& Horvath, 1957). However, D-PEN also forms a thiazolidine with PALP (Ueda, Akedo & Suda, 1960). Hence this reaction cannot explain, in rat feeding experiments (Wilson & Du Vigneaud, 1950), either the toxicity of L-PEN or the non-toxicity of D-PEN (Evered, 1963). The method of Matsuo (1957) was used to determine the stability constants of the thiazolidines formed between PALP and the various PEN isomers. The comparative results were $10^{-4} \times K = 1.4$, 1.4, 1.5 for L-, D- and DL-PEN respectively.

Two PALP-dependent enzymes, tryptophanase and mammalian alanine aminotransferase with added coenzyme, were inhibited *in vitro* by subsequent addition of L-PEN but not of D-PEN. These results were interpreted to reflect modification of the enzyme protein by L-PEN and not merely removal of coenzyme by thiazolidine formation (Ueda, Akedo & Suda, 1960).

Possible inhibitory effects of PEN isomers were tested against several aminotransferases. D-PEN (2.5 mM) did not inhibit wheat-germ aspartate aminotransferase. L-PEN inhibited this enzyme by 4%, which was not considered to be significant. Similar results were observed with pig heart aspartate aminotransferase.

Wheat-germ alanine aminotransferase was not inhibited by 2 mM D-PEN whereas 2 mM L-PEN inhibited competitively by 25%. Similar inhibition was observed between L-PEN and mammalian alanine aminotransferase (Hasenbank, Körber & Siegmund, 1968). These aminotransferases were all specific for amino acid substrates of the L configuration.

Hence the inhibitory effect of L-PEN is specific for alanine aminotransferase and not aspartate aminotransferase. A reasonable hypothesis is that the inhibitor is competing with the substrate, since L-PEN is a structural analogue of L-alanine. Therefore, the inhibition is not due to interaction of L-PEN with coenzyme or to alteration of the enzyme protein. With the wheat-germ enzymes, L-cysteine was more inhibitory to alanine aminotransferase (50%) than to aspartate aminotransferase (8%). These findings are compatible with our hypothesis.

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Protein Kinase Activity in a Cerebral Membrane Fraction: Effect of Treatment with Sodium Iodide

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Incubation of cerebral membrane fractions from the ox with [32 P]MgATP results in a time-dependent labelling of protein-bound serine phosphate groups (Rodnight & Lavin, 1966). With 1 mM-Mg-ATP and 1 mg. of protein/ml., transfer follows a curvilinear course until most of the ATP has been hydrolysed by the Mg^{2+} -effected ATPase* present in the preparations. The major part of this reaction (which may involve several systems) appears to be independent of the Na^{+} - K^{+} - Mg^{2+} -effected ATPase of membrane preparations (Decsi & Rodnight, 1965), although some evidence has been advanced for the involvement of protein-bound serine phosphate groups in the action of this enzyme (Hokin, Yoda & Sandhu, 1966).

Treatment of membrane preparations with high concentrations of sodium iodide yields a particulate preparation enriched several times in the Na^{+} - K^{+} - Mg^{2+} -effected ATPase and containing only minimal Mg^{2+} -effected ATPase activity (Nakao, Tashima, Nagano & Nakao, 1965). The sodium iodide-treated preparation has now been found to contain less than 2% of the original protein kinase activity when incubated with [32 P]ATP. Dialysis of the non-particulate fraction obtained by the treatment yielded soluble and insoluble fractions containing 2 and 10% respectively of the original activity. One explanation of the loss of activity might be that the treatment dissociates the kinase enzyme(s) from their protein substrates, but recombination experiments have not resulted in appreciable restoration of activity. Of the total protein kinase activity with phosvitin as substrate (Rodnight & Lavin, 1964), 5% was found in the sodium iodide-treated preparation and 17% and 24% in the remaining soluble and insoluble fractions respectively; the recovery of this activity is therefore considerably higher than that involving endogenous substrates. However, addition of partially purified phosvitin kinase from ox brain failed to increase transfer to the endogenous substrates.

These results confirm that the major part of the protein kinase activity in cerebral microsomes is unrelated to the Na^{+} - K^{+} - Mg^{2+} -effected ATPase, and suggests that sodium iodide treatment irreversibly dissociates at least part of the protein kinase enzyme(s) from their substrates.

* Abbreviation: ATPase, adenosine triphosphatase.

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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 mc/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