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A NOTE ON THE INORGANIC CONSTITUENTS OF NORMAL AND CATARACTOUS HUMAN CRYSTALLINE LENSES

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EFFORTS to devise some method of preventing the development of cataract in human lenses have long been pursued. Various recommendations have been made and practised, notably the application to the conjunctival surface of solutions or ointments containing soda, potash or calcium, iodine, dionine, etc., in different proportions and combinations. Electrical currents, optical contrivances, and internal medication have all had their advocates and practitioners. Faith healing has not been omitted. Quackery has flourished by such means, and unjustifiable pretensions and promises have emptied the purses of many timid and gullible patients hoping to escape from the prospect of extraction by surgical operation.

That the problem must be worked out on scientific lines is self-evident, and efforts in that direction have not been neglected, but so

far, with no very conclusive results. More precise knowledge of the conditions in which the lens is liable to lose its transparency, and the actual changes in its structure and chemical constitution are essential as a base from which to approach this problem. The investigation calls for the fullest possible knowledge of the nutrition of the normal lens, and of the possibility of modifying it by chemical or other treatment. One must enquire to what extent substances can be conveyed into the interior of the lens by absorption from the conjunctival sac, the aqueous or vitreous, or through the general circulation. Alternatively, is it possible beneficially to extract undesirable elements through the unbroken capsule of the living lens?

As a first step, it appeared to us desirable that a fresh series of observations should be made into the chemical condition of cataractous, as compared with transparent, human lenses, and that advantage should be taken of the most up-to-date methods of analysis. Very desirable also to employ for this purpose were lenses which had been extracted with intact capsules. The post-mortem room has supplied the transparent lenses; the hospital operating theatre the nontransparent group, kindly provided by colleagues who practise the intracapsular method of extraction.

Thus far, no attempt has been made by the writers of this paper to correlate the transparent lenses with the morbid condition of the cadaver from which the lens was taken.

Nor have the cataractous lenses obtained from living subjects been sufficiently numerous to classify them into special groups or varieties of cataract—only the sex and age of each patient have been noted, except in one group which was definitely known to have a traumatic origin.

The quantity of ash yielded by each lens is so small that the analysis has been carried out on groups of four lenses fortuitously placed together. It is hoped that larger supplies of lenses may make it possible and profitable to classify the lenses clinically. This, of course, can only be done through the co-operation of ophthalmic colleagues who are willing to take this extra trouble at the time of extraction and forward the clinical titles or comments along with the lenses when submitted for analysis. An appeal for such helpful team work is hereby made by the writers.

The present paper does not pretend to be more than a preliminary note intimating that this research is being conducted, and is launched in the hope of stimulating interest, support and encouragement.

Burge (1909) observed that human cataractous lenses contained much more calcium and much less potassium than did normal lenses, and this observation has been confirmed by Adams (1929). Burdon-Cooper and Lewis (1929) have obtained similar results, having shown by spectrographic analysis that the K/Ca ratio in the lens falls

with the development of cataract. The opacity of the cataractous lens has been ascribed to these changes in inorganic constituents, and especially to the increase in calcium. Thus, although Adams (1929) was unable to prove conclusively that increase in calcium content is the primary cause of the production of opacity; she found abnormal deposition of calcium in cataractous lenses, and her evidence was suggestive in other respects as well. The diminished potassium content of the cataractous lens has been made the basis of a method of treatment which has met with rather doubtful success; very similar results being obtained when sodium salts were applied instead of potassium salts. (Inglis Pollock, 1917-1923).

There are, however, other changes in the composition of the crystalline lens which has become cataractous, and although these may possibly be secondary to changes in the inorganic constituents, they indicate that alterations in the calcium and potassium are accompanied by changes in the concentration of other inorganic ions which may be equally important. Thus Lavagna (1926) has found cataractous lenses to have a lower pH than normal ones (*i.e.*, to be slightly less alkaline) a condition which would actually militate against the deposition of calcium phosphate, and to have a greater water content, as well as marked variations in the concentration of the organic constituents.

As a preliminary to further studies, we have for some time been engaged in the analysis of human lenses, normal and cataractous.

Experimental Methods and Results.—The cataractous lenses used in these analyses were removed with capsule, and placed at once in chemically clean, sterile, stoppered glass bottles, but not immersed in fluid other than the slight amount which might have adhered to them at the moment of extraction. It was not always possible to carry out the analysis at once—a number of the lenses were sent to us by post—so that it was impracticable to use the wet weight as a standard of comparison. In these circumstances we were compelled to use the dry weight* and the weight of ash. Neither of these, perhaps, is ideal, since Lavagna (1926) and others have found differences in the water content of normal and cataractous lenses. Burge (1909) too, reports a difference between the dry weight of the normal lens and that of the cataractous, but his figures are not particularly convincing, since one series of cataractous lenses (from the U.S.A.) had a smaller mean dry weight than his normal controls, while the other (from India) had a larger. Adams (1929), however, gives the average dry weight of 24 normal lenses as 53.0mg., and that of 31 cataractous lenses as 44.5mg.—probably an important difference though she states that she herself does not regard it as significant. Our own figures (Table I) show a definite diminution

* *i.e.*, the weight after drying at 105°C. until no further loss occurred.

in the dry weight of the cataractous lens. We find, in addition, that there is a tendency for the cataractous lens to contain rather less ash than the normal, but the two series overlap so completely that in spite of the difference in the mean values the figures cannot be regarded as more than suggestive. Although, on the average, a cataractous lens contains a slightly smaller weight of ash than the normal, transparent lens, the total weight of the cataractous lens is also smaller. So marked, indeed, is the diminution in size of the cataractous lens as a whole, that the *percentage* of ash in such a lens is actually greater than normal, whether the ash is expressed as percentage of dry weight or of wet weight (the latter being calculated from Adams' figures for the water content).

TABLE I.

Number of Lenses	Dry Weight in Mgs.		Weight of Ash Mgs.		Ash as percentage of *	
	Range	Average	Range	Average	Dry Weight	Wet Weight
Normal - - 18	53'7 to 67'5	60'14	1'10 to 2'22	1'59	2'64	1'04
Cataractous 27	30'0 to 50'6	44'07	1'10 to 2'40	1'41	3'20	1'44

* From Adams' (1929) figures :—

$$\frac{\text{Average Wet Weight}}{\text{Average Dry Weight}} = 2.54 \text{ for normal; } 2.21 \text{ for cataractous lenses.}$$

For analysis, the ash of four lenses (obtained by careful incineration in a platinum crucible at a temperature not exceeding 350°C.) was dissolved in a little dilute nitric acid, and the volume of solution was made up to 20 c.c. with distilled water. Aliquot portions of this solution were used for the various determinations. Potassium and calcium were determined by the methods of Kramer and Tisdall (1921*); sodium by the method of Kramer and Gittleman (1924); total sulphur by barium sulphate precipitation; total sulphate (on a separate series of single lenses) by the method of Denis and Leche (1925); phosphate by the method of Briggs (1924); and chloride by back titration with ammonium thiocyanate after addition of excess of silver nitrate.

The sulphate present in the ash includes part of the organic sulphur of the lens, as is shown by the fact that the method for

*Used also by Adams.

TABLE II.

Number and description of lenses	Dry Wt. Mg.	Ash Wt. Mg.	CALCIUM		POTASSIUM		SODIUM		CHLORINE		SULPHUR AS SO ₄		PHOSPHORUS AS PO ₄	
			% Dry Wt.	% Ash	% Dry Wt.	% Ash	% Dry Wt.	% Ash	% Dry Wt.	% Ash	% Dry Wt.	% Ash	% Dry Wt.	% Ash
<i>Normal</i>														
4, male -	215.5	5.8	0.026	0.98	0.705	26.2	0.412	15.3	0.056	2.1	1.13	42.2	0.495	18.4
4, male -	243.0	6.8	0.034	1.23	0.696	24.9	0.316	12.3	0.095	3.4	—	—	0.655	23.4
4, male -	270.1	7.0	0.031	1.19	0.487	18.8	0.189	7.3	0.150	5.8	1.28	49.4	0.511	19.7
4, male -	235.6	8.4	0.024	0.64	6.634	17.3	0.623	16.5	0.087	2.3	—	—	0.487	12.9
<i>Mean</i> -			0.029	1.01	0.635	21.8	0.385	12.6	0.097	3.4	1.20	45.8	0.537	18.6
<i>Senile Cataract Mature</i>														
4, female -	144.3	5.5	0.210	5.51	0.114	3.0	—	—	0.469	12.3	1.82	47.9	0.320	8.4
4, female -	197.8	4.5	0.118	5.25	0.025	1.1	0.622	27.3	0.084	3.7	1.24	54.4	0.355	15.6
4, male -	199.3	7.6	0.159	4.18	0.107	2.8	—	—	0.351	9.2	—	—	0.343	9.0
4, male -	116.8	5.3	0.318	7.01	0.122	2.7	—	—	0.395	8.7	1.92	41.9	0.526	11.6
<i>Mean</i> -			0.201	5.49	0.092	2.4	0.622	27.3	0.325	8.5	1.66	48.1	0.386	11.1
<i>Senile Cataract Immature</i>														
4, male -	202.5	7.4	0.084	2.31	0.309	8.45	0.603	16.5	0.272	7.4	—	—	0.303	8.3
<i>Traumatic Cataract</i>														
1, male -	—	2.4	—	1.32	—	4.4	—	—	—	3.1	—	—	—	—

inorganic sulphate gives results definitely lower than those obtained by use of the method for total sulphate. Most probably, part of the phosphate is similarly combined organically in the lens as phosphoprotein or phospholipin, and though we have no direct evidence on this point, it is noteworthy that Lavagna (1926) found fully cataractous lenses to contain less phospholipin than normal ones—just as, in our series, is the case with total phosphate. Indeed, if Lavagna's figures be accepted, phospholipin appears to account for about three quarters of the total phosphate found in the lens.

The ash appears to contain practically no carbonate, a fact which is apparently to be explained by the replacement, during the process of ashing, of the carbonate (or bicarbonate) actually present in the lens, by phosphate or sulphate from organic compounds, the carbon dioxide, of course, being driven off. The ions actually estimated account for practically the whole of the ash, and indeed, in one or two cases they total to rather more than 100 per cent.—a result which is not altogether surprising in view of the difficulty of determining accurately such small amounts. It may be concluded, however, that other inorganic substances can only be present in relatively small amounts.

The results of analysis are summarized in Tables II and III.

TABLE III.

<i>Sulphate content of lens.</i>										SO ₄ as % of dry weight.
Normal	-	-	-	-	-	-	-	-	-	0'549
Do.	-	-	-	-	-	-	-	-	-	0'561
Do.	-	-	-	-	-	-	-	-	-	0'494
Mean	-	-	-	-	-	-	-	-	-	0'535
Cataractous	-	-	-	-	-	-	-	-	-	0'567
Do.	-	-	-	-	-	-	-	-	-	0'661
Do.	-	-	-	-	-	-	-	-	-	0'671
Mean	-	-	-	-	-	-	-	-	-	0'633

Discussion.—Although it is somewhat unsafe to go very far in interpreting these results—in particular, further information is needed as to the forms in which the sulphate and phosphate of the lens exist, as well as on the presence of bicarbonate—two definite indications seem to emerge.

It is to be noted that the various inorganic constituents of the healthy lens are present in concentrations markedly different from those of the same substances in blood plasma, or—which is probably more important—in normal aqueous humour. The latter closely resembles cerebro-spinal fluid in composition, and thus does not differ very markedly from blood plasma. It contains rather more sodium than does plasma, about $5/4$ as much chlorine, slightly less potassium, and about half as much calcium. Hence, to confine our attention to these substances, ignoring the phosphate and sulphate, of whose concentration in the lens we know little, we can contrast healthy lenses and normal plasma or aqueous humour as follows:—

Normal Lens.—

Potassium greatly in excess of sodium.

Calcium very low (about 10mg. per cent., calculated on wet weight).

Chlorine less than equivalent to sodium.

Aqueous Humour.—

Sodium greatly in excess of potassium.

Calcium about 5mg. per cent.

Chlorine about equivalent to sodium.

The lens is thus strongly differentiated from the aqueous humour and from blood plasma. (The vitreous humour has been ignored, since, apart from the improbability of its being concerned in the nutrition of the lens, little is known of its inorganic constituents. Its calcium content, however, is intermediate between that of the lens and that of the aqueous humour, *i.e.*, about 7.3mg. per cent., and its chlorine content is slightly below that of the humour (O'Brien and Salit, 1929.) In the cataractous lens, however, this power of maintaining its differentiation, at any rate from plasma, has been partly lost. Whether this also applies to the differentiation from aqueous humour must be a subject of future investigation. In the cataractous lens, sodium predominates over potassium, but the latter remains higher than in blood plasma, and the chloride is increased though it remains lower than in plasma. These changes are shown graphically in Fig. 1. There is thus a *tendency on the part of the lens to return to, or take on, a composition similar to that of blood plasma*, and this tendency is shown in immature as well as mature cataracts. It appears, indeed, to be progressive, and, therefore, to be closely connected with the diseased process.

A notable exception to this, however, is the calcium, which, instead of increasing slightly as would be expected in a general

Fig. 1.

Comparison of some inorganic constituents of normal lenses, with those of cataractous lenses, and of serum. The figures for the lenses are calculated on the wet weight, using Adams' estimations of water content.

return to a plasma-like composition, is greatly increased in cataractous lenses, both absolutely, and relatively to the other ions. This argues some specific relationship of calcium to the development of cataract. Adams (1929), confirming the earlier work of Wessely (1922) and Kranz (1927), finds actual deposits of calcium (possibly as phosphate) in the cataractous lens, and relates the erratic occurrence of these deposits with the wide variability in calcium content of individual lenses. Wessely and Kranz considered that the calcium deposition was secondary to a protein degeneration and Adams states that it cannot as yet be proved conclusively that the increase in calcium is a primary factor in the production of opacity in the lens. With this conclusion we must still rest content. The immature cataractous lenses show an increase in calcium content, which, however, is less than that in mature cataract, but they also show the other divergences from the normal, again to an intermediate extent. There is thus no evidence that calcium is the first constituent to alter, as one might expect if it were the primary factor. Yet undoubtedly the difference 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.

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