups during the entire eight weeks of the experiment. The rats receiving cod-liver oil gained on an average 69 g. per rat, those receiving spinach (Group S) 79 g., whilst the controls in Group A gained no more than 45 g. Tables II and III contain the average intake, output and retention per rat of Ca and of P respectively during the three periods of the experiment. In Fig. 2 the average successive total increments of Ca and P per rat are shown plotted against the time. Table III contains the data obtained by analysis of the leg bones of the rats.

Table I. *The calcium metabolism (average per rat) of the six female rats in Exp. 1.*

GROUP C. Rats receiving basal diet and a daily ration of 3 drops of cod-liver oil per rat.						
Period	Rise in body wt. g.	Intake g.	Output			Retention g.
			Faeces g.	Urine g.	Total g.	
Preliminary (2 weeks)	47- 71					
1. (2 weeks)	71- 97	1.0672	0.7565	0.0239	0.7804	0.2868
2. (")	97-122	1.1614	0.8600	0.0238	0.8838	0.2776
3. (")	122-140	1.0676	0.8728	0.0157	0.8885	0.1791
Total (6 weeks)	71-140	3.2962	2.4893	0.0634	2.5527	0.7435
GROUP S. Rats receiving basal diet and a daily ration of 5 g. of fresh green spinach leaves per rat.						
Preliminary (2 weeks)	45- 73					
1. (2 weeks)	73-100	1.1950	0.8608	0.0195	0.8803	0.3147
2. (")	100-132	1.4175	1.1340	0.0223	1.1563	0.2612
3. (")	132-152	1.2561	1.0835	0.0161	1.0996	0.1565
Total (6 weeks)	73-152	3.8686	3.0783	0.0579	3.1362	0.7324
GROUP A. Rats receiving basal diet only.						
Preliminary (2 weeks)	47- 60					
1. (2 weeks)	60- 69	0.7976	0.6573	0.0085	0.6658	0.1318
2. (")	69- 89	0.8549	0.7037	0.0103	0.7140	0.1409
3. (")	89-105	0.8680	0.7062	0.0118	0.7180	0.1500
Total (6 weeks)	60-105	2.5205	2.0672	0.0306	2.0978	0.4227

Table II. *The phosphorus metabolism (average per rat) of the six female rats in Exp. 1.*

GROUP C. Rats receiving basal diet and a daily ration of 3 drops of cod-liver oil per rat.						
Period	Rise in body wt. g.	Intake g.	Output			Retention g.
			Faeces g.	Urine g.	Total g.	
Preliminary (2 weeks)	47- 71					
1. (2 weeks)	71- 97	1.1437	0.3661	0.5676	0.9337	0.2100
2. (")	97-122	1.2608	0.4201	0.6913	1.1114	0.1494
3. (")	122-140	1.1402	0.4488	0.5760	1.0248	0.1154
Total (6 weeks)	71-140	3.5447	1.2350	1.8349	3.0699	0.4748
GROUP S. Rats receiving basal diet and a daily ration of 5 g. of fresh green spinach leaves per rat.						
Preliminary (2 weeks)	47- 73					
1. (2 weeks)	73-100	1.2710	0.3280	0.7263	1.0543	0.2167
2. (")	100-132	1.4591	0.4519	0.8415	1.2934	0.1657
3. (")	132-152	1.2999	0.4102	0.7693	1.1795	0.1204
Total (6 weeks)	73-152	4.0300	1.1901	2.3371	3.5272	0.5028
GROUP A. Rats receiving basal diet only.						
Preliminary (2 weeks)	47- 60					
1. (2 weeks)	60- 69	0.8379	0.3419	0.3799	0.7218	0.1161
2. (")	69- 89	0.9164	0.3660	0.4461	0.8121	0.1043
3. (")	89-105	0.9457	0.3703	0.4861	0.8564	0.0893
Total (6 weeks)	60-105	2.7000	1.0782	1.3121	2.3903	0.3097

Table III. *Analyses of leg bones (femora, tibiae and fibulae) dried at 110° for 24 hours of the six female rats in Exp. 1.*

Rat No.	Diet	Body wt. g.	Dry wt. of leg bones g.	Dry wt. of bones, % of body wt. (average of 2 rats)	Ca content of bones, % of dry wt.
61	Cod-liver oil	149	0.9345	0.66	20.44
62	"	130	0.8957		19.12
65	Spinach	156	0.8339	0.56	18.74
66	"	148	0.8701		18.57
63	Basal diet only	99	0.6609	0.74	17.89
64	"	105	0.7764		17.14

At first sight it would appear from these tables that the addition of spinach to the diet has promoted the retention of Ca to as great a degree as has the ration of cod-liver oil. When however the data are carefully examined this proves not to be the case.

Fig. 1. Curves showing the increase in body weight of the rats in Exp. 1, receiving (A) basal diet only, (C) basal diet and cod-liver oil, and (S) basal diet and spinach. Each curve represents average figures for two female rats from the same litter. D is the Wistar Institute's curve of normal growth for female rats.

The rats receiving cod-liver oil had a final body weight 33 % heavier than that of the controls and their bodies contained more fat. Their leg bones (see Table III) were 25 % heavier and had a Ca content which was 12 % higher when reckoned on the dry weight. Chick and Roscoe [1926] find that the bones of rats deprived of fat-soluble vitamins contain more fat than do those of rats receiving cod-liver oil or spinach. Therefore it may be assumed that could the content of Ca in the fat-free bone be calculated, the difference in Ca content would be less.

Fig. 2. Curves of Ca retention by the rats in Exp. 1 receiving basal diet only (AC), basal diet and cod-liver oil (CC) and basal diet and spinach (SC). Curves AP, CP and SP show the respective P retentions of the three groups.

The amount of Ca retained for every 10 g. increase in body weight (see Table IV) was 15 % higher in Group C than in Group A. Figures given by Donaldson [1924] show that at this time of life in a normal rat the increase in weight of the skeleton is very nearly proportional to that of the whole body. That this was so in the rats receiving cod-liver oil is shown by the fact that during the successive periods of the experiment the retention of Ca per 10 g. increase in body weight remained level (0.110, 0.112, 0.100). The above figures show that the favourable influence of cod-liver oil upon growth and Ca retention is clearly demonstrated in this experiment.

Table IV. *The amounts of Ca and P retained per 10 g. increase in body weight by the rats in Exp. 1.*

Group	Addition to basal diet	1st fortnight		2nd fortnight		3rd fortnight		Total period	
		Ca g.	P g.	Ca g.	P g.	Ca g.	P g.	Ca g.	P g.
C	Cod-liver oil	0.110	0.075	0.112	0.060	0.100	0.064	0.108	0.068
S	Spinach	0.116	0.080	0.082	0.075	0.074	0.040	0.092	0.063
A	None	0.146	0.130	0.070	0.052	0.094	0.057	0.094	0.069

The rats receiving spinach (Group S) also grew well and were 44 % heavier than the controls (Group A) and 11 % heavier than the rats receiving cod-liver oil (Group C) at the end of the experiment. The weight of the skeleton was not however increased in proportion, the leg bones being only 18 % heavier than those of Group A and 7 % lighter than those of Group C. The content of Ca in the leg bones of Group S reckoned on the dry weight was 6 % more than in Group A; for the reasons given above it is probable that the percentage of Ca in the fat-free bone would show a smaller difference. Over the entire six weeks of the experiment the average amount of Ca retained per unit increase in body weight was the same in Groups A and S (0.094 and 0.092 g. respectively). In Group S there was a steady decrease in this ratio during the course of the experiment, indicating that the calcification of the skeleton was *declining* relatively to the increase in weight of the body. These results lead one to the conclusion that the winter spinach improved the growth both of the skeleton and of the body as a whole and produced rats stronger and more healthy than were the controls, but there is no evidence that the *degree of calcification of the bones* was improved. It seems probable from Chick's results that had summer spinach been employed the level of Ca storage might have been slightly improved.

Exps. 2 and 3 (October 1924 to January 1925). In each of these experiments four female rats from one litter were placed in pairs in metabolism cages, the two cages being treated as one unit for the purpose of collection and analysis of excreta. The rats received basal diet 2 without any addition for seven weeks. This was Period 1 during the first fortnight of which no analyses were made. During Period 2 which lasted for four weeks a daily ration of spinach was given in addition to the basal diet. The rats received as much spinach as they could be induced to eat—an average of 3.5 g. per day per rat. It was intended to follow Period 2 by a third period during which the spinach would be replaced by a ration of cod-liver oil but this proved impracticable. Such prolonged diarrhoea resulted from the administration of the oil that it was impossible to collect the excreta with a sufficient degree of accuracy.

The results of these experiments confirm those of the first experiment but are not as clearly defined. The stock from which the rats were drawn had been receiving a certain amount of egg-yolk in addition to the usual stock diet which is relatively deficient in the fat-soluble vitamin D. When

the experiments were started it was not realised that the administration of the egg-yolk to the parent animals would result in the young rats possessing such rich stores of fat-soluble vitamins as subsequently proved to be the case. The growth curves given in Fig. 3 show that in Period 1 when the rats were

Fig. 3. Curves showing increase in body weight of the rats in Exps. 2 (Curve A) and 3 (Curve B). Each represents average figures for four female rats from one litter. From X to Y the rats received basal diet alone; from Y to Z a daily ration of fresh spinach was given. Curve D is the Wistar Institute's curve of normal growth for a female rat.

receiving basal diet alone, a rate of growth not far below that of a rat fed upon a complete diet was maintained throughout the seven weeks. In both cases the addition of spinach to the diet in Period 2 distinctly stimulated the rate of growth.

Table V. *The average figures for calcium and phosphorus metabolism per rat of the four female rats in Exp. 2.*

During Period 1 basal diet only was given; during Period 2 a daily ration of from 3 to 4 g. of fresh green spinach leaves was ingested by each rat.

Period	Weeks of Exp.	Output										Rise in body wt. g.	Ca retention per 10 g. rise in body wt. g.
		Intake		Faeces		Urine		Total		Retention			
		Ca	P	Ca	P	Ca	P	Ca	P	Ca	P		
		g.	g.	g.	g.	g.	g.	g.	g.	g.	g.		
1. Basal diet only	1 and 2											26-40	
	3	0.1556	0.1701	0.0608	0.0354	0.0043	0.0647	0.0651	0.1001	0.0904	0.0700	40-48	
	4 and 5	0.3786	0.4032	0.2518	0.1480	0.0132*		0.2650		0.1136		48-66	0.079
	6 and 7	0.3614	0.4012	0.2678	0.1676	0.0108	0.1518	0.2786	0.3194	0.0828	0.0818	66-76	
2. Basal diet+3.5 g. of fresh spinach per rat per day	8											76-91	
	9 and 10	0.5332	0.5594	0.4020	0.1890	0.0154	0.2776	0.4174	0.4666	0.1158	0.0668	91-116	0.038
	11	0.2966	0.3148	0.2618	0.1154	0.0080	0.1689	0.2698	0.2843	0.0278	0.0610	116-128	

* Calculated on the assumption that 95 % of the total Ca excreted was in the faeces, 95 % being the mean of the figures obtained in the preceding and following periods (94 and 96 % respectively).

Table VI. *The average figures for calcium and phosphorus metabolism per rat of the four female rats in Exp. 3.*

During Period 1 basal diet only was given; during Period 2 a daily ration of from 3 to 4 g. of fresh green spinach leaves was ingested by each rat.

Fresh green spinach leaves was ingested by each rat.											
Period	Weeks of Exp.	Intake		Output				Retention		Rise in body wt. g.	Ca retention per 10 g. rise in body wt. g.
		Ca g.	P g.	Faeces		Urine		Total			
				Ca g.	P g.	Ca g.	P g.	Ca g.	P g.		
1. Basal diet only	1 and 2									27-45	
	3	0.1413	0.1497	0.0830	0.0393	0.0032	0.0664	0.0862	0.1057	45-53	
	4 and 5	0.3678	0.3938	0.2294	0.1200	0.0102	0.1916	0.2396	0.3016	53-71	} 0.071
	6 and 7	0.4624	0.5112	0.2802	0.1676	0.0150	0.2208	0.2952	0.3884	71-94	
2. Basal diet + 3.5 g. of fresh spinach per rat per day	8 and 9	0.6108	0.6420	0.4396	0.2102	0.0158	0.3066	0.4454	0.5168	94-123	} 0.059
	10 and 11	0.6194	0.6642	0.4920	0.2154	0.0232	—	0.5152	—	123-139	

Table V contains figures showing the average Ca and P intake, excretion and retention per rat in Exp. 2, and Table VI gives the same figures for Exp. 3. In Exp. 2 the result of the spinach ration was to raise the absolute amount of Ca retained, but the level of retention in proportion to the increase in weight was lowered from an average of 0.079 g. per 10 g. increase in body weight during Period 1 to an average of only 0.038 g. in Period 2. This shows that the stimulus to growth was not accompanied by a comparable stimulus to the process of calcification.

In Exp. 3 for some reason which it is difficult to see, the retention of Ca and P rose week by week instead of decreasing as is usually the case [see Boas, 1924]. During the first fortnight of Period 2 it remained at the same level and fell during the last fortnight. Here again, as in Exp. 2, in relation to the growth made, the level of Ca retention fell as the result of adding spinach to the diet. The Ca retention per 10 g. increase in body weight fell from 0.071 in Period 1 to 0.059 g. in Period 2.

Exps. 2 and 3 thus provide additional evidence that the inclusion of winter spinach in the diet does not raise the level of Ca retention.

2. THE INFLUENCE OF THE DIET UPON THE DISTRIBUTION OF THE CALCIUM AND PHOSPHORUS EXCRETION BETWEEN THE URINE AND THE FAECES.

There has been recently a tendency among certain investigators to regard the alimentary canal as the site of the lesion in rickets, and to attribute the disease at any rate in part to a faulty absorption of Ca and P. In consequence more Ca and P would appear in the faeces at the expense of that formerly excreted in the urine, the result of which would be to lower the concentration of these substances in the blood.

Schabad [1909] pointed out that in rickets there was more Ca and P in the faeces and less in the urine than normally. Bertram [1878] stated that in rickets the Ca in the urine was very low. Bogert and Traill [1922] and Givens [1918, 2] were able to divert Ca excretion from faeces to urine by the addition of fat-soluble vitamins to the diet in the form of butter-fat or fresh milk. Orr, Holt, Wilkins and Boone [1923] found that in three cases of rickets in infants the result of ultra-violet irradiation was to improve the retention of Ca and P and to decrease the amounts of these substances excreted in the faeces. In all three cases the amount of P in the urine rose and in two of the cases the amount of Ca also rose.

The administration of acid or of acid-forming diets has been shown to increase the Ca and P in the urine at the expense of that in the faeces by Weiske [1872], Schetelig [1880], Rüdell [1893], Givens [1917, 1918, 1], Kishi [1922] and Bogert and Kirkpatrick [1922], whilst the opposite effect has been shown to occur, when alkalies or base-forming diets are given, by Weiske [1872], Gerhardt and Schelesinger [1899], Wuertz [1913] and Bogert and Kirkpatrick [1922].

Zucker, Johnson and Barnett [1922] showed that the acid-base ratio of the diet could be an important factor in determining whether rickets would or would not develop in rats. The addition of 20 % ammonium chloride to a rickets-producing diet prevented the development of the disease, whilst sodium carbonate changed a non-rickets-producing diet into one which gave marked rickets. It was therefore suggested that in rickets the faulty absorption of Ca and P was due to insufficient acidity of the intestinal tract. Zucker and Matzner [1924] showed that the p_H of a suspension of faeces was about 7.6 in the case of rats receiving their No. 84 rickets-producing diet and that the result of administering the active principle of cod-liver oil, or of irradiation with the mercury vapour lamp was to lower the p_H of the faeces suspension to 5.7–6.4. When the active principle of cod-liver oil was injected subcutaneously no change in the acidity of the faeces took place. Soames [1924] has however shown that the intraperitoneal injection of cod-liver oil confers partial protection against a rickets-producing diet.

Jones, James and Smith [1924] have reported the production of rickets in puppies by the use of diets adequate in all respects but possessing a high potential alkalinity; they were however unable to obtain similar results with

rats. Jones [1924] described three cases of rickets in children in which HCl therapy was used with satisfactory results. Unfortunately in only one case was there no other change in the diet. Stehle [1917] claimed to have increased the storage of Ca in a dog by the administration of HCl, but Givens [1918, 1] and Givens and Mendel [1917] were unable to confirm this, whilst Bogert and Kirkpatrick [1922] concluded that the result of acid-forming diets was to decrease rather than increase the retention of Ca.

There seems therefore to be some evidence for the opinion that in rickets there may be an abnormal alkalinity of the alimentary canal. This would give rise to the formation of insoluble basic salts rather than the more soluble acid salts, and hence to a decreased absorption of Ca and P. The administration of cod-liver oil or irradiation by the mercury vapour lamp are assumed to aid absorption by restoring the normal reaction of the gut. That this is the only action of the anti-rachitic factor and that acid therapy would be as effective as cod-liver oil in the prevention and cure of rickets is difficult to believe, and the experiments of Givens and Mendel, of Bogert and Kirkpatrick and of Soames already referred to are against this view.

Experimental Results.

In the metabolism experiments described above the partition of the Ca and P excretion between the urine and faeces was observed and the data obtained are collected in Tables VII and VIII. Throughout all three experiments 95-99 % of the total Ca excreted was found in the faeces. Neither the addition to the diet of cod-liver oil nor of spinach exerted any appreciable influence upon this partition. It appears therefore that in rats only a very small amount of the Ca is passed in the urine.

Table VII. *The percentage of the total calcium and phosphorus excretion found in the faeces of the rats in Exp. 1.*

Period No.	Group C Basal diet and cod-liver oil		Group S Basal diet and spinach		Group A Basal diet only	
	Ca %	P %	Ca %	P %	Ca %	P %
1	97	39	98	31	99	48
2	97	38	98	35	99	45
3	98	44	99	35	99	43
Mean	97	40	98	34	99	45

Table VIII. *The percentage of the total calcium and phosphorus excretion found in the faeces of the rats in Exps. 2 and 3.*

Period	Exp. 2		Exp. 3	
	Ca %	P %	Ca %	P %
1. Rats receiving basal diet only	95	46	96	40
2. Rats receiving basal diet and 3-4 g. of spinach	97	41	96	40

In Exp. 1 the rats in Group A receiving basal diet alone excreted on an average 45 % of the total P excretion in the faeces. The result of adding

cod-liver oil to the ration was to lower this figure to 40 %, whilst in the rats receiving spinach an even smaller proportion of the P excretion was found in the faeces—an average of 34 %.

In Exp. 2 during Period 1 when basal diet alone was given the average percentage of the total P excretion found in the faeces was 47. This was decreased to 41 during the four weeks of spinach ingestion.

The results of Exp. 3 are not of interest. So rich was the store of anti-rachitic factor induced in the rats by the administration of egg-yolk to their parents that seven weeks' deprivation of fat-soluble vitamins proved insufficient to cause any diversion of P from urine to faeces. During both periods of the experiment the proportion of the total P excretion found in the faeces averaged 40 %, the same figure as that obtained in the rats receiving cod-liver oil in Exp. 1.

It is curious that spinach, in spite of its alkaline ash and of the fact that it has been shown to contain little anti-rachitic factor, should yet cause a diversion of P from faeces to urine. There is very little evidence bearing on this point in the literature of the subject. Hart, Steenbock, Hoppert and Humphrey [1922] substituted fresh green alfalfa for dry alfalfa hay as the diet of three milking cows. The storage of Ca and P was increased but the amount of Ca and P in the urine fell and that in the faeces rose. There was however a marked decline in the yield of milk which they attribute to the hot weather and to the presence of flies, so that the animals seem to have suffered from some metabolic disturbance. In a second experiment upon milking cows carried out by the same observers [Hart, Steenbock, Hoppert, Bethke and Humphrey, 1922] timothy hay was changed to green alfalfa hay, and in this case there was no variation in the milk yield. In all three cows the amount of P in the urine rose whilst that in the faeces fell.

It is perhaps dangerous to draw definite conclusions from the results of three metabolism experiments in which only 14 rats were involved but the results obtained offer some evidence against the theory that the anti-rachitic factor promotes calcification by lowering the p_H of the intestine. Two substances, cod-liver oil and spinach, have both been shown to favour the absorption of P as indicated by a relative decrease in the amount of P excreted in the faeces. Cod-liver oil is a rich source of vitamin D but spinach has been shown to contain only negligible quantities of this factor. It follows therefore that although a substance may be able to divert P from faeces to urine there is no ground for assuming that it necessarily possesses anti-rachitic power.

SUMMARY.

1. Direct metabolic experiments have been carried out on young rats in order to compare the influence upon the retention of Ca and P of daily additions to diets deficient in all fat-soluble vitamins of cod-liver oil and of fresh green winter spinach leaves.

2. The addition of cod-liver oil to the diet resulted in an improvement in the general health and an increase in the rate of growth of both the skeleton and the whole body. The degree of calcification of the skeleton was also improved, for there was a rise in the amount of Ca retained per unit increase in body weight.

3. The fresh leaves of winter-grown spinach added to the experimental diet caused an even greater improvement in the well-being of the rats and in the rate of growth. The weight of the skeleton was not however proportionally increased.

4. The conclusion is drawn that winter spinach contains an amount of vitamin D which is negligible compared with its content of vitamin A.

5. 95–98 % of the total Ca excretion of the rat is found in the faeces, and this partition is independent of the amount of fat-soluble vitamins in the diet.

6. Both cod-liver oil and spinach, when added to a diet deficient in fat-soluble vitamins, cause an increase in the amount of P excreted in the urine at the expense of that excreted in the faeces.

I wish to record my thanks to Prof. C. J. Martin and Dr H. Chick for their unfailing help and advice throughout the course of this investigation.

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