In vivo Incorporation of D-Glucose-U-C^{14} into Tetrasialoganglioside in Rat Brain

A ganglioside containing 4 residues of N-acetylneuraminic acid, tetrasialoganglioside GQ1 according to SVENNERHOLM[1], was found to occur in the brain of all tested vertebrates[1-3]. The metabolism of ganglioside GQ1, specially its biosynthetic pathway, is mostly unknown. The present investigation is concerned with the pattern of the vivo labelling of GQ1 in rat brain, after administration of D-glucose-U-C^{14}.

Methods. Newborn to 25-day-old Wistar rats were used, in groups of 8. Each rat was injected i.p. with 1 μCi of D-glucose-U-C^{14} (The Radiochemical Centre, Amersham; 2.9 mCi/m*M*, in sterile 0.9% NaCl); rats from each group were then decapitated at various intervals (from 1 to 12 h). The gangliosides were extracted from brain cortex, fractionated and purified on silica gel H (Merck) thin layer plates, following the procedure of TETTAMANTI et al.[3]. The pure isolated gangliosides, identified by chromatographic comparison with standard GM1, GD1a, GD1b, GT1b and GQ1 (nomenclature according to SVENNERHOLM[1]). proved to be rid of free sugar nucleotides.

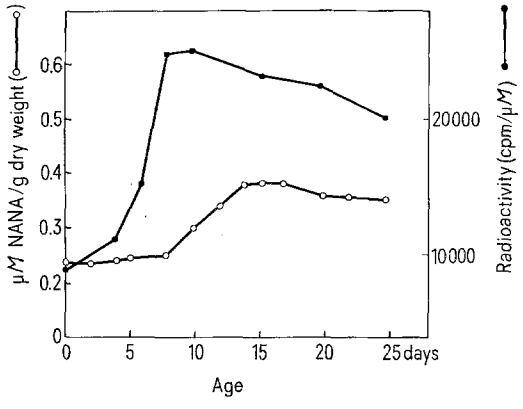

Fig. 1. Brain content of tetrasialoganglioside GQ1 in the rat (-O-O-O) and incorporation of D-glucose-U-C^{14} into the same GQ1 (-□-□-□-) as a function of age. The reported data are the mean value of 8 experiments.

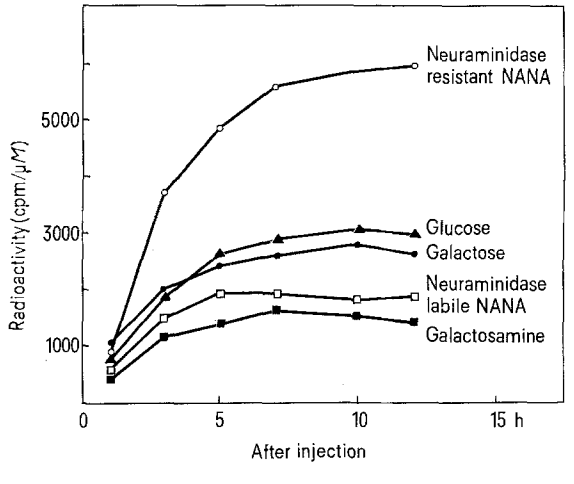

Fig. 2. Distribution of the radioactivity incorporated into the individual sugar components of rat brain tetrasialoganglioside GQ1 (neuraminidase resistant and labile NANA; glucose; galactose; galactosamine) as a function of the time after injection in 8-day-old animals. The reported data are the mean values of 4 experiments.

After location of the corresponding spot, each ganglioside was eluted from the scraped-off gel with 20 ml of chloroform/methanol/water = 2/5/2, v/v/v, the eluates evaporated to dryness and the residue used for analysis. The content of sphingosine, glucose, galactose, N-acetylgalactosamine and N-acetylneuraminic acid (NANA) was established by the chromatographic procedure of ZANETTA et al.[4]. The content of NANA was also determined by SVENNERHOLM's method[5]. The isolation of the individual sugars contained in ganglioside GQ1, (neuraminidase labile NANA, neuraminidase stabile NANA, glucose, galactose, and galactosamine), was carried out following the procedure of SUZUKI and KOREY[6], properly adapted to our experimental conditions. 80–100 μg (as lipid bound NANA) of GQ1 were used. The final separation and purification of the sugars obtained was accomplished on silica gel H thin layer plates (gel thickness: 150 μm; solvent: ethyl acetate/acetic acid/water = 6/3/2, v/v/v; 10 h run at 18–20 °C), the corresponding reference standards (glucose, galactose, galactosamine, NANA) being concurrently chromatographied. After location of the spots, each sugar was eluted from the scraped-off gel with 20 ml of: a) ethanol/0.1 *N* HCl = 75/25, v/v, in the case of galactosamine and NANA; b) ethanol/water = 75/25, v/v, in the case of glucose and galactose. After evaporation to dryness, each sugar was quantitated gaschromatographically as reported above, and the corresponding radioactivity measured with an automatic windowless gasflow Geiger Müller counter (Nuclear Chicago Corp., Model D47) (efficiency of D-glucose-U-C^{14}: 51.6%).

Results. The developmental profile (till 25 days) of the brain content of ganglioside GQ1, and of the radioactivity incorporated (at 8 h after injection) into the same GQ1, is shown in Figure 1. The content of GQ1 is, at birth, 0.23 μ*M*/g dry weight. It remains unchanged till 8 days, then sharply increases to a maximum of 0.38 μ*M*/g dry weight, reached around the 15th day, slowly decreasing later. The uptake of radioactivity, 9000 cpm/μ*M* at birth, undergoes a sharp increase after the 4th day, reaches the maximum (25,000 cpm/μ*M*) at the 8th–10th day, then slowly diminishes. Thus the rising of radioactivity uptake anticipates by a few days the accumulation of GQ1 in brain.

The distribution of radioactivity in the individual sugars of GQ1, as a function of the time after injection, is graphically shown in Figure 2. In all the sugars the incorporation of radioactivity, low after 1 h, then increases till the 7th–10th thereafter maintaining a plateau or tending to decrease. Surprisingly (after the evidence of MACCIONI et al.[7] and of HOLM et al.[8]) the radioactivity uptake in neuraminidase resistant, or stabile, NANA is maintained at a higher level (from 1- to 2-fold) than that of the other sugars, particularly neuraminidase

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labile NANA. A similar behaviour, however, was already described for gangliosides GM1, GD1a (GD1b?) and GT1b by SUZUKI and KOREY[6]. It may be provisionally explained by postulating the existence of a different pool of precursors for the resistant and the labile NANA.

The total incorporation of radioactivity into ganglioside GQ1, in 8-day-old rats, 8 h after injection, is 25,000 cpm/μM. Under the same conditions, which were reported to be optimal also for the other gangliosides, the specific radioactivities of gangliosides GM1, GD1a, GD1b and GT1b were, respectively: 13,500; 18,000; 8,600; 13,300 cpm/μM. This means that none of the above-mentioned gangliosides, which are chemically simpler, can be supposed to act as precursor of GQ1. In consequence, a specific and separate enzyme system is required for the biosynthesis of GQ1. This assumption is in agreement with the current concept[7] on the biosynthesis of gangliosides, postulating a specific biosynthetic system for each ganglioside, completely apart from the cellular structures (the neuronal membranes) into which gangliosides accumulate. According to this concept the higher specifc activity of GQ1 observed (possibly the simple expression of a higher turnover of GQ1) may be due to a higher ratio between the amount of GQ1 produced (in labelled form) by its biosynthetic system and the amount of GQ1 present in the neuronal membranes. In other words the 'dilution' of the radioactive GQ1 in the total pool of GQ1 is lower than in the case of the other gangliosides.

Riassunto. L'incorporazione di glucosio-U-C^{14}, introdotto per via i.p., nel tetrasialoganglioside GQ1 del cervello di ratto è massima all'8° giorno di vita e dopo 8–10 ore dall'iniezione. La radioattività specifica del GQ1 è notevolmente superiore a quella riscontrata nei gangliosidi GM1, GD1a, GD1b e GT1b, il che esclude che questi siano precursori del GQ1. L'acido sialico neuraminidasi stabile è più radioattivo di quello neuraminidasi labile: ciò indica un diverso impegno metabolico dei due tipi di acido sialico.

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An Electrophoretic Study of the Negative Correlation of Certain Carboxylesterases to Insecticide Resistance in *Musca domestica* L.

Carboxylesterase (E.C.3.1.1.1.), a major hydrolase has been shown to be negatively correlated with organophosphate resistance in the house-fly[1]. Several attempts have since been made to characterize it from other hydrolases and to demonstrate such differences electrophoretically[2–4]. Although the patterns from these studies indicated variation between and within strains, no clearcut differences were demonstrated between standard susceptible and low-esterase resistant strains.

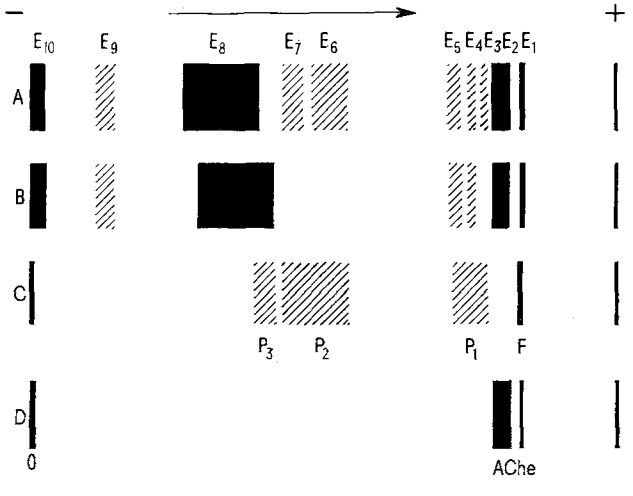

Fig. 1. Comparative zymogram of carboxylesterases (E_{1+2}-E_{10}) acetylcholinesterase (AChE) and peptidases (P_1-P_3) in a susceptible and an organophosphate resistant strain of housefly, separated in 5% gel. A) Carboxylesterases of susceptible strain, SRS. B) Carboxylesterases of resistant strain, SKA. C) and D) Peptidases and acetylcholinesterase of the susceptible and resistant strain. O, sample slot and F) indicates the marker dye front (10 cm). The arrow indicates the direction of protein migration.

An interesting strain of housefly, SKA, has been selected for several years with diazinon pressure at Rothamsted Experiment Station (Harpenden, England) and has developed resistance to a number of organophosphates and high cross-resistance to a number of chlorinated hydrocarbons[5]. My initial studies of the carboxylesterases of the SKA strain showed that the 'low esterase' activity in this strain is entirely due to carboxylesterase and not to other hydrolases such as lipase or peptidase[5]. Low carboxylesterase activity observed in the resistant strain was due to a proportionate decrease in head, thorax, abdomen and gut[6]. The present study was aimed at the further characterization and quantitation of various carboxylesterases of the SKA strain, separated electrophoretically on polyacrylamide gel. A WHO susceptible strain, SRS, obtained from University of Pavia, Italy, was used as a standard reference strain.

Materials and methods. One hundred 4-6-day-old decapitated houseflies were homogenized in cold (2–4 °C) in 4 ml of 0.1 M phosphate buffer adjusted to pH 7.0. The homogenate was centrifuged at 17,500 g for 20 min. The Table shows the results of centrifuging fly homogenates. The supernatant was used directly for electrophoresis of carboxylesterases, phosphatases and peptidases. For the separation of acetylcholinesterase, 100 fly-heads were homogenized in 4 ml of 0.1 M phosphate buffer and

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