additional to *D. melanogaster* are Townsville 5, Brisbane 3, and Melbourne 2. This is presumably a reflection of high heterogeneity of available resources in tropical habitats. In any case, temperate zone habitats are clearly more uniform for *Drosophila* resources especially in the vicinity of wineries[16].

The Darwin population, further to the north (and west) of those considered so far, appears exceptional. However, the climate is very extreme since mean maximum temperatures exceed 30 °C every month of the year including winter. In agreement is the biological indicator of only 1 additional sympatric species *D. ananassae*, known from unpublished data to be very desiccation-resistant. The extreme nature of the climate is also shown by the absence of *D. simulans* which is found in the 3 other localities, and is known to be more sensitive to environmental extremes than *D. melanogaster*[15]. In other words, Darwin must be regarded as ecologically marginal in spite of its latitude. A consequence is relatively low variability among isofemale strains at similar levels to the ecologically marginal temperate-zone Melbourne population (table), even though the means are lower in Darwin as found in the other tropical populations.

The genetic characteristics of marginal populations have been considered in the literature with varying conclusions[17,18]. For example, in certain but not all *Drosophila* species, chromosomal polymorphism levels fall towards the (geographic) margins but this is not a general result. The situation is even more obscure for allozyme frequencies[19]. Here 2 populations, Melbourne and especially Darwin, are defined as ecologically marginal or extreme, in terms of climatic and biotic factors. In such extreme populations, low genetic variability for traits of direct significance in the field occurs. This follows from the demonstration that ethanol occurs in the field, occasionally to high concentrations[16]. Such low variability may preclude the possibility of spreading into even more extreme habitats even if resources are available, although as in the Queensland fruit fly, *Dacus tryoni*, such a possibility cannot be excluded[20].

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Steady-state distribution of lithium during cultivation of dissociated brain cells[1]

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Summary. The formation of a steady-state intracellular lithium level was studied in the course of cultivation of dissociated nerve cell cultures obtained from chick embryonic brains. When lithium was given at a concentration of 2 mM, in the nutrient medium, at day 5, a steady state intracellular lithium content was achieved after about 30 min of incubation and it did not change significantly during the time of cultivation up to the 13th day in vitro.

Lithium salts have a beneficial effect in the treatment and prophylaxis of manic-depressive illnesses[2]. An uneven steady-state distribution of lithium (Li) between the red cells and plasma has been observed during long-term Li therapy[3]. In very recent years the transport mechanisms which result in this unequality of Li distribution in the blood have been characterized[4,5]. Studying the properties of Li transport across the membranes of nerve cells is of importance for an understanding of the characteristics of the regulation of Li homeostasis in the brain, and of the therapeutic mode of action of Li.

Richelson reported that Li entered neuroblastoma cells through the sodium channel and that ouabain, a potent inhibitor of Na, K ATPase, did not affect this Li uptake[6]. Tumorous lines of glial cells in culture accumulate Li, attaining an intra-/extracellular Li distribution ratio of 3–5[7]. In earlier papers we detected a Na^+-dependence of the Li uptake and an ouabain-insensitivity of the Li fluxes in primary nerve cell cultures[8,9]. In the present work we have studied the intra/extracellular Li distribution in the course of cultivation in dissociated cultures prepared from embryonic brain.

Material and methods. Primary cultures of a population of neuronal and glial cells were prepared from 7-day-old chick embryonic brains after the method of Sensenbrenner et al.[10]. The cerebral hemispheres were dissected, cleaned of their meningeal membranes, then passed through a nylon sieve (48 µm pore size). Only mechanical dissociation was used. Cells were grown in Falcon plastic Petri dishes (60 mm diameter) containing Eagle's Minimal Essential Medium supplemented with 20% fetal calf serum (Gibco). The medium was changed 3 times per week. The cultures

were checked by phase contrast and electron microscopy as described elsewhere[11]. Lithium chloride was added to the nutrient media of cultures at day 5 in vitro, at a concentration of 2 mM. The Li content of the cells was measured after different periods (10, 20, 30, 60 and 120 min) of incubation, then it was estimated every 2nd day up to the 13th day of cultivation. The cells were washed 3 times with 0.25 M sucrose, dried at room temperature and dissolved in 1 N NaOH. Lithium measurements were carried out with a Perkin Elmer atomic absorption spectrophotometer (306 Model). Protein content of the cells was determined by Lowry's method.

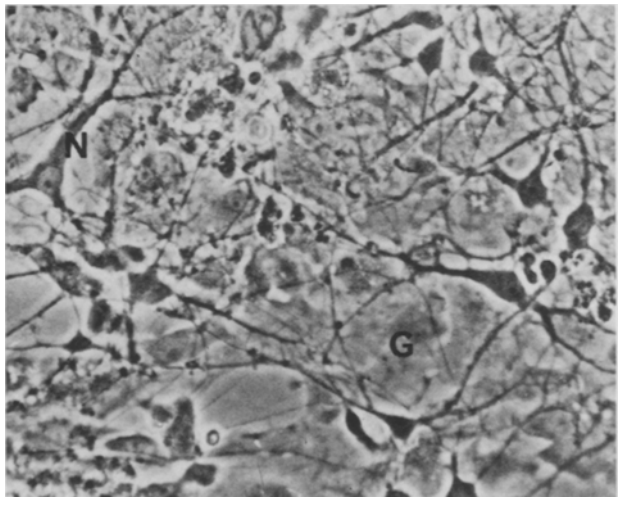

Fig. 1. Morphological features of primary culture prepared from 7-day-old chick embryonic brain. N, neuron; G, glia. 7 days in vitro. Phase contrast, × 400.

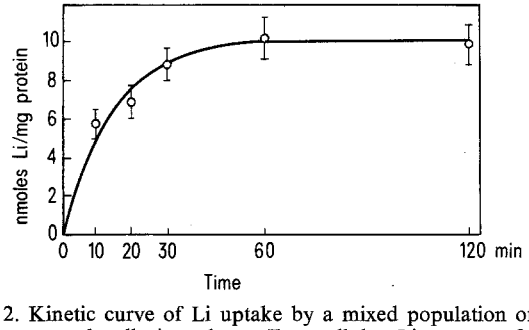

Fig. 2. Kinetic curve of Li uptake by a mixed population of glial and neuronal cells in culture. Extracellular Li content: 2 mM. Values represent means of 4 separate experiments with 3 parallels. Error bars indicate SD.

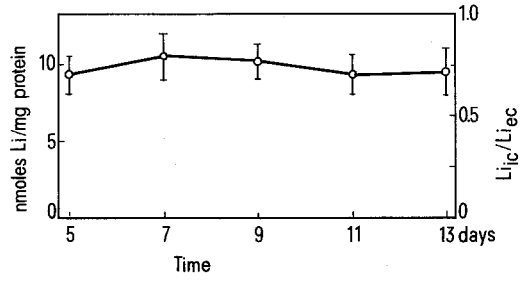

Fig. 3. Steady-state distribution of Li in nerve cell cultures during cultivation. External Li content: 2 mM. Li_{ic}/Li_{ec} means intra/extracellular Li distribution ratio. Values represent averages of 4 separate experiments with 3 parallels. Error bars indicate SD.

Results. Figure 1 presents some morphological features of the cultures used in our experiments. A mixed population of neuronal and glial cells can be achieved under the conditions of cultivation described above.

Figure 2 shows the time-curve of Li uptake in primary brain cell cultures. A steady-state distribution of Li between the intra-and extracellular spaces is attained after about 30 min of incubation. The initial linear part of the curve was used to calculate the Li uptake rate which was found to be 0.55 nmoles Li/mg protein · min^{-1}.

Figure 3 shows the intracellular Li content of the brain cells and the intra/extracellular Li concentration ratios in the course of cultivation from 5 to 13 days. During the period examined, no significant changes could be observed in the intracellular Li content. Data of Kukes et al.[12] were used to determine the intracellular Li concentration. As seen in figure 3, the steady-state Li ratio is below 1 in these primary brain-cell cultures.

Discussion. The transport mechanisms determining the uneven intra/extracellular Li distribution in the blood have been described recently[4,5]. The question arises as to whether neural elements differ qualitatively or quantitatively in their Li transport properties from red blood cells. Several papers have dealt with Li movement across the membranes of excitable cells[6,13]. Li can use the sodium channel when entering mouse neuroblastoma cells[6]. However, these cells, and rat glioma lines, could accumulate Li from a culture medium supplemented with 'therapeutic' concentrations of Li[7]. Our data obtained on primary cultures from chick embryonic brain suggest that no Li accumulation occurs in a mixed population of neuronal and glial cells in vitro and a steady-state intra/extracellular distribution of Li is achieved after about 30 min of incubation under physiological conditions. Our previous studies have shown that the rate of Li influx and the steady-state internal Li level are strongly dependent on the external sodium concentration[8,9]. Furthermore, we observed a lower rate of Li influx into glial cells in primary culture in comparison with the neuronal elements[14].

It appears that the steady-state distribution of Li between the intra- and extracellular spaces in primary nerve cell cultures is similar to that observed in human blood during prophylactic Li treatment.

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