

Table 3. Total amino sugar (hexosamine). The hexosamine (amino sugar) content of the urea extracts USF, UIF<sub>A</sub> and UIF<sub>B</sub> 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 <sub>A</sub> (%)	UIF <sub>B</sub> (%)
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<sup>16</sup> and the spraying system of Patridge<sup>17</sup>.

**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<sub>A</sub>, UIF<sub>B</sub>) 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<sub>A</sub> and UIF<sub>B</sub> 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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## The effect of thermal injury on plasma carnitine in rats

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**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<sup>1</sup>. 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.

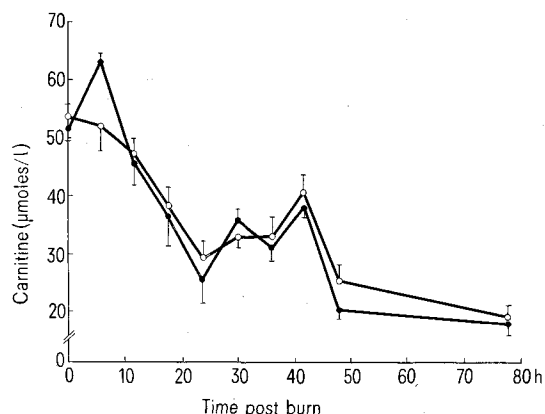
**Methods.** Hair was removed from the dorsum of anesthetized (5 mg/100 g sodium pentobarbital) male Sprague-Dawley rats weighing 190–220 g. 20% of the body surface was scalded by partial immersion in a 100 °C water bath for 15 sec<sup>2</sup>. Immediately following injury each animal received 5 ml of 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<sup>3</sup>.

**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

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skeletal muscle<sup>4</sup>. In the case of a burn however, insulin resistance and glucose intolerance increase this requirement for fat. Mobilization of fatty acids from adipose tissue occurs at 24 h after a burn<sup>5</sup>. If this mobilization



Plasma L-carnitine after scalding. ○—○, control rats; ●—●, burned rats. Means of 6 animals  $\pm$  SEM.

does not occur prior to this time then the increase in plasma carnitine at 6 h post burn may be due to an inadequate supply of fatty acids in the blood for the utilization of available carnitine.

A refractory period appears to exist for up to 24 h following injury in rats receiving a 20% body surface scald for 30 sec in an 83°C water bath<sup>6</sup>. During this period there is a decreased utilization of oxygen and energy substrates accompanied by a drop in colon temperature. Therefore, an alternate possibility is that the increase in carnitine at 6 h post burn may be a manifestation of the decreased oxidation of fatty acids rather than occurring secondary to a deficiency of plasma free fatty acids as mentioned above.

Subsequent to the 6 h interval, the fluctuations observed in plasma carnitine from one sampling interval to the next may represent a carnitine biorhythm since both control and experimental groups appear to follow the same patterns.

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## Galactosyltransferase of *Neurospora*<sup>1</sup>

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**Summary.** An enzyme, galactosyltransferase, able to catalyze the formation of galactose polymers was detected in cell-free extracts of a wild type strain of *Neurospora crassa*. Enzyme activity was found in both the supernatant and the particle fractions after centrifugation at 100,000  $\times$  g. The enzyme assayed in the 100,000  $\times$  g supernatant showed a 4fold difference in specific activity as compared to that found in the particle fraction.

**Galactosyltransferase in *Neurospora crassa*.** The role of different carbohydrate polymers in determining the morphology of *Neurospora* has been reviewed<sup>2</sup>. Much evidence exist to support the hypothesis that the cell wall components, primarily carbohydrate in nature, have an important part in the determination of the morphology of *Neurospora*<sup>3</sup>. Several independent investigators have reported the presence of galactosamine-rich polymers in the cell wall of *Neurospora crassa*<sup>4,5</sup>. It has been suggested that polymers have a role in the regulation of morphogenesis and growth in *Neurospora*<sup>6</sup>. Galactose has been shown to alter the morphology of *Neurospora* when used as the sole source of carbon in a culture medium<sup>7</sup>. In such a medium the normally filamentous wild type strain grew as tight restricted colonies. In this paper we describe an enzyme (galactosyltransferase) in *Neurospora crassa* which is able to transfer galactose from UDP-galactose to a galactose polymer.

**Materials and methods.** Strain: A wild type strain of *Neurospora crassa* RL 3-8A (Rockefeller University), was used. Cultures were grown in Vogel's minimal medium<sup>8</sup> containing 2% sucrose in shaken cultures at 26°C.

**Chemicals:** Uridine diphosphate galactose-<sup>3</sup>H(N) and Aquasol were purchased from New England Nuclear Corporation. Uridine 5' diphosphogalactose and glycyglycine were purchased from Sigma Chemical Co.

**Enzyme extraction:** *Neurospora* mycelia (36–40 h cul-

tures) were harvested by filtration using Whatman 1 and ground at 4°C with twice the wet weight of sea sand. The cell lysate was suspended in 4–5 volumes of 0.025 M glycyglycine buffer pH 7.5 and centrifuged at 1000  $\times$  g for 15 min. All centrifugations were performed at 4°C. The supernatant solution was centrifuged at 100,000  $\times$  g for 1 h. This supernatant will be referred to as supernatant II. The pellet was dissolved in 5 ml of the buffer. Both supernatant II and the pellet were assayed for enzyme activity. **Assay for galactosyltransferase:** Enzyme activity was determined by incubating an appropriate volume of supernatant II or pellet (containing 1.5–2.0 mg of protein) with 0.8 ml of 0.025 M glycyglycine buffer pH 7.5, 0.4 ml of 0.1 M MnCl<sub>2</sub>, 0.05 ml of UDP-galactose-<sup>3</sup>H(0.5  $\mu$ Ci) and 0.02 ml UDP-galactose (100  $\mu$ g) at 37°C for 60 min. The reaction was stopped by chilling the mixture in an ice bath for 10 min and adding an equal volume of 95% ethanol. The precipitate was collected on glass fibre filters (Gelman, Type A, 25 mm) and washed several times with 95% ethanol. The filters were dried and placed into vials containing 8 ml of Aquasol and counted in a Beckman LS-230 liquid scintillation counter. Protein was determined by the method of Lowry et al.<sup>9</sup>. Specific activity is expressed as cpm/mg of protein.

**Determination of galactose incorporation:** The reaction products from supernatant II and the pellet were washed several times with cold 95% ethanol and the precipitate