We are grateful to the U.S. Public Health Service for Grant no. NB 05502-05 for support.

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Inherited Characteristics Affecting Neurotropic Behaviour of Scrapie

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A genetic influence has been shown to exert part control on the incidence of scrapie disease in sheep (Dickinson, Stamp, Renwick & Rennie, 1968). The clinical stage of the disease is caused by neurotropic damage, namely neuronal vacuolation and astrocytic hypertrophy. Whatever may be the nature of the agent (see Gibbon & Hunter, 1967) this lethal degeneration can be considered as initiated by an interaction between agent and nervous tissue producing malfunction in a chain of metabolic processes. The distribution of lesions in brains from affected sheep is variable and related to the combination of breed and scrapie type. A similar situation has been found in scrapie passaged in mice; the effects are highly dependent on the mouse strain and the source of the scrapie inoculum (Dickinson, Meikle & Fraser, 1968). In terms of the extent of development of lesions, each mouse within a random-bred strain appears to have an individual response with time after inoculation (Mould, Dawson, Slater & Zlotnik, 1967). With inbred strains the spread is much smaller. Changes in enzymic activity in brain tissue during the long period of development of the disease also show these individual variations. For example, the pattern of decrease in brain acetylcholinesterase with time differs between a random-bred Swiss White strain and an inbred C57 strain. It is probable that reported changes in other constituents would show such variability.

Recent work has confirmed that in lymphoid tissue, e.g. spleen and salivary gland, an appreciable increase in scrapie activity can appear at an earlier stage before comparable activity is reached in the brain. No histopathological change has been described in these tissues. This raises the possibility that there may be genetically controlled conditions determining the passage of scrapie from lymphoid tissue to the nervous system. Genetic control, manifest in the presence or absence of specific components, almost certainly influences the final

process of brain damage in any individual susceptible animal. These characteristics need to be considered in speculations as to the nature of the agent.

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Development of Metabolic Compartmentation in Rat Brain

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The maturation of the cerebral cortex takes place in the rat within a short well-defined period when morphological, electrophysiological and biochemical changes are observed (cf. Himwich, 1962). The capacity for rapid conversion of [^{14}C]glucose into glutamate develops in the rat brain between 10 and 21 days after birth (Gaitonde & Richter, 1966). This phenomenon reflects the final co-ordination of the different pathways of energy metabolism characteristic of the adult brain. It has also been reported that the signs of compartmentation of glutamate develop in the cat neocortex 3–4 weeks after birth (Berl, 1965). In the present work we investigated whether or not the development of these two characteristics of the adult brain coincides in one species. Labelled leucine was used since there was evidence that compartmentation of glutamate can be observed after administration of [^{14}C]leucine (Roberts & Morelos, 1965).

Porton rats (newborn to 35 days old) were injected subcutaneously with [$\text{U-}^{14}\text{C}$]leucine ($10\text{ }\mu\text{C}/100\text{ g}$. body wt.; specific radioactivity $31\text{ mC}/\text{m-mole}$), and at 10 min. after the injection the free amino acids of the brain were separated and analysed. The specific radioactivity of alanine relative to that of glutamate was very low at each age (about 0.1); thus conversion of [^{14}C]leucine into glucose, which might enter the brain via the circulation, was probably negligible, and the incorporation of ^{14}C into amino acids was mainly through conversion of [^{14}C]leucine into [^{14}C]acetyl-CoA in the tissue. The index used for the metabolic compartmentation of glutamate was the high incorporation of ^{14}C into glutamine (relative specific radioactivity > 1) relative to glutamate. The relative specific radioactivity of glutamine was found to be about 0.5 up to the age of 9 days; it reached a value of 1.0 at 15 days and

rose to about 2.5 at 21 days, which is comparable with the value observed in the adult brain. The development of the compartmentation of glutamate in rat brain therefore coincided approximately with that of the rapid conversion of [^{14}C]glucose into glutamate.

The fate of [^{14}C]leucine in the adult brain was further investigated at 5–120 min. after intraperitoneal administration of [^{14}C]leucine. The maximum specific radioactivity was attained at first by glutamine, then by glutamate and considerably later by aspartate. The curves for the specific radioactivities as a function of time did not intersect; the relative specific radioactivity of glutamine even at 2 hr. after the injection was about 1.5.

The present results are consistent with the entry of [^{14}C]acetyl-CoA derived from [^{14}C]leucine into a metabolic compartment containing a relatively small pool of glutamate, which is connected to a relatively large pool of glutamine in the brain (cf. reviews by Berl & Clarke, 1968; Van den Berg, 1968). Evidence was also obtained that the co-ordination of the energy metabolism develops in parallel with metabolic compartmentation in the rat brain.

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The Effect of Thyroid Hormones on the Biochemical Maturation of the Rat Brain

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The growth and maturation of the brain are associated with changes of metabolic pattern resulting from changes in the activity and the co-ordination of different enzyme systems. The rapid incorporation of glucose into the free amino acids, which is characteristic of the adult brain, has been shown to develop in the rat between the ages of 10 and 21 days (Gaitonde & Richter, 1966). The development of this metabolic pattern was used as an index of maturation in studying the action of thyroid hormones.

Rats of Sprague-Dawley strain (10–19 days old) were killed 20 min. after an injection of 10–20 μC /

100 g. body wt. of [^{14}C]glucose of specific radioactivity 10 $\mu\text{C}/\mu\text{mole}$. The amino acids in the acid-soluble fraction were separated from neutral compounds and carboxylic acids as described by Gaitonde & Richter (1966). In the control 10-day-old rats, about 25% of the acid-soluble ^{14}C was recovered in the amino acids. By the age of 19 days the value had increased to 60%, which is close to the value obtained in the adult.

Tri-iodothyronine treatment (Khamisi & Eayrs, 1966) advanced the onset of rapid incorporation of glucose carbon into amino acids in the rat brain. At 12 days the rate of incorporation in the thyroid-hormone-treated animals was 64% higher than in normal control rats, whereas in neonatal radiothyroidectomized animals the incorporation was decreased: at 10 days it was 72% and at 19 days 54% of the normal control value.

The results give evidence that the biochemical maturation of the brain is retarded in thyroid deficiency. Conversely, tri-iodothyronine treatment results in a premature development of the adult metabolic pattern. There is an apparent correlation between the advancement of innately organized behaviour (e.g. startle response; Khamisi & Eayrs, 1966) and the biochemical maturation of brain: in animals treated with thyroid hormone. The behavioural changes observed after neonatal thyroidectomy are apparently associated with the retardation of the biochemical maturation of the brain and structural changes involving a hypoplasia of the cortical neurophil (Eayrs, 1966).

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Solubilization of Mitochondrial Hexokinase from Ox Cerebral Cortex with Triton X-100

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The mitochondrial hexokinase of rat and guinea-pig brain has been solubilized by osmotic shock in the presence of ATP (Bachelard, 1967) or glucose 6-phosphate (Rose & Warms, 1967) or by treatment with Triton X-100 in the presence of high salt concentrations (Teichgräber & Biesold, 1968). The hexokinase of purified mitochondria from ox cerebral cortex, prepared by sucrose-density-gradient centrifugation, was found to be relatively resistant to extraction by the methods involving osmotic shock, but was readily solubilized with Triton X-100, with the apparent activation observed in the previous studies. The mitochondria,