naphthalene daily for eight days. Retinal exudates and peripheral striae were present on the second day. The exudates rapidly enlarged and became confluent; by the ninth day the entire periphery of the retina had atrophied. The lens striae developed during 20 days; on the 24th true lens opacities were present; complete cataract of both eyes subsequently developed about one month after the last dose of naphthalene.

(e) Rabbit number 50:—Black, weight 2,010 grams. Received two grams of naphthalene daily for two days. The ocular lesions developed rapidly. Retinal exudates and peripheral lens striae were present on the second day; by the fifth day the exudates had become confluent and the lens striae were rapidly extending towards the centre; by the ninth day the lens striae had still further increased and the affected areas of the retina had become atrophic. The animal died on the 10th day after having manifested all the signs of severe toxæmia including loss of appetite, weakness, torpor, and severe diarrhoea. The sound of the breathing indicated the presence of fluid in the lungs. There was a very rapid decrease in weight, amounting to a total of 700 grams.

(f) Rabbit number 51:—Grey, weight 1,900 grams. Received five two-gram doses of naphthalene. The ocular lesions were extensive and developed rapidly. The retina was covered with large patches of watery exudate on the second day; these rapidly became confluent, and by the fifth day, the whole retina was atrophic. Peripheral lens striae were present on the second day; these increased and by the ninth day, had extended throughout the lens; on the 14th day true lens opacities were present. The animal died on the 19th day, having developed anorexia and having lost 350 grams in weight.

BIBLIOGRAPHY

- Adams.—*Brit. J. of Ophthalm.*, Vol. XIV, pp. 49 and 545, 1930.
 Bouchard and Charrin.—*Compt. rend. Soc. de Biol.*, Vol. VIII, p. 614, 1886.
 Curatulo.—Il Morgagni. Parte I, p. 105, 1889.
 Bourne and Campbell.—*Biochem. J.*, Vol. XXVI, p. 183, 1932.
 Hess.—*Ber. über die 19 Versammlung der Ophthalm. Gesell.*, Rostock, 1887.
 Kolinski.—*Arch. f. Ophthalm.*, Vol. XXXV (2), p. 29, 1889.
 Michael and Vancea.—*Compt. rend. Soc. de Biol.*, Vol. XCVII, p. 1569, 1927.
 Panas.—*Rev. gén. d'Ophthalm.*, Vol. VI, p. 7, 1887.
 Panico.—*Annali di Ottal. e Clin. Ocul.*, Vol. LVI, p. 799, 1928.
 Salfner.—*Arch. f. Ophthalm.*, Vol. LIX, p. 520, 1904.

THE RÔLE OF CALCIUM IN NAPHTHALENE CATARACT

BY

MARGHERITA COTONIO BOURNE

AND

DOROTHY ADAMS CAMPBELL

FROM THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY,
UNIVERSITY COLLEGE, LONDON

THE theory that a relationship exists between calcium and the production of cataract arises from two independent sources.

In the first place, a number of observers have found that the calcium content of cataractous lenses is higher than normal. Burge, in 1909, first reported an increased proportion of calcium in the ash of cataractous human lenses. This was confirmed by Adams (1929) who found that the calcium in the lens in human senile cataract is greater than normal. Recently, Mackay, Stewart, and Robertson (1932) also found the calcium in human cataractous

lenses to be markedly increased; and these authors were led to conclude that, "the differences between the behaviour of calcium and that of the other inorganic ions during the development of cataract points to some special connection of the former with the production of opacity."

Secondly, the high incidence of cataract in diseases which involve a disturbance of calcium metabolism has long been known. For example, cataract may be associated with tetany (Graefe-Saemisch Handbook II, 1911), a condition which is now generally thought to be induced by a fall in blood calcium, and the accompanying cataract has been attributed to the same cause. Donald Hunter in a Goulstonian Lecture (1930) drew attention to the frequency with which cataract has been described in endemic tetany, post-operative tetany, rickets, and spontaneous hypoparathyroidism. He and his associates were able, by means of the slit-lamp, to demonstrate opacities in one case of tetany with steatorrhea, one case of renal rickets, and one case of osteitis fibrosa of the focal type. All of these conditions are characterized by a faulty calcium metabolism.

There is abundant evidence to show that hypocalcaemia and cataract are both part of the syndrome of parathyroid deficiency. Cole (1930) reviews the relation of cataract to parathyroid tetany and describes one new case; 38 other cases, previously recorded are discussed. O'Brien (1932) reviews 42 published cases of cataract in post-operative tetany, and describes three new cases from his own experience. Cataract has been produced experimentally in dogs and in rats (Luckhardt and Blumenstock, 1923; Erdheim, 1906) by the removal of the parathyroid gland; and it has been independently demonstrated by many observers (Peters and van Slyke, 1931, page 816) that in the absence of the parathyroid hormone the blood calcium in all animals is considerably lowered.

Although such observations as these make plausible the suggestion that calcium is somehow involved in the production of cataract, it should be remembered that at the present time there is no experimental evidence whatever to justify the conclusion that a low blood-calcium or even a disturbance of calcium metabolism is the *cause* of cataract. It is quite possible to explain the increased calcium content of cataractous lenses as being due to the calcification of a tissue damaged by some other agency; and this is, in fact, the present hypothesis (Mackay *et al.*, 1932) in relation to senile cataract. The occurrence of low blood-calcium and cataract in hypoparathyroidism may be a matter of pure coincidence.

It occurred to us that some light might be shed on this problem through the medium of the experimental naphthalene cataract.

As is well known, cataract may be produced in rabbits by the administration of naphthalene; and it seemed likely that a study of the changes in blood-calcium and in the urinary excretion of calcium during naphthalene dosing might yield some information concerning the possible relationship between calcium and cataract.

One of us (Adams, 1930) had previously observed that there is a slight fall in the blood-calcium of rabbits after a single dose of naphthalene; it seemed possible, on the basis of this observation, that the cataract produced by repeated doses of naphthalene might actually be due to the initial fall in blood-calcium. Naphthalene was administered to rabbits over long periods, and the calcium of blood and urine was determined in order to find out whether any significant variations from the normal were produced, and in what way any changes which might occur were related to the development of cataract.

Experimental

In planning our experiments it was necessary to consider two important factors: (1) The blood-calcium in normal rabbits is known to depend upon the diet (Bourne and Campbell, 1932); (2) it is possible to produce cataract by naphthalene only under certain dietary conditions (page 210).

With these conditions in mind, two diets were selected: (a) a diet of oats and cabbage, since cabbage is known to cause increased calcium absorption and to protect the lens from damage by naphthalene; (b) a diet of bran and carrots, which provides a low level of calcium absorption, and on which cataract is readily produced by naphthalene.

Experiments were arranged as follows:

- I. Control determinations of serum and urine calcium in normal rabbits;
 - (a) On oats and cabbage (high calcium) diet.
 - (b) On bran and carrots (low calcium) diet.
- II. Determination of serum and urine calcium in rabbits during daily dosing with naphthalene;
 - (a) On oats and cabbage diet, where the blood-calcium was high and where crystals were laid down on the retina, but where no cataract could be produced.
 - (b) On bran and carrots diet, where the blood-calcium was lower, and cataract was produced within about 30 days.
 - (c) Determination of serum calcium only on bran and carrots, with large doses of naphthalene, where the animal suffered severe toxæmia and died from the effects of the dose.

Methods

Blood samples:—These were obtained from the marginal ear vein; between eight and ten c.c. were withdrawn at each bleeding. An interval of from five to seven days was allowed for recovery between each bleeding, and haemoglobin estimations were made at frequent intervals as a check upon the effect of haemorrhage. The blood samples were always taken after feeding and before the administration of the daily dose of naphthalene.

Serum calcium:—The Clark and Collip (1925) modification of the Kramer and Tisdall method was used. The blood was allowed to clot overnight, and after the addition of the oxalate the tubes were again allowed to stand overnight to ensure complete precipitation.

Urinary calcium:—This was determined by the method of McCrudden (1911) on 24 hour samples of the urine, which had been collected free from faeces in a metabolism cage. If the urine was alkaline it was made slightly acid to litmus with HCl and filtered. The calcium was precipitated from 10 c.c. of the filtered urine, redissolved in sulphuric acid and determined by permanganate titration.

Naphthalene administration:—Pure crystalline naphthalene was dissolved in about 15 c.c. of warmed liquid paraffin and administered by means of a tube introduced into the stomach.

Eye lesions:—These were examined daily by means of an ophthalmoscope.

Results

1. *The effect of diet on the serum and urine calcium of the normal rabbit:*—In man the blood-calcium is remarkably constant; even by the feeding of calcium-rich foods or by the administration of calcium salts it is difficult to alter it, and it remains steadily fixed between 9 and 11 mg. per 100 c.c. serum under normal conditions and also in a wide range of pathological conditions. The rabbit, on the contrary, seems to be peculiarly susceptible to variations in blood-calcium. Even in the normal animal the blood-calcium level and the amount of calcium excreted in the urine vary considerably from day to day, and are markedly influenced by the character of the diet upon which the animal is feeding. We have previously found (Bourne and Campbell, 1932) that in rabbits feeding exclusively on cabbage the blood-calcium is high (14 to 19 mg. per 100 c.c. serum) and a large amount of calcium is excreted in the urine. If, however, the diet is changed to oats and water, the blood-calcium falls to a lower level (11 to 15·8 mg. per 100 c.c. serum) and the calcium either disappears altogether from the urine or is present in only very small amounts.

In view of the known effect of diet it was necessary to determine the normal levels of blood- and urine-calcium on the diets which we proposed to use before proceeding to test the effect of naphthalene. Therefore, as controls to this investigation, two healthy rabbits were selected and each placed in a metabolism cage of suitable size. One, number 47, was given a diet of oats and cabbage; and the other, number 36, was given bran moistened with water, and carrots. In each case the food was liberally supplied and the animals were allowed to feed at will. They were bled at intervals of from four to seven days; and calcium was determined in the serum.

The results are given in Tables I and II. As will be seen from Table I, on a diet of oats and cabbage the serum calcium varied between 14.1 and 15.7 mg. per 100 c.c., while the urine calcium was high but variable, between 114 and 498 mg. being excreted in 24 hours.

TABLE I

Serum and Urine Calcium of a Normal Rabbit on Oats and Cabbage Diet.

Rabbit Number 47. Colour: Black. Weight: 2585 grams.

Day.	Serum Calcium mg./100 c.c.	Urine Calcium mg./in 24 hours.	Weight (grams.).
1	15.7	257	2585
2		392	
3		314	
4		374	
5		370	
6		157	
7	14.2	160	
8		114	
9		472	
10		477	
11		254	
12		235	
13	15.2	145	2725
14		240	
15		250	
16		297	
17		358	
18		114	
19	15.9	151	2697
20		190	
21		498	
22		318	
23		291	
24		270	
25	15.7	352	2830
26		213	
27		331	
28		163	
29		207	
	14.1		2860

On the bran and carrots diet (Table II), the blood-calcium was equally variable but the level was much lower, between 8·6 and 10·7 mg. per 100 c.c. serum. At the same time the amount of calcium excreted in the urine was remarkably small, only between 4 and 28 mg. being excreted in 24 hours.

TABLE II

Serum and Urine Calcium of a Normal Rabbit on Bran and Carrots Diet.

Rabbit Number 36. Colour: Brown. Weight: 2440 grams.

Day.	Serum Calcium mg./100 c.c.	Urine Calcium mg./in 24 hours.	Weight (grams.).
1	10·5	28	2440
2		7	
3		24	
4		8	
5		4	
6	8·6	6	
7		8	
8		8	
9		7	
10		11	
11	8·8	9	2450
12		26	
13		25	
14		7	
15		7	
16	10·7	12	2380
17		14	
18		11	
19		4	
20		12	
21	9·3	12	2415
22		8	
23		4	
24		10	
25		4	
26	8·8	6	2445
27		24	
28		15	
29		8	
30		8	

The high blood-calcium and the large urinary excretion of calcium which results from the ingestion of the oats and cabbage suggests that on this diet there is an increased absorption of calcium; while the lower blood-calcium and the smaller urinary excretion which accompanies the bran and carrots diet point to a much lower level of calcium absorption.

II. Effect of naphthalene on blood- and urine-calcium:—A large number of experiments on different animals were performed in which blood- and urine-calcium were determined after naphthalene.

For the sake of brevity, details of only two typical experiments, one in which the diet consisted of oats and cabbage, and the other in which the diet was bran and carrots, are given here. In these we continued the dosing for a long time; and were thus able to test all the possible effects of naphthalene on calcium.

Two healthy rabbits of the same age and weight were placed in separate metabolism cages. One, number 40, was given diet of

TABLE III

Serum and Urine Calcium of Rabbit receiving Two Grams. of Naphthalene Daily on Oats and Cabbage Diet.

Rabbit Number 40. Colour: Brown. Weight: 2070 grams.

Day.	Serum Calcium mg./100 c.c.	Urine Calcium mg. in 24 hours.	Ocular Lesion.	Remarks.
1	14.1	104		
2		150		
3		196	Peripheral lens striae	
4		266	Few small retinal exudates	Some loss of appetite ; wt. 2020 gm.
5		187	Crystals on retina	
6		279		
7	14.7	279		
8		156	Crystals increased	
9		207		
10		290	Striae sl. increased	
11		497		
12		284	Crystals larger	
13		335		
14		465		
15	15.2	455	Lens clearer	Wt. 2060 gm.
16		232	Retrogression of striae	
17		160		
18		143		
19		326		
20		114		
21		485	Crystals increased	Appetite recovered ; wt. 2060 gm.
22		275		
23		302		
24	16.7	413		
25		234		
26		146		
27		114		
28		205		
29		161	Crystals increased	
30	16.2	112		
31		257		Wt. 2090 gm.
32		250		
33	16.2	465		
34		455		
35		410	Lens and retina no further change	
36	17.1	172		Wt. 2100 gm.; healthy and lively

oats and cabbage; while the other, number 41, received moist bran and carrots. Two grams of naphthalene dissolved in a little warm liquid paraffin, was administered daily to each rabbit. The experimental period was in each case 36 days.

The results are given in Tables III and IV. On the oats and cabbage diet the serum calcium was found to vary between 14.1

TABLE IV

Serum and Urine Calcium of Rabbit receiving Two Grams. of Naphthalene Daily on Bran and Carrots Diet.

Rabbit Number 41. Colour: Brown. Weight: 2070 grams.

Day.	Serum Calcium mg./100 c.c.	Urine Calcium mg. in 24 hours.	Ocular Lesion.	Remarks.
1	11.4	37		Weight: 2070
2		11	Small retinal exudates	
3		21	Peripheral lens striae	Appetite good; wt.
4		17		1912 gm.
5		16	Exudates increased	
6		17	Lens striae increased	
7	10.2	34		
8		26	Exudates enlarged	
9		27		
10		23		
11		24	Striae progressed	
12		13		
13		12	Lens hazy	
14		18		Eating less; wt.
15	8.6	11		1850 gm.
16		4		Hair falling out; wt.
17		22	Lens changes progressing	1810 g.m.
18		22		
19		15		
20		13		
21		33		
22		16	Lens opacities	Appetite recovered;
23		23		wt. 1810 gm.
24	10.9	17		
25		16		
26		7		
27		13	Early cataract right eye	
28		21		
29		30		
30	10.3	19		
31		39		Flank bare of hair;
32		23		wt. 1805 gm.
33		11		
34	12.4	10		
35		15		
36	9.1	14	Complete cataract right eye; early cataract left eye	Wt. 1800 gm; appetite good

SERUM AND URINE CALCIUM OF NAPHTHALINIZED

RABBITS COMPARED WITH NORMAL

and 17.1 mg. per 100 c.c., and the calcium excreted in the urine in 24 hours was between 112 and 495 mg. On the bran and carrots diet serum-calcium values between 8.6 and 12.4 mg. were found, while the urine-calcium was between 7 and 39 mg. for the 24 hours.

These figures, in the case of both diets, so closely approximate the normal for each diet that no effect either on the blood-calcium or on the urinary excretion of calcium can be attributed to the naphthalene. Figs. I and II illustrate this point remarkably clearly.

Even in those cases where the toxic effects of naphthalene were much more severe, *i.e.*, where the eye lesions developed rapidly and the animal died within a short time, the blood-calcium remained within normal limits. Results of three experiments of this kind are given in Table V. In each of these experiments the animal was given a daily dose of three grams of naphthalene; the diet

TABLE V
Serum Calcium in Rabbits Dying from Effects of Naphthalene Poisoning.

Rabbit Number.	Dose.	Day.	Serum Calcium mg./100 c.c.	Ocular Lesion.	Remarks.
25*	3 gm. daily	1	10.6	Confluent exudates Advanced lens striae	Weak and ill Refusing all food Dead
		3	15.0		
		5	11.7		
		6			
26*	3 gm. daily	1	11.7	No lesion	Refusing food Dead
		2	9.2		
		3			
27	3 gm. daily for 5 days	1	9.9	Lens hazy	Refusing food Weak and ill Dead
		3	12.5		
		5	10.3		
		14			

in each case consisted of bran and carrots. All of these rabbits exhibited signs of severe toxæmia; there was complete loss of appetite after the first dose; the animal became lethargic, weak and ill and speedily died. At autopsy the gastro-intestinal tract was found to be inflamed and congested; there were gastric ulcers; the intestines were filled with mucus and bile.

III. Relation of blood-calcium to ocular lesions after naphthalene:—The striking effect of diet on the nature of the ocular lesions produced by naphthalene has already been described (page 210); and from Tables III and IV it will be seen that this influence of diet on the ocular lesions occurring after naphthalene, bears an interesting relation to the blood-calcium.

In rabbits on a diet of oats and cabbage we were unable to produce cataract by naphthalene; crystals were laid down on the retina, but the lens seemed to be protected from damage. Table III shows that this protective effect goes hand in hand with a high blood-calcium and a high level of calcium excretion. On the other hand cataract and the signs of general toxæmia are produced in rabbits feeding upon bran and carrots, in association with a low blood-calcium and a low level of urinary calcium excretion. As will be seen from Table IV, rabbit number 41 developed complete cataract while its average serum-calcium, although not abnormally low for this diet, was about five milligrammes below the average level for the oats and cabbage diet, and the amount of calcium excreted in the urine was almost negligible.

The retinal crystals which occur after naphthalene only in rabbits feeding upon oats and cabbage diet (see page 212) have been shown by micro-chemical analysis to be crystals of a calcium salt, probably calcium-oxalate (Adams, 1930). Where the level of calcium absorption is high, the blood and all the body fluids must be saturated with calcium; under these conditions one might expect to find such a deposition of a calcium salt in a damaged tissue.

Discussion

Our experiments show clearly that naphthalene does not cause any fundamental disturbance of the calcium metabolism in rabbits. The slight fall in blood-calcium previously observed by Adams (1930) in the first 24 hours after a single dose of naphthalene is probably explained by the effects of this substance on the gastrointestinal tract. The cessation of food intake which results from a loss of appetite, together with the more profound irritative effects of naphthalene on the gastric mucosa, in all probability interfere with peristaltic movements and diminish the absorption of calcium. In none of Adams' experiments nor in a larger series of our own in which the experiments of Adams were repeated, did a fall below the lower normal limits occur. Thus the cataract which naphthalene produces cannot be attributed to an upsetting of the normal calcium metabolism; nor can it, like the cataract of tetania parathyropraevia, be associated with an abnormal lowering of the blood-calcium.

However, the suggestion of another possible rôle for calcium emerges from our work. We find that naphthalene is unable to produce cataract when the blood-calcium is maintained at a high level as the result of the ingestion of a calcium rich food such as cabbage, while on the calcium poor diet, such as bran and carrots, naphthalene may produce the most extensive eye lesions including complete cataract. There is no evidence that the presence

or absence of an excess of calcium in the blood stream is the factor which determines the difference in the behaviour of naphthalene on these two diets, but the possibility suggests itself.

The cataract of tetania parathyropraevia is definitely associated with a blood-calcium lower than normal, but it seems unlikely that this is *per se* the cause of the cataract. Possibly, when the calcium falls to a low level, some poison, normally detoxicated by calcium, is set free and is able to cause lens damage.

Senile cataract, like naphthalene cataract, occurs in the presence of a normal calcium metabolism. In experiments on patients with senile cataract in which the intake of calcium and its excretion in faeces and urine was measured, Kirby (1931) found a normal calcium balance. Adams (1929) found that in senile cataract the blood-calcium is normal or slightly raised. All attempts to produce cataract by the artificial raising or lowering of the blood-calcium have failed (Adams, 1929; and Eisman and Luckhardt, 1927).

In conclusion, the weight of evidence seems to be in favour of the view that cataract, whether experimentally produced by naphthalene or occurring in human beings, is not the result of either a lowering of the blood-calcium or a disturbance in calcium metabolism. But the possibility still remains that an excess of calcium may be able to protect the lens against toxic substances, whether foreign, as in the case of naphthalene, or produced as in the case of man by some pathological process.

Summary

I. The blood-calcium and the urinary excretion of calcium in rabbits was found to be fundamentally unchanged by prolonged naphthalene dosing. Even in those cases where cataract was produced or where the toxic effects of the naphthalene caused the rapid death of the animal, no significant variations in the blood-calcium were observed.

II. Although naphthalene was found to be without effect on the calcium metabolism, there is the reverse possibility that the blood-calcium may play a part in determining the effect of naphthalene on the eye; for an oats and cabbage diet, which protects the lens from damage by naphthalene and prevents the development of cataract, was found to cause a high blood-calcium (14.1 to 15.7 mg. per 100 c.c. serum) and a large urinary excretion of calcium (114 to 498 mg. daily); while a bran and carrots diet, on which cataract and toxic symptoms may be produced by naphthalene, is associated with a much lower level of blood-calcium (8.6 to 10.7 mg. per 100 c.c. serum) and a very small urinary excretion of calcium (4 to 39 mg. daily).

III. The occurrence of retinal crystals, believed to be crystals

of calcium oxalate, was found to be associated with the maintenance of a high blood-calcium. This explains why such crystals are seen only when the naphthalene is administered in connection with oats and cabbage diet.

IV. The relation of these results to the general problem of the rôle of calcium in cataract formation is discussed.

We wish to express our thanks to the Committee on the Physiology of Vision of the Medical Research Council for a grant held by one of us (M. C. B.), and to Professor Drummond for his advice and helpful criticism.

BIBLIOGRAPHY

- Adams.—*Biochem. Jl.*, Vol. XXIII, p. 902, 1929.
 ——— *Brit. Jl. of Ophthal.*, Vol. XIV, pp. 49 and 545, 1930.
 Bourne and Campbell.—*Biochem. Jl.*, Vol. XXVI, p. 183, 1932.
 Burge.—*Arch. of Ophthal.*, Vol. XXXVIII, p. 447, 1909.
 Clark and Collip.—*Jl. Biol. Chem.*, Vol. LXIII, p. 461, 1925.
 Cole.—*Lancet*, Vol. I, p. 13, 1930.
 Erdheim.—*Mittel. a. d. Grenzgeb. d. Med. u. Chir.*, Vol. XVI, p. 632, 1906.
 Eisman and Luckhardt.—*Proc. Soc. Biol. Med.*, Vol. XXV, p. 6, 1927.
 Graefe-Sameisch *Handbuch der Augenheilk.*, II Teil., *Path. u. Ther. des Linsensyst.* Julius Springer, 1911.
 Hunter.—*Lancet*, Vol. I, p. 903, 1930.
 Kirby.—*Arch. of Ophthal.*, Vol. V, p. 868, 1931.
 Luckhardt and Blumenstock.—*Amer. Jl. of Physiol.*, Vol. LXIII, p. 409, 1923.
 Mackay, Stewart and Robertson.—*Brit. Jl. of Ophthal.*, Vol. XVI, p. 193, 1932.
 McCrudden.—*Jl. Biol. Chem.*, Vol. X, p. 187, 1911.
 O'Brien.—*Arch. of Ophthal.*, Vol. VII, p. 71, 1932.
 Peters and Van Slyke.—*Quantitative Clinical Chemistry*, p. 816. Ballière, Tindall & Cox, London, 1931.

RETROBULBAR INJECTIONS AND SEVENTH NERVE BLOCK

BY

K. KOMAN NAYAR, D.O.M.S.

AND

G. J. NARAYANASWAMY NAYUDU

GOVERNMENT OPHTHALMIC HOSPITAL, MADRAS

IN the March, 1932, number of the *Brit. Jl. of Ophthal.*, on p. 166, under the heading "Iris anaesthesia by retrobulbar injection and paralysis of the orbicularis oculi by seventh nerve block," Dr. L. R. Somerville Large has given a detailed description of the technique of, and the complications resulting from the above injections as practised at the Moorfields Hospital during his period of residency there.