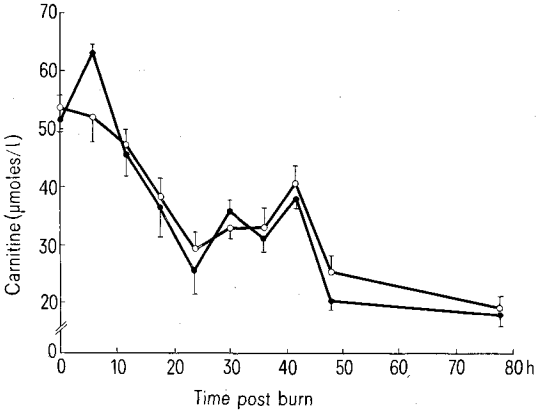
skeletal muscle[4]. In the case of a burn however, insulin resistance and glucose intolerance increase this requirement for fat. Mobilization of fatty acids from adipose tissue occurs at 24 h after a burn[5]. If this mobilization does not occur prior to this time then the increase in plasma carnitine at 6 h post burn may be due to an inadequate supply of fatty acids in the blood for the utilization of available carnitine.



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

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[6]. 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[1]

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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 × g. The enzyme assayed in the 100,000 × 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[2]. 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[3]. Several independent investigators have reported the presence of galactosamine-rich polymers in the cell wall of Neurospora crassa[4,5]. It has been suggested that polymers have a role in the regulation of morphogenesis and growth in Neurospora[6]. Galactose has been shown to alter the morphology of Neurospora when used as the sole source of carbon in a culture medium[7]. 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[8] containing 2% sucrose in shaken cultures at 26 °C.

Chemicals: Uridine diphosphate galactose-^{3}H(N) and Aquasol were purchased from New England Nuclear Corporation. Uridine 5′ diphosphogalactose and glycylglycine were purchased from Sigma Chemical Co.

Enzyme extraction: Neurospora mycelia (36–40 h cultures) 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 glycylglycine buffer pH 7.5 and centrifuged at 1000 × g for 15 min. All centrifugations were performed at 4 °C. The supernatant solution was centrifuged at 100,000 × 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 glycylglycine buffer pH 7.5, 0.4 ml of 0.1 M $MnCl_2$, 0.05 ml of UDP-galactose-^{3}H(0.5 μCi) and 0.02 ml UDP-galactose (100 μg) 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.[9]. 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

Comparison of incorporation of UDP-galactose-^{3}H in supernatant and pellet fractions and the effect of different concentrations of crude extract

	Protein (mg)	CPM
Assay mixture without crude extract	0	249
Assay mixture without UDP-galactose-^{3}H with supernatant II	4.5	99
Complete assay mixture with supernatant II	0.9	5426
	1.8	45025
	4.5	48483
	9.0	70850
Complete assay mixture with pellet	1.16	5502
	2.32	14430
	5.8	26900
	11.6	35764

Incubation was carried out as described in the methods. Each assay was brought to a constant volume with 0.025 M glycylglycine buffer pH 7.5.

was subjected to hydrolysis in 3N HCl at 100 °C for 2 h. The HCl was evaporated and the residue dissolved in 0.1 ml of H_2O. Descending chromatography was carried out on Whatman 1 paper in ethyl acetate-pyridine-water (8:2:1 v/v) for 19 h at 25 °C. The chromatogram was developed with diphenylamine-aniline[10] and 1-cm wide strips were cut into squares and counted in a Beckman LS-230 liquid scintillation counter with toluene-phosphor (Fisher). The following standard monosaccharides were used for chromatography: D-glucose, D-galactose, n-acetylglucosamine and n-acetylgalactosamine.

Results. These studies provide evidence for the presence of an enzyme in Neurospora crassa that is able to catalyze the transfer to galactose from UDP-galactose to a galactose acceptor. This enzyme will be referred to as galactosyltransferase. Incorporation of UDP-galactose-^{3}H was greater in the supernatant II than in the pellet as shown in the table. Incorporation was shown to increase in a linear manner when the amount of protein added to the assay mixture was increased. These data are presented in the table. The specific activity of the enzyme in the supernatant II fraction increased linearly with a maximum of 25,014 cpm/mg protein. The maximum activity shown in the pellet fraction was 6220 cpm/mg. Thus there was a 4fold difference in the specific activity of the 2 fractions. The enzyme showed an absolute requirement for manganese for its activity. The radioactive carbohydrate incorporated was identified by descending chromatography. All radioactivity was shown to cochromatograph with galactose in the supernatant II fraction only.

Discussion. The restricted growth exhibited by wild type Neurospora when grown in a culture medium containing galactose as the sole carbon source suggests the importance of galactose in the morphogenesis of Neurospora[7]. A possible regulatory role for galactose polymers in Neurospora morphogenesis has been previously shown[6]. However, the biochemical nature of the effect of galactose has not yet been elucidated.

The present investigations describe a galactosyltransferase that is able to effect the formation of galactose polymers in Neurospora. Further studies would be of interest in determining the precise role of galactose and galactosyltransferases in the growth and morphology of Neurospora. One approach to such future investigations would involve the ability of galactosyltransferase to confer concanavalin A agglutinability to certain cell types[11]. Concanavalin A, a lectin obtained from Jack beans, is known to agglutinate slime mutant of Neurospora[12]. This observation would offer the opportunity for a biochemical genetic analysis of galactosyltransferase by selecting for mutants of this enzyme due to their inability to agglutinate in the presence of concanavalin A.

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(Ca-Mg)ATPase activity of human erythrocyte membranes: Influence of incubation buffer[1]

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Summary. Activity of the (Ca-Mg)ATPase of human red blood cell membranes is highly dependent on the specific buffer used in the ATPase assay. Activity is highest in histidine and/or imidazole buffers and is lowest in HEPES buffer.

There is now general agreement that the (Ca-Mg)ATPase of the human erythrocyte (RBC) membrane is associated with the active transport of Ca from the cell[2]. There is less agreement, however, on the magnitude of the (Ca-Mg)-ATPase activity of seemingly comparable preparations

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