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Incorporation of Radioactivity from U-^{14}C-Glucose into Oligodendrocytes and Myelin of Quaking Mice and Their Littermate Controls

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Abstract. Oligodendrocytes and myelin were purified from the cerebra of quaking mice and their littermate controls (11–60 days of age) after injecting the animals intraperitoneally with U-^{14}C-glucose. A peak of incorporation of radioactivity in the lipid extract of oligodendrocytes of both quaking and normal mice at 16–18 days of age was found, suggesting that the onset of myelination in the cerebra starts approximately at the same time for quaking mice and their littermate controls. Nevertheless the level of incorporation per cell was lower in the oligodendrocytes of quaking mice (50% of the control). The pattern of incorporation into myelin during development was similar between the two strains, but the specific activity as measured in dpm/mg protein was higher in the myelin of young quaking animals (up to16 days). Peaks of incorporation were found in cerebrosides and sulfatides of oligodendrocytes and myelin in normal controls at 18 days. In the quaking mice these peaks were absent in oligodendrocytes and much delayed in the myelin of the mutant. The results would suggest that the defect in the quaking mutant in respect to myelination is in oligodendrocyte metabolism and thus in an early stage of the assembly of the myelin membrane.

The quaking mouse is characterized by a severe myelin deficiency in the central nervous system (CNS) [33, 35, 37]: the myelin content is only 5–10% that of normal as determined by electron microscopy [14] and by fractionation and purification [17]. Quaking and other neurological mutants have been recently reviewed [2, 21]. It has been shown that in the brain of the quaking mouse, the synthesis of myelin-typical lipids (fatty acids, cerebrosides and sulfatides in particular) is severely depressed [1, 4, 5, 11, 16, 38]. However, the synthesis of myelin proteins has been reported to be normal at the micro-

somal level [7, 18, 19]. Morphologically, oligodendrocytes in quaking mice appear to be normal in size and in the time of appearance but increased in numbers [15]. It has been shown that glucose is a substrate for lipid synthesis by rat brain oligodendrocytes [23]. In the rat, metabolic activity as determined by glucose uptake increases specifically in oligodendrocytes during the period of myelination [10]. Thus the incorporation of radioactive glucose into oligodendrocytes and myelin of quaking mice was investigated together with their littermate controls throughout the development of the animals with particular reference to the incorporation into cerebrosides and sulfatides.

Materials and Methods

D-(U-^{14}C)-glucose (specific activity 250 mCi/mol) was purchased from Commissariat à l'Energie atomique, France. Trypsin (type III) and bovine serum albumin (Cohn fraction V) were purchased from Sigma. All other chemicals were of laboratory analytical grade unless specified.

The mutant quaking strain (B6-CBA) of mice was bred in the laboratory of Dr. Baumann (Inserm U-134) in Paris. Groups of 10 animals from 3–4 age-matched litters were used for each experiment. Animals were injected intraperitoneally with 1 μCi/g body weight of radioactive glucose (10 μl) and were sacrificed 2 h after by cervical dislocation. Oligodendrocytes and myelin were prepared from the cerebra of the mice according to the method of Chao and Rumsby [9] with slight modifications. Briefly, the brain tissue was minced and incubated with 0.1% (w/v) trypsin in 10 m*M* potassium dihydrogen orthophosphate-sodium hydroxide buffer containing 5% glucose, 5% fructose and 1% bovine serum albumin (HAP) at pH 7.5 for 1 h under oxygen. The softened tissue was sieved through nylon and stainless steel sieves, suspended in 0.9 *M* sucrose in HAP and layered onto a sucrose density gradient of 0.9 over 1.55 *M* sucrose and centrifuged at 3,300 *g* for 10 min. The crude oligodendrocyte fraction was collected from the 0.9/1.55 interphase and diluted to approximately 10 times its volume with HAP and reconcentrated by centrifugation at 200 *g* for 1 min. This crude oligodendrocyte fraction was left on ice for 2 h and then resuspended in HAP to be layered onto a second sucrose gradient of 1.0 over 1.75 *M* sucrose in HAP and centrifuged at 7,500 *g* for 20 min. Purified oligodendrocytes were collected from the 1.0/1.75-*M* interphase. Crude myelin was collected from above the 0.9-*M* sucrose after the first gradient centrifugation for the isolation of oligodendrocytes and was further purified by osmotic shock and gradient centrifugation as described by Norton and Poduslo [31]. Purified myelin was collected from the 0.32/0.8-*M* sucrose interphase. Cells were counted using a hemocytometer under phase contrast microscopy. Quantitation of proteins was carried out according to the method of Lowry et al. [25].

Lipids of the samples were extracted with chloroform:methanol (2:1) according to Folch et al. [13] and the separation of lipids was performed on thin-layer chromatography (HPTLC Merck) using the solvent system chloroform:methanol:water (70:30:4 by volume). Bands were visualized with I_2 vapor.

The lipid spots on silica gel plates were scraped directly into the scintillation vials without any extraction and 10 ml of scintillation mixture was added. The same mixture was used for the lipid extract. Quenching curves were prepared with ^{14}C-hexadecane standard purchased from the Radiochemical Centre, Amersham (England) and all counts were converted to dpm using the quenching curves. Detection of radioactivity was carried out on the Packard Liquid Scintillation System type Tricarb 460.

Experiments were performed at least three times. Statistical analysis was performed using Student's t test.

Results

Recovery of Oligodendrocytes

The yield of oligodendrocytes obtained from the mouse cerebrum was $1–4\times10^6$ cells per brain with a purity of 73.4±7.2%. This large range included brain from controls and mutants at different ages. The purity [28] of the preparations was very similar, whatever the age of the animal, in both controls and mutants.

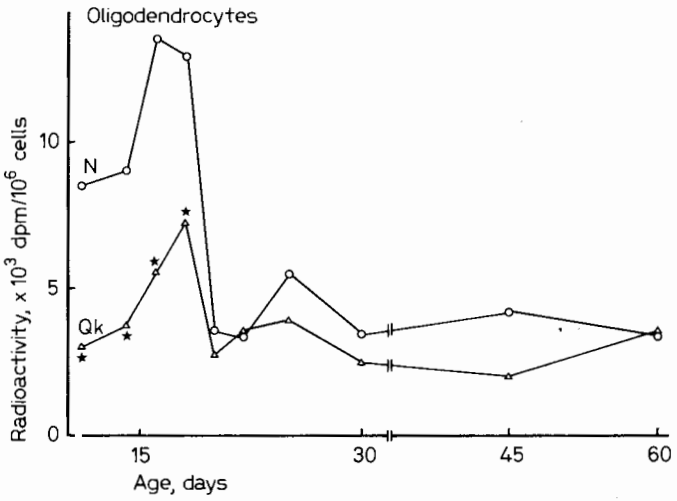

Fig. 1. Incorporation of radioactivity into lipids of oligodendrocytes of quaking mice (△) and their littermate controls (○). Each point is the mean value from 3 experiments. Each mouse was injected intraperitoneally with 1 μCi/g body weight of U-^{14}C-glucose and sacrificed 2 h later. Oligodendrocytes were isolated from the cerebra and radioactivity counted. * Statistically different from control ($p<0.05$ at least). NS=Not significant.

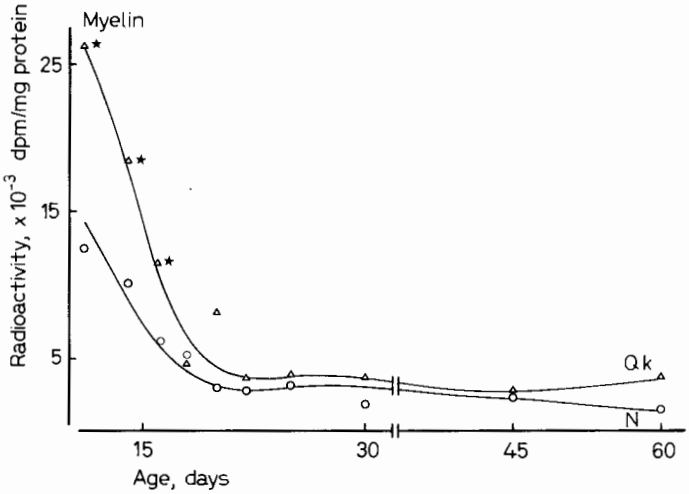

Fig. 2. Incorporation of radioactivity into myelin lipid extract of quaking mice (△) and their littermate controls (○). Myelin was purified by sucrose density gradient centrifugation as described in the text. Other experimental conditions are the same as in figure 1.

Incorporation of Radioactivity into Lipids of Oligodendrocytes and Myelin

The pattern of incorporation of radioactivity from U-^{14}C-glucose into oligodendrocytes of quaking and normal mice is shown in figure 1. Peaks of incorporation were found at 16 days for normal controls and 18 days for quaking mice. For the normal controls, the incorporation of radioactivity into oligodendrocytes at 16 days of age was 4 times that at 60 days of age. For the quaking mutant, the difference was only twice that between 16 and 60 days. In general, the incorporation of radioactivity into oligodendrocytes was lower in quaking than in the normal controls, but the difference was only significant in the young animals (up to 18 days). At the peak of incorporation, the radioactivity of quaking oligodendrocytes was only approximately 50% that of the normal controls.

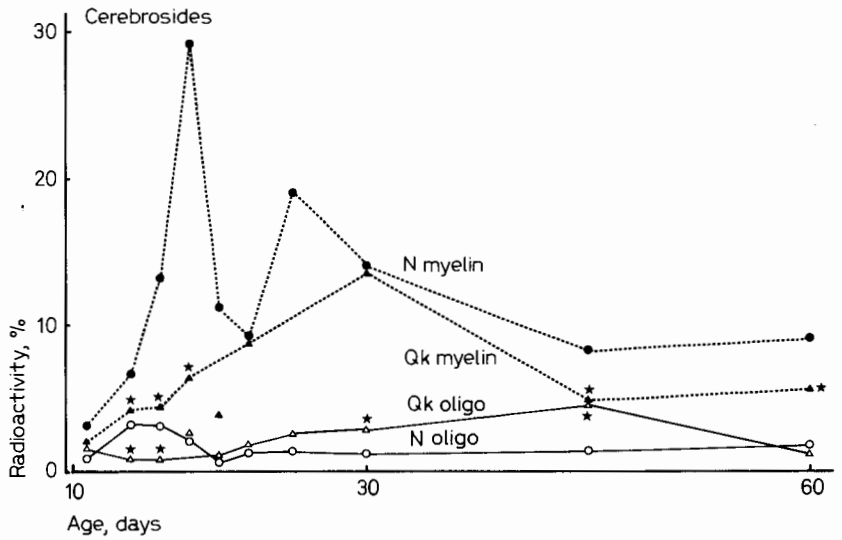

Fig. 3. Incorporation of radioactivity into cerebrosides of myelin (●, ▲) and oligodendrocytes (○, △) of quaking mice (▲, △) and their littermate controls (●, ○). Experimental conditions are the same as in figure 2. Lipids were extracted with chloroform:methanol (2:1) and separated by thin-layer chromotography. See figure 1 for statistical significance. Results are expressed in percent of total lipid extract.

The incorporation of radioactivity in myelin decreased with age for both the quaking mice and their littermate controls (fig. 2). The specific activity (dpm/mg protein) of quaking myelin after U-^{14}C-glucose injection was approximately twice that of myelin from normal controls up to 16 days (after 16 days the differences between controls and quaking mice were not significant).

Incorporation of Radioactivity into Cerebrosides and Sulfatides

Radioactivity incorporated into cerebrosides compared to that incorporated into the total lipids of oligodendrocytes and myelin of quaking and normal control mice is shown in figure 3. With cerebrosides from normal mice, a peak of incorporation was found in oligodendrocytes at 14–16 days of age, at 18 and 25 days in normal myelin. At this age, 30% of the radioactivity present in the myelin lipids of normal control mice were cerebrosides. In the quaking mouse, these peaks were absent. However, at 30 days of age, a peak of radioactivity was observed in the quaking myelin cerebrosides and at this age, 12% of the radioactivity present in the myelin lipids of quaking mice was recovered in cerebrosides. At all ages, the recovery of radioactivity from cerebrosides was lower in the myelin of quaking mice than in that of their littermate controls and this was statistically significant up to 35 days.

The pattern of incorporation into sulfatides (fig. 4) was similar to that of cerebrosides. Peaks were found in the sulfatides of oligodendrocytes and myelin from normal control mice during the 10- to 20-day period

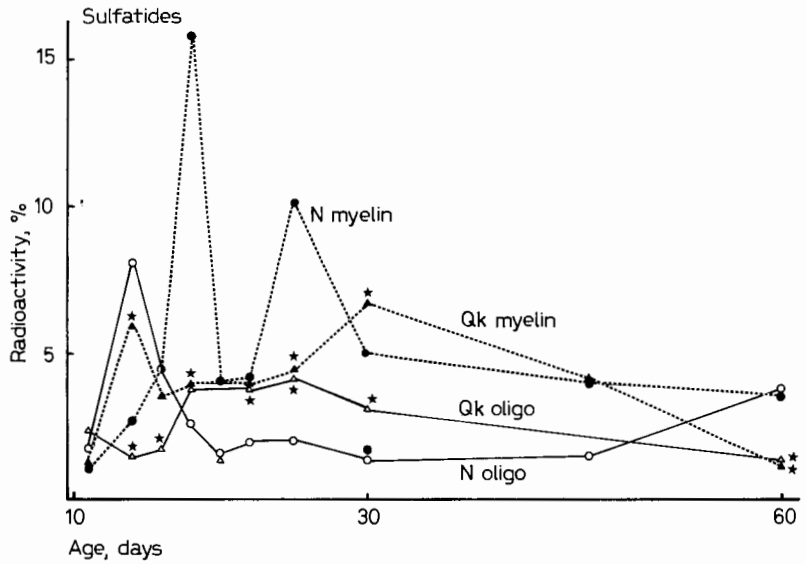

Fig. 4. Incorporation of radioactivity into sulfatides of myelin (●, ▲) and oligodendrocytes (○, △) of quaking mice (▲, △) and their littermate controls (●, ○). Experimental conditions and figure representation are the same as in figure 3. See figure 1 for statistical significance.

with the peak in oligodendrocytes just before that in myelin. Such peaks were either absent or delayed in the quaking mutant (fig. 4). As for cerebrosides, at 30 days of age, a peak of radioactivity was observed in the quaking myelin sulfatides.

Discussion

In the quaking mouse, the lack of myelin parallels the deficiency in galactolipids, especially in cerebrosides [3, 20, 22], myelin markers [27, 29, 32]. There are several ways in which the observed lack of cerebrosides in myelin could arise: (i) a decrease in the rate of synthesis of cerebrosides in oligodendrocytes; (ii) a defect in the incorporation of cerebrosides into the myelin sheath leading to a build up of cerebrosides in oligodendrocytes and inhibition of further synthesis of the lipid, and (iii) an increase in the metabolic breakdown of cerebrosides.

In the quaking mouse, it is unlikely that the deficiency of cerebrosides is due to an increase in catabolism as no increase in the turnover of cerebrosides was found [29]. It has been shown that galactosyl ceramide transferase is enhanced during the period when myelination is rapid in normal controls, but is very much reduced in the mutant [1, 11, 12, 38]. The reduction in galactosyl ceramide transferase activity cannot wholly explain the deficiency of cerebrosides and myelin in the quaking mouse [11, 12, 38]; the primary focus of the gene defect in the quaking mutant does not seem to be at the level of the galactocerebroside synthesis, since the enzymatic

activity is reduced, but not absent. The reduction in enzyme activity is 65% in quaking brain compared to controls [11, 37] whilst the cerebroside content is only 60% of normal at 16 days and 24% of normal at 30 days [20] and 10% of normal in myelin [17].

It is generally accepted that just before myelination, oligodendrocytes are in some yet unknown way stimulated to synthesize lipids and proteins for the formation of myelin [27, 34]. In the quaking mouse, we found that such stimulation does occur and the oligodendrocytes respond in a similar manner as in the normal controls, as shown by the increased glucose incorporation. However, the response of quaking oligodendrocytes is much reduced (fig. 1). Such abnormality of quaking oligodendrocyte metabolism has been suggested by others [26, 30] and oligodendrocytes in the quaking brain are indeed morphologically abnormal [29].

This work suggests that the defect in the quaking mutant in respect to myelination is in oligodendrocytes. However, it is not excluded that oligodendrocytes gained by the same method in normals and mutants are different oligodendrocyte subpopulations and, by this, their metabolic activity cannot be directly compared.

The decrease in glucose incorporation calculated in oligodendrocytes from quaking mice was not due to a smaller than normal percentage of oligodendrocyte isolated from the mutant (the purity of oligodendrocyte in control and quaking mice were similar). The pleiotypic nature of the quaking mutation could affect the specific carrier system for glucose across the blood brain barrier. As intraperitoneal injections were performed, this could affect the level of the injected glucose incorporated into oligodendrocytes of quaking mice but it is highly improbable.

The higher radioactivity of myelin in quaking mice, as compared to their littermate controls, that we found after labelling with radioactive glucose, probably suggests that the 'pathway' from oligodendrocytes to myelin is likely to be normal, i.e. the late assembly of myelin in the quaking mutant. If the lack of myelin in the quaking mouse was due to retarded myelin assembly, the radioactivity of myelin would be lower and not exceed labelling in the control. The fact that in quaking mice the radioactivity of myelin was higher and of oligodendrocytes was lower when compared to controls further suggests that the problem is at the level of an early stage of myelin assembly. This is in agreement with others who proposed that the deficiency of myelin in the quaking mouse was due to abnormalities of myelin assembly [1, 7, 18, 19], based on their double-labelling studies. However, the rationale behind the interpretation of the double-labelling technique results, as designed by Wiggins et al. [36], will only be applicable if the myelin composition of the brain in quaking mice is similar to the myelin composition of normal controls, which is not true in quaking mice.

When uniformly labelled ^{14}C-glucose was used to label brain cerebrosides and sulfatides, the specific activity of these two lipids was higher in the myelin of quaking mice than in myelin of normal controls. Such experimental results could only be explained if the defect of dysmyelination in the quaking mutant was localized in the oligodendrocyte cell itself. Our results showed that there was a specific increase of incorporation of radioactivity into cerebrosides and sulfatides of oligodendrocytes between 11 and 17 days of age in normal mice (fig. 3, 4). The absence of such peaks in the quaking mutant suggested that their synthesis was abnormal and re-

duced. This could also explain the finding that the amount of cerebrosides and sulfatides was drastically reduced in the pre-myelin material of quaking mouse brains as isolated by zonal centrifugation [6].

The functional role of cerebrosides and sulfatides in the myelin sheath is yet unknown. Proposals have been made that these lipids are essential for the assembly and compaction of the myelin sheath [23]. We propose that the defect in the quaking mutant in respect to myelination is a synthetic abnormality localized in the oligodendrocytes, the myelin-forming cell [8], which provokes a defective myelin assembly at a very early stage.

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