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THE EFFECT OF UNILATERAL COMMON CAROTID OCCLUSION AND OF ACUTE UNILATERAL PREGANGLIONIC CERVICAL SYMPATHOTOMY ON THE ANIONS OF THE AQUEOUS HUMOUR

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The demonstration (Linnér, 1952) that the relative differences in blood flow through the two eyes of the rabbit after unilateral ligation of the common carotid artery and after acute unilateral preganglionic cervical sympathotomy are paralleled by changes in the concentration of ascorbate in the aqueous humours implies that these operations have no effect on the rate of flow of aqueous humour. On the other hand, the work of Bárány (1947) and of Davson & Matchett (1951, 1953) indicated that carotid occlusion decreases, and acute sympathotomy increases, the rate of flow of aqueous humour.

In an attempt to reconcile these divergent opinions, the concentration of lactate in the aqueous humour after carotid occlusion and after sympathotomy has been studied. This metabolite is present in higher concentration in the aqueous humour than in the plasma owing to its continuous formation by the lens and cornea (Fischer, 1931; Kinsey, 1950, 1953; Langham, 1952); a decrease in the rate of renewal of the aqueous humour might therefore be expected to cause an increase in the concentration of lactate and vice versa. The effect of carotid occlusion and of sympathotomy on the lactate of the aqueous humour when the concentration of ascorbate in the plasma was above the saturation level has also been investigated.

The concentration of lactate in the anterior aqueous after carotid occlusion and after sympathotomy showed well-defined changes, the nature of which it was difficult to explain on the basis of changes in the rate of flow of aqueous. An alternative explanation supported by a study of the chloride and bicarbonate of the anterior aqueous and the lactate of the posterior aqueous was founded on differences in the rate at which substances exchanged with the plasma across the iris capillary walls. These experiments led to an extension of Linnér's original work on ascorbate.

METHODS

The rabbits used in this work were taken at random from adult stock and weighed for the most part between 2·5 and 3·5 kg. They were fed on diet 18 pellets (Association London Flour Millers) supplemented with hay, and water *ad lib.* Albinos were used when aqueous samples from the posterior chamber were required. Operative procedures were carried out some 20 hr before the aqueous samples were taken. Pentobarbitone sodium (Nembutal, Abbott Laboratories, 0·4 ml./kg) anaesthesia maintained with ethyl chloride was employed.

Where necessary the saturation level of ascorbate in the plasma was exceeded for at least 6 hr (i.e. long enough to yield steady state conditions in the eye) by a series of intraperitoneal injections of a 6% (w/v) solution of sodium ascorbate (pH 7·4).

Lactate was estimated by the method of Barker & Summerson (1941).

Sendroy (1937) was followed in the estimation of chloride.

The standard manometric technique of van Slyke was employed in the estimation of 'total CO_2'.

The pH of the aqueous was obtained by using a standard pH meter in conjunction with a small glass electrode assembly (Cambridge Instruments Ltd.).

Ascorbic acid was estimated by titration with 2:6-dichlorophenolindophenol (Mindlin & Butler, 1937).

RESULTS

Lactate. The steady-state concentrations of lactate in the aqueous humour and plasma of normal rabbits were $7{\cdot}38 \pm 0{\cdot}34$ m-equiv/kg H_2O (10) and $4{\cdot}32 \pm 0{\cdot}38$ m-equiv/kg H_2O respectively, giving a distribution ratio of $1{\cdot}79 \pm 0{\cdot}13$ (10).

The effects of carotid occlusion and of sympathotomy on the lactate concentration of the anterior aqueous are recorded in Table 1. Carotid occlusion resulted in a statistically significant fall in the concentration of lactate in the

TABLE 1. The effect of unilateral common carotid occlusion and of acute unilateral preganglionic cervical sympathotomy on the lactate concentration of the aqueous humour of the rabbit at normal and raised ascorbate levels. Also included are Linnér's values for the ascorbate of the aqueous humour. LA = lactate; AA = ascorbate. The subscripts H and C refer to the homolateral and contralateral eye respectively.

	Lactate in aqueous humour (m-equiv/kg H_2O)		Lactate Right aqueous / Left aqueous	Ascorbate (Linnér, 1952) Right aqueous / Left aqueous
Normal	$7{\cdot}36 \pm 0{\cdot}22$ (22)		$0{\cdot}99 \pm 0{\cdot}008$ (22)	$1{\cdot}00 \pm 0{\cdot}004$ (23)
	$[LA]_H$	$[LA]_C$	$[LA]_H/[LA]_C$	$[AA]_H/[AA]_C$
Unilateral carotid occlusion	$7{\cdot}46 \pm 0{\cdot}28$ (22)	$6{\cdot}60 \pm 0{\cdot}25$ (22)*	$1{\cdot}13 \pm 0{\cdot}022$ (22)*	$0{\cdot}83 \pm 0{\cdot}01$ (86)*
Unilateral carotid occlusion and saturation with sodium ascorbate	$7{\cdot}75 \pm 0{\cdot}35$ (19)	$7{\cdot}54 \pm 0{\cdot}31$ (19)	$1{\cdot}03 \pm 0{\cdot}016$ (19)	$1{\cdot}01 \pm 0{\cdot}008$ (19)
Unilateral preganglionic cervical sympathotomy	$6{\cdot}05 \pm 0{\cdot}175$ (22)*	$6{\cdot}88 \pm 0{\cdot}18$ (22)	$0{\cdot}87 \pm 0{\cdot}012$ (22)*	$1{\cdot}11 \pm 0{\cdot}01$ (42)*
Unilateral preganglionic cervical sympathotomy and saturation with sodium ascorbate	$7{\cdot}17 \pm 0{\cdot}20$ (18)	$7{\cdot}28 \pm 0{\cdot}22$ (18)	$0{\cdot}99 \pm 0{\cdot}012$ (18)	$1{\cdot}01 \pm 0{\cdot}01$ (18)

* Significantly different from the normal value (i.e. $P < 0{\cdot}05$).

aqueous humour of the contralateral eye ($P < 0{\cdot}01$). The homolateral eye was normal in this respect. After sympathotomy the concentration of lactate in the homolateral aqueous decreased ($P < 0{\cdot}01$), while the concentration of lactate in the contralateral aqueous was unaltered ($0{\cdot}1 < P < 0{\cdot}2$). Mock operations caused no changes in the concentration of lactate in the aqueous humour.

Linnér (1952) found that the difference in concentrations of ascorbate in the two eyes, caused by carotid occlusion or sympathotomy, vanished if the animal was saturated with ascorbic acid. The effects of this treatment on the concentrations of lactate in the two eyes of operated animals are also shown in Table 1; it will be seen that, as with ascorbic acid, the difference between the two eyes disappeared.

TABLE 2. The effect of unilateral carotid occlusion and of unilateral preganglionic cervical sympathotomy on the concentration of lactate in the aqueous humour of the anterior and posterior chambers

	Anterior aqueous (m-equiv/kg H_2O)			Posterior aqueous (m-equiv/kg H_2O)		
Normal (13) expts.	$9{\cdot}68 \pm 0{\cdot}36$		Homolateral side	$8{\cdot}77 \pm 0{\cdot}30$		Homolateral side
	Homolateral side	Contralateral side	Contralateral side	Homolateral side	Contralateral side	Contralateral side
Unilateral carotid occlusion (16 expts.)	$9{\cdot}42 \pm 0{\cdot}48$	$8{\cdot}59 \pm 0{\cdot}46$	$1{\cdot}10 \pm 0{\cdot}02$	$9{\cdot}01 \pm 0{\cdot}54$	$8{\cdot}83 \pm 0{\cdot}54$	$1{\cdot}02 \pm 0{\cdot}03$
Unilateral preganglionic cervical sympathotomy (14 expts.)	$8{\cdot}26 \pm 0{\cdot}33$	$9{\cdot}47 \pm 0{\cdot}41$	$0{\cdot}88 \pm 0{\cdot}02$	$8{\cdot}24 \pm 0{\cdot}37$	$8{\cdot}62 \pm 0{\cdot}41$	$0{\cdot}96 \pm 0{\cdot}02$

In Table 2 the effects of the operative procedures on the concentrations of lactate in the aqueous humour taken from both the anterior and posterior chambers of the eye are shown; the results indicate that it was essentially the aqueous humour in the anterior chamber that was affected by carotid occlusion or sympathotomy.

The values for the concentration of lactate in the aqueous humour reported in Table 2 are considerably higher than those reported in Table 1. The principal reason for this is probably that the animals used to obtain the values in Table 2 were not fully grown and weighed only about 1·7 kg. Since the lens of the young animal is more actively glycolytic than that of the adult (Bellows, 1944, p. 218) a higher concentration of lactate might be expected in the aqueous humour of the young animal.

Neither carotid occlusion nor sympathotomy had any effect on the lactate concentration in the lens which was found to be $12{\cdot}41 \pm 0{\cdot}74$ m-equiv/kg wet tissue.

Chloride. The normal concentration of chloride in the aqueous humour was found to be 109 ± 1 m-equiv/kg H_2O; neither carotid occlusion nor sympathotomy had any effect on the concentration in either eye.

Bicarbonate. The proportion of the 'total CO_2' in the aqueous humour present as bicarbonate depends on the pH of the fluid and the first dissociation constant of carbonic acid. The latter may be assumed to be unaffected by the operations; the former was shown to be unchanged, a value of 7·68 being recorded. In these circumstances the ratio of the 'total CO_2' in the fluids may be taken as the ratio of the concentrations of bicarbonate. The results of the operative procedures on normal and ascorbic acid saturated animals are shown in Table 3. Carotid occlusion produces a definite difference between the two

TABLE 3. The effect of unilateral carotid occlusion and of acute unilateral preganglionic cervical sympathotomy at normal and raised plasma ascorbate levels on the total CO_2 of the aqueous humour of the rabbit

	Total CO_2 (m-mole/kg H_2O)		Right eye / Left eye
Normal	$32{\cdot}3 \pm 0{\cdot}8$ (10)		$1{\cdot}00 \pm 0{\cdot}01$ (10)
	Homolateral eye	Contralateral eye	Homolateral eye / Contralateral eye
Unilateral carotid occlusion	$34{\cdot}3 \pm 0{\cdot}9$ (14)	$31{\cdot}0 \pm 0{\cdot}7$ (15)	$1{\cdot}07 \pm 0{\cdot}02$ (12)
Unilateral carotid occlusion and saturation with sodium ascorbate	$29{\cdot}5 \pm 1{\cdot}1$ (14)	$27{\cdot}6 \pm 0{\cdot}9$ (17)	$1{\cdot}06 \pm 0{\cdot}03$ (14)
Unilateral preganglionic cervical sympathotomy	$32{\cdot}3 \pm 1{\cdot}0$ (13)	$33{\cdot}5 \pm 0{\cdot}9$ (13)	$0{\cdot}96 \pm 0{\cdot}01$ (13)
Unilateral preganglionic cervical sympathotomy and saturation with sodium ascorbate	$28{\cdot}7 \pm 1{\cdot}4$ (12)	$30{\cdot}7 \pm 1{\cdot}3$ (12)	$0{\cdot}94 \pm 0{\cdot}01$ (12)

eyes, the concentration being higher on the side of occlusion; sympathotomy has the reverse effect. Saturation of the animal with ascorbate is without influence. Although these experiments establish the existence of a difference in concentration between the two eyes it is not possible to state with any certainty whether the change is in the homolateral or the contralateral eye.

Ascorbate. The concentration of ascorbate in the aqueous humour of the rabbit is a very variable quantity. For this reason absolute concentrations have been avoided wherever possible in this section. In the normal rabbit there is the same concentration of ascorbate in the anterior aqueous of the two eyes (Linnér, 1952; Langham, 1955). This result has been confirmed incidentally in the course of the present work; it was also shown that this identity applied also to the posterior aqueous. The mean ratio between the eyes was $0{\cdot}99 \pm 0{\cdot}05$ (15).

The results of carotid occlusion and of sympathotomy on the ascorbate concentration of the fluids of the posterior and anterior chambers are presented in Table 4. It is seen that the ratio of the concentrations in the posterior chamber fluids was the same as that in the anterior chamber fluids.

In the second group of experiments in this section an examination of the concentration of ascorbate of the anterior and posterior chamber fluids at normal and elevated plasma levels was made. Kinsey (1953) reported the ratio of the concentration of ascorbate in the anterior aqueous to that in the posterior as 0·75 with a range from 0·53 to 1·17. The present author found the ratio to be 0·82 ± 0·03 (20) derived from a value of 1·30 ± 0·10 m-equiv/kg H_2O (20) in the anterior aqueous and 1·58 ± 0·10 m-equiv/kg H_2O (20) in the posterior aqueous.

TABLE 4. The effect of unilateral carotid occlusion and unilateral preganglionic cervical sympathotomy on the concentration of ascorbate of the aqueous humour in the anterior and posterior chambers of the rabbit eye. (AA = ascorbate, the suffixes H and C refer to the homolateral and contralateral eyes respectively)

	$[AA]_H/[AA]_C$		Posterior aqueous—anterior aqueous (m-equiv/kg H_2O)	
	Anterior aqueous	Posterior aqueous	Homolateral side	Contralateral side
Unilateral carotid occlusion	0·82 ± 0·02 (14)	0·83 ± 0·04 (13)	0·36 ± 0·06 (13)	0·50 ± 0·09 (13)
Unilateral preganglionic cervical sympathotomy	1·12 ± 0·02 (13)	1·15 ± 0·02 (13)	0·69 ± 0·08 (13)	0·57 ± 0·09 (13)

After a 6 hr period of saturation with sodium ascorbate the ratio rose to 0·95 ± 0·02 (16). The absolute values were 3·36 ± 0·19 m-equiv/kg H_2O (16) for the anterior aqueous and 3·52 ± 0·19 m-equiv/kg H_2O (16) for the posterior aqueous. The increase in the ratio after saturation is largely a reflexion of the higher absolute values from which it is derived. More significance is to be attached to the observation that the loss of ascorbate from the anterior chamber by diffusion was slightly greater in the normal animal than in the saturated animal.

DISCUSSION

The concentration of lactate in the aqueous humour is determined by four main factors:

(i) Influx in the primary secretion from the ciliary body, presumably in a concentration comparable with that in the plasma.

(ii) Liberation from the metabolizing tissues, in particular the lens.

(iii) Outflow in bulk from the anterior chamber through the canal of Schlemm.

(iv) Diffusion, principally from the anterior chamber into the vessels of the iris.

An important consideration in deciding which of these factors is involved in the lactate changes is the finding that such changes are confined to the anterior chamber fluid, the concentration of lactate in the posterior aqueous remaining at the normal level (Table 2). If exactly compensating changes be rejected, it eliminates factor (i), casts strong doubts on the implication of factor (iii) (since

any changes in the rate of flow of aqueous humour might be expected to be discernible in the posterior chamber) and suggests that there are no changes in the rate of lactate production by the tissues adjacent to the posterior chamber. The results are, however, consistent with the view that carotid occlusion and sympathotomy increase the ease with which lactate diffuses into the vessels of the iris, carotid occlusion exerting its effect on the contralateral eye and sympathotomy on the homolateral eye.

The same explanation may be applied to the effects on the steady-state distributions of the other anions studied. Thus bicarbonate is present in higher concentration in the aqueous than in the plasma (Kinsey, 1953; Lee, 1955); an increased facility for diffusion across the iris capillary walls should therefore result in a lowered concentration at steady-state so that carotid occlusion may be expected to cause the ratio Concentration on occluded side/Concentration on non-occluded side to become greater than unity. This has been found. The concentration of chloride in the aqueous humour of the rabbit is normally rather less than that in the plasma (Davson, Matchett & Roberts, 1952), the difference is so small, however, that we should not expect any significant change in the steady-state level on altering the facility for diffusion out of the anterior chamber, and this, too, has been found. In the case of ascorbate, which is secreted across the blood-aqueous barrier, the effects of the increased facility of diffusion are masked by the difference in concentrations of ascorbate in the aqueous humours imposed by the unequal blood supply to the two eyes (Linnér, 1952). However, reference to Table 4 will show that the difference between the concentration of ascorbate in the posterior chamber fluid and that in the anterior chamber fluid (i.e. that amount which may be considered as having diffused out of the anterior chamber) is greater in the contralateral eye after carotid occlusion and in the homolateral after sympathotomy. Thus a single hypothesis will suffice to explain the changes found at normal plasma ascorbate levels.

The underlying reason for the difference between the eyes with respect to the ease with which the anions diffuse into the iris vessels is probably to be found in vascular changes consequent upon carotid occlusion and sympathotomy. It is generally accepted that unilateral carotid ligation in the rabbit gives rise to differences between the eyes in both blood flow and pressure. Similar interocular differences may be expected to follow unilateral preganglionic sympathotomy since the sympathetic innervation of the eye is wholly vaso-constrictive (Duke-Elder, 1932). It is therefore suggested that the improved conditions of exchange between the anterior aqueous and the plasma are to be associated with the comparative hyperaemia of the iris of the affected eye. It is not possible to say whether the effect is due primarily to differences in blood pressure or in the rate of blood flow, since both may be expected to be present in hyperaemia.

Direct measurements have been made of the rate of penetration of substances into the aqueous humour after carotid occlusion (Davson & Matchett, 1951, 1953). The results were not consistent with the hypothesis that 'penetration from the iris consisted of a flow, i.e. an ultrafiltration', but the movement of solutes across a membrane can occur independently of the movement of water. Thus, although the iris may be of little importance for the transfer of fluid it could, nevertheless, be important in the diffusional exchange of solutes between the anterior aqueous and the blood. Kinsey's observations (1953) on the composition and osmotic pressure of the posterior and anterior chamber fluids lend support to this point of view.

The fact that saturating the animal with ascorbate abolishes the effects of the operative procedures so far as the differences in lactate and ascorbate are concerned, but leaves them unchanged so far as the bicarbonate changes are concerned, is not easy to explain. Ascorbic acid apparently plays an important part in the economy of the extracellular components of the capillary wall (Reid, 1943; Reppert, Donegan & Hines, 1951) which dominate the permeability characteristics of the capillary endothelium (Chambers & Zweifach, 1947). It is possible then that saturation with ascorbic acid maintains the capillary permeability at a minimal value (cf. Krogh, 1929, p. 315). This could explain the lactate results. In the case of ascorbate the disappearance of the effects of the operative procedures after saturation is probably partly due to the saturation of the transfer mechanism and partly to a reduced permeability of the capillary wall to ascorbate. The latter effect is demonstrated by the finding that the difference between the concentrations of ascorbate in the posterior and anterior chamber fluids is less after saturation (in the otherwise normal animal) than at normal plasma ascorbate levels, in spite of a steeper concentration gradient from aqueous to plasma in the saturated animal. However, such a hypothesis leaves the behaviour of the bicarbonate ion unexplained unless one postulates that bicarbonate uses a predominantly intracellular route in its passage across the capillary wall.

Finally, what remains to be said of Linnér's proposal to use ascorbic acid as a test substance to measure relative changes in the rate of blood flow through the ciliary processes? First, there can be little doubt that Linnér's arguments apply strictly only to the posterior aqueous. His use of them in connexion with the anterior aqueous involves the assumption that the ascorbate concentration of the posterior aqueous is in no way modified during its sojourn in the anterior chamber, an assumption it would be difficult to sustain. However, an examination of the ascorbate concentration of the posterior aqueous after carotid ligation and after sympathetic section has shown that the posterior chambers yield the same ratio as the anterior chambers. This identity is probably fortuitous but in any case highly convenient, for the anterior aqueous is more readily accessible and abundant than the posterior aqueous.

Secondly, Linnér's treatment of the problem and its subsequent re-examination from a slightly different point of view by Langham (1955) have turned largely on the question of whether or not there are post-operative changes in the rate of flow of aqueous. The question remains unanswered in so far as the crucial experiment has not yet been performed, but the evidence presented in this work, albeit circumstantial, speaks in favour of there being no change in the aqueous flow rate after carotid ligation or sympathotomy.

SUMMARY

1. The effect of unilateral common carotid occlusion and of acute unilateral preganglionic cervical sympathotomy on the principal anions of the aqueous humour of the rabbit have been studied with a view to examining further the proposal (Linnér, 1952) to use ascorbic acid as a test substance to measure relative differences in the rate of blood flow through the ciliary processes.

2. There was a relative increase in the lactate and bicarbonate of the anterior aqueous of the homolateral eye after carotid occlusion and of the anterior aqueous of the contralateral eye after sympathotomy. The operations were without effect on the concentration of chloride in the aqueous.

3. The concentration of lactate in the lens was unaltered.

4. The concentration of lactate in the aqueous humour of the posterior chamber was not affected by the operations.

5. Post-operative saturation with sodium ascorbate eliminated the changes in lactate concentration but had no effect on the bicarbonate changes.

6. The relative changes in the concentration of ascorbate in the posterior aqueous after carotid occlusion and after sympathotomy were found to be numerically equal to those found by Linnér (1952) in the ascorbate concentration of the anterior aqueous.

7. Saturation with sodium ascorbate was shown to decrease the permeability of the iris capillaries to the ascorbate ion.

8. The conclusions drawn were:

(i) That it was unlikely that carotid occlusion or sympathotomy had any marked effect on the rate of flow of aqueous humour.

(ii) That one of the effects of carotid occlusion and of sympathotomy is an interocular difference in the conditions governing the exchange of substances between the anterior aqueous and the plasma.

(iii) That, when the concentration of ascorbate in the plasma is constant, a relative change in the concentrations of ascorbate in the aqueous humours is indicative of a similar change in the rate of blood flow through the ciliary processes.

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