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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liver[11] after X-irradiation. Since no attempt has been made to follow testicular MAO activity after X-irradiation, we undertook these investigations to determine if irradiation alters testicular MAO activity and if so, how such changes are related to testicular function as evidenced by changes in testicular weight.

Materials and methods. 56 male rats (Holtzman strain) were irradiated with 250 R of whole-body X-irradiation (12 weeks of age), using previously described methods[12]. 56 comparable animals were sham irradiated to serve as controls. Prior to and after irradiation the aminals were housed in our small environment controlled animal laboratory with feed and water given ad libitum. 3 animals were sacrificed from both the control and the irradiated groups according to the following schedule: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 42, 52, 68, 80 and 153 days after treatment. At sacrifice the animals were weighed, killed with ether anesthesia, and their testes removed, chilled in ice-cold 0.1 *M* phosphate buffer[12], trimmed, weighed, and decapsulated.

Seminiferous tubules were subsequently teased from the interstitial cells[12], and aliquots of either teased-tubules or homogenized testicular tissue preparations were assayed for MAO activity[3]. MAO activity was measured by the amount of radioactively labeled serotonin that was converted into 5-hydroxyindole acetic acid which was then extracted into ethyl acetate, isolated by thin layer chromatography, and counted in a liquid scintillation counter. A mean comparison analysis was used to test for statistical significance between groups of animals for a given time period.

Results. Testicular MAO activity of the irradiated animals (Table) was higher than control values from 7–16 days after treatment and lower from day 19 to 31. Testicular weights significantly declined ($P < 0.05$) from day 7 to 52 (Figure 1). MAO activity of the seminiferous tubules (Figure 2) was depressed from 42–68 days after treatment. MAO activity was increased 80 days after irradiation concomitant with a significant increase in testicular weight and regeneration of the seminiferous tubules. MAO activity at 153 days after treatment was not significantly different between the two groups of animals. MAO activity of the control animals declined from 42 through 153 days after irradiation and preceded the noticeable decline in testicular weight after 152 days of age (68 days after treatment).

Discussion. The increase in testicular MAO activity in the treated animals, noted from 7 through 16 days after irradiation agrees with findings of other workers who have observed increased 5-HIAA excretion in the urine

Changes in rat homogenized whole testicular monoamine oxidase (MAO) activity at various time intervals after 250 R of X-irradiation

Treatment	Days after irradiation	Mean testicular MAO activity (C/10 m × 10^6)	*P* value
Control	1	57.20	$P > 0.50$
Irradiated	1	69.00	
Control	4	175.70	$P > 0.50$
Irradiated	4	156.50	
Control	7	158.80	$P < 0.05$[a]
Irradiated	7	259.19	
Control	10	55.70	
Irradiated		160.64	
Control	13	62.62	$P < 0.10$[a]
Irradiated	13	89.64	
Control	16	20.24	
Irradiated	16	40.90	
Control	19	50.78	$P < 0.05$[a]
Irradiated	19	19.53	
Control	22	28.06	
Irradiated	22	8.71	
Control	25	15.71	
Irradiated	25	9.58	
Control	28	22.45	
Irradiated	28	11.94	
Control	31	39.05	
Irradiated	31	11.80	
Control	42	20.07	$P > 50.0$
Irradiated	42	17.92	

[a] Group mean comparison of irradiated animals compared to controls.

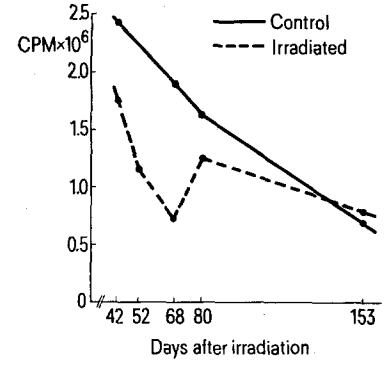

Fig. 2. Changes in Monoamine oxidase activity of seminiferous tubules after treatment of male rats with 250 R of whole-body X-irradiation expressed on a per animal basis.

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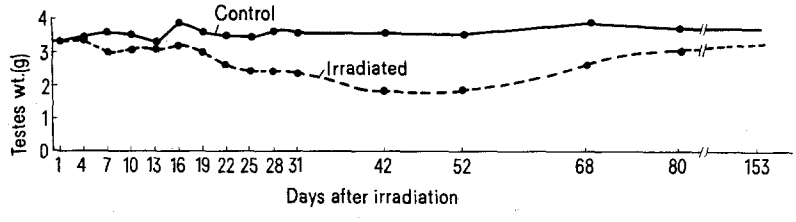

Fig. 1. Changes in testicular weight after treatment of male rats with 250 R of whole-body X-irradiation.

of rats 1 week after irradiation[9,10]. Melatonin synthesis is also elevated from 4–19 days after irradiation[12] and melatonin can elevate MAO activity in vitro[13] and in vivo[13,14]. Both melatonin synthesis and MAO activity were elevated by feed deprivation in rats[14], while androgen synthesis and testicular weights were depressed. Our data suggest that the increase in testicular MAO activity noted after irradiation could be due to an increase in melatonin production.

The decline in MAO activity noted for the seminiferous tubules of the 126–137-day-old control animals from 4 through 153 days after treatment is consistent with the decline in MAO activity of rat testes associated with senescence of this organ[6]. The increase in MAO activity noted on day 80 and the return of this activity to control levels is thought to be due to the repair process and repopulation of the germinal epithelium that was evidenced by an increased testicular weight.

These data offer additional evidence for a possible direct effect of melatonin on the testis and for a functional role of the pineal gland in the radiation syndrome confirming earlier observations from our laboratory that the pineal gland may be important in determining the extent of damage to the testis after irradiation and the influence of light and dark schedules on this phenomenon[12].

Résumé. Chez le rat l'activité de l'oxydase testiculaire monoaminée est augmentée d'une manière significative 7 à 10 jours après une irradiation-X de 250 R du corps entier. Elle s'est abaissée au-dessous de la valeur du contrôle pendant les 153 jours qui suivirent le traitement.

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Lag Period of Action of 25-Hydroxycholecalciferol on Bone Collagen Metabolism in Vitamin D Deficient Rats

Vitamin D undergoes various transformations within the body. Among its known metabolites, two compounds exhibit considerable biological activity: 25-hydroxycholecalciferol (25-HCC) and 1,25-dihydroxycholecalciferol (1,25-DHCC)[1]. Intestinal calcium absorption is more rapidly stimulated by 25-HCC than by D_3[2]. The lag of action of 1,25 DHCC on the gut is even shorter than that of 25-HCC, and 1,25-DHCC is more effective in lesser dose than either D_3 or 25-HCC[3,4]. It is less certain whether similar differences exist in Vitamin D dependant metabolic processes of bone. In bone tissue culture, the calcium mobilization of bone was unchanged when D_3 was added to the medium; it was increased following 25-HCC administration, and the effect of 1,25-DHCC exceeded that of 25-HCC[5,6]. The latter, however, was more than twice as active as the former in curing rickets in the rat[4]. To elucidate the biological significance of 25-HCC and 1,25-DHCC in bone metabolism, further studies on their effects on different metabolic processes of bone seem to be necessary.

The cholecalciferol status of the organism has an appreciable effect on bone collagen metabolism[7–9]. The total hydroxyproline release from rachitic rat bone in vitro was found to be higher than that of normal bone, and cholecalciferol supplementation, given 1 day before sacrifice, resulted in a further significant increase of of hydroxyproline release[10]. In a previous study we observed that 25-HCC given 9 h before sacrifice to rachitic rats influenced bone collagen metabolism, while vitamin D_3 was ineffective[11].

In this report we provide evidence that the lag period of action of 25-HCC on collagen metabolism of rachitic rat bone is significantly shorter than that of cholecalciferol.

Materials and methods. 102 inbred rats (R Amsterdam) of both sexes, 23 days old, were used; 8 rats received a semisynthetic normal diet (0,8% Ca, 0.5% P, 1 µg/100 g vitamin D_3) for 28 days and served as controls. The others received a vitamin D deficient, high Ca low P rachitogenic diet (1.2% Ca, 0.1 P) for 25 days, then a diet low in Ca and P, and lacking vitamin D (0.2% Ca, 0.1 P) for the 3 days previous to killing as reported earlier[11]. They were fasted for the last night. All the rats consuming the rachitogenic diet had widened metaphyses and hypocalcemia when killed. On the 29th day, the rachitic rats were divided into 3 groups: group D_3 received 2.5 µg cholecalciferol, group 25-HCC received 2.5 µg 25-HCC, group R received the solvent only. The supplements were given i.v. 4, 6, 10, 13, 15, 19, and 24 h after the injection 4–6 rats of each group were killed by decapitation and their bones were used for the in vitro study. The normal controls were killed together with the '10 h supplement' groups.

25-HCC (kindly donated by Dr. E. KODICEK and P. BELL of the Dunn Nutritional Laboratory, Cambridge) and cholecalciferol (Philips Duphar, Amsterdam) were dissolved in ethanol and further diluted before injection with the solution used for the incubation of bone.

Immediately after killing, pieces of proximal tibia metaphysis and of distal femur metaphysis 4 mm long were taken, split into 2 halves and cleaned of soft tissue and epiphyseal cartilage. Bone fragments of each rat were incubated in a separate Warburg vessel at 37 °C, for 4 h

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