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THE USE OF ASCORBIC ACID TO MEASURE THE RATE OF FLOW OF PLASMA THROUGH THE CILIARY PROCESSES

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It is well known that the concentration of ascorbic acid in the aqueous humour of man and many animals exceeds that in the plasma. The origin of this excess has been attributed to various causes (see Bellows, 1944; Linnér, 1952) but only two justify consideration, namely, an active transfer of ascorbic acid from the blood stream into the eye and a synthesis of ascorbic acid within the eye. In rabbits the secretion of ascorbic acid into the aqueous humour reaches a maximum at a plasma concentration of 2–3 mg/100 ml. and gives a mean concentration of 50–70 mg/100 ml. in the aqueous humour (Kinsey, 1947; Langham, 1950; Linnér, 1952; Bárány & Langham, 1955). In guinea-pigs the ascorbic acid in the aqueous humour is derived entirely from the blood, but in rabbits the possibility still remains that a concentration of 10–14 mg/100 ml. is due to synthesis within the eye (Bárány & Langham, 1955).

The effect of a change in blood flow through the uveal circulation on the concentration of ascorbic acid in the aqueous humour of the rabbit has been studied by Linnér (1952). He observed that ligation of one common carotid artery led to a decrease in the concentration of ascorbic acid in the aqueous humour on the same side which was equivalent to the percentage decrease in the flow of blood through the uveal tract. On raising the concentration of ascorbic acid in the plasma (mean value 13 mg/100 ml.) the concentration in the aqueous humours of the two eyes became equal. It was concluded that when the amount of ascorbic acid transferred across the blood-aqueous barrier was not maximal, the concentration of ascorbic acid in the aqueous humour was determined by the amount reaching the site of transfer. Linnér therefore proposed the use of ascorbic acid to estimate the rate of flow of plasma through the ciliary processes in a manner analogous to the use of *p*-aminohippuric acid in studies of renal function.

The work reported in this paper is confined to rabbits with a unilateral ligation of the common carotid artery. It has been undertaken to investigate if any of the ascorbic acid in the aqueous humour is formed within the eye, and whether the relation between the concentrations of ascorbic acid in the plasma and the aqueous humour of operated animals supports the concept of Linnér.

METHODS

Experimental procedure. Adult rabbits of both sexes weighing between 1·8 and 2·5 kg were used. They were fed diet 18 pellets (Associated London Flour Millers), water *ad lib.* and hay once a week. Unilateral ligation of the common carotid artery was performed on animals anaesthetized with intravenous Nembutal (pentobarbitone sodium B.P.). Samples of aqueous humour from the conscious animal were taken 20–30 hr after the operation, following the instillation of a 1% solution of pantocaine into the conjunctival sac. In some experiments the aqueous humour was removed immediately after the administration of a general anaesthetic. All samples of aqueous humour were tested for the presence of protein by the addition of an equal volume of a solution of 8% trichloroacetic acid and results were rejected if more than the normal faint clouding was observed.

The ascorbic acid for injections was dissolved in water, partially neutralized with a solution of sodium hydroxide to pH of 6·5–7·2 and made up to an isotonic concentration. Raised concentrations of ascorbic acid in the plasma were maintained by an initial intravenous injection followed by hourly intramuscular injections for 6 hr. A period of 6 hr is sufficient to allow a steady state to be reached in the aqueous humour to within a few per cent.

Analytical. Ascorbic acid in the aqueous humour was determined by a modification of the osazone technique of Roe, Mills, Oesterling & Damron (1948). The concentration of ascorbic acid in the plasma was analysed by a modification of the electrometric titration procedure of Mindlin & Butler (1938) in which a solution of 2:6-dichlorophenol-indophenol is used as oxidant. The analytical procedures followed those described in a previous paper (Bárány & Langham, 1955).

RESULTS

The concentrations of ascorbic acid (AA) in the aqueous humour of the two eyes of a normal rabbit are equal. In twenty-six adult rabbits the mean ratio of the concentrations of AA in the aqueous humour of the two eyes was $1{\cdot}00 \pm 0{\cdot}011$ (arithmetic mean $\pm$ S.E. of the mean). The effect of unilateral ligation of the common carotid artery on the concentration of AA in the aqueous humour was determined in twenty-five rabbits on normal diet. The measurements were made 24–30 hr after recovery from the anaesthetic. The mean concentration of ascorbic acid in the aqueous humour on the operated and unoperated sides was $20{\cdot}5 \pm 0{\cdot}99$ and $25{\cdot}15 \pm 1{\cdot}47$ mg AA/100 ml. respectively. The mean ratio of the concentration of AA in the aqueous humour on the operated side to that on the unoperated side was $0{\cdot}80 \pm 0{\cdot}035$. This agrees with the value of $0{\cdot}83 \pm 0{\cdot}025$ reported by Linnér (1952) in similar experiments.

The concentration of the transformation products of ascorbic acid, dehydroascorbic (DHA) plus diketogulonic acids (DKG), was determined on six operated animals (see Table 1).

A series of twenty-nine operated rabbits was fed on diets deficient in or

supplemented with AA, and a range of aqueous humour AA concentrations of 15–40 mg/100 ml. was observed. The experimental results which are recorded in Table 2 have been divided into two groups according to whether the concentrations did or did not exceed 25 mg/100 ml. The mean ratios in the two groups do not differ ($P > 0{\cdot}10$), but the difference in the concentration of AA between the two eyes of the two groups is significantly different ($P < 0{\cdot}001$). These values are consistent with the view that in this range the operation results in a constant percentage fall in the AA concentration in the two eyes.

TABLE 1

	AA concentration (mg/100 ml.)	DHA plus DKG concentration (mg/100 ml.)
Operated side	24·27±2·67 (6)	0·65±0·03 (6)
Unoperated side	30·45±2·42 (6)	0·70±0·04 (6)
Ratio: Op/Unop	0·78±0·05 (6)	0·99±0·008 (6)

TABLE 2. The concentration of AA in the aqueous humour of the two eyes after a unilateral ligation of the common carotid artery.

Group	$Aq._1$	$Aq._2$	Mean $Aq._2$–$Aq._1$	Mean ratio $Aq._1/Aq._2$
1	17·1±0·92 (15)	20·5±1·00 (15)	3·41±0·37 (15)	0·83±0·02 (15)
2	24·7±1·10 (14)	32·5±1·21 (14)	7·73±0·69 (14)	0·76±0·02 (14)

$Aq._1$ represents the AA concentration in the aqueous humour on the operated side and $Aq._2$ that on the unoperated side. Concentrations are expressed as mg/100 ml.

TABLE 3. The effect of ascorbic acid administered intramuscularly on the concentration of ascorbic acid in the aqueous humour in rabbits with a unilateral ligation of the common carotid artery.

Group	Injections	$Aq._1$	$Aq._2$	$Aq._2$–$Aq._1$	Ratio
1	Saline	16·81±0·9	20·56±1·07	3·75±0·68	0·82 ±0·03 (10)
2	5 mg	24·3 ±1·30	33·58±2·83	9·28±1·80	0·725±0·037 (6)
3	10	31·64±2·41	42·99±2·84	11·35±2·33	0·727±0·058 (6)
4	20	40·77±0·96	52·30±3·17	11·53±2·60	0·79 ±0·056 (6)
5	30	58·53±2·79	62·19±3·04	3·66±0·92	0·945±0·014 (6)
6	40	65·56±2·95	70·67±2·81	5·11±2·25	0·928±0·029 (6)
7	50	67·40±2·47	67·71±2·55	0·31±0·54	0·994±0·007 (9)

Aqueous humour 1 is taken from the eye on the operated side and aqueous humour 2 from the eye on the unoperated side. After an initial injection of twice the subsequent dosage, injections were made hourly for a period of 5 hr and the aqueous humour removed 1 hr later. Concentrations are expressed as mg/100 ml.

The effect of increasing doses of AA on the concentration of AA in the aqueous humour of operated rabbits is shown in Table 3. It will be observed that neither the absolute difference in AA concentrations between the two eyes nor the mean ratios are constant in the seven groups. The mean ratios of the concentrations of AA in the two eyes in groups 1–4 do not differ significantly, although the concentration of AA in the aqueous humour of group 4 is approximately $2\frac{1}{2}$ times as great as in group 1. The ratios in groups 5–7 do, however, differ significantly from those in earlier groups and the value of unity found in

group 7 is the same as found in eyes of normal rabbits. The mean difference in the absolute concentrations of AA in the aqueous humour of groups 1–4 shows a tendency to increase with the AA concentration in the aqueous humour. This is in keeping with the constancy of the percentage difference in AA concentration found in these groups. In groups 5–7 there is a very significant reduction in the concentration difference between the two eyes which in group 7 drops to a value similar to that observed between the two eyes of a normal rabbit. The mean concentrations of ascorbic acid in the aqueous humour of the eyes on the unoperated sides in groups 5–7 do not differ significantly from the maximum of $67{\cdot}5 \pm 2{\cdot}5$ mg AA/100 ml. in normal animals when the plasma concentration exceeds 2–3 mg/100 ml. (Bárány & Langham, 1955).

The minimal concentration of AA in the plasma required to produce a maximal rate of transfer of AA in the eyes of operated animals was determined in separate experiments. Conscious rabbits of approximately 2·5 kg were used and the injections made intramuscularly except for an additional initial intravenous injection; blood samples were withdrawn from the fully dilated marginal ear vein. The results, which are shown in Fig. 1 and Table 4, fall into two groups. In the first four rabbits the ratio of the concentrations of AA in the two eyes is not significantly different from the value of $0{\cdot}80 \pm 0{\cdot}035$ observed in untreated animals, and the AA concentration in the aqueous humour on the operated side is well below the mean maximum of approximately 70 mg/100 ml. observed when the secretory mechanism is operating at its maximal rate. In the remaining rabbits the AA concentrations in the aqueous humour of both eyes lie within the range of maximal values and here the ratios approach unity. It will be observed that a maximal transfer of ascorbic acid on the unoperated side occurred with a plasma concentration of 3–5 mg/100 ml. which is higher than the value of 2–3 mg/100 ml. found for normal rabbits (Bárány & Langham, 1955). It is, however, doubtful whether this is a significant difference in view of the small number of experiments and the observation that the titration method gives consistently 0·5–1·0 mg/100 ml. more ascorbic acid in the plasma than indicated by the osazone method used in studies on normal rabbits (Bárány & Langham, 1955).

DISCUSSION

The results of these experiments may be considered from two aspects. First, the transfer of ascorbic acid from the plasma into the aqueous humour; secondly, the measurement of ciliary blood flow. The experimental results are in agreement with the suggestion that the transfer of ascorbic acid from the blood stream into the aqueous humour could be dependent on the rate of blood flow through the ciliary processes. The transfer of ascorbic acid in

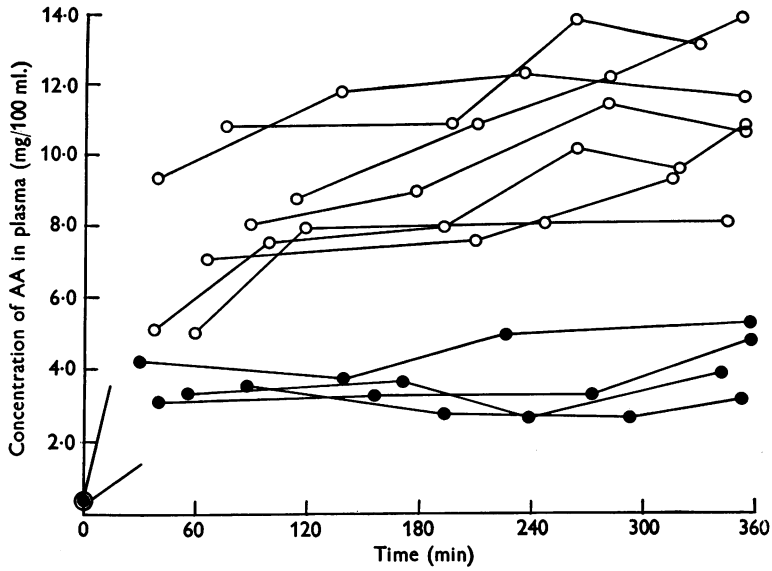

Fig. 1. The concentration of ascorbic acid in the plasma required to produce a maximal transfer of ascorbic acid into the aqueous humour of rabbits with a unilateral ligation of the common carotid artery. A solution of ascorbic acid (pH 7·2) was administered intramuscularly at hourly intervals and an additional intravenous injection was made at $t=0$ min. The upper set of results represented by the open circles led to a maximal transfer of ascorbic acid in both eyes. The concentration of ascorbic in the plasma of the lower group represented by filled-in circles was insufficient to produce a maximal rate of transfer in either eye (see text).

TABLE 4. The relation of the concentration of ascorbic acid in the plasma to that in the aqueous humour of rabbits with a unilateral ligation of the common carotid artery. The plasma concentrations were analysed at time of the paracentesis; the full plasma curves are given in Fig. 1.

Rabbit	Plasma	$Aq._1$	$Aq._2$	Ratio: $\frac{Aq._1}{Aq._2}$
1	3·1	46·3	55·5	0·83
2	4·7	42·5	52·0	0·82
3	5·2	45·8	70·0	0·65
4	3·8	43·0	54·0	0·80
5	10·5	59·0	61·0	0·97
6	13·8	80·5	85·0	0·95
7	11·6	78·0	82·5	0·95
8	13·0	62·0	63·0	0·98
9	9·2	64·0	65·0	0·98
10	8·0	67·6	68·0	0·99
11	10·7	71·0	70·5	1·00

Aqueous 1 is taken from the eye on the operated side and Aqueous 2 from the unoperated side. Concentrations are expressed as mg/100 ml.

normal rabbits and in animals with unilateral carotid ligation is similar in several respects. Equal concentrations of ascorbic acid in the aqueous humour of all animals were observed when the rate of transfer was at a maximum. Similarly, the plasma concentration needed to produce a maximal transfer on the unoperated side was approximately the same as for normal eyes. On the operated side, however, where there is a decreased blood flow, the plasma concentration had to be raised to produce maximal transfer. If the concentration in the aqueous humour is limited by the rate at which the compound reaches the site of transfer it might be expected that to maintain a constant transfer it would be necessary to increase the plasma concentration by an amount proportional to the drop in blood flow. A 50% drop in blood flow would require the plasma concentration to be doubled. On the basis of the present experiments the change in blood flow is between 15 and 40%: whereas a plasma concentration of 5 mg/100 ml. is adequate to produce maximal transfer in the unoperated eye, a plasma concentration of 6–8 mg/100 ml. is necessary on the operated side. A decrease in ciliary blood flow of 15–40% is in general agreement with the observation that there is a decrease of 20% in blood flow through the vortex veins following unilateral carotid ligation (Linnér, 1952). It is therefore possible that the difference of 20% in the concentration of ascorbic acid between the two eyes is an approximate measure of the difference in ciliary blood flow. It is certainly not due to alterations in intraocular metabolism of ascorbic acid: the concentrations of the transformation products of ascorbic acid were found to be equal in the two eyes and to be less than 3% of the total ascorbic acid present.

No accurate method has yet been devised to measure the rate of blood flow through the ciliary processes. If it is accepted that the ligation of one common carotid artery leads to a change in this rate of flow and in the concentration of ascorbic acid in the aqueous humour, the validity of Linnér's suggestion that ascorbic acid might be used as an index of the former, in a manner similar to clearance techniques in the study of renal function, is of unusual interest.

The balance between the amount of ascorbic acid entering and leaving the aqueous humour can be expressed in the form:

$$Fl_{pl.}(C_{art.} - C_{ven.}) = C_{aq.} k_{AA}, \quad (1)$$

where $Fl_{pl.}$ is the rate of flow of plasma, and $C_{art.}$ and $C_{ven.}$ the concentrations of ascorbic acid in arterial and venous samples of plasma flowing through the ciliary processes: k_{AA} is the rate of loss of ascorbic acid from the aqueous humour and $C_{aq.}$ the concentration of ascorbic acid in the aqueous humour. Since it has been shown (Bárány & Langham, 1953, 1955) that the relation between the ascorbic acid concentrations in the aqueous humour and the plasma is of such a form that when extrapolated back to zero plasma concentration the intercept on the aqueous concentration axis is certainly not

negative, it would seem that the venous ascorbic acid concentration in the ciliary body must either be very small or nearly proportional to the arterial concentration. It is difficult to see why, as the concentration of ascorbic acid in arterial plasma rises, the arteriovenous concentration difference should rise in proportion: it might be more readily expected to decrease. It seems most probable, therefore, that the explanation of the relation between arterial and aqueous concentration is that the venous concentration is negligible. If this be so, then equation (1) can be rewritten

$$Fl_{pl.} \cdot C_{art.} = C_{aq.} k_{AA} \qquad (2)$$

and the question of the validity of Linnér's hypothesis resolves itself into the question of the constancy of k_{AA}.

This question will have to remain open, since it has recently been shown (Bárány, 1953) that the drainage of the anterior chamber is, at least in part, an active process which could presumably be modified by the changes in composition of the aqueous which accompany carotid ligation, or by any change in blood supply.

SUMMARY

1. The concentration of ascorbic acid in the aqueous humour and plasma of rabbits after a unilateral ligation of the common carotid has been studied. The concentration of ascorbic acid in the aqueous humour of the ligated side was found to be approximately 20% lower than on the unligated side. The mean ratio of the concentration of ascorbic acid in the aqueous humour of the two eyes of $0{\cdot}80 \pm 0{\cdot}035$ agrees with the value of $0{\cdot}83 \pm 0{\cdot}025$ observed by Linnér (1952). This ratio has been found constant in a range of aqueous humour ascorbic concentration of 15–40 mg/100 ml.

2. On raising the concentration of ascorbic acid in the plasma the concentrations of ascorbic acid in the aqueous humour of the two eyes rose to a maximal value of $67{\cdot}7 \pm 2{\cdot}6$ and $67{\cdot}4 \pm 2{\cdot}5$ mg/100 ml.; these values do not differ significantly from that of $67{\cdot}5 \pm 2{\cdot}1$ mg/100 ml. observed in similar experiments on normal rabbits (Bárány & Langham, 1953, 1955).

3. The concentration of ascorbic acid and its transformation products, dehydroascorbic and diketogulonic acids in the aqueous humour of operated animals has been determined. The concentration of the transformation products was approximately the same in both eyes and accounted for less than 3% of the total ascorbic acid.

4. The concentration of ascorbic acid in the plasma required to produce a maximal transfer of ascorbic acid across the ciliary epithelium on the unligated side is 3–5 mg/100 ml. On the ligated side saturation of the transfer mechanism occurs at a value which exceeds 5 mg/100 ml. but is less than 7–8 mg/100 ml.

5. It is concluded that the present results support the contention of Linnér (1952) that the rate of transfer of ascorbic acid across the ciliary processes is determined by the amount reaching the site of transfer and is modified by changes in the rate of flow of blood through the eye. The use of ascorbic acid to measure the rate of blood flow cannot, however, be fully evaluated until it is known if the rate of loss of ascorbic acid in the aqueous humour is affected by the rate of transfer of ascorbic acid.

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