INSTABILITY OF CEREBRAL PROTEINS

Abel Lajtha and Jeno Toth

New York State Research Institute for Neurochemistry
and Drug Addiction, Ward's Island, New York, N. Y.
and Department of Biochemistry, College of Physicians
and Surgeons, Columbia University, New York, N. Y.

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It has been shown in a number of laboratories that proteins of brain, like proteins of other organs, are in a dynamic state (Lajtha, Furst, Gerstein, and Waelsch, 1957; Gaitonde and Richter, 1956). The average half-life was estimated as about 14 days (Lajtha, 1964; Richter, 1959). These studies also established that the turnover rates of cerebral proteins are heterogeneous, some proteins having short half-lives, others turning over slowly (Lajtha, 1959; Waelsch and Lajtha, 1961). The rate of turnover, that is, the proportion of the protein fraction that turns over rapidly, decreases during development (Lajtha, 1959; Palladin, Poliakova, and Silitch, 1957; Roberts, Flexner and Flexner, 1959), and one fraction, proteolipids (Folch and Lees, 1951), has a rate of turnover that is considerably lower than average (Furst, Lajtha and Waelsch, 1958). It was pointed out that this fraction, or part of it may be stable throughout the lifetime of the organism (Davison, 1961; Gaitonde, 1961).

The dynamic state and the heterogeneity of turnover rates in the brain are not restricted to proteins; experiments in which all cerebral constituents were labeled (Thompson and Ballou, 1956; Buchanan, 1961) also showed metabolic heterogeneity. In these experiments a significant portion of cerebral constituents had a low rate of turnover (54% with a half-life of 150 days in one study (Thompson and Ballou, 1956), or 32% over 100 days in another (Buchanan, 1961)), but since in these experiments lipids, nucleic acids, and carbohydrates as well as proteins were labeled, it was not clear whether any proteins were included among the compounds with the low turnover. Similar experiments with non-lipid material of brain particulate fractions showed that about 21% of the initial label remained stable in nuclei, corresponding to the amount of nuclear DNA, but that very little significant retention of label occurred in mitochondria and microsomes (Khan and Wilson, 1965).

The above studies established the dynamic state of some of the cerebral proteins, but the question remained whether all proteins turn over or whether some proteins after their deposition remain stable throughout the lifetime of the organ. In the experiments reported below we chose conditions under which all cerebral proteins could be expected to be labeled and then followed the release of the label as a measure of cerebral protein turnover.

METHODS AND MATERIALS

A slurry was prepared from Rockland mouse breeder diet and water; C^{14}-labeled L-lysine was mixed with it, and the food was dried. This food contained labeled lysine with a specific activity of 30 counts/min/μmole. After adult Swiss mice were given this food exclusively for a few days, the specific activity of plasma lysine approximated that in the food. After one week the animals were mated, and the same food was given to the mothers throughout pregnancy and lactation and to the offspring until two months of age. During their entire period of development, the young experimental animals thus had access to lysine of constant specific activity only. As expected, the specific activity of lysine in brain proteins in these two-month old animals was the same as that in the food (30 counts/min/μmoles, see "0 time," Table II).

When the animals on the radioactive diet reached 60 days of age, their food was changed to a diet containing only unlabeled lysine, and the decrease of radioactivity in the brain with time was measured. At the end of the experimental period, at various times after the replacement of labeled food, the animals were killed by decapitation; the brains were frozen in dry ice, weighed, and homogenized in 3% perchloric acid; and the acid-soluble fraction and the proteins were prepared as described previously (Lajtha, Furst, Gerstein and Waelsch, 1957; Lajtha, 1959). An aliquot portion of the perchloric acid extract was used for the determination of radioactivity of the acid-soluble fraction in a liquid scintillation spectrometer. The proteins were suspended with the aid of 5% thixotropic gel in the same counting fluid as the acid-soluble fraction was (Lajtha and Toth, 1965), and the radioactivity was determined the same way.

RESULTS

Table 1 shows that after the replacement of the labeled food the radioactivity in the proteins of the brain progressively decreases. The drop during the first 30 days to 29 per cent of the original activity is compatible with the average half-life of about 14 days derived from the incorporation studies. Since a significant portion of the released label may be reincorporated into proteins in the brain, the true turnover may proceed

at a higher rate than our measurements of decrease of label would indicate. Acid-soluble radioactivity is significant even after 60 days (Table I), making some reincorporation from the amino acid pool likely. Measurements of the release of label therefore gave a minimal turnover time; the true turnover may be somewhat more rapid. Also, it must be emphasized that the 14 day value is an average of heterogeneous turnover rates.

TABLE I

Decrease in Protein-Bound Radioactivity in the Brain with Time

Experimental time, days	Protein-bound activity		Acid soluble activity	
	counts/min/ 100 mg protein	% of original activity	counts/min/ g fresh tissue plasma	brain
0*	1680	100	94	170
30	492	29	49	65
60	143	8.5	8.4	43
150	81	4.8	0	0

*0 experimental time: 60 day-old Swiss mice just before replacing the labeled with the unlabeled diet.

Part of the reincorporated radioactivity is due to the incorporation of amino acids other than lysine, as C^{14} from uniformly labeled lysine appears in other amino acids such as glutamic acid if C^{14}-lysine is fed to the living animals over an extended time. The rate of decrease of the specific activity of protein-bound lysine therefore gives a closer estimate of protein metabolism than the decrease of total radioactivity in the proteins. In order to measure the specific activity of lysine, a portion of the cerebral proteins was hydrolyzed in 6 N HCl at 110° for 15 hours, the lysine was separated on AG 50 columns (Stein and Moore, 1950), its concentration was measured by the quantitative ninhydrin method, and the radioactivity of lysine and other fractions of the hydrolysate was determined (Lajtha and Toth, 1965). With longer experimental time a decreasing portion of the protein-bound radioactivity is found to be in lysine (Table II), therefore the radioactivity of lysine decreases at a somewhat higher rate than that of the proteins. These results also show that the major portion of the protein-

bound labeled lysine was rapidly replaced - over 90 per cent in 60 days. Although the remaining label after 150 days could not be estimated accurately, it seems that only a small fraction of the label originally incorporated in the proteins remained there after 150 days.

TABLE II

Decrease in the Specific Activity of Protein-Bound Lysine with Time

Experimental time, days	% of protein-bound counts in lysine	Counts/min μmoles/lysine	% of original activity left
0	88	30	100
30	86	8.8	29
60	71	2.1	7.0
150	42	0.7	2.4

Our earlier studies measuring decrease in rates of incorporation in longer term experiments indicated that only a small proportion of cerebral proteins has a considerably higher than average turnover rate (Lajtha, Furst, Gerstein and Waelsch, 1957; Lajtha, 1959). While the present experiments do not measure cerebral protein turnover rates accurately, they do indicate that proteins with a considerably lower than average turnover rate may also comprise only a relatively small fraction of brain proteins. It seems then that the great bulk, at least 90 per cent and perhaps more, of the cerebral protein, shows an average half-life between 10-20 days.

The replacement of most of the protein-bound lysine within 150 days (Table II) in brain in which all proteins were initially labeled indicates that at least in mice only a very small fraction, if any, of the cerebral proteins which are laid down before 60 days of age are stable throughout the lifetime of the animals. Such instability of cerebral proteins, although not in contradiction, must be taken into account in any theories describing the permanent storage of memory by means of proteins.

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