XXIII. VARIATIONS OF SERUM-CALCIUM IN THE RABBIT.

By MARGHERITA COTONIO BOURNE AND
DOROTHY ADAMS CAMPBELL.

*From the Department of Physiology and Biochemistry,
University College, London.*

(Received 23rd December, 1931.)

IN continuing the investigation of the relation of blood-calcium to the formation of cataract in rabbits, we found that there are certain conditions under which the serum-calcium shows considerable variations. It is true that one of us [Adams, 1930] previously obtained satisfactory control experiments in which only very slight variations were observed; but, with repeated experiments, it became apparent that the level of serum-calcium may vary considerably in individual rabbits, over long periods, and in particular with different diets.

The evidence of other observers is conflicting. Thus Davies, Dickens and Dodds [1926] state that in individual rabbits the serum-calcium does not vary more than 1.5 mg. per 100 cc. serum. Ssewerin [1927] states that the serum-calcium level remains unchanged when cabbage is fed to a starving rabbit. He gives no figures. On the other hand, Ipponsugi [1927, 1], in fifteen control rabbits, found the serum-calcium varying from 13.5 to 18 mg. per 100 cc. and in ten other controls he found variations from 12 to 17 mg. per 100 cc. Harnes [1928] determined the serum-calcium in eighty rabbits throughout one year and found variations from 14.5 to 18.5 mg. per 100 cc. Culhane [1927] reports variations, in six rabbits fed on oats and cabbage, from 14.31 to 17.12 mg. Ca per 100 cc. serum. Kapsinow and Underhill [1929] observed considerable daily variations in the serum-calcium level, usually between 10 and 15 mg. per 100 cc. serum, with one figure as high as 21 mg. per 100 cc.

In addition there exists a further uncertainty as to the effect of diet¹ on the serum-calcium in this animal. Culhane [1927, 1930] reported that cabbage causes a rise in the serum-calcium, but Kapsinow and Underhill [1929] were unable to confirm this. Cannon and Greenwood [1930] found that a diet of sweet clover caused the serum-calcium of young rabbits to decrease.

¹ After this paper had been sent to press, we received the copy of the *Journal of Biological Chemistry* for December, 1931, containing an article by Dupré and Semeonoff [1931] in which the effects of diet on serum-calcium and phosphorus in the rabbit are described. The results obtained by these authors are in agreement with those which we report in this paper.

Because of the importance of the rabbit as an experimental animal, and the uncertainty which seems to exist regarding the normal serum-calcium in this animal, it seemed worth while to attempt to determine more exactly the conditions under which the serum-calcium is variable, and to ascertain how these variations are related to diet.

EXPERIMENTAL.

Our investigations may be summarised as follows.

I. Normal variations.

- (a) A general comparison of estimations of serum-calcium from a number of animals over a considerable period.
- (b) A comparison of estimations of serum-calcium of animals which had fasted for 16 hours before the taking of the blood sample.

II. A study of the effect of diet on

- (a) The serum-calcium of fasting animals.
- (b) The reaction and calcium content of the urine.
- (c) The calcium and inorganic phosphorus of the serum and urine.

III. Estimations of haemoglobin, as a control on the effect of haemorrhage, whenever an animal was bled repeatedly.

Methods.

The methods used were as follows.

Blood samples. These were taken from the marginal ear vein. The ear was warmed gently, and a small slit made with a flat surgical needle of the Hagedorn type. The size of the slit was regulated to the amount of blood required. The blood flowed quickly and the bleeding was easily stopped when the required amount of blood had been obtained.

Calcium. The Clark and Collip [1925] modification of the Kramer and Tisdall method was used in all cases. The blood was allowed to clot overnight, and after the addition of oxalate the tubes were again allowed to stand overnight to insure complete precipitation. Care was taken that all experimental details were as constant as possible.

Inorganic phosphate in serum. The method of Fiske and Subbarow [1925] was used. The blood for this determination was drawn into a centrifuge-tube. As soon as it had clotted, the clot was loosened from the side of the tube, and the whole centrifuged at high speed for about 10 minutes. 2 cc. of clear serum were used for each determination.

Urinary calcium. This was determined by the method of McCrudden [1911]. If the urine was alkaline, it was made slightly acid to litmus with HCl and filtered. The calcium was precipitated from 10 cc. of the filtered urine, redissolved in sulphuric acid and determined by permanganate titration.

Inorganic phosphate in the urine. This was determined by the Fiske and Subbarow [1925] method, a portion of the slightly acidified filtered urine being used.

Haemoglobin. A Salhi haemoglobinometer was used.

RESULTS.

I. *Normal variations.*

(a) *General comparison of estimations from different animals.* We have collected the results of our experience with 18 buck rabbits over a period of about one year. These animals were kept in separate cages and were well housed and cared for. They were given a plentiful supply of oats and a variable amount of cabbage once a day. The figures obtained show considerable and irregular variations in the serum-calcium of individual rabbits over a period of time and also variations among different individuals bled at approximately the same time. These results were obtained on blood samples taken at random from normal animals without reference to diet or to the length of time elapsing after feeding.

The lowest value obtained was 12.4 mg. calcium per 100 cc. serum, and the highest 18.6 mg. per 100 cc. serum. The maximum variation for a single animal was 5.6 mg.

(b) *A comparison of estimations from animals which had fasted for 16 hours before the taking of the blood sample.* In order to find the effect of more constant conditions on the serum-calcium of rabbits, we tried withholding food from the animals for 16 hours before taking the blood sample for calcium determination. The routine was that the animals were allowed to feed freely for 8 hours during the daytime; all food was removed from the cages in the evening, and the animals fasted for 16 hours. The blood samples for calcium determination were taken on the following morning, at the end of the fast and before the beginning of the day's feeding. The results are given in Table I. They show that under these conditions the serum-calcium is more nearly constant than in blood samples taken at random from animals presumed to be continuously feeding.

The lowest value obtained in the fasting animals was 12.3 mg. per 100 cc. serum (No. 15, Table I), and the highest, 15.3 mg. per 100 cc. serum (No. 10). The maximum variation among 10 animals under the fasting conditions described was therefore 3 mg. as contrasted with a variation of 6.2 mg. among the feeding animals.

The maximum variation for an individual rabbit under the fasting conditions was 1.6 mg. (No. 18, Table I), whereas among the feeding rabbits, the maximum variation for a single individual was 5.6 mg.

In Table I we have indicated the type of diet fed prior to the 16-hour fasting period. The diet was varied, sometimes oats alone or cabbage alone, or a mixture of oats and cabbage was given. As will be seen from Table I, the type of diet fed prior to the fast does not appear to have any striking effect on the

Table I. *Showing the relative constancy of the fasting blood-calcium level.*

Rabbit No.	Weight g.	Date	Haemoglobin % initial	Serum-calcium mg. per 100 cc.	Diet prior to fasting 16 hrs.
7	2000	5. v. 31	—	15.3	Oats and cabbage
		6. v. 31	—	13.8	Oats and water
		7. v. 31	—	14.2	Oats and water
		9. v. 31	—	13.9	Cabbage
9	1625	28. iv. 31	100	15.2	Oats and cabbage
		29. iv. 31	100	14.9	Oats and water
		30. iv. 31	—	14.4	Oats and water
		27. v. 31	100	13.9	Oats and cabbage
		29. v. 31	—	13.7	Oats and cabbage
		4. vi. 31	94	13.6	Oats and cabbage
10	1875	28. iv. 31	—	15.2	Oats and cabbage
		29. iv. 31	—	15.3	Oats and water
		30. iv. 31	—	13.9	Oats and water
15	1625	14. iv. 31	100	13.8	Oats and cabbage
		15. iv. 31	100	13.0	Cabbage
		16. iv. 31	96	13.7	Oats and water
		17. iv. 31	88	12.3	Cabbage
		26. v. 31	100	13.5	Oats and cabbage
		29. v. 31	—	13.4	Cabbage
		4. vi. 31	113	13.5	Cabbage
16	2125	6. v. 31	—	14.1	Oats and cabbage
		7. v. 31	—	14.6	Oats and water
		8. v. 31	—	13.3	Cabbage
17	1725	14. iv. 31	100	13.9	Oats and cabbage
		15. iv. 31	97	13.5	Cabbage
		16. iv. 31	92	13.7	Oats and water
		17. iv. 31	97	13.6	Cabbage
		13. v. 31	100	14.8	Cabbage
		14. v. 31	—	14.2	Cabbage
		15. v. 31	86	13.5	Cabbage
		18. v. 31	—	14.3	Cabbage
		19. v. 31	80	14.2	Cabbage
		22. v. 31	81	14.6	Oats and water
18	2375	13. v. 31	100	13.9	Oats and water
		14. v. 31	—	14.4	Oats and water
		15. v. 31	83	13.6	Oats and water
		19. v. 31	77	14.5	Oats and water
		21. v. 31	—	15.1	Oats and cabbage
		22. v. 31	70	15.2	Cabbage

fasting blood-calcium level, although in one case (No. 18) there appears to be a definite rise in the fasting level when cabbage is fed after an oats diet of 4 days' duration.

In the experiments on fasting animals, and in all subsequent work where repeated determinations were made on the same animal, haemoglobin estimations were included as a check on the effect of bleeding. The evidence in the literature as to the possible effect of blood loss on the serum-calcium is conflicting. Kapsinow and Underhill [1929] found a fall in the haemoglobin content as a result of repeated withdrawal of blood samples for calcium determination, and suggested that this might materially affect the serum-calcium value. Clark [1920] showed that extensive haemorrhage caused a fall in the serum-

calcium of rabbits, whereas Stewart and Percival [1927] found that a withdrawal of 30 to 48 cc. of blood, causing a 20 to 42 % reduction in the haemoglobin content, caused an 8.6 to 20 % reduction in the serum-calcium. Adams [1930] obtained a variation of not more than 1 mg. in four successive estimations in 24 hours, in any one rabbit, 6 to 8 cc. being taken for each sample.

In this work we have taken the precaution to avoid possible complicating factors due to haemorrhage by avoiding too frequent bleeding of the same animal, and in addition we have included control estimations of the haemoglobin content of the animal's blood.

II. *The effect of diet.*

The fact that the serum-calcium was found to be higher and more variable in the feeding than in the fasting animals, together with the statement by Culhane [1927, 1930] that cabbage causes a rise in the serum-calcium of rabbits, led us to study systematically the effect of diet on serum-calcium, in the hope of finding an explanation of the extraordinary variability of the blood-calcium level in the rabbit.

(a) *The effect of a meal of oats and of cabbage on the serum-calcium of fasting animals.* Table II shows the effect of cabbage and of oats on the serum-

Table II. *Showing the effect of cabbage and of oats on the serum-calcium of fasting rabbits.*

Rabbit No.	Date	Cabbage.	Serum-calcium, mg. per 100 cc.	
			Fasting	2-2½ hrs. after food
7	7. v. 31		14.2	16.8
9	27. iv. 31		14.4	16.4
10	27. iv. 31		13.9	16.1
14	9. iv. 31		13.3	15.9
14	10. iv. 31		11.3	15.9
15	10. iv. 31		13.9	15.2
15	16. iv. 31		13.7	14.8
16	10. iv. 31		12.5	13.9
17	13. iv. 31		13.9	15.2
17	16. iv. 31		13.9	14.3
18	25. iv. 31		14.7	16.8
		Oats.		
7	4. v. 31		15.3	14.7
9	27. iv. 31		15.2	15.1
10	28. iv. 31		15.2	14.4
16	5. v. 31		14.1	14.2
16	8. v. 31		13.9	12.4

calcium of fasting animals. In each case the first determination was made after a fast of 16 hours, and the second in 2 to 2½ hours after a liberal meal of cabbage or of oats. In order to compensate for the dryness of the oats, animals on this diet were allowed access to water.

In all the eleven experiments with cabbage there was a rise in the serum-calcium 2 hours after feeding. The maximum rise observed was 4.6 mg. in rabbit

No. 14 (Table II); the minimum, 0.4 mg., in rabbit No. 17. The average rise 2 hours after cabbage was 1.8 mg.

Of the five animals to which oats were given instead of cabbage, four showed a fall in serum-calcium 2 hours after feeding, while one showed a rise of 0.1 mg. The maximum fall was 1.5 mg. and the minimum, 0.1 mg. The average fall on the oats diet was 0.62 mg. as contrasted with an average rise on the cabbage diet of 1.8 mg.

(b) *The effect of diet on the reaction and calcium content of the urine.* The animals were placed in small metabolism cages, and the urine was collected separately from the faeces. On a diet of cabbage only, a large volume of strongly alkaline urine was excreted. This urine was of a pale yellow colour, and contained varying amounts of white precipitate. The precipitate went into solution with the evolution of much CO_2 when the urine was acidified with HCl. Such urine invariably contained large amounts of calcium, probably excreted in solution as calcium bicarbonate, but precipitating out as calcium carbonate when CO_2 was lost as a result of allowing the urine to stand exposed to the air.

On a diet of oats alone, a small volume of acid urine was excreted. The urine varied in colour from pale straw to dark brown, and almost always contained a quantity of gelatinous material. The calcium content of the urine was much less on the oats than on the cabbage diet.

Typical results from two animals are given in Table III. The total volume of urine excreted by rabbit No. 18 on a cabbage diet in 24 hours varied from 275 to 632 cc. In rabbit No. 17, on an oats diet, the volume varied between

Table III. *Effect of diet on the reaction and calcium content of the urine.*

Rabbit No.	Date	Total calcium mg. in 24 hrs.	Volume cc. in 24 hrs.	Reaction to litmus	Food eaten during 24 hrs. g.
18	13. v. 31	279	286	Alkaline	400 Cabbage
	14. v. 31	437	275	"	500 "
	15. v. 31	Lost	486	"	500 "
	16. v. 31	1000	535	"	500 "
	17. v. 31	788	632	"	500 "
	18. v. 31	1060	420	"	500 "
	19. v. 31	884	320	"	520 "
	20. v. 31	985	564	"	600 "
	21. v. 31	905	324	"	400 Cabbage
					and 145 Oats
	22. v. 31	125	81	Sl. alk.	58 "
17	13. v. 31	54	51	Acid	47 Oats and water
	14. v. 31	32	—	"	58 "
	15. v. 31	38	56	"	74 "
	16. v. 31	40	53	"	62 "
	17. v. 31	7	10	"	101 "
	18. v. 31	32	40	"	118 "
	19. v. 31	37	25	"	44 "
	20. v. 31	0	0	"	89 "
	21. v. 31	169	102	Sl. acid	22 Oats
					and 200 Cabbage
	22. v. 31	795	292	Alkaline	400 "

0 and 57 cc. The animals did not thrive on the diet of oats alone, even when free access to water was allowed. They soon showed loss of appetite, and in some cases developed complete anuria.

The total calcium excretion varied, on the cabbage diet, from 279 to 1060 mg. in 24 hours. On the oats diet the calcium excretion was between 0 and 54 mg. in 24 hours.

(c) *The effect of diet on the calcium and phosphorus content of serum and urine.* As an extension of the foregoing observations, the effects of diet on the serum and the urine were studied simultaneously. As the metabolism of calcium is now known to be related to that of phosphorus in some fundamental way, determinations of the inorganic phosphorus of serum and urine were made in addition to the determinations of serum- and urine-calcium.

The animals were placed in small metabolism cages. They were kept supplied with oats or with cabbage and allowed to feed at will. Six animals were used, and these were divided into two groups. (1) Three were kept on a diet of cabbage only for a period of 6 days, and were then given oats only for a period of 7 or 9 days. (2) The other three were given oats only during the first period and cabbage only during the second period.

The urine was collected separately from the faeces, and the total excretion of calcium and of inorganic phosphorus was estimated from the 24-hour sample.

The calcium and inorganic phosphorus of serum were estimated in blood samples withdrawn at the same time, and these were obtained at any convenient time during the day.

Determinations of calcium and phosphorus in the urine were made every day. Determinations of serum-calcium and phosphorus were not made daily but at intervals in order to avoid too frequent bleeding of the animal. Control estimations of haemoglobin were made in all cases; variations in the haemoglobin content of more than 10 % of the initial value were not found, except in one case (rabbit No. 20, Fig. 3), when there was a fall to about 44 % of the initial value.

The space at our disposal does not permit us to present the results both graphically and in the form of a complete table. We have therefore summarised the results briefly below, and in addition we have selected four experiments, representative of all types of results obtained, which are given in detail in the form of diagrams (Figs. 1 to 4). The diagrammatic form emphasises the interesting relationship between calcium and phosphorus which exists both in the serum and in the urine.

The results of all six experiments may be summarised as follows:

Cabbage diet.

		Maximum	Minimum
Serum	Calcium (mg./100 cc.)	19.3	14.3
	Phosphorus (mg./100 cc.)	4.3	1.5
Urine (alkaline)	Calcium (total in 24 hrs.)	2200 mg.	342 mg.
	Phosphorus (total in 24 hrs.)	Trace	Nil

Oats diet.

Serum	Calcium (mg./100 cc.)	Maximum	Minimum
	Phosphorus (mg./100 cc.)	15.8	11.0
Urine (acid)	Calcium (total in 24 hrs.)	654 mg.	0 (anuria)
	Phosphorus (total in 24 hrs.)	225 mg.	0 (anuria)

Charts showing the effect of diet on calcium and inorganic phosphorus of serum and urine.

Fig. 1. (Rabbit No. 9.)

Fig. 2. (Rabbit No. 19.)

Fig. 3. (Rabbit No. 20.)

Fig. 4. (Rabbit No. 21.)

In those animals to which cabbage was fed during the first period (see Figs. 1 and 4), there was a rise in the serum-calcium within the first 24 hours from a fasting level (13–14 mg. per 100 cc.) to 16 or 17 mg. At the same time the serum-phosphorus fell. Throughout the period of cabbage feeding, the serum-calcium, although variable, was maintained at a high level. The serum-phosphorus, although also variable, was maintained at a low level and showed a tendency to fall as the serum-calcium rose. The excretion of phosphorus in the urine fell rapidly on the cabbage diet, until, after 2 or 3 days of cabbage feeding, none or only a trace could be detected in the urine. The excretion of calcium on the cabbage diet was variable, but consistently great.

When the diet was changed from cabbage to oats, the serum-calcium rapidly fell and at the same time the serum-phosphorus rose. Throughout the period of oats feeding, the serum-calcium remained at a low level, while the serum-phosphorus was raised; both calcium and phosphorus varied considerably. The urinary excretion of phosphorus was greatly increased on the oats diet, while the excretion of calcium was very much diminished.

In those animals which were given oats during the first period and cabbage during the second (see Figs. 2 and 3), the same effects of diet were observed.

The effect of a change of diet manifested itself more quickly in the serum than in the urine. In shifting from the cabbage to the oats diet, the serum-calcium fell within 24 hours, but there was a lag period of 1 or 2 days during which the urinary excretion of calcium gradually fell and the urinary excretion of phosphorus gradually rose. Conversely, in changing from the oats to the cabbage diet, the phosphorus excretion fell off gradually during 1 or 2 days, while the calcium excretion gradually rose.

It is interesting to note that in the case of both diets the calcium and phosphorus are being influenced in exactly opposite directions. But, whereas the cabbage causes the calcium to increase and the phosphorus to diminish in serum and in urine, the oats diet has exactly the reverse effect.

The fact that diet can affect the reaction of the urine has been known since Claude Bernard [1846] demonstrated that the clear, acid urine of carnivorous animals, and the turbid urine of herbivorous animals result from the character of their diet and not from some fundamental difference in their mode of assimilation and digestion. But only a few detailed studies of the variable effects of different kinds of diet in herbivora are to be found in the literature. In connection with our results, the following observations are of interest.

Warth and Ayyar [1930] observed that cattle excreted an alkaline urine containing large amounts of CO_2 when they were fed on green fodders. Underhill and Bogert [1916] found that rabbits on a diet of oats and corn excreted an acid urine of high phosphorus content. Ipponsugi [1927, 2] found that the excretion of calcium in the urine of the rabbit is variable, and that it is dependent upon the calcium content of the food eaten. He was unable to find any relation between the calcium excretion in the urine and the serum calcium.

1022

In the experiments of Harnes [1928], who studied the variations in serum-calcium and inorganic phosphorus in rabbits during 8 months, the inorganic phosphorus fell as the serum-calcium increased and *vice versa*.

DISCUSSION.

The chief result of this work is a demonstration of the importance of dietary factors in determining the serum-calcium level in rabbits. It has been shown that the ingestion of cabbage by a fasting rabbit may cause a definite rise in the serum-calcium. In fasting rabbits, and in rabbits feeding upon oats, the serum-calcium was found to lie between 11 and 15.8 mg. per 100 cc., while on a diet of cabbage, values between 14 and 19 mg. per 100 cc. were obtained.

Variations in serum-calcium, such as we have recorded (Section I(a)) will probably be observed whenever the blood samples for calcium determination are taken at random, without reference to diet or to feeding-time. In these experiments the animals were fed on oats, and were given a variable amount (50–500 g.) of cabbage once a day. The blood samples were collected either before, or at some unknown interval after, the cabbage meal, with the result that wide variations in the calcium level occur.

The more constant values previously obtained by one of us [Adams, 1930] must be ascribed to fortuitous experimental conditions; possibly chiefly to the coincidence that blood samples were never taken after a heavy cabbage meal.

The effect of cabbage on serum-calcium in rabbits was first noted by Culhane [1927, 1930], who reported that cabbage causes a transitory rise in serum-calcium provided the initial value is not already high. Kapsinow and Underhill [1929] interpreted the findings of Culhane as suggesting that cabbage contains a calcium-raising substance analogous to that found in the parathyroid gland. They attempted to confirm her observation, but, although their experiments gave some indication that cabbage may cause a rise in serum-calcium, this was not sufficient to enable them to conclude that cabbage has the same effect as the parathyroid hormone on serum-calcium. These authors observed considerable daily variations in the serum-calcium level, and they considered that the daily withdrawal of the 5 cc. of blood necessary for calcium determination may possibly interfere with a consistent effect produced by cabbage.

Our results confirm the observation of Culhane [1930] as to the calcium-raising effect of cabbage. But we do not think that this necessarily means that cabbage contains a substance analogous to the parathyroid hormone. It is probable that the raised blood-calcium is the result of an increased absorption of calcium from the intestine. The very great increase in the urinary excretion of calcium, which we have observed on a cabbage diet, suggests that large amounts of calcium are being absorbed.

It is true that in man it is very difficult to alter the blood-calcium level by feeding of calcium salts or foods rich in calcium, but there is evidence to show that in the rabbit this may be more easily accomplished. For example,

McGowan, Cunningham and Auchinachie [1931] found a raised blood-calcium and an increased urinary excretion of calcium, together with a diminished urinary excretion of phosphorus, when 8 % CaCO_3 was added to the stock ration given to a rabbit.

The absorption of calcium seems to depend upon the relative amount of phosphorus present, among other factors. When the diet is high in calcium and low in phosphorus, the phosphates are precipitated in the intestine and excreted by the bowel as insoluble calcium phosphate, thus creating a phosphorus deficiency [Peters and Van Slyke, 1931, p. 1107]. The excess calcium is absorbed into the blood to be later excreted by the kidney in some soluble form, such as bicarbonate [Cushny, 1928].

An analysis of the available data on the calcium and phosphorus contents of cabbage and of oats indicates that the Ca/P ratio in these two foodstuffs is widely different.

Source of data	Calcium %		Phosphorus %		Ca/P ratio	
	Cabbage	Oats	Cabbage	Oats	Cabbage	Oats
Our own analyses	0.161*	0.077	—	—	—	—
Sherman [1927]	0.045†	0.069‡	0.029†	0.392‡	1.55	0.17
Kestner and Knipping [1926]	0.200	0.100§	0.085	0.401§	2.35	0.25
Gregory [1928]	0.051¶	—	—	—	—	—
$\text{Ca}_3(\text{PO}_4)_2$	—	—	—	—	1.93	—

* Outside leaves, such as were given to rabbits.

† Edible portion. ‡ Oatmeal. § Oatflakes.

|| Given as PO_4 , recalculated by us as P.

¶ Average for various New Zealand cabbages.

It appears from these figures that cabbage is relatively rich in calcium, while oats are relatively rich in phosphorus, and this may be the basis of our observations. On a cabbage diet the phosphate present might be precipitated and excreted by the bowel as $\text{Ca}_3(\text{PO}_4)_2$, causing the phosphate of blood and urine to be markedly diminished. The excess of calcium which the cabbage appears to contain might be absorbed into the blood-stream and eventually excreted in the urine as bicarbonate. Under these circumstances, the regulation of the blood-calcium level would depend upon the rate of excretion of calcium by the kidney, and, in cases of rapid absorption, a transitorily raised blood-calcium might be expected to occur.

The oats diet, on the other hand, with its relatively high phosphorus content, favours the excretion of calcium by the bowel. The absorption of phosphorus and its subsequent excretion in the urine would be increased.

We are inclined to take the view that dietary factors, particularly the ratio of calcium to phosphorus in the diet, may be of great importance in determining the upper limit of the blood-calcium level. But before a hypothesis can be formulated more complete data, such as could be obtained from metabolism experiments, are required.

SUMMARY.

1. The serum-calcium of rabbits may be very variable. Values from 12.4 to 18.6 were found in 18 buck rabbits bled at various times over a period of about one year.

2. If the animals were starved for 16 hours before the taking of the blood sample, the range of variation was narrower, being between 12.3 and 15.3 mg. per 100 cc. serum.

3. The serum-calcium level was found to depend upon the diet.

(a) Two hours after the giving of cabbage to fasting rabbits a maximal rise of 4.6 mg., and an average rise of 1.8 mg. per 100 cc., occurred in the serum-calcium. Oats, given to fasting rabbits, caused a maximal rise of 0.1 mg. and an average fall of 0.62 mg. per 100 cc. serum.

(b) During continuous feeding on cabbage alone, values between 14.3 and 19.3 mg. calcium per 100 cc. serum were observed. During continuous feeding on oats, the serum-calcium values were between 11 and 15.8 mg. per 100 cc.

4. Simultaneous determinations of calcium and inorganic phosphorus in serum showed that the concentrations of these two substances bear a reciprocal relation to each other. On a diet of cabbage, the serum-calcium increased, while the inorganic phosphorus of the serum decreased. Conversely, on oats a fall in the serum-calcium was accompanied by a rise in the inorganic phosphorus of the serum.

5. The urine excreted by rabbits on a cabbage diet was strongly alkaline to litmus, and contained large amounts of calcium, but no phosphorus, or only a trace.

6. On an oats diet, an acid urine rich in phosphorus and containing relatively little calcium was excreted.

7. The volume of urine excreted in 24 hours on a cabbage diet was more than 10 times as great as that excreted in the same time on a diet of oats and water.

8. It is suggested that dietary factors, such as the calcium/phosphorus ratio in the diet, may be of great importance in determining the blood-calcium level in rabbits.

Our thanks are due to Prof. Drummond for his interest and advice, and to the Committee on Physiology of Vision of the Medical Research Council for a grant held by one of us (M. C. B.).

REFERENCES.

- Adams (1930). *Brit. J. Ophthal.* **14**, 546.
Bernard (1846). *Compt. Rend. Acad. Sci.* **22**, 534.
Cannon and Greenwood (1930). *J. Dairy Sci.* **13**, 424.
Clark (1920). *J. Biol. Chem.* **43**, 89.
—— and Collip (1925). *J. Biol. Chem.* **63**, 461.
Culhane (1927). *Biochem. J.* **21**, 1015.
—— (1930). *J. Biol. Chem.* **86**, 113.
Cushny (1928). *Pharmacology and therapeutics*, 10th ed., p. 592. (Churchill, London.)
Davies, Dickens and Dodds (1926). *Biochem. J.* **20**, 695.
Dupré and Semeonoff (1931). *J. Biol. Chem.* **94**, 341.
Fiske and Subbarow (1925). *J. Biol. Chem.* **66**, 375.
Gregory (1928). Unpublished data.
Harnes (1928). *J. Exp. Med.* **48**, 549.
Ipponsugi (1927, 1). *Mitteil. allgem. Path.* **3**, 216.
—— (1927, 2). *Mitteil. allgem. Path.* **3**, 244.
Kapsinow and Underhill (1929). *J. Biol. Chem.* **82**, 377.
Kestner and Knipping (1926). *Die Ernährung des Menschen*, 2nd ed., p. 116. (Julius Springer, Berlin.)
McCrudden (1911). *J. Biol. Chem.* **10**, 187.
McGowan, Cunningham, and Auchinachie (1931). *Biochem. J.* **25**, 1295.
Peters and Van Slyke (1931). *Quantitative clinical chemistry*, p. 1107. (Ballière, Tindall and Cox, London.)
Sherman (1927). *Chemistry of food and nutrition*, 3rd ed., p. 587. (Macmillan, New York.)
Ssewerin (1927). *Biochem. Z.* **190**, 326.
Stewart and Percival (1927). *Biochem. J.* **21**, 302.
Underhill and Bogert (1916). *J. Biol. Chem.* **27**, 161.
Warth and Ayyar (1930). *Biochem. J.* **24**, 1595.