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Phospholipid Metabolism in Nervous Tissue

3. THE ANATOMICAL DISTRIBUTION OF METABOLICALLY INERT PHOSPHOLIPID IN THE CENTRAL NERVOUS SYSTEM

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The metabolic inertness of a proportion of the phospholipids of nervous tissue has been demonstrated in earlier experiments by incorporating ^{32}P and ^{14}C into the brain, spinal cord and peripheral nerve of animals during early development, and by following the fate of the labelled material for periods from 6 months to a year after injection (Davison & Dobbing, 1960; Davison, Morgan, Wajda & Wright, 1959). It was suggested that this persistence was not due to any general metabolic inertness of particular cerebral phosphatides such as sphingomyelin, but rather that their metabolic stability was associated with their presence in certain anatomical structures, predominantly in the myelin sheaths. It follows from this hypothesis that it should be possible to incorporate labelled substances into the lipids of the myelin sheath only during the period of myelinogenesis. On the other hand, in adult animals after injection of ^{32}P virtually all the labelled phospholipid should undergo turnover.

In the preceding paper (Davison & Dobbing, 1960) the fate of ^{32}P in the phospholipids of the nervous system has been compared in developing and adult rats. It was found that a disproportionate persistence of acid-soluble ^{32}P in the adult blood compared with that in the young made the comparison of cerebral-phosphatide metabolism difficult. It was therefore decided to approach the problem from an anatomical standpoint.

Although the white matter of the brain contains as many cells as grey matter, much of the central-nervous-system myelin is located in the white matter. If the uptake of ^{32}P into white- and grey-matter phospholipid is expressed as specific activity, then in adults the white-matter specific activity should be lowered by the presence there of the inert proportion representing the fully developed myelin sheaths, and any isotope incorporated into phospholipids should not predominate in the more myelinated areas. Conversely, if the present hypothesis be true, that part of the phospholipid ^{32}P which persists in the brain after injection into developing animals should be located predominantly in the white matter. In this way it should be possible to detect a different anatomical pattern of distribution of phospholipid ^{32}P in the two age groups several weeks after injection of ^{32}P, while the radioactivity is still easily measurable. If metabolically inert phospholipid were found to be mainly situated in white matter, this would be a result similar to that previously obtained with [4-^{14}C]cholesterol (Davison, Dobbing, Morgan & Wright, 1959) and would be further presumptive evidence in favour of a metabolically inert myelin sheath.

METHODS

Two groups of rabbits of the same strain (Copenhagen White) were injected with ^{32}P. The first group consisted of 11 animals from two litters, each injected intraperitoneally when 16 days old with 250 μc of ^{32}P as phosphate. This represented a dose of about 1 μc of ^{32}P/g. body wt. at the time of injection. The second group of six adult rabbits, of 3 kg. average weight, each received intravenous injections of 500 μc of ^{32}P. Animals were killed singly with intravenous pentobarbitone, up to 180 days after injection, except for the final point in the developing series when two animals were killed together and their tissues were combined.

Brains were removed immediately after death and placed in formalin–0·9% sodium chloride soln. (10:90, v/v) for fixation for 24 hr. They were then dissected by a procedure already described (Davison, Dobbing, Morgan & Wright, 1959). Briefly, this involved cutting the whole brain into coronal slices about 2 mm. thick and then dividing the whole of each slice grossly into 'white' and 'grey' parts. In the forebrain and cerebellum this presented little difficulty. Wherever grey and white matter is not easily distinguishable, for example in much of the brain stem, equal amounts were allocated to the 'white' and 'grey' specimens. The result is a predominantly white and a predominantly grey specimen, each inevitably contaminated by the other, the two comprising the whole brain when added together.

Lipid extraction and counting procedure. The methods for the extraction of phospholipid and for the estimation of phosphorus were as previously described (Davison & Dobbing, 1959, 1960). Radioactivity was determined by liquid β counting up to 85 days after injection and by scintillation counting thereafter as reported in the preceding paper (Davison & Dobbing, 1960).

RESULTS

The method of dissecting the brains produced reasonably uniform samples, as can be seen from Table 1, in which the white fraction is expressed as a percentage of the whole brain. In the developing series white matter forms an increasing proportion of the wet weight and its phospholipid phosphorus content also rises during the first 100 days (the period during which myelin is being laid down).

The distribution of phospholipid ^{32}P between white and grey fractions up to 180 days after injection into adult animals is shown in Table 2. The specific activity in the white fraction is below or equal to that in the grey at all times except at the 180-days point, where it shows a slight excess. Towards the end of this period, however, the low radioactivity of the phosphorus increases the experimental error, and the difference between white and grey fractions is probably not significant.

The fate of phospholipid ^{32}P in 'white' and 'grey' fractions of brain in animals injected with inorganic ^{32}P when 16 days old is also shown in Table 2. With these animals, the specific activities have been individually corrected for the increments in phospholipid phosphorus occurring during growth, these levels in the 17-day-old rabbit being used as a reference point. As might be expected, phospholipid phosphorus increased in both fractions but disproportionately more so in the white matter (Table 1). Failure to make such corrections for growth would result in an apparent depression of the later phospholipid radioactivity and a spurious suggestion of loss, particularly in the white fraction. Table 2 shows that in about the first 40–50 days after injection, a turnover of phospholipid ^{32}P is taking place more in the grey fraction than in the white. Subsequently the decline in radioactivity up to 174 days after injection takes place almost wholly in the grey matter—that is, the white matter shows much greater persistence.

This relatively greater persistence of phospholipid-^{32}P radioactivity in the white fraction of the younger series is best shown by expressing the results as the ratios of white-matter to grey-matter specific radioactivity. In this way variability of dosage, concentrations of ^{32}P in the blood and biological variation between animals are eliminated. Fig. 1 shows that this ratio remains virtually at unity in the animals injected during adult life. In

Table 1. *Changes in weight and phospholipid-phosphorus content of 'white' matter in developing and adult rabbits*

White-matter fraction is expressed as a percentage of whole brain in young and old rabbits when dissected according to the method described in the text.

Age (days)	Time after injection (days)	Wet wt. of 'white' matter as % of whole brain	Phospholipid P in 'white' matter as % of total brain-phospholipid P
		Developing animals	
17	1	33	41
23	6	29	34
30	14	33	43
46	30	35	37
67	51	33	45
100	84	48	56
101	85	49	51
149	133	45	55
182	166	47	56
190	174	43	57
		Adult animals	
	7	55	56
	14	52	50
	23	57	63
	51	56	57
	85	47	66
	180	47	56

Table 2. *Specific activity of [^{32}P]phospholipid in 'white' and 'grey' matter in developing and adult rabbits*

Specific activity (counts/min./mg. of P) has been determined as described in the text. Results for developing rabbits have been corrected for phosphorus increments during growth. Figures for 174 days after injection are the means of two animals.

Time after injection (days)	Specific activity in rabbits injected with ^{32}P when 16-days old		Time after injection (days)	Specific activity in adult rabbits injected with ^{32}P	
	'White' matter	'Grey' matter		'White' matter	'Grey' matter
1	2 610	2 640	—	—	—
6	7 725	10 400	7	992	1310
14	9 930	10 500	14	1510	2240
30	13 150	16 300	23	2090	2480
51	9 930	7 220	51	1870	2190
84	12 560	5 810	—	—	—
85	11 200	7 040	85	1015	1035
133	7 830	4 080	—	—	—
166	7 450	2 290	—	—	—
174	8 800	2 110	180	1200	1060

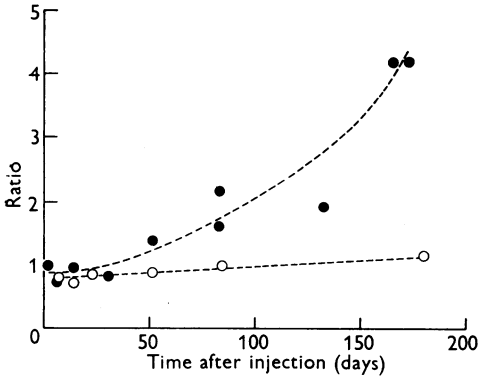

Fig. 1. Ratio of 'white'- to 'grey'-matter specific activity. Specific activities were determined by methods described in the text. Figures for 'white' matter from young rabbits have been corrected for increments of P during growth as reported in the text. Results show the ratios for young (●) and adult (○) rabbits.

those injected neonatally, the white : grey ratio increases from about 50 days after injection until, at 174 days the corrected specific radioactivity of phospholipid phosphorus in the white matter is four times that in the grey.

DISCUSSION

It is known that different phospholipids 'turnover' at widely different rates in the brain (Ansell & Dohmen, 1957), and also that any particular phospholipid may have different apparent rates of metabolism in animals of different ages (Ansell & Spanner, 1959; Miani & Bucciante, 1957). It seems equally probable that in an organ so complex structurally as the brain, the rate of metabolism of such substances may depend as much on their location in tissue elements as on their chemical constitution. This heterogeneity takes an extreme form when the substance is metabolically inert in some components of the organ while subject to turnover in others, as might happen were it present in myelin or in cells. During the growth and maturation of the central nervous system, the position is further complicated by the fact that the spectrum of turnover rates for particular phospholipids may change with increase in the age of the animal. Any valid comparison of the phospholipid metabolism of animals at different stages of development thus presents a number of difficulties, only some of which have been solved.

When labelled materials are incorporated into the brain during development, their persistence in any metabolically inert structure may be seriously underestimated if results are expressed as specific activities, or in any other way as concentrations, since these labelled compounds are progressively diluted by unlabelled compounds taken up during normal growth. To obviate this source of error, our results have previously been expressed in terms of whole organs.

If it is desired to study relative persistence in the various gross anatomical divisions of the brain, results could be expressed by taking whole cerebrum, whole cerebellum, etc. (see Fries & Chaikoff, 1941*a*, *b*). On the other hand, any investigation of metabolism or persistence in a microscopical structural component in the tissues, such as the myelin sheath, which is common to all the major anatomical divisions of the central nervous system, is at present possible only by dissecting the whole brain as nearly as possible into its 'white' and 'grey' matters. This unfortunately makes it impossible to express results in terms of 'whole structure', and necessitates the use of specific-activity-correction devices to compensate for the effects of growth. The method of compensation employed here for the series of immature rabbits has been to correct each phospholipid-phosphorus specific-activity value for growth increments of phospholipid phosphorus, using the first 17-day-old animal as an arbitrary reference point. This correction factor, which is based on brain-phospholipid phosphorus at various ages, never exceeds 3·5. This procedure introduces several theoretical objections, the chief of them being the implied assumption that subsequent growth increments have not added to the radioactivity of those extracted phospholipids by further incorporation of additional labelled precursor. However, in the post-injection period, the levels of circulating precursor ^{32}P fall more rapidly in developing than in mature animals so that errors from this source are unlikely (Davison & Dobbing, 1960). Moreover, the present investigation is principally concerned with the location of the persisting phospholipid ^{32}P long after the blood levels in both immature and adult rabbits have become insignificant.

From this present study on phospholipid metabolism in the brain, two facts seem to emerge. First that [^{32}P]phospholipid persists in the white matter and not in the grey matter after injection of ^{32}P into young rabbits. This finding confirms earlier work (Davison, Morgan, Wajda & Wright, 1959) with [3-^{14}C]serine in which it was demonstrated that sphingomyelin and cephalins undergo little turnover in the brain up to 250 days after injection. In that paper it was also shown that in an animal killed after 250 days, four times as much radioactive sphingomyelin and cephalin was present in the 'white' as in the 'grey' matter. Secondly, a comparison of the ratio of radioactivity in 'white' and 'grey' matter obviates many errors and Fig. 1 shows how large are the differences that develop

with time in this ratio for the metabolism of ^{32}P when injected into developing or mature rabbits. This work is therefore fully consistent with the hypothesis that the lipids of the myelin sheath are metabolically inert. Furthermore, it ought to be possible, by more refined anatomical and biochemical techniques, to establish the hypothesis in even greater detail.

SUMMARY

1. ^{32}P has been injected into developing and adult rabbits and its fate investigated in the phospholipids of white and grey matter of brain.

2. Those phospholipids which have been found to persist are predominantly located in the white matter.

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Metabolism of Myosin and Life Time of Myofibrils

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The concept of the dynamic state of body proteins is now generally admitted, but recent work has made some reappraisal necessary. Proteins may undergo three types of metabolic patterns. Some turn over at a rather fast rate, but the turnover is the result of a secretion out of the cells: such is true of digestive enzymes or of the plasma proteins as studied by Miller, Bly, Watson & Bayle (1951). Others remain in the cells and undergo turnover; Dreyfus, Schapira & Kruh (1956) have shown that this is so with muscle aldolase. On the other hand Shemin & Rittenberg (1945) demonstrated that haemoglobin does not turn over during the life of the cell. Later this concept was applied by Neuberger, Perrone & Slack (1951) and by Robertson (1952) to collagen, which might remain inert during the whole life of the animal. Thompson & Ballou (1956) have emphasized the quantitative importance of apparently inert proteins. Shemin & Rittenberg (1944) showed that the muscle proteins can be divided into two groups, one of which is rapidly metabolized and the other slowly metabolized. Myosin belongs to the slowly metabolized group (Bidinost, 1951).

Neuberger (1952) suggested that 'the metabolic activity of actin or myosin in the muscle fibre, or that of collagen, for example, is not a continuous function, but varies in a discontinuous manner and is dependent on morphological changes of the structures with which these proteins are associated'. This idea has never been experimentally checked for muscle proteins. In this work we have tried to determine whether myosin is in a dynamic equilibrium or whether it is inert enough to permit the determination of the life span of the cell or of a part of the cell. A preliminary note has been previously published (Dreyfus, Kruh & Schapira, 1957).

METHODS

Injection of radioactive glycine. White male rats of pure Wistar strain, weighing 200–250 g., fed with an identical diet, were used in each series of experiments. In these conditions, the specific activity of radioactive proteins from two animals given the same diet did not differ by more than 15%. The animals were given two types of diet, a 'standard' diet and a 'protein-rich' diet. The standard diet was a commercial one containing (%): oats (3), corn (5), wheat (30), molasses (2), wheat and barley seeds (8), lucerne flour (4), defatted peanut meal (5), defatted soya meal (11), defatted flax meal (5), skimmed-milk powder (3), meat flour (4), fish flour (4) and brewer's yeast (3), together with salts and vitamins. This standard diet contained approx. 25% of protein. The 'protein-rich' diet was pre-