Effects of Environmental Changes on the Incorporation of Carbon Atoms of Pyruvate into Mouse Brain and Liver[1]

Prolonged exposure of animals either to isolation or to an 'enriched' environment is accompanied by a wide variety of behavioral changes, including altered aggressive[2] and sexual[3] behaviors as well as altered levels of emotional arousal[4]. Also, it has been claimed that slight, though significant, increases in both brain weight and volume can be caused by exposure of animals to enriched environments[5-7]. However, no information is presently available which relates directly the effects of environment to cerebral energy metabolism. In this note the effects of prolonged 'isolation' or 'aggregation' of mice on the entry of carbon atoms of pyruvate into brain and liver are presented. In addition, since injections of lithium chloride (LiCl) cause increased glucose entry into[8] and glycogen formation by[9] brain tissue, the possible interaction between the effects of this compound and the environmentally-produced changes was also studied.

Two sets of experiments were conducted using weanling, male, littermate (C-57B) mice (Indianapolis Laboratory Supply Corp.). Preliminary results indicated that these animals were less susceptible than Swiss albino mice to the toxic effects of LiCl. Isolated mice were housed individually in small (6″ × 8″ × 10″) cages in a quiet room and their littermates were kept in larger cages (12″ × 14″ × 24″) in groups of 25 (aggregated). No 'enrichment' was provided for the aggregated animals in order to simplify the interpretation of results.

In the first set of experiments mice were kept under isolated and aggregated conditions for 5 weeks and then fasted for 12–18 h prior to receiving s.c. injections of 0.1 μC/g of 1-^{14}C-pyruvate (New England Nuclear Corp.; 5.9 mC/mmole) in 0.154 *M* NaCl solution. Animals were sacrificed 30 min later by immersion in liquid nitrogen and their brains (above the level of the colliculi and excluding the cerebellum) were excised rapidly, homogenized in 3.0 ml of 80% ethanol solution and extracted. Radioactivity was monitored in aliquots of the homogenates and extracts using a Packard Model 2002 scintillation counter. Corrections for quenching and conversion of cpm to dpm were made by a method of channels-ratio. The striking changes in the incorporation of pyruvate carbon into brain caused by exposure to the different environments are shown in Table I. The data indicate also that 30–38% of the ^{14}C in brain is not readily extractable, i.e., that this portion may be incorporated into macromolecules or lipids.

In the second set of experiments animals were isolated or aggregated for 10–11 weeks and were not fasted prior to experimentation. Three h before s.c. injection of the labelled pyruvate, all animals were injected i.p. with either NaCl, 550 mg/kg, or LiCl, 200 or 400 mg/kg. The mice were sacrificed by decapitation 30 min after receiving pyruvate and their brains and livers were homogenized. Results shown in Table II confirm the difference in cerebral radioactivity found between isolated and aggregated mice (Table I) and also indicate that injections of LiCl cause increased incorporation of radioactivity (of pyruvate) into the brains of isolated mice. The values shown for mice receiving the larger dose of LiCl were indistinguishable from those of aggregated (control) animals receiving NaCl. Since similar results were obtained with liver (Table II) it appears that the effect of isolation on the disposition of carbon atoms of pyruvate, as well as the reversibility of this effect by Li^+, may be a more widespread phenomenon than was expected.

It is noteworthy that injections of LiCl exerted no obvious effects on the entry of pyruvate carbon atoms into

Table I. Effects of environmental changes on the incorporation of carbon atoms of 1-^{14}C-pyruvate into mouse brain

	Radioactivity; dpm/g frozen brain (× 10^{-3})	
	Isolated	Aggregated
Whole homogenate	29.9±1.93 (8)	49.3±1.66 (6)[a]
Ethanolic extract	17.0±0.57 (7)	34.5±0.60 (6)[a]

Animals (isolated or aggregated for 5 weeks) were fasted for 12–18 h prior to experiments, and brains were dissected out in the frozen state after 'quick-freezing' the whole animals in liquid N_2. Means ± standard errors, of the means; numbers of mice in parentheses; [a] indicates $p \ll 0.001$ with respect to isolated mice (Student's *t*-test; two-tailed).

Table II. Effects of injections of LiCl on the incorporation of carbon atoms of 1-^{14}C-pyruvate into the brains and livers of isolated and aggregated mice

Injection	Radioactivity in tissue extracts; dpm/g fresh weight (× 10^{-3})	
	Isolated	Aggregated
Brain		
Control; NaCl (550 mg/kg)	18.1±0.96 (4)	26.7±3.23 (6)[b]
LiCl (200 mg/kg)	23.4±2.61 (4)	28.8±3.43 (6)
LiCl (400 mg/kg)	29.2±3.52 (4)[a]	27.9±2.48 (5)
Liver		
Control; NaCl (550 mg/kg)	87.7±6.55 (4)	123.4±13.47 (6)
LiCl (200 mg/kg)	73.0±7.54 (4)	149.5±21.87 (6)[c]
LiCl (400 mg/kg)	105.4±5.15 (4)[a]	128.2±15.62 (5)

Animals (isolated or aggregated for 10–11 weeks) were not fasted, and tissues were dissected out at room temperature. Means ± standard errors, of the means; numbers of mice in parentheses; [a] indicates $p<0.05$ with respect to control isolated group; [b] indicates $p<0.025$ with respect to isolated group; [c] indicates $p<0.05$ with respect to the isolated group which received the same dose of LiCl (Student's *t*-test; one-tailed).

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the brains and livers of aggregated animals and that the total radioactivity found in brain extracts was quite similar in fasted (Table I) versus non-fasted (Table II) animals. We do not know at this time whether the increased ^{14}C found in the brains of aggregated mice or in those of isolated mice which received injections of LiCl is distributed uniformly among intermediary metabolites or whether it is incorporated specifically into certain pharmacologically-active substances (e.g., the amino acids glutamate and GABA).

Résumé. L'incorporation des atomes radioactifs de 1-^{14}C-pyruvate dans le cerveau des souris est plus marquée quand les animaux ont soumis à un isolement prolongé.

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Pancreatic Islet Cell Damage in Mice Produced by Coxsackie B_1 and Encephalomyocarditis Viruses[1]

It has been suspected that viral infection can cause diabetes mellitus in man[2–5], based on clinical evidence with isolation of viruses and determination of rising titers of neutralizing antibodies to certain viruses. Nevertheless, the pathogenesis of diabetes mellitus by viruses needs clarification.

In experimental studies, CRAIGHEAD et al.[6,7] reported diabetes mellitus or diabetes mellitus-like syndrome associated with islet cell damage of pancreas in mice infected with encephalomyocarditis (EMC) virus suggesting that the virus acts on the islets of Langerhans to reduce the mass of functional β-cells. But viral crystals have never been shown directly in the damaged β-cells of the islets of Langerhans. In our studies we located and demonstrated both EMC and Coxsackie B_1 virus crystals in β-cells of the islets of Langerhans in the pancreas of mice infected with these two viruses. The findings of significant islet cell necrosis with demonstration of viral crystals in the damaged β-cells of the islets of Langerhans of mice proved that viruses do produce islet damage.

The source of our EMC virus has been previously reported[8]. The Coxsackie virus B_1 was a virus stock received from the Communicable Disease Center in 1959. It has been passed once in KB cells and twice in monkey kidney cells.

Three groups of a random breed strain of HaM/ICR mice of different ages (new-born, 8-day-old and young adult) were used for EMC virus inoculation and 3 other groups of the same strain of mice (new-born, 14-day-old and young adult) were used for Coxsackie virus B_1 inoculation.

The mice were injected i.p. with 0.05 ml to 0.2 ml of EMC virus culture fluid with a titer of 10^{-6} $TCID_{50}$/ml or with 0.1 ml to 0.2 ml of Coxsackie virus B_1 culture fluid with a titer of 10^{-4} $TCID_{50}$/ml. Control mice were injected i.p. with the same amounts of virus-free culture fluids.

The EMC virus infected mice were killed 1 to 3 days and the Coxsackie virus B_1 infected mice were killed 1 to 8 days after viral inoculation. Control mice for each group were killed at the same times as the infected animals.

Histologically, all mice infected with either virus showed mild to severe pancreatic islet cell degeneration and necrosis, usually beginning on the first day after inoculation. The acinar cells also showed damage associated with interstitial inflammation of the pancreas. As time elapsed the lesions became more extensive and widespread. Generally, the acinar cell damage of the EMC virus infected mice was less severe than that of the Coxsackie virus B_1 infected mice. Atrophic changes of islets of Langerhans were noted in the Coxsackie virus B_1 infected mice 4 days after inoculation.

Electron microscopically, crystals of EMC and Coxsackie B_1 viruses were demonstrated in β-cells as well as in acinar cells of the pancreas along with significant ultrastructural changes. In most mice the damage to the pancreatic cells was so severe that it was virtually impossible to identify the cell type. In the identifiable β-cells of mice infected with EMC virus the damage was usually severe, whereas the damage to the β-cells of mice infected with Coxsackie B_1 virus was mild to moderate. With EMC virus infection, vacuoles and vesicles of unknown nature formed and the RER usually became dilated. There was a decrease in β-granules in some of the severely damaged β-cells. With Coxsachie virus B_1 infection, the damage was usually restricted to that portion of the cell in which the viral crystal was present. In the area surrounding the viral crystals, vesicles and vacuoles formed. The mitochondria appeared swollen and there was evidence that the β-cell granules had become dissolved and that the granule core was condensed. The pancreatic tissues of the control animals were normal.

That EMC virus infected mice have islet cell damage and develop diabetes mellitus-like syndrome has been shown[6,7]. Coxsackie virus B_1 infected mice showed islet cell damage similar to that of mice infected with EMC virus, but it is not known whether diabetes mellitus could also occur in Coxsackie virus B_1 infected mice. Biochemical studies of this nature are in progress in this laboratory.

The Coxsackie virus B_1 is one of the viruses known to infect man. Clinically, some diabetic patients have shown

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