the somewhat high manganese content in the tissues of the intestinal tract might similarly be attributed to manganese present as part of the peptidase system.

SUMMARY

1. The manganese contents of rabbit tissues have been determined, and the results compared with those obtained by previous workers.

2. The range covered by the results is narrow,

especially as some of the tissues relatively rich in manganese contain much of it in dialysable form.

3. It is suggested that manganese participates in general cell metabolism while attached to the cell-wall protein.

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Manganese in Eye Tissues

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As part of a programme of research on the biochemistry of vision the manganese contents of the eye tissues of the ox have been determined. The only similar study appears to be that of Tauber & Krause (1943), who used an extension of the periodate method (Wiese & Johnson, 1938) on samples obtained by taking tissues from the eyes of a large number of oxen. The method used here is the modified catalytic method (Fore & Morton, 1952a); it is sufficiently sensitive to be applicable to the tissues from single eyes.

EXPERIMENTAL

Left and right eyes from each of two oxen were studied separately. After removing muscle, etc., attached to the sclera, each eye was dissected using carefully cleaned stainless-steel instruments. Separation of the tissues was complete except perhaps for the conjunctiva, and minute fragments of iris tissue were sometimes present in the sample of vitreous humour. The optic nerve was cut out of the sclera, leaving no nerve tissue in the sclera sample, from which choroid pigment was carefully removed. The cornea, lens, sclera, optic nerve and conjunctiva were washed with glass-distilled water and touched on filter paper (Whatman no. 50) before weighing. The retina, choroid and the vitreous and aqueous humours were placed directly into crucibles.

The iris, which adheres to the delicate membrane retaining the vitreous humour, was touched on filter paper to remove traces of vitreous humour.

In a later experiment a retinal homogenate was prepared from about forty ox retinas, toluene added and the brei dialysed against changing glass-distilled water. The Mn contents of samples removed from the cellophan bag at 70 and 180 hr. were determined by the modified catalytic method.

RESULTS

The results of the manganese determinations of the eye tissues are given in Tables 1 and 2. In Tables 3–5 the tissues are arranged in order of decreasing manganese content alongside the values obtained by Tauber & Krause, who did not examine the aqueous humour nor the contents on an ash-weight basis.

The results of the dialysis experiment are given in Table 6. The weight of the homogenate used for dialysis expressed as dry weight (*) was calculated using the percentage dry weight (not given) obtained for two samples of the homogenate. Then, from the manganese content of these samples and the calculated dry weight, the absolute amount of manganese in the homogenate used for the dialysis (†) was obtained.

Table 1. *Experimental data on ox eye tissues*

Tissue	First experiment				Second experiment			
	Wt. of fresh tissue (g.)	Dry matter (%)	Ash on fresh tissue (%)	Mn from tissue (μ mg.)	Wt. of fresh tissue (g.)	Dry matter (%)	Ash on fresh tissue (%)	Mn from tissue (μ mg.)
Conjunctiva	1.10*	20	0.39	286	0.169	19	1.0	21.0
	0.601*	30	0.55	164	0.085	19	0.83	19.6
Cornea	0.723	18	0.57	33.4	0.666	22	0.95	26.3
	0.788	18	0.72	Lost	0.644	23	0.89	27.8
Aqueous humour	1.25	1.3	0.85	12.8	1.62	1.2	0.94	21.8
	0.937	1.3	0.86	14.3	1.63	1.2	0.88	15.7
Iris	0.678	16	0.77	71.0	0.860	18	0.92	113
	0.821	14	0.93	104	0.838	18	1.2	91.0
Lens	2.54	34	0.68	31.6	2.41	35	0.70	16.7
	2.39	40	0.73	29.1	2.36	36	0.71	24.8
Vitreous humour	13.8	1.2	0.86	110	16.8	1.1	0.81	83.5
	12.2	1.3	0.87	163	16.2	1.1	0.82	104
Retina	0.624	11	0.87	112	0.760	12	0.90	76.8
	0.656	11	0.93	91.5	0.715	13	1.0	71.7
Choroid	0.514	18	1.07	69.1	0.480	21	1.3	49.8
	0.429	18	1.21	57.8	0.445	20	1.4	45.3
Sclera	5.15	27	0.48	675	5.58	30	0.43	1010
	5.00	29	0.54	1130	5.42	30	0.53	920
Optic nerve	0.463	28	1.06	36.8	0.639	28	1.2	9.0
	0.399	32	1.15	39.8	0.962	27	1.2	12.9

* Possibly contaminated with sclera.

Table 2. *Manganese and ash contents of ox eye tissues determined in the present work and compared with the findings of Tauber & Krause (1943)*

(Figures in brackets are the extreme values for individual determinations.)

Tissue	Ash on fresh tissue (mean) (%)	Manganese (p.p.m.) of				Ash
		Fresh tissue		Dried tissue		
		Present work	Tauber & Krause	Present work	Tauber & Krause	
Conjunctiva	0.70	0.23 (0.13-0.27)	0.10 (0.069-0.11)	1.0 (0.68-1.3)	0.28 (0.23-0.37)	39 (12-67)
Cornea	0.80	0.044 (0.04-0.046)	0.11 (0.094-0.13)	0.24 (0.18-0.29)	0.44 (0.37-0.49)	6.4 (4.2-8.2)
Aqueous humour	0.89	0.012 (0.010-0.015)	—	0.98 (0.78-1.2)	—	1.5 (1.1-1.8)
Iris	0.93	0.12 (0.11-0.13)	0.24 (0.21-0.29)	0.73 (0.62-0.89)	0.92 (0.79-1.2)	13 (9-14)
Lens	0.71	0.010 (0.007-0.012)	0.18 (0.14-0.22)	0.031 (0.02-0.037)	0.52 (0.41-0.63)	1.5 (1-1.8)
Vitreous humour	0.85	0.008 (0.005-0.013)	0.031 (0.019-0.042)	0.69 (0.44-1.1)	1.5 (0.93-2.1)	0.95 (0.6-1.5)
Retina	0.93	0.13 (0.10-0.18)	0.32 (0.24-0.39)	1.1 (0.8-1.6)	2.6 (2.0-3.2)	15 (10-21)
Choroid	1.2	0.12 (0.10-0.14)	0.21 (0.17-0.32)	0.63 (0.50-0.76)	0.71 (0.59-1.1)	9.9 (5.6-13)
Sclera	0.50	0.18 (0.13-0.23)	0.09 (0.078-0.13)	0.61 (0.48-0.77)	0.32 (0.25-0.42)	36 (27-43)
Optic nerve	1.1	0.052 (0.013-0.10)	0.26 (0.22-0.31)	0.18 (0.049-0.31)	1.0 (0.83-1.2)	4.7 (1.1-8.7)

Table 3. *Order of decreasing manganese content (ox eyes) based on fresh tissue*

First ox		Second ox		Tauber & Krause	
Tissue	p.p.m.	Tissue	p.p.m.	Tissue	p.p.m.
Conjunctiva	0.27	Conjunctiva	0.18	Retina	0.32
Sclera	0.18	Sclera	0.18	Optic nerve	0.26
Retina	0.16	Iris	0.12	Iris	0.24
Choroid	0.14	Retina	0.10	Choroid	0.21
Iris	0.12	Choroid	0.10	Lens	0.18
Optic nerve	0.090	Cornea	0.041	Cornea	0.11
Cornea	0.046	Optic nerve	0.014	Conjunctiva	0.099
Aqueous humour	0.012	Aqueous humour	0.012	Sclera	0.090
Lens	0.012	Lens	0.009	Aqueous humour	—
Vitreous humour	0.011	Vitreous humour	0.006	Vitreous humour	0.031

Table 4. *Order of decreasing manganese content based on dried tissue*

First ox		Second ox		Tauber & Krause	
Tissue	p.p.m.	Tissue	p.p.m.	Tissue	p.p.m.
Retina	1.4	Aqueous humour	0.95	Retina	2.6
Conjunctiva	1.1	Conjunctiva	0.94	Aqueous humour	—
Aqueous humour	1.0	Retina	0.82	Vitreous humour	1.5
Vitreous humour	0.87	Iris	0.67	Optic nerve	1.0
Iris	0.78	Sclera	0.59	Iris	0.92
Choroid	0.75	Vitreous humour	0.50	Choroid	0.71
Sclera	0.62	Choroid	0.50	Lens	0.52
Optic nerve	0.30	Cornea	0.19	Cornea	0.44
Cornea	0.29	Optic nerve	0.050	Sclera	0.32
Lens	0.036	Lens	0.025	Conjunctiva	0.28

Table 5. *Order of decreasing manganese content (ox eye tissues) based on ash*

First ox		Second ox	
Tissue	p.p.m.	Tissue	p.p.m.
Conjunctiva	58	Sclera	37
Sclera	34	Conjunctiva	20
Retina	18	Iris	12
Iris	14	Retina	11
Choroid	12	Choroid	6.7
Cornea	8.2	Cornea	4.5
Optic nerve	8.1	Aqueous humour	1.4
Lens	1.8	Optic nerve	1.2
Aqueous humour	1.5	Lens	1.1
Vitreous humour	1.2	Vitreous humour	0.70

Table 6. *Dialysis of a retina homogenate prepared from ox eyes*

Sample	Sample (%)	Dry wt. (g.)	Ash wt. (mg.)	Mn (μ mg.)	Mn content (p.p.m.) in	
					Dry tissue	Ash
Homogenate portions used for control	—	0.186	12.8	248	1.33	19.4
	—	0.174	12.2	183	1.05	15.0
To dialysis	—	2.23*	—	2650†	—	—
70 hr. samples	27	0.534	10.0	783	1.47	78.3
	28	0.521	8.1	909	1.74	112
180 hr. samples	23	0.393	6.8	799	2.03	117
	22	0.368	4.8	689	1.88	143
Totals	100	—	—	3180	—	—

* Estimated dry weight of homogenate used for dialysis.

† Mn content of homogenate used for dialysis calculated from analysis of portion used for control.

DISCUSSION

As both eyes in an animal receive the same blood supply a difference between the manganese contents of a given tissue from the two eyes is not to be expected. Such differences were, however, observed, and in one or two cases were probably real and not due to experimental error. For the majority of the tissues the mean values for each of the two animals showed greater differences. In no case where a different value is given (Table 3) for the two oxen did the values for the two eyes from each overlap.

This apparent difference between individual oxen cannot be used to explain the poor agreement between the values obtained in this laboratory and those of Tauber & Krause. With the exceptions of the sclera and conjunctiva the values obtained by the American workers are several times larger. The values for the conjunctiva given in the present paper are to be considered with caution as there is some doubt as to whether conjunctiva alone was included in the samples.

In regard to which of the fresh tissues contain the greatest concentrations of manganese there is fair agreement with the American results if the sclera and conjunctiva be omitted.

The retina appeared to be one of the richest tissues with respect to manganese and, as this tissue was also the site of the chemical visual processes, fractionation was attempted. When dialysis was applied to retinal homogenate more manganese was recovered in the non-dialysable material than was present (by calculation) in the original homogenate. No explanation of this can be offered since rigorous

precautions against contamination were taken. However, it appears from the experiment that little of the manganese in the retina is dialysable.

The manganese in retina appeared to be attached to the retinal tissue and the latter was therefore fractionated, using a well defined technique in use in this laboratory, to give preparations containing most of the rods, the photoreceptors of scotopic vision. There appeared to be a smaller concentration of manganese in the rods than in the remainder of the retina. Hence there is so far no evidence that the manganese present in the retina is selectively or specifically concerned with visual biochemistry. The functions of manganese present in the eye tissues may well be displayed at other sites in the body (see Fore & Morton, 1952b, on rabbit tissues).

SUMMARY

1. The manganese contents of the tissues of single ox eyes have been determined.
2. The results have been compared with those of Tauber & Krause (1943) obtained using large samples from several hundred ox eyes.
3. Preliminary experiments on the fractionation of retinas have indicated that manganese in this tissue is non-dialysable and not specifically concerned in the visual process.

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The Determination and Distribution of Phosphocreatine in Animal Tissues

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Phosphocreatine has been described for over 20 years and methods for its determination in tissues generally depend upon the estimation of the phosphorus moiety which can be split off readily in acid solution. A much-used technique is that described by LePage & Umbreit (1945), whereby the inorganic P is determined by the method of Fiske & Subbarow (1925) after its precipitation from an alkaline solu-

tion as a barium or calcium salt. The difference between this value and that determined by the same method prior to precipitation, is assumed to give the phosphocreatine P. This method, which lacks the sensitivity desirable if low phosphocreatine concentrations are present, has the further disadvantage that its accuracy depends on the quantitative precipitation of inorganic P under the recom-