the light emission. The high quantum yield of bioluminescence requires a protection from collisional deactivation. This could be achieved by a spacial arrangement in which the active centre is not accessible to the large enzyme molecule.

The observation reported in this study should also be considered of practical relevance for the employment of the firefly bioluminescence as an analytical tool for the determination of very small quantities of ATP, ADP, creatine phosphate and other energy-rich organic phosphates in biological extracts. Since the extracts may contain appreciable amounts of compounds (e.g. azide, cysteine, mercaptoethanol, glutathione, alcohols) which interfer with the light emission, these assays should be interpreted with caution unless the appropriate controls have been performed.

Carbohydrate composition of the human cataractous lenses

J. F. Alao[1,2]

College of Medicine, University of Lagos, Biochemistry Department, P. M. B. 12003, Lagos (Nigeria), 15 July 1976

Summary. Specific chemical assays, including carbohydrate, hexosamines and hexuronic acid, were determined on the lens insoluble albumoid. It was noticed that the carbohydrate composition varies with age. The significance of carbohydrate in the lens is discussed.

The structural composition of the insoluble albumoid of the human cataractous lenses has not been clearly defined. It has been hypothesized[3] that cataract formation is associated with the conversion of low molecular weight protein to high molecular weight aggregates, the heaviest of which corresponds to the insoluble protein fraction. Various authorities[4,5] have suggested that the presence of high molecular protein aggregates in the lens can lead to increased scattering of light as a consequence of loss of transparency. Others[6–8] indicated that calcium or glucose may influence the aggregate state of the subunits of α-crystallin protein. The carbohydrate composition of the insoluble albumoid in the human cataractous lens is mostly unknown. The present investigation is concerned with the pattern and composition of the carbohydrate in cataractous lenses removed at surgery.

Materials and methods. Human lenses were obtained from patients following cataract operation, usually within 2 h of removal. 8 human cataractous lenses were used in each age group. The procedure for extracting insoluble albumoid and its subsequent treatment with urea has been fully described[9].

Hexuronic acid. Hexuronic acid was determined by a modification of Bitter and Muir[10] of the method of Dische[11]. Hexosamine. Weighed samples in 1 ml were hydrolysed in vacuo in sealed tubes at 110 °C. Total hexosamine was determined by the method of Muir and Jacobs[12] and the distillation procedure of Cessi and Peliego[13]. Hexose. The procedure was that described by Grant and Jackson[14] using Dowex 50 (H^+ form) resin hydrolysis. After hydrolysis neutral sugar and amino sugars were separated according to the method of Anastassiadis and Common[15].

Table 1. Carbohydrate composition (quantitative). The neutral sugar content of the urea extracts USF, UIF_A and UIF_B from the human cataractous lenses aged 20–91 years were estimated by the orcinol sulphuric acid reagent method of Tillman and Phillip[18]. Glucose was used to plot a calibration curve

No.	Average age of patient	Protein USF (%)	UIF_A (%)	UIF_B (%)
1	22 ± 2 years	ND	1.00	0.975
2	38.5 ± 0.5 years	0.800	1.208	0.980
3	43 ± 2 years	0.700	1.182	1.00
4	53 ± 3 years	0.500	1.396	0.900
5	65 ± 2 years	0.698	1.533	1.100
6	74 ± 4 years	0.425	1.428	1.108
7	82 ± 2 years	0.500	1.864	1.621
8	90.5 ± 0.5 years	0.500	2.118	1.968
9	Adult bovine	0.799	1.150	0.969

ND, not detectable.

Table 2. Distribution of carbohydrate in the insoluble lens extract of human cataractous lenses, average age 20–90 years; using paper chromatography

Protein	Age (years)	Galactose	Glucose	Mannose	Fucose	Xylose
USF	20	+	+	ND	ND	ND
	30	+	++	ND	ND	Trace
	40	+	++	ND	ND	Trace
	50	+	++	ND	ND	ND
	60	+	++	ND	ND	Trace
	70	+	++	ND	ND	ND
	80	+	++	ND	Trace	ND
	90	+	++	ND	ND	ND
UIF_A	20	+	+++	++	+	+
	30	+	+++	++	++	+
	40	++	+++	++	++	+
	50	++	+++	++	++	+
	60	++	++++	++	++	+
	70	++	++++	+++	++	+
	80	++	++++	+++	++	+
	90	++	+++++	++++	++	+
UIF_B	20	+	++	+	ND	+
	30	+	++	+	ND	+
	40	+	++	+	ND	+
	50	+	++	++	ND	+
	60	+	++	++	Trace	+
	70	+	++	++	+	+
	80	+	+++	++	+	+
	90	+	+++	++	++	++

+ Indicates the relative intensity of the spot on the chromatogram after dipping in sodium thiosulphate; quantitative value for + 8%. ND, not detectable.

Table 3. Total amino sugar (hexosamine). The hexosamine (amino sugar) content of the urea extracts USF, UIF_A and UIF_B from the human cataractous lenses aged 20–91 were determined. Glucosamine hydrochloride was used to plot a calibration curve

No.	Average age of patient	Protein USF (%)	UIF_A (%)	UIF_B (%)
1	22 ± 2 years	ND	2.967	ND
2	38.5 ± 0.5 years	ND	4.100	0.868
3	43 ± 2 years	ND	3.000	1.068
4	53 ± 3 years	ND	4.800	0.890
5	65 ± 2 years	ND	5.300	0.986
6	74 ± 4 years	ND	6.000	1.000
7	82 ± 2 years	ND	4.814	1.160
8	90.5 ± 0.5 years	ND	6.240	0.615
9	Adult bovine	ND	4.40	ND

ND, not detectable.

Paper chromatography was performed on Whatman No. 1 chromatography paper, using the method of Gaillard[16] and the spraying system of Patridge[17].

Results and discussion. The percentage composition of carbohydrate in the urea extract (table 1) is not constant; it varies considerably. Comparatively the percentage composition of carbohydrate in the urea-soluble fraction (USF) decreases with age, whereas that of the urea-insoluble fractions (i.e. UIF_A, UIF_B) increases. The carbohydrates identified by paper chromatography (table 2) are galactose, glucose, mannose, fucose and xylose. The predominant sugar units are glucose and mannose; the relation of these forms of carbohydrate to the albumoid and their stability is not yet established. However, the presence of glucose and galactose in the urea-soluble fraction (USF) (table 1) is of considerable interest. The absence of fucose and mannose, and the occurrence of trace amounts of xylose, tend to suggest that one of the functions of the glucose is to induce aggregation of the (USF) molecule. The UIF_A and UIF_B also contain some amino sugar (table 3); this indicates that the urea-insoluble fractions contain some glycosamino glycan linked to a protein, a type of glycoprotein or proteoglycan.

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3 S. Zigman, J. Schultz and T. Yulo, Exp. Eye Res. *10*, 58 (1970).
4 G. B. Benedek, Appl. Optics *10*, 459 (1971).
5 S. G. Waley, in: The Eye, vol. 1, p. 308. Acad. Press, New York and London 1969.
6 J. A. Jedziniak, J. H. Kinoshita, E. M. Yates, L. O. Hocker and G. B. Benedek: Invest. Ophthal. *11*, 905 (1972).
7 A. Spector, T. Freuidt, L. K. Li and R. C. Augusteyn, Invest. Ophthal. *10*, 677 (1971).
8 A. Spector, R. Augusteyn, L. K. Li, A. Schneider and T. Frend, Biochem. J. *124*, 337 (1971).
9 J. F. Alao, Ph. D. Thesis, University of Salford, Salford, England (1975).
10 T. Bitter and H. Muir, Analyt. Biochem. *4*, 330 (1962).
11 Z. Dische, J. biol. Chem. *167*, 189 (1974).
12 J. Muir and S. Jacobs, Biochem. J. *103*, 367 (1967).
13 L. Cessi and F. Peliago, Biochem. J. *7*, 508 (1960).
14 M. E. Grant and D. S. Jackson, Biochem. J. *108*, 559 (1968).
15 R. A. Anastassiadis and R. H. Common, Can. J. Biochem. Physiol. *36*, 413 (1958).
16 B. D. E. Gaillard, Nature, Lond. *171*, 1160 (1953).
17 S. M. Patridge, Nature, Lond. *164*, 443 (1949).
18 J. Tillman and K. Phillip, Biochem. Z. *15*, 326 (1929).

The effect of thermal injury on plasma carnitine in rats

E. L. Van Alstyne, Celia D. Sladek and D. C. Smith

Department of Biological Sciences, State University College, Brockport (New York 14420, USA), 7 December 1976

Summary. The plasma concentration of L-carnitine in scalded rats was determined to be greater ($p \leq 0.05$) than that of control rats at 6 h following the administration of a 20 % body surface, full-thickness burn produced by scalding in a 100 °C water bath for 15 sec.

L-carnitine is essential for the transport of activated long-chain fatty acids into mitochondria[1]. Following a burn, carnitine may play a crucial role in the ability of tissues to oxidize fat. In this study, the plasma carnitine values of scalded rats suggest an increased release of carnitine by the liver or a decreased utilization of carnitine in fatty acid metabolism.

Methods. Hair was removed from the dorsum of anesthetized (5 mg/100 g sodium pentobarbitol) male Sprague-Dawley rats weighing 190–220 g. 20% of the body surface was scalded by partial immersion in a 100 °C water bath for 15 sec[2]. Immediately following injury each animal received 5 ml of a 0.9% sterile saline solution i.p. Blood samples were collected from the animal's tails. Carnitine was determined by a modification of the method described by Cederblad and Lindstedt[3].

Results and discussion. The effect of a 20% body surface, full-thickness scald on the plasma concentration of carnitine is shown in the figure. There is a significant ($p \leq 0.05$) post burn increase in the mean plasma carnitine of the traumatized animals with respect to that of the controls.

Increased energy requirements following burn injury are met largely by the increased utilization of fat. Fatty acid oxidation normally contributes at least one-half of the oxidative energy in heart muscle, liver, kidney and resting

1 N. R. Marquis and I. B. Fritz, J. biol. Chem. *240*, 2193 (1965).
2 H. L. Walker and A. D. Mason, Jr, J. Trauma *8*, 1049 (1968).
3 G. Cederblad and S. Lindstedt, Clin. chim. Acta *37*, 235 (1972).