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Comparative Investigations of Catalase Activity in Different Ocular Tissues of Cattle and Man

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Abstract. As previously published, ocular catalase activity was measured by Warburg's respirometer, the examined material being taken from the local slaughterhouse and keratoplasty-donor eyes. The results were given in µl O_2 per mg soluble protein (Biuret's solution) and showed an extensive parallelism between human and bovine eyes: They were in a decreasing order of catalase activity, i.e., conjunctiva > retina > vitreous > sclera > iris > choroid > cornea > aqueous humor > serum > lens. In the latter, catalase activity was a sensitive indicator of aerobic metabolism. Oxidative processes in the vitreous merit more consideration.

Zusammenfassung. In der gleichen Weise wie früher veröffentlicht, ließ sich die Katalaseaktivität mit Hilfe des Warburg-Respirometers messen. Das untersuchte Material entstammte bovinen Oculi aus dem städtischen Schlachthof und Keratoplastik-Spender-Bulbi. Die Ergebnisse in µl O_2 pro mg lösliches Protein (Biuret) zeigten eine weitgehende Parallelität zwischen menschlichen und bovinen Augen; sie lauteten in abnehmender Reihenfolge: Bindehaut > Netzhaut > Glaskörper > Sklera > Iris > Chorioidea > Hornhaut > Kammerwasser > Serum > Linse. In letzterer erwies sich die Catalase als empfindlicher Indikator für den aeroben Stoffwechsel, wie es aus der Bakteriologie bekannt ist. Daneben verdienen oxydative Vorgänge im Glaskörper mehr Beachtung.

Previous results (Mayer, 1969) have shown that lens epithelium represents a normally respiring tissue with a respiratory quotient of 1.04, its metabolic needs being mainly supplied by glycolysis, and in tissue culture oxygen consumption of lens epithelium remains at a constant level until death in the fortieth subculture (Mayer and Sames, unpublished results).

Catalase activity has proved to be independent of human age but showed to be correlated with the degree of cataract (Mayer and Schmidt-Götz, 1977). This correlation was reproducible in incubated calf lenses (Mayer, 1970). In

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discussions of ocular catalase function (Theorell, 1948; Chance, 1961) the enzyme always seems linked to aerobic metabolism: Bacteriologists have long been aware of this fact (Mayer and Zimmermann, 1962; Mayer, 1962).

These results suggested measuring catalase activity in different tissues of bovine eyes and comparing them to equivalent human tissues.

Material and Methods

As previously published, catalase activity was measured by Warburg's respirometer. The examined material was taken from the local slaughterhouse and keratoplasty-donor eyes. Soluble protein was obtained by Biuret's solution and evaluated photometrically.

Results

Table 1 gives the measured data in µl O_2 per mg soluble protein in human eyes. It seemed striking that catalase was almost as active in the vitreous as in the iris, but these results are not statistically significant.

In examining bovine eyes, larger sample pools were compared, as can be seen in Table 2. The activity of conjunctiva showed a conspicuously high level (373) while the activity in the vitreous was higher than that in the uvea. The

Table 1. µl O_2 per mg protein in different ocular tissues. n = Number of samples

Man		
Lens	3.6 ± 0.7	(n=8)
Iris	64 ±20	(n=2)
Vitreous	58 ±18	(n=2)
Retina	14.5 ± 0.5	(n=2)
Serum	25 ± 4	(n=99)

Table 2. µl O_2 per mg protein in different ocular tissues
n = Number of samples

Cattle		
Conjunctiva	373 ±20	(n=3, pool of 5)
Sclera	144 ±20	(n=5, pool of 5)
Cornea	46 ±15	(n=8, pool of 5)
Aqueous humour	23 ±15	(n=3, pool of 10)
Total lens	3.4 ± 1	(n=3, pool of 7)
Iris	37 ±20	(n=3, pool of 3)
Uvea	42 ±20	(n=3, pool of 3)
Vitreous	132.7 ±40	(n=40)
Retina	140 ±30	(n=3, pool of 3)
Serum		
Calf	4.0 ± 1	(n=3)
Cow	0.15 ± 1	(n=3)

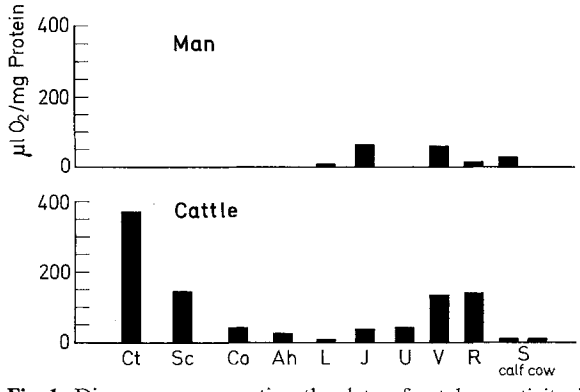

Fig. 1. Diagramm representing the data of catalase activity in ocular tissues of man and cattle. *ct* conjunctiva, *sc* sclera, *co* cornea, *ah* aqueous humour, *l* lens, *i* iris, *u* choroid, *v* vitreous, *r* retina, *s* serum. The amount for human serum is taken from a publication by Kirsch and Burmeister, 1966

other data show a decreasing order of activity: retina; sclera; cornea; aqueous humor; serum; lens. Figure 1 is a diagram comparing the results of Tables 1 and 2.

In tissues represented by only three single values, each sample was extracted from a pool of several specimens of the same tissue taken from different eyes; but the pigmented tissues produced some problems. While measuring catalase in the Warburg's respirometer was easy, the photometric evaluation of soluble protein was not. Decoloration of the pigment by H_2O_2 before adding Biuret's solution was not possible without denaturation of the color reaction. In these cases the extraction of the soluble protein by Biuret's solution was done in spite of the pigment granules, the latter being centrifuged and the supernatant examined by Eppendorf's photometer.

Discussion

Other enzymatic investigations have shown extensive parallelism between human and bovine eyes, similar to the results above, so that it would seem reasonable to compare them using other cytological and enzymatic investigations. Individual differences are current, even to the extent that Takahara (1952) described nine cases of progressive oral gangrene due to an absence of blood catalase (acatalasemia).

All the above data concerning the lens agrees with that generally known (Klethi and Nordmann, 1975; Friedburg and Moog, 1967; Reim et al., 1976; Hockwin et al., 1974; Iwata et al., 1976). Kinoshita and Merola (1973) have described how incubated rabbit lenses become opaque within a few hours after the addition of cyanide because of a direct alteration of lens protein. However, an inactivation of catalase might also have been possible due to the sensitivity of the trivalent iron nucleus to cyanide, the iron content of the lens nucleus having been described by Gerhard and Calmé (1976). Hoffmann and Wurster

(1974) found a similar enzymatic pattern in the vitreous as in other connective tissues and conclude that there might be a minimal but many-sided vitreous metabolism. Considering all the above findings, oxygen metabolism of vitreous seems to be noteworthy. Jakobi and Driest (1965) described an augmentation of vitreal oxygen consumption from the center to the cortex and a prepapillary maximum in the latter. This supports the conclusion that the vitreous sustains a differentiated oxydative metabolism.

Until now comparative data have only been published by Bhuyan et al. (1973). They found slightly more catalase in lens epithelium than in corneal epithelium, and both values were about one-fiftieth of those values measured in the blood. Titrimetrical examination by the method of Bonnichsen et al. (1947) showed catalase activity of the following tissues in decreasing order: Lens capsule with adherent epithelium; corneal epithelium; corneal endothelium; vitreous; lens fibers; corneal stroma; and aqueous humor. In the latter three the amount was about zero. These data confirm those of Reim et al. (1976), who examined peroxidase in eyes of cattle and rabbits. The measured activities were in decreasing order: Corneal epithelium; conjunctiva; retina; and lens. The fact that redox systems are important for the status of the hyaluronic acid molecule in the vitreous has been demonstrated by Schmut and Hoffman (1976), catalase inhibiting the reduction of its viscosity by substances such as $FeSO_4$, ascorbate, and cystein. It, therefore, is supposed, that H_2O_2 and free radicals may originate in reactions, which might alter the hyaluronic acid molecule (Laser, 1952; v. Pirie, 1965; Varma et al., 1977).

In summary it can be said that catalase seems to be of importance in the oxidative metabolism of ocular tissues, and that oxidative processes in the vitreous merit more consideration. This has already been demonstrated in the lens, where catalase activity has been shown to be a sensitive indicator of aerobic metabolism. Any disturbance of the latter seems linked to cataractogenesis, as shown in earlier experiments. This agrees with the results of Bhuyan et al. (1973). Where catalase activity was reduced to 39% of he normal value after application of the herbicide and cataractogenic poison amizol. The finding of Kinoshita and Merola (1973) also showed that cyanide inhibited catalase and made the lens opaque. These results emphasize the thesis that catalase is a very important lens enzyme.

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