The content of nonsulfated chondroitin sulfate isomer increased with the advance of age. It should be of interest to know that the amount of the nonsulfated isomer is quite the reversed of that of chondroitin sulfate A. This may be interpreted by our previous finding that chondroitin sulfate A and chondroitin coexist independently or form dependently undersulfated chondroitin sulfate A in normal urinary AGAG[7]. There was no significant change in oversulfated chondroitin sulfate with advancing age.

These age-dependent changes of urinary AGAG in normal subjects would reflect the effect of age on the distribution of AGAG in body connective tissue. For example, KAPLAN and MEYER[13], and MATHEWS and GLAGOV[14] reported that in human cartilage chondroitin sulfate A decreased significantly with advancing age, whereas the C-isomer decreased moderately. Consequently, the ratio of the A-isomer to the C-isomer in the human cartilage decreased with the advance of age. Thus, the age-related changes of urinary AGAG, chondroitin sulfate isomers in particular, should be an important parameter with respect to age-dependent function of AGAG in body connective tissue[15].

Résumé. Le sulfate A de chondroitine est un acide glycosaminoglycanes principal chez les enfants normaux. Mais cette substance diminue avec l'age, tandis que le sulfate C de chondroïtine a une tendance à augmenter. Il en résulte une diminution, de l'action du sulfate A sur le sulfate C. La prépondérence du sulfate C de chondroïtine se manifeste après l'adolescence. Il augmente continuellement au cours de la vie.

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Changes in Density of Organelles from *Neurospora*

In the course of a study of the biochemical characteristics of the 'glyoxysome-like' particles (GLPs) isolated from *Neurospora crassa*[1], we observed that their density was affected by the growth conditions and by the isolation procedure. If overlooked, these factors might produce a cross-contamination of the organelles, leading to erroneous conclusions regarding their true enzyme content. On the other hand, such alterations might provide a valuable tool for improving the separation of the organelles and gaining information on the binding of enzymes on the particles. The purpose of this report is to assess the effect of: 1. the carbon source provided in the growth medium, 2. the tonicity of the medium in which the particles are suspended, on the mean densities of both mitochondria and GLPs.

Material and methods. Neurospora crassa (wild type, Lindegren +) is grown at 30 °C in a shaken (200 strokes/min) liquid medium[2]. Derepression of the glyoxylate cycle enzymes is achieved either by transfer to a similar basal medium containing 40 m*M* acetate as the only carbon source, or by growing the mold in a complete medium supplemented with acetate (110 m*M*) and with a decreased sucrose concentration (14 m*M*). Homogeneization is performed as described elsewhere[1], in a complex medium made 0.4 *M* in sucrose. Crude particulate pellets are obtained by 3 successive centrifugations at $500 \times g$ (10 min), $3{,}000 \times g$ (30 min) and $10{,}000 \times g$ (45 min). Even though the bulk of the mitochondria sediments at $3{,}000 \times g$, the pellet of the last centrifugation (P 3–10 K) was chosen for this study, after it was shown that the mitochondria sedimenting in both pellets exhibit the same density pattern. The P 3–10 K pellet is suspended in various concentrations of sucrose, 1 m*M* in EDTA. The suspension (10–12 mg protein) is either layered on the surface of a 32–60% (W/W) linear sucrose gradient (1 m*M* in EDTA), or layered within the gradient at a point corresponding to its own density. The isopycnic centrifugation is carried out at $100{,}000 \times g$ for 8 to 16 h in a Spinco SW 27 rotor. The gradient is collected in 1 ml fractions and enzyme activities are measured by established procedures. As a rule, isocitrate lyase (IL) and NAD isocitrate dehydrogenase (NAD IDH) were used as marker enzymes for GLPs and mitochondria respectively.

Results and discussion. The first set of experiments (Table) shows that there is a correlation between the level of IL derepression and the average density of the

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Table I. Correlation between the derepression of isocitrate lyase (IL) and the mean densities of particles isolated from *Neurospora crassa*

Growth conditions	IL activity	Density of:	
		Mitochondria	GLPs
58 m*M* sucrose	0.70	1.182	1.205
Transfer to 40 m*M* acetate, 7 h	5.00	1.184	1.210
Transfer to 40 m*M* acetate, 24 h	10.2	1.194	1.219
Mixture 14 m*M* sucrose – 110 m*M* acetate	12.0	1.205	1.215

IL activity in μmoles of L (+)-isocitrate cleaved per h and per mg protein; densities in $g \times cm^{-3}$ at 20 °C

particles. The equilibrium density of both mitochondria and GLPs is affected by the nature of the carbon source. The same growth conditions which are known to derepress the glyoxylate cycle enzymes and some of the Krebs cycle enzymes[3,4] bring about an increase of the apparent density of both organelles. We do not know of any published report demonstrating a heterogeneity of either peroxisomes or glyoxysomes. The heterogeneity of populations of rat liver mitochondria appears to be associated with an alteration of the balance between enzymes of the Krebs cycle, suggesting that an enzyme deficit may alter significantly the membrane-lipid to protein ratio of an organelle, and thus decrease its net density[5]. However, mitochondria extracted from repressed cultures of *Saccharomyces cerevisiae* are both denser and

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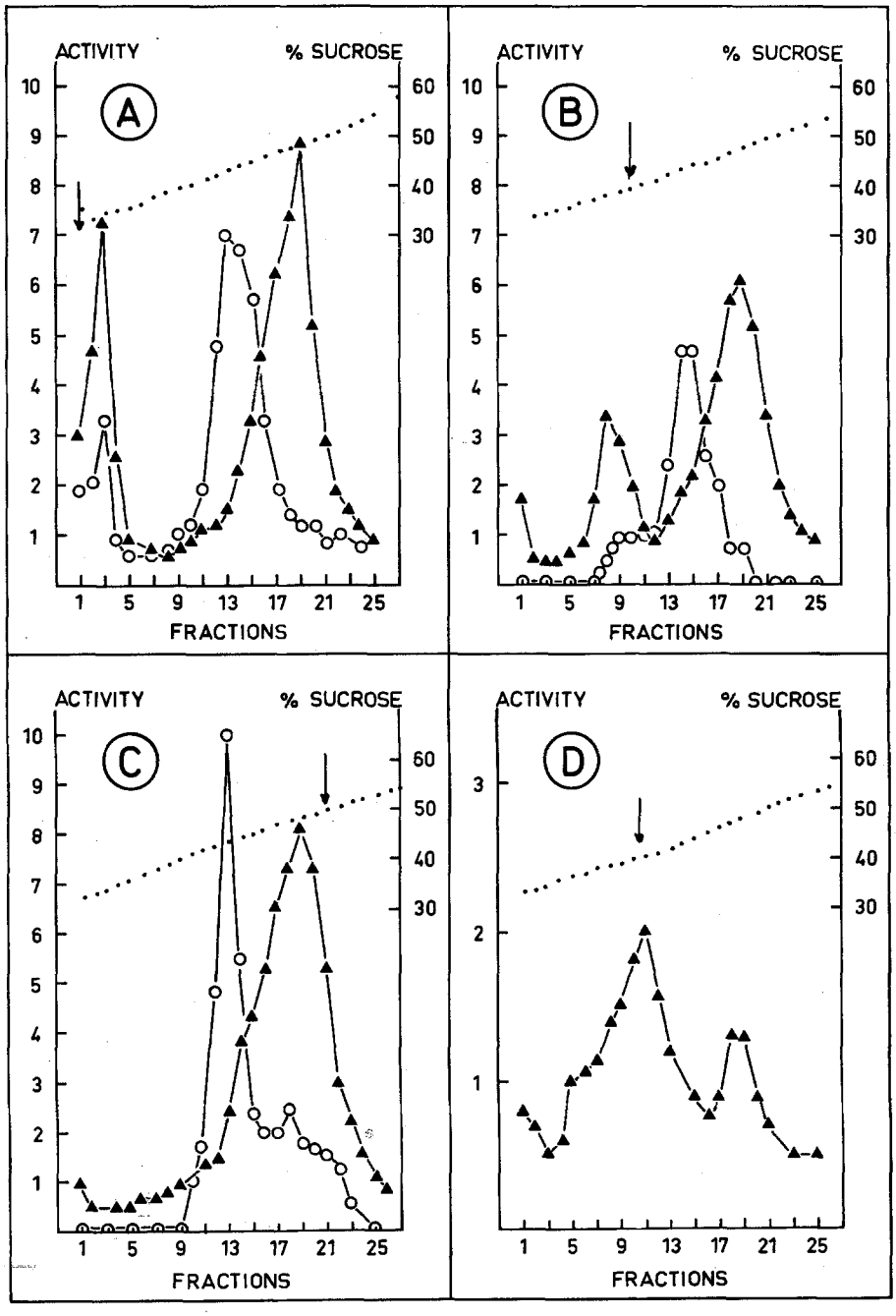

Activity profiles of isocitrate lyase (—▲—) and NAD isocitrate dehydrogenase (—○—) in sucrose gradients after isopycnic centrifugation of particulate fractions (3,000–10,000 × *g*) isolated from *Neurospora crassa*. The pellets are suspended in various concentrations of sucrose (A) 32%; (B) 40%; (C) 50%, and layered in the gradients as indicated by the arrows. Profile D: GLPs from gradient C diluted to 39.5% sucrose and layered as indicated by the arrow. Activities in μmoles of isocitrate converted per h and per fraction (1 ml); % sucrose concentration in the gradients measured at 20 °C.

larger than those isolated from cultures derepressed by ethanol[6]. This contrasts with the density pattern characterizing the mitochondria from *Neurospora*. This discrepancy results probably from the active lipid and phospholipid synthesis associated with the formation of new mitochondria triggered in the yeast by catabolite derepression[7].

The second set of experiments (Figure) shows the effect of various concentrations of sucrose on the organelles. P 3–10 K pellets were prepared from a culture derepressed by transfer to 40 m*M* acetate for 1 day. Each pellet was suspended in 32% (1.137 $g \times cm^{-3}$), 40% (1.176 $g \times cm^{-3}$) and 50% (1.230 $g \times cm^{-3}$) sucrose (1 m*M* in EDTA), and the suspensions were subjected to isopycnic centrifugation. The fractionation profile of the pellet suspended in 32% sucrose shows 2 discrete peaks of IL activity, at 1.143 $g \times cm^{-3}$ and 1.219 $g \times cm^{-3}$ respectively (Figure A). The centrifugation pattern of the pellet in 40% sucrose indicates that the 'light' peak of IL is now shifted to an apparent density of 1.169 $g \times cm^{-3}$, whereas the major peak remains unaffected (Figure B). Finally, in the gradient obtained from the pellet suspended in 50% sucrose, all of the IL activity is confined to a single peak with a mean density of 1.219 $g \times cm^{-3}$ (Figure C). Recentrifugation of this band after dilution to 39.5% (1.173 $g \times cm^{-3}$) sucrose (1 m*M* in EDTA) gives the IL profile plotted in Figure D. The previously homogeneous band is now split in two; the first one retains its original density, the second one equilibrates at 1.180 $g \times cm^{-3}$.

The value of 1.219 $g \times cm^{-3}$ represents the typical density of GLPs isolated from derepressed cultures. Treatment with lower sucrose concentrations yields an atypical population of bodies whose densities closely approximate to the density of the suspending medium. In contrast to the GLPs, the density of the mitochondrial stroma is unaffected by these change in tonicity of the medium. Regardless of the sucrose concentration, the NAD IDH peaks consistently around 1.195 $g \times cm^{-3}$. However, our results do not preclude the possibility that more subtle alterations of the mitochondria might take place, for instance a stripping of the outer membrane.

This shift of density affecting the GLPs upon dilution remained unnoticed at the time we reported that a NAD malate dehydrogenase was associated with them[1]. We now understand that this result was an artefact resulting from our purification procedure. Complete separation of GLPs from mitochondria by rate centrifugation demonstrates that the particulate malate dehydrogenase and citrate synthetase are specifically associated with the mitochondria (to be reported elsewhere).

The lability of microbodies in homogenization media is a difficulty experienced by many investigators[1,8]. From our data, it appears that GLPs exhibit a high sensitivity to the tonicity of the surrounding medium, which is apparently associated with the suppression of a permeability barrier maintaining the density characteristic of the intact particles. Once this permeability barrier is abolished, the GLPs assume the density of the surrounding medium. However, even these altered particles do not release rapidly the bulk of their enzymatic content as they do, for instance, upon aging for 3 h at room temperature, or upon treatment with TRITON X-100.

In conclusion, the apparent density of the GLPs isolated from *Neurospora* under our experimental conditions is affected by 2 independent factors: 1. the nature of the carbon source in the growth medium; 2. the tonicity of the suspending medium. The density of the mitochondria, however, is dependent only on the growth conditions.

Résumé. Des mitochondries et des particules possédant certaines caractéristiques des peroxysomes ont été isolées du *Neurospora crassa* par centrifugation isopycnique. Lorsqu'elles sont extraites d'une culture réprimée, les mitochondries ont une densité apparente de 1,182 $g \times cm^{-3}$, les particules peroxysomales, de 1,205 $g \times cm^{-3}$. Isolées d'une culture complètement déréprimée, les densités respectives sont de 1,205 $g \times cm^{-3}$ et de 1,219 $g \times cm^{-3}$. La densité apparente des particules peroxysomales, mais non celle du stroma mitochondrial, est fortement affectée par des chocs hypotoniques ménagés.

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Inhibition of Rat Testicular Monoamine Oxidase Activity after 250 R of Whole-Body X-Irradiation[1]

Monoamine oxidase, a deaminating enzyme, (MAO, Monoamine:O_2oxidoreductase(Deaminating) EC.1.4.3.4.), and endogenous 5-hydroxytryptamine (serotonin, 5-HT), a substrate of testicular MAO are both normally present in rat testes[2–6] and have been shown to undergo changes with maturation of this organ[6]. 5-HT, a radioprotective agent, is decreased in the hypothalamus[7], blood, and spleen[8], after irradiation. Increased amounts of 5-hydroxyindole acetic acid (5-HIAA) (deaminated product of 5-HT metabolism by MAO) appear in the urine[9,10] and

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