Biochem. J. (1962) 85, 332

Fatty Acid Composition of Lipids Present in Different Parts of the Ox Eye

By W. BARTLEY, RUTH VAN HEYNINGEN, BRENDA M. NOTTON AND A. RENSHAW
Department of Biochemistry, University of Oxford and Nuffield Laboratory of Ophthalmology, University of Oxford

(*Received* 25 *April* 1962)

There have been several studies on the lipids of the eye (see Pirie & van Heyningen, 1956) but little on the fatty acid composition. Each tissue of the eye has a specialized function which is reflected in its chemical make-up. This paper shows, as expected, that each tissue of the eye has a different fatty acid content both in amount and composition.

MATERIALS AND METHODS

Cattle eyes were obtained from the abattoir, stored in ice and dissected as soon as possible. The eyes were from calves 2–14 days old or from oxen 1·5–3 years old. Sexes were equally divided and the breeds were mixed. No evidence was found in this study that these variables affected the fatty acids of the eye. Weighed samples of the tissues were saponified in 5 ml. of a solution containing 20 ml. of 10N-KOH, 50 ml. of methanol, 0·1 g. of quinol and 30 ml. of water. Saponification was allowed to proceed for 3 days at 30°. The extraction of the fatty acids, their methylation and the separation of the methyl esters on the gas chromatograph were as described by Getz & Bartley (1961).

The approximate quantities of material in one cattle eye are as follows: aqueous humour, 2 g.; lens, 2 g.; ciliary body plus iris, 0·38 g.; retina, 0·58 g.; choroid, 1 g.; sclera, 8 g.; vitreous body, 18 g.; corneal stroma, 0·55 g.; corneal epithelium, 0·06 g. About 0·5 g. of each tissue was usually sufficient to give a reliable estimate of total ester present. With the vitreous body, 43 ml. was evaporated to dryness and saponified. The analysis of lens capsule was on material taken from about twenty-five cow lenses. The capsules were scraped free from epithelial tissue.

The identification of acids was mainly by comparison with known acids run on the gas chromatograph. In some cases bromination or hydrogenation was used to confirm that a particular acid was unsaturated. We have found the use of the 'carbon number' a very convenient and reliable index of the degree of unsaturation of a fatty acid (Woodford & van Gent, 1960).

RESULTS

Comparison of the fatty acids of brain, retina, optic nerve and sciatic nerve. Table 1 compares the fatty acid profiles of retina and optic nerve with those found by Baker (1961) for human brain and sciatic nerve and that found by Biran & Bartley (1961) for rat brain. The two conducting tissues were similar in their high content of unsaturated acids (optic nerve 61·9 % and sciatic nerve 59·8 %), which consisted mainly of oleic acid. Optic nerve had a higher content of polyunsaturated fatty acid than had sciatic nerve (13·8 % compared with 8·6 %) but the distribution of the polyenes was quite different. In spite of the differences from sciatic nerve, the fatty acid composition of optic nerve was closer to that of sciatic nerve than it was to brain. This is illustrated by the very high content of oleic acid in both nerves.

The fatty acid profile of retina resembles that of brain cortex more than that of whole brain. Thus both cortex and retina have a lower proportion of oleic acid (22 % and 17·3 %) than whole brain (32·6 %). Both cortex and retina have a much higher content of C_{20} and C_{22} polyenes than whole brain. Retina is distinguished by having up to 30 % (mean value 22·9 % from three determinations) of a single long-chain polyunsaturated fatty acid (see also Hörhammer, Wagner & Wolff, 1960). This on hydrogenation gave behenic acid. The position on the gas chromatograph is consistent with a C_{22} hexaene. This type of fatty acid has been described in the phospholipids extracted from mitochondria and mitochondrial particles (Macfarlane, Gray & Wheeldon, 1960; Bartley, Getz, Notton & Renshaw, 1962; Getz, Bartley, Stirpe, Notton & Renshaw, 1962). Mammalian retinal tissue is rich in mitochondria (Sjöstrand, 1953).

Comparison of the fatty acids of lens capsule, corneal stroma and sclera. These three tissues are similar in that they are all tough structures composed mainly of collagen or collagen-like protein. The pattern of the fatty acids in the tissues is also somewhat similar, particularly in the main acids (Table 2), but the relative content of stearic acid was comparatively low in the lens capsule.

Fatty acids of the corneal epithelium. The corneal epithelium is characterized by its very high proportion of oleic acid and its overall high content of unsaturated fatty acids (69·8 %), the highest proportion of any tissue of the eye (Table 3). It has been shown (Cogan & Kuwabara, 1955; Hill, Kinoshita & Kuwabara, 1959) that rabbit cornea

in vitro will produce fat (sudanophilic) globules when incubated with serum and oleic acid.

Fatty acids of aqueous humour and vitreous body. The aqueous humour contains a higher concentration of fatty acids than the vitreous body (0·068 μmole/g. wet wt. compared with 0·02 μmole/g. wet wt.) (Table 4). The acid profiles are also quite different. The vitreous body contained more un-

Table 1. *Comparison of fatty acids of brain, optic nerve, sciatic nerve and retina*

The samples of ox optic nerve (one sample) and ox retina (mean from three analyses) analysed contained 76·2 and 22·6 μmoles of fatty acid/g. wet wt. of tissue respectively. The values for rat brain are abstracted from Biran & Bartley (1961) and for the human tissues from Baker (1961). 22–(1–4) indicates all C_{22} acids with from 1 to 4 double bonds, and 22–(5, 6) C_{22} acids having 5 or 6 double bonds. Values are the percentage of the total area of chromatogram peaks that was occupied by each individual peak.

Fatty acid	No. of C atoms and double bonds	Percentage of each fatty acid: Whole rat brain	Whole human brain	Human cortex	Ox retina	Ox optic nerve	Human sciatic nerve
Myristic acid	14–0	—	—	—	1·1	1·3	4·2
	15–1	—	—	—	—	2·6	—
Palmitic acid	16–0	23·9	24·0	28·7	24·9	21·3	19·2
Palmitoleic acid	16–1	1·3	3·2	—	1·8	1·0	8·3
	16–2	—	—	—	—	—	1·5
	17–1	—	—	—	1·2	2·5	—
	17–2	—	—	—	—	2·5	—
Stearic acid	18–0	22·8	23·0	15·5	17·3	16·3	5·0
Oleic acid	18–1	30·0	32·6	22·0	17·3	40·7	49·7
Linoleic acid	18–2	1·4	—	3·1	—	—	8·6
	20–1	3·7	3·9	5·9	—	3·1	—
Arachidonic acid	20–4	9·5	6·5	6·0	7·9	1·8	—
	22–(1–4)	—	3·6	6·4	—	2·6	—
	22–(5, 6)	7·6	2·0	11·8	22·9	2·2	—
Percentage of total unsaturated acids	—	53·3	51·8	55·2	51·1	59·0	68·1
Percentage of total polyunsaturated acids	—	18·4	12·1	27·3	30·8	9·1	10·1

Table 2. *Comparison of the fatty acids of lens capsule, corneal stroma and sclera*

The corneal stroma contained 2·94 and the sclera 3·39 μmoles of fatty acid/g. wet wt. of tissue. Insufficient lens capsule was available to obtain accurate values for the fatty acid content. The percentages of fatty acids were calculated as in Table 1.

Fatty acid	No. of C atoms and double bonds	Percentage of each fatty acid: Lens capsule	Corneal stroma	Sclera
Myristic acid	14–0	4·0	3·7	2·3
	14–1	1·3	—	—
	15–0	—	2·3	—
	15–1	—	—	2·2
Palmitic acid	16–0	30·8	24·4	25·7
	16–1	7·4	6·5	1·2
	17–1	—	2·3	1·3
Stearic acid	18–0	9·7	22·2	18·5
Oleic acid	18–1	34·4	31·3	33·4
Linoleic acid	18–2	—	—	3·4
	20–1	—	2·1	—
Arachidonic acid	20–4	2·6	—	6·2
	20–6	5·7	3·0	1·1
	24–2	—	1·0	1·3
Percentage of total unsaturated acids	—	51·4	46·2	50·1

saturated fatty acid than the aqueous humour (61 % compared with 49·5 %). Particularly striking is the large proportion of linoleic acid in the vitreous body, almost three times that in the aqueous humour. Oleic acid is also present in the vitreous body in twice the proportion present in the aqueous humour. On the other hand, the aqueous humour has a higher proportion of polyenes of chain length C_{20} and greater than the vitreous body.

Fatty acids of the ciliary body plus iris and of the choroid. These structures are all highly vascularized pigmented tissues and have similar fatty acid profiles (Table 5). However, the choroid has a higher content of fatty acids than the ciliary body plus iris (8·8 μmoles/g. wet wt. compared with 4·8 μmoles/g. wet wt.). The main differences are that myristic acid was not detected in the choroid and acids, presumably a C_{20} triene and a C_{20} diene, appear in the ciliary body plus iris but not in the choroid. On the other hand, a C_{24} monoene appears in the choroid only. The rabbit iris contains a long-chain unsaturated hydroxy fatty acid (Ambache, 1957, 1959), extractable by water without saponification.

Fatty acids of the lens. Table 6 gives the mean values and range for the fatty acid content of six individual lenses. Minor components less than 1 % of the total fatty acids are not listed. The lens is a tissue having a very low oxygen uptake and no blood circulation and thus is in complete contrast with the retina. Although the lens contains about the same proportion of unsaturated fatty acids as the retina, the main unsaturated fatty acid is

Table 3. *Fatty acids of the corneal epithelium*

Insufficient material was available to make an accurate quantitative determination of the fatty acid content. The percentages of fatty acids were calculated as in Table 1.

Fatty acid	No. of C atoms and double bonds	Percentage of each fatty acid
Myristic acid	14–0	1·7
	14–1	1·2
Palmitic acid	16–0	13·7
Palmitoleic acid	16–1	5·7
	17–1	1·1
	17–2	4·9
Stearic acid	18–0	9·3
Oleic acid	18–1	51·3
Arachidonic acid	20–4	2·8
	*24–2	1·0
	*24–6	1·8
Percentage of total unsaturated acids	—	69·8

* Indicates tentative indentification.

Table 4. *Fatty acids of vitreous body and aqueous humour*

The aqueous humour contained 0·068 and vitreous body 0·02 μmole of fatty acid/ml. respectively. The percentages of fatty acids were calculated as in Table 1.

Fatty acid	No. of C atoms and double bonds	Percentage of each fatty acid: Aqueous humour	Percentage of each fatty acid: Vitreous body
	12–0	—	0·4
Myristic acid	14–0	1·3	2·5
	14–1	—	0·4
	15–0	—	5·8
	15–1	—	2·0
Palmitic acid	16–0	33·8	20·9
Palmitoleic acid	16–1	1·6	5·9
	17–1	—	1·7
	17–2	—	1·3
Stearic acid	18–0	15·4	12·0
Oleic acid	18–1	9·9	16·9
Linoleic acid	18–2	7·5	19·9
	20–0	—	1·8
	20–2	1·2	—
Arachidonic acid	20–4	11·4	5·3
	*21–1	10·6	—
	*21–2	5·1	3·5
Percentage of total unsaturated acids	—	47·3	56·9

* Indicates tentative identification.

Table 5. *Fatty acids of the ciliary body plus iris and of the choroid*

The ciliary body plus iris contained 4·82 and the choroid 8·75 μmoles of fatty acid/g. wet wt. respectively. The percentages of fatty acids were calculated as in Table 1.

Fatty acid	No. of C atoms and double bonds	Percentage of each fatty acid: Ciliary body + iris	Percentage of each fatty acid: Choroid
Myristic acid	14–0	1·0	—
	15–1	1·8	1·4
Palmitic acid	16–0	28·3	22·8
Palmitoleic acid	16–1	4·4	1·0
	17–1	1·4	1·4
Stearic acid	18–0	13·1	15·2
Oleic acid	18–1	28·7	23·0
Linoleic acid	18–2	4·4	5·7
	*20–3	1·4	—
Arachidonic acid	20–4	9·8	13·0
	22–1	1·0	1·3
	24–1	—	2·9
	24–2	1·2	6·7
	24–3	1·3	—
Percentage of total unsaturated acids	—	55·4	56·4

* Indicates tentative identification.

Table 6. *Fatty acids of the lens*

Results were obtained from the analysis of six lenses. The mean content of fatty acid of the lens was 3·16 μmoles/g. fresh wt. (range 2·06–3·83). The percentages of fatty acids were calculated as in Table 1.

Fatty acid	No. of C atoms and double bonds	Percentage of each fatty acid	
		Mean	Range
Myristic acid	14–0	3·08	2·38–3·64
Palmitic acid	16–0	35·4	33·5–39·3
Palmitoleic acid	16–1	4·5	2·95–5·4
	16–3	1·48	1·08–1·89
	17–1	5·7	4·6–7·2
Stearic acid	18–0	4·3	3·2–4·9
Oleic acid	18–1	36·5	33·3–41·7
	20–1	1·4	1·14–1·87
Arachidonic acid	20–4	3·48	2·28–5·04
Percentage of unsaturated acid	—	53·06	—

oleic acid in about the same proportion as that found in the optic nerve. Only about 7 % of the fatty acids of the lens were polyenes whereas 30 % of the retinal fatty acids contained more than one double bond. In both retina and lens linoleic acid is virtually absent. Although it might be expected from the common embryological origin that the corneal epithelium and the lens would have a similar pattern of fatty acids, there is no marked similarity in the mature tissues (contrast Tables 2 and 6).

DISCUSSION

The fatty acids of the eye are found mostly in the form of phospholipid (see, for example, Krause, 1934; d'Asaro, Young & Williams, 1954; Brolin, 1958). Our values for the fatty acids are about half those of d'Asaro *et al.* (1954) for lens and corneal stroma and about twice their value for retina. It is clear from the amounts of phospholipid found in various tissues of the eye (Krause, 1934; Brolin, 1958) and our measurements of the amounts of fatty acids that much of the phospholipid must be in the form of plasmalogen. It would be expected from the two sets of measurements that some 50 % of the phospholipids would be in this form. Aldehydes were measured only in the retina where they were present in somewhat less amounts than the fatty acid (aldehydes 13·7 μmoles/g. fresh wt., acids 12·6 μmoles/g. fresh wt.), but Tronche & Mandel (1958) have detected acetal phosphatides in the lens. Our values for the fatty acid content of the various tissues of the eye expressed as mg./g. dry wt. are as follows: retina, 48; lens, 2·8; optic nerve, 74; corneal stroma, 5·3; sclera, 3·7; aqueous humour, 1·82; vitreous body, 5·3; ciliary body plus iris, 7·8; choroid, 12·5.

The fatty acid patterns of the tissues of the eye are characteristic for each tissue. The only measurements of individual fatty acids of the eye so far known to us are those of Hörhammer *et al.* (1960) on the pig retina. Our values are in essential agreement with theirs.

SUMMARY

1. The fatty acid patterns of the different tissues of the eye (retina, lens, optic nerve, lens capsule, corneal stroma, sclera, corneal epithelium, aqueous humour, vitreous body, ciliary body plus iris, and choroid) have been measured.

2. Optic nerve had the highest content of fatty acids (76·2 μmoles/g. wet wt.) whereas retina had 22·6 μmoles/g. wet wt. The other tissues ranged between 8·8 μmoles/g. wet wt. (choroid) and 0·02 μmole/g. wet wt. (vitreous body). Insufficient amounts of corneal epithelium and lens capsule were available to estimate the quantity of fatty acids they contained.

3. The retina was characterized by the high content of a C_{22} polyunsaturated acid (up to 30 % of the total fatty acid). About 50 % of the total fatty acids of the eye were unsaturated.

REFERENCES

Ambache, N. (1957). *J. Physiol.* **135**, 114.
Ambache, N. (1959). *J. Physiol.* **146**, 255.
Baker, R. W. R. (1961). *Biochem. J.* **79**, 642.
Bartley, W., Getz, G. S., Notton, B. M. & Renshaw, A. (1962). *Biochem. J.* **82**, 540.
Biran, L. A. & Bartley, W. (1961). *Biochem. J.* **79**, 159.
Brolin, S. E. (1958). *Acta chem. scand.* **12**, 110.
Cogan, D. G. & Kuwabara, T. (1955). *Arch. Path.* **59**, 453.
d'Asaro, B. S., Young, R. G. & Williams, H. H. (1954). *A.M.A. Arch. Ophthal.* **51**, 596.
Getz, G. S. & Bartley, W. (1961). *Biochem. J.* **78**, 307.
Getz, G. S., Bartley, W., Stirpe, F., Notton, B. M. & Renshaw, A. (1962). *Biochem. J.* **83**, 181.
Hill, K., Kinoshita, J. H. & Kuwabara, T. (1959). *A.M.A. Arch. Ophthal.* **61**, 361.
Hörhammer, L., Wagner, H. & Wolff, P. (1960). *Hoppe-Seyl. Z.* **319**, 7.
Krause, A. C. (1934). *The Biochemistry of the Eye.* Baltimore: Johns Hopkins Press.
Macfarlane, M. G., Gray, G. M. & Wheeldon, L. W. (1960). *Biochem. J.* **77**, 626.
Pirie, A. & van Heyningen, R. (1956). *Biochemistry of the Eye.* Oxford: Blackwell.
Sjöstrand, F. S. (1953). *J. cell. comp. Physiol.* **42**, 45.
Tronche, P. & Mandel, P. (1958). *Acta XVIII Concilium ophthalmologicum belgica*, **1**, 699.
Woodford, F. P. & van Gent, C. M. (1960). *J. Lipid Res.* **1**, 188.