XCIX. THE RÔLE OF CALCIUM IN SENILE CATARACT.

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(*Received July 25th, 1929.*)

THE SERUM-CALCIUM OF PATIENTS WITH SENILE CATARACT.

SENILE cataract is a condition which develops slowly, and it may, therefore, be due to a gradual change in some normal constituent of the blood, and, since it occurs at an age when the activity of the endocrine organs is said to be decreasing, it is possible that one of them is responsible for an alteration in the blood. A suggestion of this kind has been made by Fischer and Triebenstein [1914], viz. that senile cataract is due to parathyroid deficiency. Their suggestion is based on their observation that 88·2 % of patients whom they examined showed physical signs of "latent tetany." If such a hypothesis is correct, senile cataract might be considered to be analogous to that type of juvenile cataract which is associated clinically with tetany. The latter disease is one of the few in which a definitely low blood-calcium is known to occur, and which is generally attributed to a deficiency in the secretion of the parathyroids.

Following on this suggestion, Tron [1926], A. and S. v. Pellathy [1927] and Polichova [1928] investigated the serum-calcium in patients with senile cataract, and found only slight differences from the normal. Their results may be summarised as follows.

Tron, using de Waard's method, investigated 10 normals of ages 42–59, and found a range of blood-calcium from 12·3 to 13·7 mg. per 100 cc. (av. 13·1). In 25 patients with senile cataract, values ranged from 11·12 to 13·70, giving an average of 12·25, *i.e.* only 0·84 mg. per 100 cc. less than the normal. Polichova obtained an average for normals of 13·5 mg. per 100 cc. and for 14 cases of cataract of 13·8 mg. per 100 cc. A. and S. v. Pellathy used Kramer and Tisdall's method [1921, 2] to investigate 48 cases of cataract, from 45 to 75 years old, and found a range of 9·1–12·0 mg. per 100 cc. In a few older patients they obtained lower values, *e.g.* 9·1–9·4 mg. per 100 cc. A small number of normals, "one for each 5 years," showed a range from 9·8 to 11·7 mg. per 100 cc.

All these workers have agreed that there is no support for the theory of parathyroid deficiency. There is, however, considerable variation in their results, both in their actual figures, and in the differences found between "cataract" and normal values. Further, the normal blood-calcium has now been proved to be surprisingly constant between 9·5–10·0 mg. per 100 cc. [Kramer and Howland, 1920; Kramer and Tisdall, 1921, 1; Percival and Stewart, 1926; Fraser, 1927], and by this standard the values given by Tron and by Polichova both for cataracts and normals are very high. Further, on inspection of their actual figures Pellathy's results suggest that the blood-calcium in cataract is definitely raised, *e.g.* of 48 cases only 6 were below 10·0 and the average of the remainder was 10·75 mg. per 100 cc.

In view of these discordant results it was considered worth while to repeat the estimation of the blood-calcium in a series of normal and cataractous subjects of the same age.

Method. Clark and Collip's modification [1925] of Kramer and Tisdall's method was used. Blood was taken from the arm vein at about midday, when the subject was in a fasting condition. Samples from normal and cataractous patients were estimated concurrently. A duplicate estimation was made in every case. Owing to the difficulty of obtaining volunteer normal subjects of the right age, it was considered justifiable to use surgical patients in a convalescent stage, and in good general condition as "normal" controls. A record was made of the vision of patients with senile cataract, while care was taken to see that the controls were free from cataract and from any condition which would affect the blood-calcium abnormally.

The results are shown in Table I.

Table I. *A comparison of serum-calcium values in* (*a*) *normal subjects, and* (*b*) *senile cataract.*

(a)				(b)					
							Vision		
No.	Sex	Age	Serum-calcium mg. per 100 cc.	No.	Sex	Age	R.E.	L.E.	Serum-calcium mg. per 100 cc.
1	M.	32	11·00	1	M.	46	Hm.	6/9	11·40
2	F.	36	9·82	2	M.	51	p.l.	6/60	11·87
3	F.	39	8·32	3	F.	52	6/24	6/60	12·85
4	M.	45	9·58	4	F.	54	p.l.	aphakic	12·35
5	F.	47	9·72	5	F.	54	6/18	5/60	12·23
6	M.	49	9·68	6	M.	56	—	—	10·60
7	F.	49	9·67	7	F.	58	6/9	3/60	11·63
8	M.	50	9·90	8	F.	59	Hm.	6/12	11·97
9	M.	52	8·85	9	F.	60	—	—	10·90
10	M.	54	9·01	10	M.	61	Hm.	6/18	11·78
11	F.	57	9·72	11	M.	62	6/9	6/9	9·72
12	M.	57	11·37	12	M.	62	6/36	6/60	11·58
13	M.	58	9·32	13	F.	64	c.f.	6/9	11·57
14	M.	58	11·22	14	M.	65	p.l.	6/36	11·76
15	M.	58	9·53	15	F.	65	6/60	6/60	10·81
16	F.	58	9·10	16	M.	68	—	—	10·67
17	M.	61	9·09	17	M.	68	p.l.	6/60	11·00
18	M.	66	9·70	18	M.	71	6/18	c.f.	10·90
19	M.	68	10·66	19	M.	73	6/36	6/36	11·22
20	F.	68	9·81	20	F.	76	6/60	p.l.	11·59
21	M.	72	9·84						
		Av.	9·76					Av.	11·46

(1) In 21 controls of ages ranging from 32 to 72, the serum-calcium varied from 8·32 to 11·39 mg. per 100 cc. Of these, 2 were below 9·0, and 4 were above

10·0 mg. per 100 cc. (av. 9·76). There was no apparent relationship between the serum-calcium and the nature of the surgical affection in any of the cases examined.

(2) In 20 subjects with senile cataract, of ages 46–76, the range in serum-calcium was 9·72 to 12·85 mg. per 100 cc. (av. 11·46).

It may be concluded therefore that the serum-calcium is appreciably raised in patients with senile cataract and that there is no age-variation in the serum-calcium of either normal subjects or of those with senile cataract.

THE RELATION OF CALCIUM TO THE CATARACTOUS LENS.

The results are certainly against the theory that senile cataract is associated with a low blood-calcium or with a parathyroid deficiency. There remains the more difficult task of explaining the occurrence and influence of a raised blood-calcium. The first and obvious suggestion that the latter is a mere exaggeration of an ordinary senile process is improbable in view of the fact that there is no age-variation either in normal subjects or in those with cataract.

In the hope of gaining further information as to the possible action of a high blood-calcium on the lens *in vivo*, some subsidiary experiments were designed to investigate the following points.

(1) The action of calcium salts on lenses *in vitro*.

(2) The influence on the lens of a high blood-calcium produced experimentally.

(3) The estimation of the calcium content of human lenses, both normal and cataractous.

(4) An examination of the histological evidence of calcium deposition in senile cataract.

(1) *The action of calcium salts on lenses* in vitro. It is generally accepted [Duke Elder, 1926] that solutions of calcium salts have no action on fresh animal lenses *in vitro* except in concentrations far exceeding the physiological range. The evidence quoted is that of two observers, namely Stoeltzner [1906], who observed that pig lenses became clouded on the addition of 3·37 % calcium chloride to the isotonic solution of sodium chloride in which they were immersed, and Nelson [1923], who found that 2·25 % calcium chloride was the lowest concentration at which clouding occurred.

In my own experiments fresh ox lenses were immersed without capsules in solutions of calcium chloride which had been buffered to the normal body p_H (7·5) with 0·001 *N* NaOH or with Ringer's solution. The solutions ranged from 0·025 to 5·0 % calcium. From 0·7 % upwards the lenses showed very superficial areas of cortical opacity which increased in extent according to the concentration of calcium. These did not resemble the opacity of cataract in any way, and seemed rather to be a fine precipitation on the surface of the lens. The same changes occurred, though more slowly, in lenses with intact capsules. It was only in hypertonic and unphysiological solutions, *e.g.* in unbuffered solutions above 2·5 % calcium, that a definite cortical opacity developed which was a true coagulation of lens-protein.

As an interesting comparison to Burge's observation [1914] that calcium chloride sensitises lens-protein to ultra-violet radiation, it was found that exposure to ultra-violet rays up to 3 hours, of lenses immersed in calcium solutions of the concentrations already quoted, and kept at 15°, caused only a slight increase in opalescence. Jess and Koschella [1923] reported that ultra-violet radiation of a lens in 0·02 % calcium carbonate solution did not cause it to become opaque. It was noticed also that sodium chloride appeared to have an antagonistic action to solutions of calcium salts, in that it favoured the maintenance of transparency in the lens.

It thus appears to be a correct conclusion that calcium salts acting *per se* on the normal lens are unable to cause opacity except when they are present in such a concentration or at such a p_H as would not occur in life. Neither does it seem that ultra-violet radiation could have much effect in life (the exposures used by Burge were very prolonged). There are, however, certain facts in favour of the theory postulated by Burge [1914] "that calcium is one of the substances which may play an important rôle in the production of cataract" in that it may "modify the lens-protein in such a way that ultra-violet radiation can precipitate it." For instance, a dialysed solution of the lens-protein α-crystallin, prepared according to the method described previously [Adams, 1925] shows a fine flocculation proportional to the amount of a calcium salt added to it. In addition, solutions of both α- and β-crystallin show marked flocculation following half an hour's exposure to ultra-violet radiation in the presence of a calcium salt. These changes are not strictly applicable to those which may occur in the whole lens *in vivo*, but are related to the physico-chemical problem of the denaturation and agglutination of protein solutions. Their significance in regard to the eye has been discussed recently by Duke Elder [1926, 1927].

(2) *The influence of a high blood-calcium produced experimentally.* By giving calcium lactate by mouth to a rabbit it is possible to raise the blood-calcium appreciably, and for a considerable length of time. I have found that repeated doses of this substance, equivalent to 1 g. of calcium per day, and continued for 5–6 weeks, cause no trace of cataract, although within 2 hours of each dose the serum-calcium rises 6–7 mg. and after 8 hours is still 2 mg. above normal, while it only regains the normal level in 20–24 hours.

It is possible to prove that a high serum-calcium actually leads to a corresponding rise in the calcium of the aqueous humour, by estimating the calcium of the serum and of the aqueous humour in the same animal (rabbit) under normal conditions and following a dose of calcium lactate (see Table II).

Additional dosage with calcium lactate does not in any way accelerate the development of cataract which is readily caused by the administration of naphthalene to rabbits.

Table II.

Date	Time	Exp.	Serum-calcium mg. per 100 cc.	Aqueous humour calcium mg. per 100 cc.
25. x. 27	1.30 p.m.	Bled from ear vein	14·03	—
	2.30	Aqueous humour withdrawn from both eyes	—	11·7
	2.40	Bled	14·04	—
1. xi. 27	12.30	Bled	14·04	—
	12.40	Ca lactate *per os* (≡ 1·0 g. calcium)	—	—
	3.10	Bled	21·17	—
	3.20	Aqueous humour withdrawn ...	—	13·2
	3.45	Bled	20·48	—

These observations are in accordance with those of Eiseman and Arno [1927] who were unable to produce cataract in dogs in conditions which would favour the deposition of calcium, *e.g.* by the injection of calcium lactate and the administration of sodium dihydrogen phosphate *per os* or *vice versa.*

(3) *The calcium content of normal and cataractous human lenses.* The only analyses recorded previously seem to be those of Burge [1909] who found an increase in the calcium content and a marked decrease in the potassium content of human cataractous lenses, in comparison with normal lenses. His figures for these substances were as follows:

	Av. dry wt. of one lens mg.	Av. wt. of ash of one lens mg.	% wt. of ash to dry wt.	K % in ash	Ca % in ash
Normal adult human lens	58·99	1·40	2·30	38·80 (≡ 0·92 % dry wt.)*	?
Embryo human lens	15·47	0·25	1·60	30·80	?
Cataract human lens (United States)	34·42	0·58	1·68	9·80	12·50 (≡ 0·21 % dry wt.)*
Cataract human lens (India)	92·30	1·52	1·64	5·81	6·00 (≡ 0·09 % dry wt.)*

* Addenda, D.R.A.

It appears that Burge found the amount of calcium in a normal lens too small to estimate. The recent introduction of micro-methods [Kramer and Tisdall, 1921, 2] has made it possible to repeat these analyses on a smaller scale. As a preliminary attempt the following average values were obtained from several estimations on a number of human lenses which had been ground together to a fine powder and dried to constant weight *in vacuo.*

Normal lenses 0·0516 g. Ca per 100 g. dry weight.

Cataractous lenses 0·1265 g. ,, ,, ,, (cf. Table IV).

These figures serve to illustrate the gross difference in the calcium content of normal and cataractous lenses, but give no indication of individual variation. A number of micro-estimations were therefore carried out on single lenses, both normal and cataractous, which yielded satisfactory results at least for purposes of comparison. Care was taken to make the experimental error as small as possible. Each lens was weighed as soon as possible after removal from the eye: *e.g.* a normal lens within 15 minutes; a cataractous lens usually within 24 hours. It was dried to constant weight *in vacuo* and incinerated in a platinum crucible. After extraction and precipitation of Ca as oxalate at the neutral point of methyl red in a small centrifuge tube, the precipitate was washed with 2 % ammonia, redissolved and estimated *in situ*, by titration against 0·01 N $KMnO_4$ (microburette graduated in 0·01 cc.).

For the purposes of calculation the wet weight of the lens, especially of a cataractous lens, was regarded as a variable quantity, since it was impossible to standardise the time which elapsed between extraction and weighing. Neither was the ash content considered a suitable basis for estimation of the calcium, since, according to Burge, the other mineral constituents are known to vary. This was confirmed in the case of potassium, as follows. In 8 normal lenses, of ages ranging from 22 to 79, the potassium content of individual lenses varied from 0·4968 to 0·7859 % dry weight, with an average of 0·6025 %. On the other hand, the amount of potassium in a single cataractous lens was too small to estimate even by the sensitive method of Kramer and Tisdall [1921, 1] (in which 1 cc. 0·01 N $KMnO_4$ is equivalent to 0·071 mg. potassium as potassium cobaltinitrite). The average value for potassium in a number of cataractous lenses was approximately 0·02 % dry weight.

Table III. *Analysis of normal human lenses.*

No.	Age	Cause of death	Wet wt. g.	Dry wt. g.	Ca, % wet wt.	Ca, % dry wt.
1	23	—	0·1578	0·0510	0·0125	0·0387
2	23	—	0·1534	0·0484	0·0123	0·0389
3	25	Pulm. tuberculosis	0·0904	0·0292	0·0155	0·0478
4	41	Cardiac failure	0·0914	0·0350	0·0153	0·0399
5	41	,,	0·0934	0·0356	0·0161	0·0421
6	42	Mitral stenosis	0·1002	0·0412	0·0135	0·0327
7	42	,,	0·1136	0·0408	0·0144	0·0400
8	45	Carcinoma of pharynx	0·1298	0·0490	0·0154	0·0408
9	45	,,	0·1372	0·0498	0·0168	0·0582
10	46	Broncho-pneumonia	0·1046	0·0404	0·0291	0·0753
11	46	,,	0·1202	0·0440	0·0316	0·0864
12	51	Carcinoma of larynx	0·1328	0·0494	0·0241	0·0648
13	51	,,	0·1430	0·0528	0·0238	0·0644
14	57	,,	0·1330	0·0512	0·0230	0·0598
15	58	Hyperpyaesia	0·1240	0·0459	0·0195	0·0527
16	58	,,	0·1246	0·0480	0·0178	0·0463
17	59	Carcinoma of tongue	0·1562	0·0568	0·0169	0·0465
18	59	,,	0·1562	0·0563	0·0166	0·0461
19	60	Carcinoma of pharynx	0·1648	0·0568	0·0138	0·0401
20	60	Intestinal obstruction	0·1416	0·0518	0·0204	0·0557
21	60	,,	0·2176	0·0736	0·0142	0·0419
22	62	Carcinoma of colon	0·1534	0·0542	0·0183	0·0516
23	66	Carcinoma of jaw	0·1494	0·0574	0·0201	0·0523
24	66	,,	0·1465	0·0529	0·0242	0·0671
		Av.	0·1348	0·0530	0·0185	0·0512

The calcium content was therefore estimated on the basis of the constant dry weight of the lens. As will be seen in Table III, this method of calculation gave very satisfactory agreement in the analysis of pairs of normal lenses, and since the range and average of the constant dry weights of normal and of cataractous lenses were substantially the same (see Table IV) it was also the most suitable basis for purposes of comparison.

Table IV. *A comparison of normal and cataractous lenses.*

	Range in wet wt. g.	Range in dry wt. g.	Average wet wt.	Average dry wt.	Ratio Av. wet wt. / Av. dry wt.	Average Ca % wet wt.	Average Ca % dry wt.
A	0·0904–0·2176	0·0292–0·0736	0·1348	0·0530	2·54/1	0·0185	0·0512
B	0·0680–0·1284	0·0209–0·0898	0·0987	0·0445	2·21/1	0·0653	0·1367

A, 24 normal lenses, ages 23–66. B, 31 cataractous lenses, ages 50–77.

Figs. 1 and 2 illustrate the range in the calcium content of individual lenses with their corresponding dry weights.

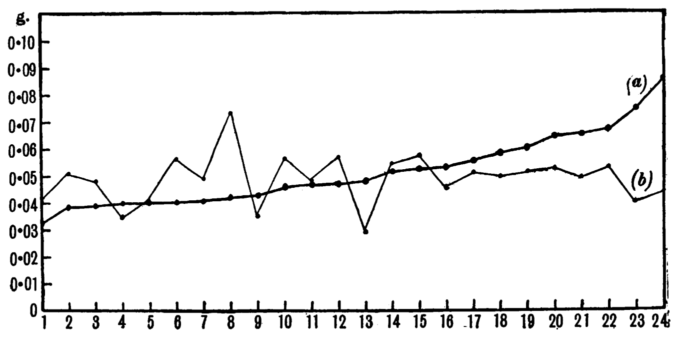

Fig. 1. The individual variation in the calcium content of normal lenses. (*a*) Calcium, % dry weight. (*b*) Corresponding dry weight.

The results of the estimations may be summarised as follows.

1. No age-variation was found in the calcium content either of normal or of cataractous lenses.

2. The statement of Burge is confirmed, that the average calcium content of cataractous lenses is much greater than that of normal lenses, while the potassium content is much less.

3. A much wider variation is apparent in the calcium content (% dry weight) of cataractous lenses, than in normal lenses. In the former it cannot be attributed to a corresponding fall in the dry weight (see Fig. 2).

4. The fact that the calcium content of 14 of the cataract lenses examined was below 0·1 % with an average of 0·0748 % dry weight, while the remaining 17 lenses gave an average content of 0·1990 %, suggests that the deposition of calcium in senile cataract is an erratic process, and occurs more extensively in some lenses than in others.

(4) *Histological evidence of calcium deposition in senile cataract.* In the course of examining a series of frozen sections from a number of senile cataractous

lenses, it was noticed that one lens in every 10 or 15 showed comparatively large circular deposits of crystals. They were visible to the naked eye as small white points in the section, and microscopically were seen to be isolated groups of radiating crystals, which were strongly doubly-refractive. They occurred in the inner layers of the cortex and there were usually 5 or 6 deposits in one section, each being surrounded by a very narrow homogeneous layer.

They have already been described by Wessely [1922], and Kranz [1927] has also referred to them as "spheroliths" in his account of the other types of doubly-refractive deposits which are to be found in senile cataract.

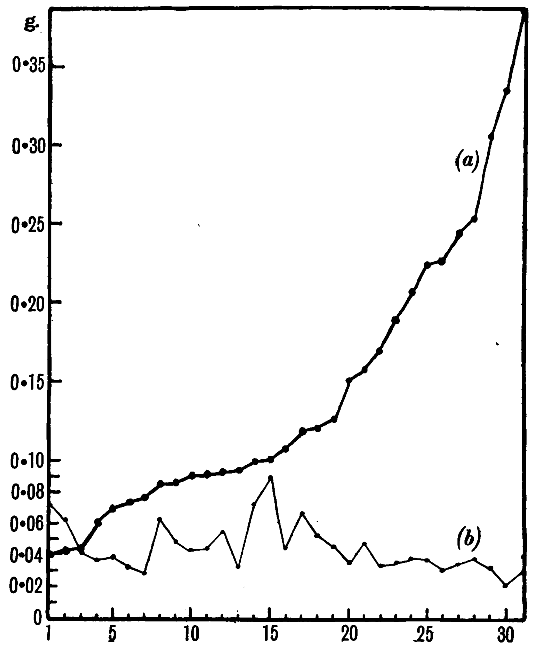

Fig. 2. The individual variation in the calcium content of senile cataractous lenses.
(a) Calcium, % dry weight. (b) Corresponding dry weight (g.).

From a series of microchemical tests Wessely concluded that the crystals are calcium phosphate, and that they represent a local deposition of calcium in areas where a preceding "colloid" or protein degeneration has already occurred, and Kranz held a similar view. If such is indeed the case, then these deposits may be regarded as the histological evidence of the erratic deposition of calcium which was presupposed from the analyses of individual cataractous lenses. Wessely noted that the lenses in which crystals were found were all from subjects over 70. The deposits are certainly large enough and sufficiently numerous to cause a markedly high calcium content in the particular lens which contains them, but the relative occurrence of such lenses is too small to account for all the high results (*e.g.* those above 0·1 %) recorded in Fig. 2. A possible explanation of this discrepancy may be that crystallisation is only a late stage in the process of localised deposition of calcium, and that it is governed by local conditions, which are not always favourable. The early stages of the change, though invisible histologically, would also cause a rise in the calcium content of a lens.

One is reminded of the similarity between these changes and those in atheroma where a combined deposition of calcium and cholesterol occurs locally in the intima, another avascular tissue. In senile cataract the cholesterol content of the lens is markedly increased. Goldschmidt [1922], in studying the age-variation of lipoids in normal lenses, found a range in cholesterol from 0·52 to 1·71 % dry weight, while by the Myers and Wardell method [1918] I obtained an average for cataract lenses of 2·579 % dry weight from several estimations on a number of lenses ground together. Since the crystalline deposits described above are wholly insoluble in organic solvents, there does not appear to be any such immediate association between calcium and cholesterol deposition in cataract as is found in atheroma. Indeed, minute spherocrystals which Kranz identified as cholesterol can be seen as independent structures, and occur very frequently in cataractous lenses.

An attempt was made to verify the nature of the circular deposits. It was found easy to confirm the presence of calcium but the tests for phosphates were much less convincing. From the general behaviour and appearance of the crystals (see below) and from the commonly known characteristics of other calcium salts, however, it must be concluded that phosphate is the most likely salt to be deposited.

The frozen sections used for microchemical tests were carefully prepared according to the method advocated by Herbert [1928] in order that substances such as soaps or fats, which might be in association with the calcium deposits, should not be affected. The lenses were fixed in neutralised calcium-free 10 % formalin and frozen in pure distilled water for cutting, and were then examined immediately. Sections to be stained were taken through neutral alcohol up to 70 % before mounting in glycerol.

The crystals were practically colourless and did not melt on being heated on the warm stage up to 180°. They were insoluble in organic reagents and only slowly soluble without effervescence in dilute ordinary mineral acids and in acetic acid. With cold concentrated H_2SO_4 they lost their double-refraction and formed an amorphous white mass, while on treatment with dilute H_2SO_4 and a little alcohol, fine needles of calcium sulphate separated out.

Tests for amino-acids, uric acid, urea, and for metallic radicles other than calcium were negative.

Specific tests for phosphate with (1) a nitric acid solution of ammonium molybdate, (2) quinol reagent (Eggleton) and (3) $FeCl_3$ and acetic acid were only weakly positive. As the crystals dissolved in the acid in each case it was difficult to be sure that the colour was not due at least partly to phosphate distributed throughout the section. Kossa's test, which Wessely used, namely the development of a yellow-brown precipitate with silver nitrate which later turned black, was strongly positive, but neither this nor the tests of Roehl or Stoeltzner, which Wessely also employed, can be considered specific for phosphate.

The crystals remained unstained by haematoxylin, van Gieson's stain, Sudan III, Nile blue, alizarin and iodine, while they gave a patchy positive staining with "alumin-alizarin." A direct Benda stain for fatty acids and soaps was negative for the whole section, but after keeping these sections for 2 or 3 months the crystals acquired an olive green tinge in the manner described for bone and calcium-containing tissues. Thus, to a certain extent, the staining reactions also confirm the presence of calcium.

Summary.

1. In patients with senile cataract the serum-calcium is appreciably higher than normal.
2. There is no age-variation in the serum-calcium either of normal subjects or of those with senile cataract.
3. Calcium salts acting on fresh ox lenses do not cause opacity except in unphysiological concentrations, nor do they act as "sensitisers" in the production of opacity by ultra-violet radiation.
4. Dialysed solutions of the lens proteins, α- and β-crystallin, exhibit a certain sensitivity to solutions of calcium salts.
5. The experimental production of a persistently raised blood-calcium in rabbits does not cause cataract, nor accelerate the development of naphthalene cataract, although it causes a corresponding rise of calcium in the aqueous humour.
6. There is no age-variation in the calcium content of normal or of cataractous human lenses.
7. The calcium content of cataractous lenses is much greater than that of normal lenses, while the potassium content is considerably less.
8. Individual cataractous lenses show a wide variation in calcium content, which may be attributed to an erratic local deposition of calcium, for which there is also some histological evidence.
9. Although there is an increase of calcium in the blood of patients with senile cataract, and an abnormal deposition of calcium in the lens, it cannot as yet be proved conclusively that the increase in calcium is a primary factor in the actual production of opacity in the lens.

My thanks are due to Sir John Parsons, to Prof. Choyce and to Dr Barnard for a supply of clinical material, and to Miss Quick and C. Vann for technical assistance.

The experiments were performed for the Committee of Vision of the Medical Research Council, to whom I am greatly indebted for a research grant.

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