A BIOCHEMICAL AND MORPHOLOGIC STUDY OF MYELINATION AND DEMYELINATION*

I. Lipide Biosynthesis in Vitro by Normal Nervous Tissue

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As part of a study of metabolic change during demyelination, with particular reference to lipogenesis, it was necessary to use a number of different preparations of nervous tissue. The present paper describes such preparations (grey matter, white matter, and peripheral nerves) obtained from normal animals, and provides comparative data on their ability to incorporate different isotopically labelled building blocks into lipide. Since myelination is a function of growth, the study was extended to nervous tissue from animals of different ages. All preparations were examined histologically, with a view to correlating morphologic and biochemical data.

Material and Methods

Male rats of the Wistar, Hisaw, or Sprague-Dawley strains weighing 230 to 280 gm. were used unless otherwise stated.

Peripheral nerves were obtained by rapid dissection after decapitation. Two preparations were used: that referred to as "sciatic" which included the tibial branch, was about 50 mm. in length and weighed 30 mg., and the brachial plexus which averaged about 20 mm. in length and 28 mg. in weight. In early experiments the four nerves from one rat were pooled and used as a single specimen; later they were equally divided between two incubation flasks.

Grey Matter.—A cross section of rat hemisphere shows a surprisingly thick cortex: the average is 1.9 mm. for young male rats (1). Human cortex is 1 to 4.5 mm. thick (2). The white matter in rats is limited to a deep layer, far thinner than the cortex (Fig. 1 A), making it easy to obtain grey matter free of white matter, providing one can cut reproducible slices of this very soft tissue. Attempts with the Stadie-Riggs microtome were not entirely successful; the yield of grey matter was low and the operation time-consuming. Many slices tended to be "smeared." A tissue slicer based on a different principle was therefore constructed. This has been described elsewhere (3). With this apparatus, cortex preparations of known thickness

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(0.23 ± 0.016 mm.) were obtained rapidly with little trauma. Each hemisphere yielded two surface slices, a large one from the lateral aspect, and a smaller one from the dorsal, giving a total weight of 25 to 30 mg. The two slices were pooled and studied as a single specimen. Deeper slices had different metabolic characteristics (see below). The time required for one preparation was 4 to 6 minutes from the death of the animal to completion of the slices. Representative histological sections of the first and second slices are shown in Figs. 1 B and 1 C.

Adequate samples of *white matter* could be secured only from the spinal cord. The vertebral column was excised, and the vertebral canal was opened with a small bone rongeur. The cord was transferred onto a filter paper moistened with saline, on the lid of a closed Petri dish filled with ice. The dura was then slit open and ripped off. The cord was cleaved into a right and a left half, starting at the cervical end. The cleavage plane was easily found, provided one started on the ventral side. After the two halves had been dissected apart over 5 to 6 mm., they could be seized with two forceps and pulled apart. Each half was laid on the filter paper, medial surface up. The grey matter appeared clearly as a thin streak in the midline, and was wiped out with small firm pads of cotton moistened in saline. Gradually, as the grey matter was removed, the white matter was spread out as a flat ribbon. A thin grey line, closer to one edge, corresponded to the posterior horn, which reaches deep into the white matter and could not be wiped off. This edge of the ribbon was eliminated by a longitudinal cut using an ordinary scalpel (Figs. 1 D and 1 E); two transverse cuts removed the ends of the strips, which had been traumatized. The final preparation weighed 50 to 70 mg., and measured 5 to 6 cm. in length, 3 mm. in diameter, and 0.4 mm. in thickness. The time required for the completion of both strips was 15 to 18 minutes from the death of the animal. Judging from measurements of oxygen uptake and lipogenesis, this preparation was highly reproducible.

Prior to incubation all tissues were kept on filter paper moistened with Krebs-Ringer phosphate medium on the bottom of a chilled Petri dish. They were then blotted gently and the fresh weight determined to the nearest 0.2 mg. on a torsion balance. *Dry weights* were determined by desiccation at 110°C. to constant values. The tissues were transferred directly from the animal to the balance, or, in the case of nerves, were briefly stored under the skin of the donor animal to prevent loss of water.

Incubation was carried out in a Warburg microrespirometer at 37°C. The medium was Krebs-Ringer phosphate solution adjusted to pH 7.4, with glucose as a source of energy (10 μM/ml.). Ordinarily the flasks were prepared as follows: Main chamber—2.8 ml. of medium containing glucose; side arm—0.2 ml. of medium containing the radioactive lipide precursor; center well—0.2 ml. of 20 per cent NaOH. The average amount of tissue per flask was 70 to 100 mg. The contents of the flasks were equilibrated with oxygen for 10 minutes. The side arms were then tipped and after an additional period of equilibration (10 minutes) the first manometric readings were taken. Subsequent readings were taken at 30 minute intervals for 4 hours, unless otherwise stated.

After incubation the tissue was removed from the flasks, blotted on filter paper, rinsed 10 minutes in 10 ml. of Krebs-Ringer phosphate solution, again blotted and ground with chloroform-methanol 2:1 (4) in a motor-driven glass homogenizer similar to the Potter-Elvehjem model but conical in shape. The *extraction of lipide* and washing to remove non-lipide contaminants were carried out essentially according to the method of Folch *et al.* (4, 5). The washing procedure was performed four times and was shown to be effective in removing radioactive water-soluble substances as well as contaminants deliberately added to the lipides of nervous tissue (reference *cf.* 5). In the case of experiments using P^{32}, traces of non-radioactive inorganic phosphate were included in the washing fluid to facilitate removal of extraneous radioactivity. The lower phase after the final washing was evaporated to dryness under N_2 in a water bath (56–60°), and the residue was taken up in 2 cc. of chloroform-methanol at 55–60°C. and filtered. The filter was washed three times with small amounts of solvent and the filtrate was

again evaporated to dryness at 56–60°C. The residue was dissolved in 0.5 cc. of chloroform and plated on tared stainless steel planchets containing a disc of lens paper. Complete transfer from the centrifuge tube was ensured by washing the tube 3 to 5 times with small aliquots of chloroform and transferring these to the planchet. The solvent was evaporated under a heat lamp and the planchets placed in a vacuum desiccator over $CaCl_2$ overnight. The desiccator contained also a small beaker of flaked paraffin wax. The planchets were weighed again to obtain the weight of lipide isolated, and counting was carried out in a windowless gas-flow counter in the proportional range as described previously (6, 7).

The *radioactive substrates* used were the following: acetate-1-C^{14}, synthesized by a standard method (8); DL-glycerol-C^{14}, prepared as described previously (9); choline-1,2-C^{14} obtained from the California Research Foundation, and glucose-C^{14} (uniformly labelled) from the Nuclear Corporation, Chicago. Radioactive phosphate was obtained from Oak Ridge and was used as potassium phosphate. The labelled organic substrates, except glucose, were used at a concentration of 2 mg. per flask. Glucose and phosphate incorporation into lipide were studied at the concentration of the Krebs-Ringer medium mentioned above. These concentrations of substrate were found to give optimal results under the conditions used. In most experiments each Warburg flask contained from 2×10^5 C.P.M. to 1×10^6 C.P.M.

RESULTS

The data to be presented were derived from about 50 experiments. A typical experiment consisted of 12 flasks containing four types of sample, each in triplicate. The dry weights, lipide content, and respiratory data for all preparations used are shown in Table I.

After incubation in the presence of glucose, all the tissues showed histological evidence of edema, slight in peripheral nerves and grey matter, more pronounced in white matter. When glucose was not present in the medium there was a moderate increase in the degree of edema in peripheral nerves and grey matter, and damage to white matter was observed even on a gross level. The samples of white matter recovered from the glucose-free medium had often disintegrated into small flakes. Histologically, swelling and fragmentation of fibres was notable, while control preparations incubated with glucose (3, 10, and 30 μM per ml.) remained comparatively intact. The effect of glucose on respiration of the tissues is shown in Text-fig. 1.

Effect of Various Experimental Conditions on Respiration and Lipide Biosynthesis.—The time which elapsed between the death of the animal and the first manometric reading varied between 25 and 55 minutes. Only one phase of processing was involved in the delay; *i.e.*, storage in chilled medium. The extent of the errors related to this variation was tested by storing samples of peripheral nerve in chilled medium for different periods before incubation. Respiration was decreased by 11 per cent after storage for 30 minutes and by 20 per cent after storage for 60 minutes (*cf.* reference 10, 11). Incorporation of acetate into lipides followed a similar trend. A study was also made of the deterioration of nervous tissue *during* the incubation period, and the results are shown in Text-fig. 2.

Several investigators have reported (12) that nerves cut into small segments

respire more than intact nerves. This observation suggested that the penetration of lipide building blocks might be improved by the same procedure. Accordingly, normal nerves were compared with nerves cut into 10 to 12 segments each. Respiration was not measurably affected, but acetate incorporation fell by 25 per cent when the nerves were cut into segments.

It was thought that differences might exist in composition and metabolic activity along the length of a peripheral nerve. Thus, sciatic preparations were arbitrarily divided into three parts: distal, middle, and proximal (the tibial nerve, and the lower and upper halves, respectively, of the sciatic trunk proper). Homologous nerve segments from different rats were pooled and incubated

TABLE I

*Characteristics of Preparations of Rat Nervous Tissue**

Tissue‡	Dry weight§	Lipide‖	O_2 uptake¶	Q_{O_2}**
	per cent	*per cent*	*μl.*	
Grey matter	19.5	3.7	218	11.2
White matter	34.5	13.6	86	2.5
Peripheral nerve	31.0	9.0	32.5	1.1

* Adult animals weighing 250 to 280 gm. were used.

‡ The preparation designated in this column is described in the section on Material and Methods.

§ Expressed as a percentage of the fresh weight of the tissue.

‖ Expressed as a percentage of the fresh weight of the tissue prior to incubation.

¶ Expressed as μl./hour and 100 mg. fresh tissue, for the 1st hour of incubation.

** Expressed as μl./hour and milligram of dry tissue, for the 1st hour of incubation.

with C^{14}-acetate. Text-fig. 3 illustrates the results of three experiments, each with triplicate samples, using male rats weighing 350 to 500 gm. In general, it may be seen that there was a drop in the figures obtained, from the proximal to the distal end, with the exception of the value for dry weight.

In view of the small amount of grey matter obtained from one rat as surface slices, the question arose whether one might use also second slices pooled with the first (Figs. 1 B, 1 C). Quadruplicate samples of each preparation were cut from the hemispheres of two rats, and incubated with radioacetate. The results are shown in Table II. When second slices were compared with surface slices, it was found that their lipide content was slightly greater, while lipogenesis from acetate was only 40 per cent of that of surface slices and oxygen uptake was not significantly different (*cf.* reference 10). In view of these findings, further experiments were carried out with surface slices only.

Comparative Aspects of Lipide Biosynthesis.—In each of a series of experiments, peripheral nerves, grey matter, and white matter were incubated with one of five labelled lipide precursors. The purposes were: (*a*) to compare the various preparations with respect to the degree of incorporation of each substrate, and (*b*) to compare the various substrates with respect to their degree

of incorporation into lipides. The results are presented in Tables III and IV respectively.

A number of experiments were carried out to determine how much of the C^{14}-containing substrates was oxidized to CO_2, compared with the amount

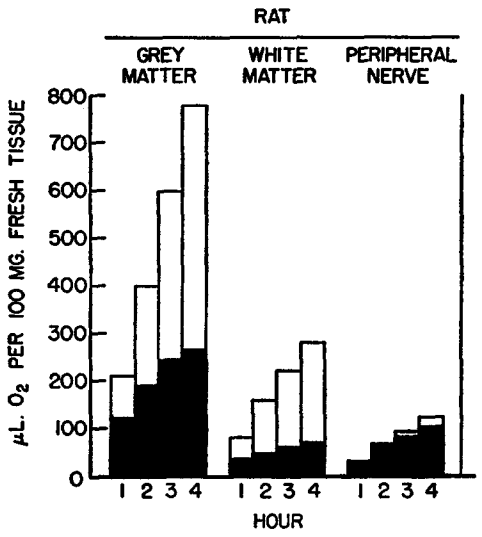

FIG. 1. Oxygen uptake of 3 preparations after 1, 2, 3, and 4 hours of incubation in the presence (total height of column) and in the absence of glucose (shaded areas).

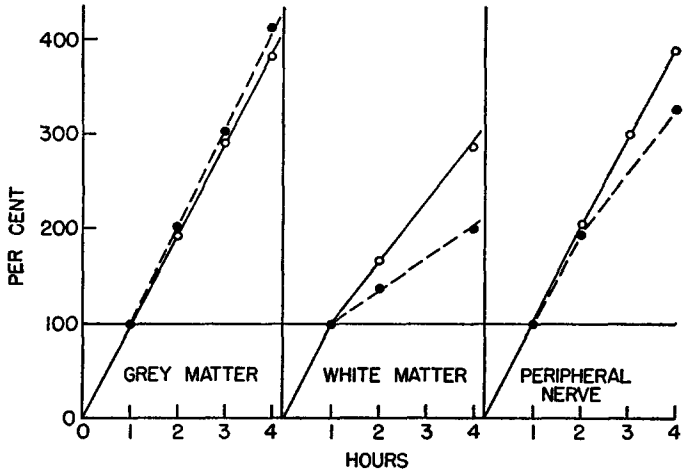

FIG. 2. Maintenance of activity *in vitro* of three preparations of nervous tissue. ○, oxygen uptake; ●, incorporation of radioactivity into lipide. In the case of grey matter the precursor was P^{32}-labelled phosphate; for white matter and peripheral nerve it was C^{14}-labelled acetate. All results at the end of the 1st hour were set at 100, and the curves thus represent cumulative values expressed as a percentage of the values for the 1st hour.

incorporated into lipide. It may be seen (Table V) that for a given amount of substrate-carbon converted to CO_2, choline is enormously more active with respect to its incorporation into lipide than is acetate or glycerol. Further, in the case of both acetate and choline there is a great disparity between the lipide-synthesizing activity of grey matter and peripheral nerve.

Biochemical and Morphologic Changes Related to Age.—It became apparent, early in this work, that both oxygen uptake and lipogenesis of peripheral nerves were closely dependent upon the age of the animal. This relationship was made the subject of more detailed study, as described below. For the sake of compari-

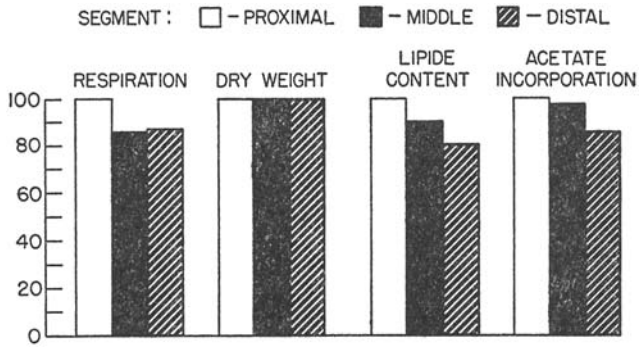

FIG. 3. Gradients along the sciatic preparation. The values for the proximal segment are set equal to 100. The data were obtained from 12 measurements of each function. Respiration was measured as μl. O_2/hour and 100 mg. fresh weight; dry weight as percentage of fresh weight; lipide as milligrams extracted per 100 mg. fresh weight and acetate incorporation as C.P.M./100 mg. lipide.

son, similar data were collected for grey matter, on which considerable information is already available (13, 14).

Samples of peripheral nerves were collected from a total of 300 rats of ten age groups (1, 5, 10, 15, 20, 30, 60, 157, 266, and approximately 500 days). Some difficulty was experienced in preparing the nerves of the newborn animals, which appeared as delicate, semitransparent threads weighing 1 to 1.5 mg. each. The use of Swiss watchmaker's forceps was of great help in minimizing trauma. Three or more samples from each age group were incubated with C^{14}-acetate; triplicate samples of 6 age groups were kept for the determination of dry weight. The results are presented in Text-figs. 4, 5, and 6. The effect of age on the incorporation of phosphate into the lipides compared with that of acetate was determined using two groups of littermates, 21 and 56 days old respectively. In the older group, the incorporation of both substrates was only 41 to 43 per cent of that found in the younger group.

Samples from animals of all age groups were fixed in 10 per cent formalin, embedded in paraffin, and stained with hematoxylin and eosin, or treated by the methods of Adams, Thomas, and Davenport (15) for myelin and for axis cylinders. The results are presented in Figs. 2 and 3. In addition, the number of nuclei per microscopic field was counted in nerves of 12 age groups on longitudinal sections stained with hematoxylin and eosin. Because of diffi-

TABLE II

*Comparison of the First and Second Slices of Brain Cortex**

	O_2 uptake‡	Lipide content§	Lipide activity‖
	μl.	*mg.*	C.P.M.
First slice	222 ± 5	2.98 ± 0.19	1830 ± 173
Second slice	233 ± 5	3.7 ± 0.16	1081 ± 69
P	<0.2	<0.02	<0.01

* The data refer to quadruplicate samples.

‡ Expressed as μl. O_2 per 100 mg. fresh weight and hour.

§ Expressed as milligrams lipide per 100 mg. fresh tissue.

‖ Expressed as counts per minute and 100 mg. lipide.

TABLE III

Comparison of Three Preparations of Rat Nervous Tissue with Respect to the Incorporation of Various Substrates into the Lipides

The activity incorporated into the lipides of each preparation has been referred to the activity incorporated into the lipides of peripheral nerve. The specific activity (s.a.) of the lipides of the peripheral nerve has been set at 100 c.p.m./100 mg. lipide. Relative total activity in the lipide of each preparation (t.a.) is obtained by multiplying the lipide specific activity by the lipide content, and has also been set at 100 c.p.m. for peripheral nerve. Comparisons may be made horizontally only.

Labelled substrate	Grey matter		White matter		Peripheral nerve	
	s.a.	t.a.	s.a.	t.a.	s.a.	t.a.
Acetate	25	10	10	15	100	100
Choline	576	237	65	98	100	100
Glucose	221	91	10	15	100	100
Glycerol	628	260	50	76	100	100
Phosphate	760	312	49	74	100	100

TABLE IV

Comparison of Four Substrates with Respect to the Degree of Their Incorporation into the Lipides of Three Preparations of Nervous Tissue

For each substrate, the activity incorporated into the lipides is referred to the activity incorporated from labelled acetate, when the value for this substrate was set at 100. Comparisons may be made horizontally only.

Tissue	Substrate			
	Phosphate	Glycerol	Choline	Acetate
Grey matter	1825	502	230	100
White matter	294	100	65	100
Peripheral nerves	60	20	10	100

culty in establishing the identity of each type of cell, only total counts were performed (it may be assumed that the Schwann cells greatly outnumber all other types of cells). The progressive decrease in thickness of the nuclei (see Fig. 3), which could account for a slight over-

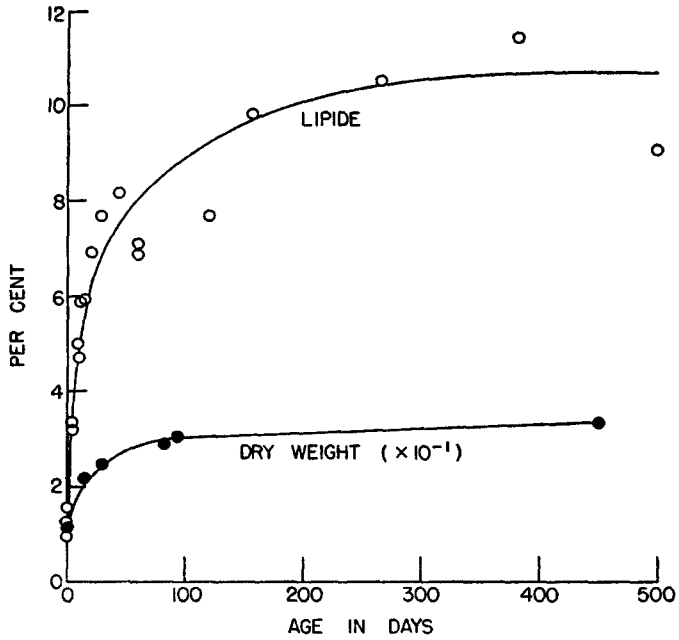

FIG. 4. Dry weight and lipide content of rat peripheral nerves at various ages, expressed as a percentage of the fresh weight.

TABLE V

The Conversion of the Carbon of Some C^{14}-labelled Substrates to CO_2 Compared with Incorporation into Lipide

Results are expressed as total activity (C.P.M.) in the lipide per 100 C.P.M. in CO_2 recovered as $BaCO_3$ from center well.

Tissue	Substrate		
	Acetate	Glycerol	Choline
Grey matter	2	86	622
White matter	55	—	2,700
Peripheral nerve	71	91	10,900

estimate (16) in the younger groups, was disregarded. The results are plotted in Text-fig. 9. The possibility also existed that the nerves of younger animals, which have a greater water content, might undergo a greater shrinkage in the process of embedding, and thus affect the validity of the nuclear counts. To examine this question, sciatics of rats 1, 20, and 60 days old were measured in length and width before and after fixation in 10 per cent neutral formalin and again after paraffin embedding. The shrinkage caused by formalin was 1 to 3 per cent in

length and 15 to 19 per cent in width, irrespective of age; the total shrinkage in paraffin-embedded nerves was 6 to 9 per cent in length and 52 to 55 per cent in width, again irrespective of age.

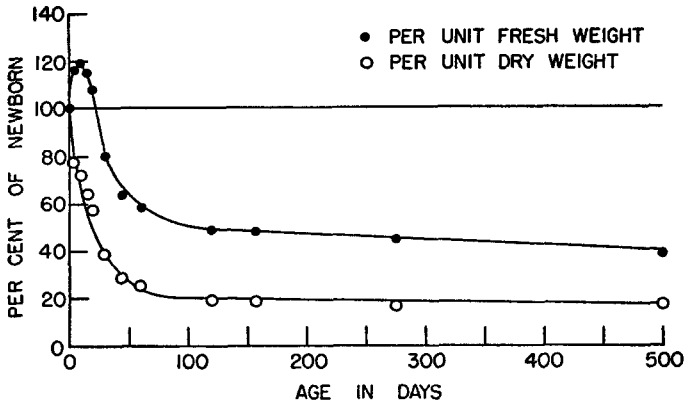

FIG. 5. Respiration of rat peripheral nerves at various ages. Results are expressed as a percentage of the values obtained for newborn animals (71 μl. per 100 mg. fresh weight per hour).

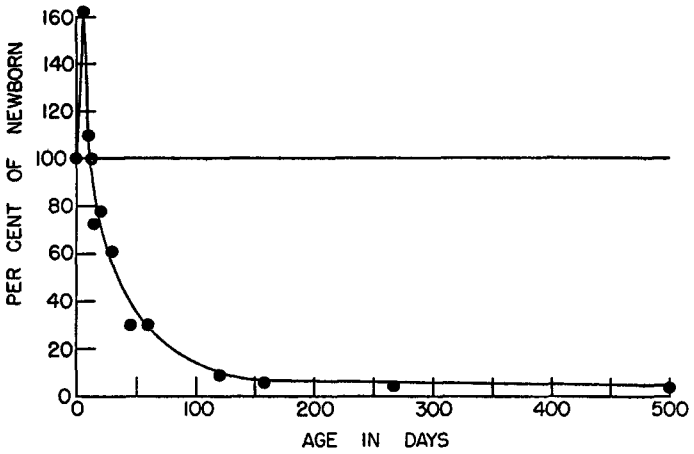

FIG. 6. Incorporation of acetate-1-C^{14} into the lipides of rat peripheral nerves at various ages. Results are expressed as a percentage of the specific activity (C.P.M./100 mg.) of nerve lipides from newborn animals. There was no overlap in values obtained from six experiments for each of the first three points on the curve.

Surface slices of cerebral cortex were prepared from 50 rats belonging to five age groups. Dry weight and lipide content were determined and samples were also incubated with radioacetate. For the newborn rats, the yield of ten hemispheres was pooled to obtain one sample of 25 mg. Text-figs. 7 and 8 summarize the results obtained for grey matter.

Comparison of Nerves with Other Tissues with Respect to Lipide Biosynthesis.—The nerves of six fed rats, belonging to two age groups, were compared with liver slices from the same animals. It was found that in the younger rats the incorporation of acetate (expressed as specific activity of lipides) was greater in the nerves than in liver slices, whereas the opposite was true for the older

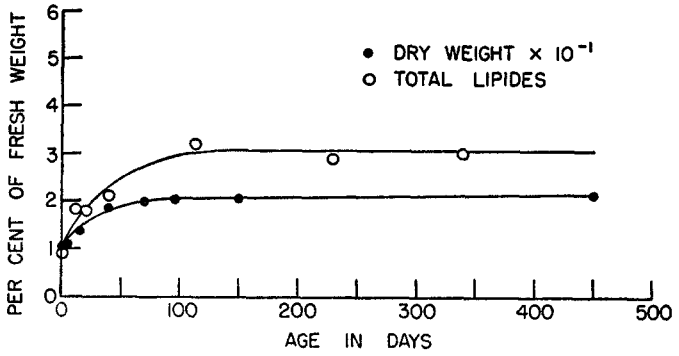

FIG. 7. Dry weight and lipide content of rat grey matter at various ages.

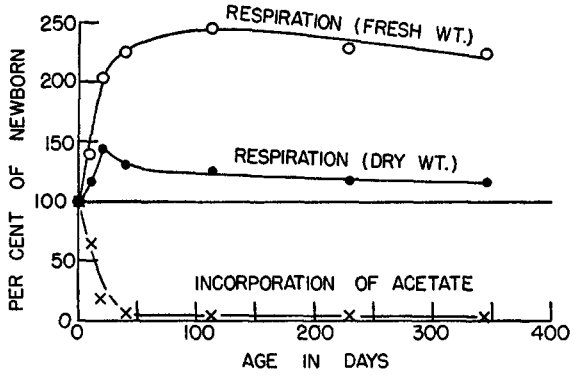

FIG. 8. Rat grey matter: respiration and incorporation of acetate-1-C^{14} into lipides at various ages. Results are expressed as in Text-figs. 5 and 6. The absolute value of oxygen uptake in the newborn was 78 μl. per 100 mg. fresh weight per hour.

animals. This was readily understood when it was found that respiration and lipogenesis did not change appreciably with age in the liver, whereas both functions dropped markedly in the nerves (Table VI). Nerves of six adult rats were also compared with adipose tissue obtained by excising fragments of suitable thickness from the omentum and from the testicular adipose body. The oxygen uptake was 2.5 times greater, and incorporation of acetate (measured as specific activity of lipides) was also 3 to 5 times greater in nerves than in adipose tissue (Table VI).

DISCUSSION

In discussing the lipogenetic properties of "grey matter," "white matter," and "peripheral nerves," it should be appreciated that reference is made to the particular preparations used in this study. While each preparation may be assumed to be representative of one tissue, local variations are to be expected. This is particularly true for grey matter, as becomes obvious when first and second slices are compared (Table II). It is also assumed that the lipide extract represented material derived from the nervous components of the preparation. A significant source of extraneous lipide—adipose tissue—could conceivably

TABLE VI

A comparison of the Lipogenetic Activities of Peripheral Nerve, Liver Slices and Adipose Tissue

The substrate was acetate-1-C^{14}. Tissues were incubated under identical conditions, *i.e. ca.* 100 mg. of tissue in 3.0 ml. medium containing 2 mg. acetate. Results are expressed as counts per minute per 100 mg. lipide in each comparative experiment, and as a percentage of the specific activity of the lipides of peripheral nerve. A, B, C and D refer to four separate experiments.

Animal		Peripheral nerve		Liver		Adipose tissue	
		C.P.M.	*per cent*	C.P.M.	*per cent*	C.P.M.	*per cent*
Young rats*	A	3530 ± 260	100	1568 ± 133	44	—	—
	B	1440 ± 230	100	—	—	173 ± 12	12
Older rats‡	C	1031 ± 258	100	1531 ± 526	149	—	—
	D	27,367 ± 3,700	100	—	—	8465 ± 415	30

* 235 to 300 gm.

‡ 350 gm. or over.

have been present only in the case of peripheral nerves. However, in the rat nerves used here, fasciculation was minimal, endoneurial adipose tissue was not present, and the few fat cells occasionally attached to the epineurium could be disregarded. "Lipogenesis" in this paper refers to the incorporation of radioactively labelled building blocks into total lipide. Two functions are discussed:—(*a*) specific activity of the extract, *i.e.* counts per minute per unit weight of lipide, and (*b*) total incorporation of C^{14} into lipides by the tissue, *i.e.* percentage weight of lipide in the tissue × specific activity.

It is readily apparent from Text-fig. 2 that the three preparations which have been used were not equal in their capacity to survive *in vitro*. The degree of metabolic deterioration closely parallelled histological evidence of damage. Grey matter was the sturdiest of all, with peripheral nerves next in resistance. The time curve of lipogenesis from acetate shown in Text-fig. 2 (peripheral nerve) was observed over 7½ hours; three samples of nerves taken at this

point showed that the incorporation was still proceeding at an almost linear rate. Rat nerves are exceptionally well suited for incubation; their small caliber and cylindrical shape make good diffusion possible (12) while the number of cells damaged by excision is minimal. White matter was the most adversely affected by prolonged incubation.

Mechanical trauma previous to incubation was detrimental to the metabolic functions of all tissues, and especially of white matter. In general, it was observed that lipogenetic activity was more sensitive than respiration. In the course of incubation, for example, the rate of lipogenesis from acetate dropped faster than that of oxygen uptake (Text-fig. 2). This also occurred as a result of trauma during preparation, or of storage in the cold for periods longer than 1 hour. An extreme case was that of homogenization, which abolished lipogenesis with a relatively small drop in respiration. A similar trend was observed by Dawson, on brain suspensions, using P^{32}-phosphate (17). It would appear that the mechanisms of lipogenesis, and by inference, of myelination, are more easily disturbed than respiration.

The measurement of oxygen uptake, though not essential to our study, was a valuable indicator of the behavior and viability of the preparations. The data obtained (Text-figs. 1 and 2; Table I) are in agreement with those of earlier workers (14, 18–21). The Q_{O_2} for brain is in the low range; this is attributed to the presence of calcium ion in our medium (11). Text-fig. 2 offers a direct comparison of grey matter, white matter, and peripheral nerves from the same animals, under identical conditions. The relative oxygen uptakes in mature rats are in the proportion of about 7:3:1 (on the basis of fresh weight) and 11:2.5:1 (on the basis of dry weight). Few comparative data of this kind are available (19). In particular, there has been a considerable dearth of studies on pure white matter *in vitro*, possibly because of the lack of a sampling technique applicable to small laboratory animals.

We were particularly interested in comparing white matter and peripheral nerves, in view of their structural similarity. It has been stated that the higher oxygen uptake of the former (22) may reflect a property of the glial cells (23). A rough estimate of the relative number of cells in the two preparations was obtained by counting the nuclei in comparable microscopic fields, on longitudinal sections stained with hematoxylin and eosin. It was found that white matter contains one-third as many nuclei as peripheral nerve. Hence, in order to explain its higher oxygen uptake, one must assume either that glial cells respire 6 to 8 times more than Schwann cells, or that central axons respire more than the axons of peripheral nerves.

Biosynthesis of Lipides.—The relative amount of labelled substrate incorporated into the total lipides varied to a large extent, depending on the preparation (Table III) and on the substrate (Table IV). It may be seen from Table I that the three preparations, when compared in terms of specific activity of

the lipides, rank in the inverse order of lipide content; when the comparison is made in terms of total activity (which measures the relative amount of lipides synthesized by 100 mg. of fresh tissue during the experimental period) the differences are attenuated. If white matter and peripheral nerves are compared, it appears that white matter—with a Q_{O_2} which is more than twice that of peripheral nerves—builds considerably less lipide per unit of time, and its pattern of substrate preference is different, particularly with regard to phosphate. These data bring out a further dissimilarity between two tissues, which though related in structure and function, have previously been found to differ in many respects, including chemical composition (24, 25), metabolic pathways (10), oxygen uptake, and physiologic behavior (22). Peripheral nerves cannot be considered as "peripheral white matter."

The specific activity of the lipides was always greater for grey matter than for other preparations, with one notable exception; *i.e.*, when acetate was the substrate (Table III). The behavior of acetate was unique among the building blocks examined, in that nerves utilized it to a greater extent than both preparations of central nervous system. A clue to this situation is perhaps to be found in the fact that brain cholesterol of adult animals does not become labelled when radioacteate is used as the substrate, either *in vivo* or *in vitro* (26, 27), whereas the label does appear in the cholesterol of nerve (28).

The incorporation of P^{32}-phosphate into different fractions of brain slices was carefully studied by Strickland (29) and by Dawson (30). When radiophosphate was injected *in vivo* (30), grey matter incorporated more activity than did white matter. These findings are in agreement with the present observations. Since both tissues lie beyond the blood-brain barrier, it seems reasonable that the relative rates of incorporation might be similar *in vivo* and *in vitro*. The *in vitro* incorporation of labelled glycerol into brain lipides was studied by Hokin and Hokin (31), and by Pritchard (32). Gidez and Karnovsky (33), in experiments on intact rats observed that the activity of brain lipides, after intraperitoneal injection of radioglycerol, was second only to that of the liver lipides. The present data also indicate that glycerol carbon is extensively incorporated into brain lipides.

In Table VI, peripheral nerves are compared with liver and adipose tissue with respect to lipogenesis from acetate. If the lipide content of each tissue is taken into account (10 per cent, 3 per cent, and 40 per cent for nerves, liver, and adipose tissue, respectively), the nerves of both young and older animals exceed the liver (with respect to total incorporation of activity into lipide per unit weight of tissue) by more than twofold. Adipose tissue is almost as active as peripheral nerve.

Gradients along the Sciatic Preparation.—The demonstration of several gradients along the sciatic preparation (Text-fig. 3) is of particular interest. Morphological differences between the proximal and distal ends of a nerve

should, of course, be expected. In branches of the sciatic, dichotomy of the fibres has been found to occur with increasing frequency towards the periphery (34, 35), while the axons tend to taper and the thickness of the myelin decreases (36). In the phrenic nerve of the rabbit, on the other hand, no tapering and no branching were observed (37). Several concentration changes were observed along the frog sciatic by Gerard and Tupikova; *e.g.*, a decrease in total phosphorus (38). These authors, however, point out very pertinently that such changes, "observed for the whole nerve, might be quantitatively accounted for by altered relations of its components, such as diminishing axon diameter and myelin thickness, or increasing interfibrillar space in the distal portion." In the giant axon of the squid, Gasteiger and collaborators found a progressive drop in the concentration of homarine (39). Differences in oxygen uptake were not found along the sciatic nerve of the frog (12) whereas in the optic nerve of *Limulus polyphemus* a progressive increase from the retinal to the central end with an over-all difference of about 30 per cent has been reported (40, 41). The direction of the change found here along the sciatic nerve is opposite to that reported for *Limulus* optic nerve.

The 20 per cent drop in lipide content reported in Text-fig. 3 probably depends upon the progressive decrease in thickness of the myelin layer. The drop in O_2 uptake (13 per cent) cannot be given a definitive interpretation. The number of nuclei, counted in the upper and lower segments, showed fluctuations with no apparent trend; changes in the relative amount of collagen, if present, escaped histological observation.

In contrast there seems to be no doubt that the 15 per cent drop in lipide-specific activity reflects a functional change of the nerve fibres. If it were an artifact, dependent upon a difference in the rate of diffusion of acetate into the fibres, then the proximal segment—which is thicker, and surrounded by a thicker epineurium—should have a lower, not a higher incorporation. If a comparison is made in terms of total lipogenetic activity, then the observation becomes even more striking since the proximal part contains more lipide of higher specific activity than the distal end.

These gradients, which suggest a decrease in metabolic functions along the nerve, recall to mind the characteristic anatomical distribution of the conditions referred to as "peripheral neuropathies," in which a lesion similar to Wallerian degeneration originates at the distal tip of a nerve and progresses centripetally. It has been customary to associate the peculiarity of these lesions with the concept of "axon flow." It is tempting to suggest an additional explanation: a depressing influence, acting on the nerve as a whole, might cause greater and earlier damage (*i.e.* demyelination) at the distal tip, where lipogenesis is lowest. It should be added that while the stepwise differences shown by our figures are relatively small (13 to 20 per cent), the determinations were made on three large segments of the nerve. Thus the true drop from the origin to the tip of the nerve is probably much greater.

Biochemical and Morphologic Changes in Peripheral Nerves and Grey Matter with Age.—Changes related to growth are particularly marked in the nervous system. Whereas the morphologic events of growth have been thoroughly investigated both in the central and in the peripheral nervous system, metabolic studies have been largely confined to the brain (*cf.* reference 13). These considerations prompted our study of lipogenesis in the growing peripheral nerves. A few comparative data were also obtained on grey matter.

In peripheral nerves, oxygen uptake at birth is almost as high as in the preparation of cerebral cortex at the same age. It rises for about 10 days (Text-fig. 5), then drops progressively for 5 months, with only slight change thereafter. The biphasic shape of the curve disappears if the oxygen uptake is referred to dry weight. While other tissues are known to respire less with increasing age (42, 43), the drop is not as great as that reported here for nerves, with the possible exception of striated muscle (43).

Lipogenesis from acetate (expressed as specific activity) also follows a biphasic course (Text-fig. 6): it rises to 162 per cent between the 1st and the 5th day, returns to the initial value by 12 days, then drops to 4 per cent of the initial value in the course of 500 days. The greater part of this drop takes place during the first 5 months. A similar trend was obtained using P^{32}-phosphate as a lipide precursor. This finding may be considered to confirm the growth effect here described as actually referring to the lipides of nerve, and not to those of extraneous adipose tissue, in which the proportion of phosphatides would be low.

Correlation with the histological findings (Fig. 2) shows that the initial rise in lipide-synthesizing activity corresponds to the period during which myelin becomes first demonstrable. No myelin could be demonstrated at birth in the sciatics (*cf.* reference 44), using methods based on osmic acid. A few myelinated fibres could be found, however, in the brachial plexus, which resembled, at this stage, a sciatic at the age of 3 days. This is in agreement with previous findings, indicating that maturation and myelination of the nervous system tend to proceed in a craniocaudal direction (45–47). At 6 days most of the visible fibres are myelinated. Thereafter the myelin sheaths become thicker, and the axons larger, while the lipide content rises from 1 to 10 per cent (Text-fig. 4).

From the curves depicted in Text-figs. 4 to 6 one may surmise that the nerves attain relative maturity only towards the 150th day. If it is considered that the "250 gram male rat" ordinarily used in laboratories is only 9 to 10 weeks old, and therefore in full growth period, it becomes obvious that metabolic studies on rat nerves must be accurately standardized with respect to age.

Our growth curves are in close agreement with data derived from histologic measurements (48–50). Hatai, counting the myelinated fibres in the ventral roots of rat spinal nerves, concluded that maturity was reached between 100 and 200 days (48). Physiological properties, such as conduction velocity, attain adult characteristics at about 3 months in the cat (51), which has a life expect-

ancy several times greater than the rat, and at 4 to 5 years in man (52). This is relatively earlier than the plateaux for metabolic and structural properties discussed above.

It is interesting that there should be a sharp postnatal rise in the activity of lipogenesis (Text-fig. 6), at a time when myelin is becoming histologically demonstrable. Histological (45) and analytical chemical studies (53) of the developing central nervous system have shown that there are "pulses, or waves, of medullation" (45), and that birth, even if premature, seems to speed the process of myelination enormously (45).

While the nerves are undergoing myelination, respiratory and lipogenetic activities change in the same direction. Such is not the case in the developing brain, in which lipogenesis drops while oxygen uptake increases (Text-fig. 8). The different relationship in brain is interesting, because the divergent course of the curves for respiration and lipogenesis led Sperry and Waelsch to propose a mechanism by which lipides may be synthesized at a more rapid rate in the young brain (27, 54). It was postulated that the competition between oxidative processes and lipogenesis for available acetate regulated myelin synthesis. The data here presented with regard to peripheral nerves are not consistent with this hypothesis.

Since myelination in peripheral nerves is essentially a function of the Schwann cells (55), an attempt was made to correlate the changes in lipogenesis with the relative number of Schwann cells present in the sample. Only one Schwann cell is present per internode; as the fibre grows, the internode increases in length, and the cells become more and more widely spaced along the axon (56) (Fig. 3). In order to reach an estimate of the relative amount of lipide synthesized by a single Schwann cell at various ages, two steps were necessary. First, the number of cells per microscopic field was counted. This decreased with age, as expected, and again the curve tended to level off towards 5 months (Text-fig. 9). Second, the relative amount of lipide synthesized at each age by 100 mg. of tissue was obtained as earlier indicated. If one now divides the total lipide activity by the relative number of nuclei for a given age, one obtains a figure which indicates the relative amount of C^{14} incorporated into the lipides by a single Schwann cell at that age. This quantity varies as shown in the upper curve of Text-fig. 10. If the assumptions on which this calculation is based are legitimate, the amount of lipide synthesized by each cell is actually at its lowest in the newborn rat; the greatest activity is reached at about 20 days. It seems reasonable to conclude that myelination in rat peripheral nerve is most active between 15 and 25 days of age.

The pattern of metabolic changes in grey matter (Text-fig. 8) is sharply different from that of peripheral nerve. Oxygen uptake rises to 225 per cent of the initial value in 40 days, with no apparent change thereafter (*cf.* reference 14). While oxygen uptake increases, lipogenesis from acetate falls very steeply

(Text-fig. 8). The drop reaches a value of 6 per cent in 40 days, by which time it is almost complete, which possibly indicates that the very thin fibres present in the grey matter have acquired the greater part of their lipide sheath. A decline

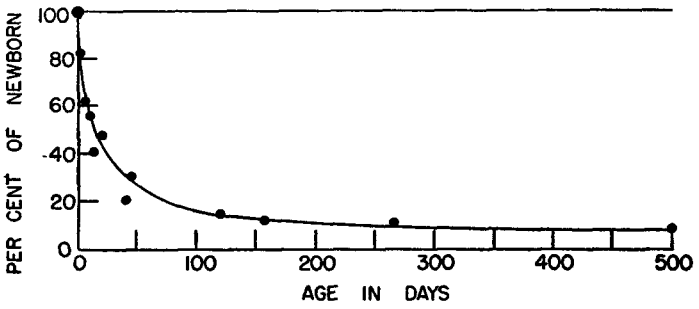

FIG. 9. Relative number of nuclei per unit volume of rat sciatic nerve at different ages. Value for newborn set at 100.

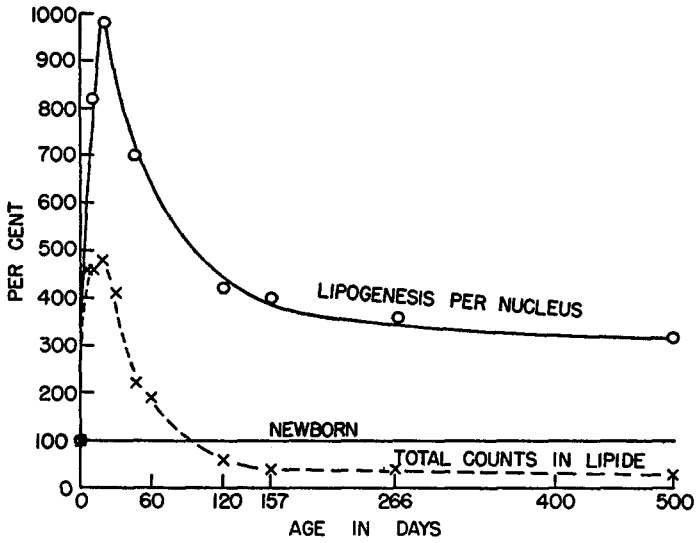

FIG. 10. Total incorporation of activity into lipides of rat sciatic *nerves* at different ages (lower curve), and incorporation per Schwann cell nucleus. Values for newborn set at 100. Further explanations in text.

of lipide biosynthesis by brain tissue with age has been found in other isotope studies, using P^{32}-phosphate (57) and deuterated water and fatty acids (58).

Growth Curves of Brain and Nerves.—Since the experiments describe above made available the weights of a large number of preparations of sciatic nerve from animals of different ages, these were plotted, together with the brain and

body weights of the same animals (Text-fig. 11). It can be seen that the nerves, unlike the brain, steadily follow the growth curve of the body as a whole. Our data agree with those of other investigators (54, 59, 60).

If the weight curves of brain and nerves in Text-fig. 11 are compared with the metabolic activities of the same tissues (Text-figs. 5, 6, 8), it becomes apparent that each organ develops in its own characteristic way. Brain showed an early period of rapid change and a sharp levelling off of weight and activity at about 40 days; nerves showed a protracted period of change and did not reach a plateau until 5 months. The two metabolic functions, respiration and lipogenesis, show alterations with age which parallel the growth curves of the relevant organs.

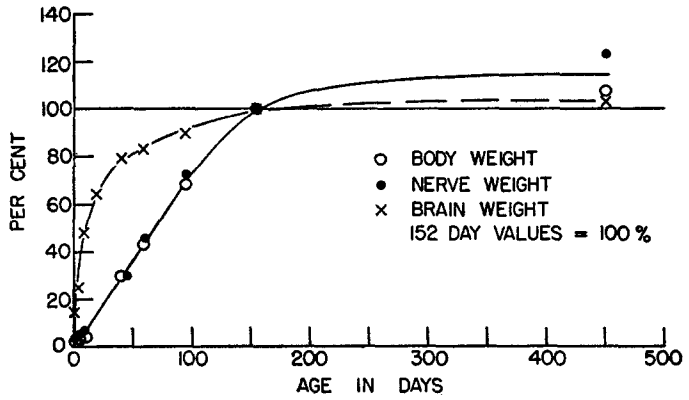

FIG. 11. Growth patterns of the brain, peripheral nerves, and whole body of male albino rats. The curves refer to weights at various ages. The values corresponding to rats 152 days old are set at 100 per cent.

SUMMARY

Samples of normal grey matter, white matter, and peripheral nerves obtained from rats were incubated in Warburg vessels with glucose and a labelled lipide precursor (acetate, phosphate, choline, glycerol, glucose). The total lipides were then extracted and their radioactivity measured.

The preparations were compared with respect to dry weight, lipide content, O_2 uptake, and ability to incorporate the various substrates into the lipides. Grey matter was found to be the least damaged by incubation, white matter the most. Damage to the tissue depressed lipogenesis to a greater extent than respiration.

Five substrates were compared with respect to their degree of incorporation into the lipides of the various preparations. White matter, which had a greater oxygen uptake than peripheral nerves, showed the lowest degree of incorporation for most of the substrates studied. The results suggest that there are con-

siderable quantitative differences in the metabolism of central and peripheral myelin.

In the sciatic preparations, oxygen uptake and lipogenesis from acetate were found to decrease from the proximal to the distal end of the nerve. This finding may be relevant to the pathogenesis of peripheral neuropathies.

The growth and metabolic activity of peripheral nerves were studied in rats aged 1 to 500 days, and the biochemical and histological findings were correlated. The results indicated that the lipogenetic activity of the Schwann cell was lowest in the newborn animal, and reached its peak at about 20 days. Comparative data were also obtained from the cerebral cortex.

The growth pattern of peripheral nerves was distinctly different from that of the brain. With respect to changes in tissue weight, respiration, and lipogenesis, growing peripheral nerve correlated with body weight, while the brain matured much more rapidly.

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EXPLANATION OF PLATES

PLATE 42

FIG. 1. Histology of the preparations of rat nervous tissue. Scale, 100 micra.

A. Hemisphere of adult rat. Frontal section, midway between frontal and occipital poles. The cortex (right) is several times thicker than the underlying layer of white matter. Paraffin-embedded; stained with gentian violet.

B and C. Cross-sections of a first (B) and second (C) slice of cortex, shaved off with the hand microtome (3) set at 330 micra. The slices were not incubated. The first slice corresponds closely to the plexiform layer (extreme right on A). Paraffin-embedded; stained with gentian violet.

D and E. Two stages in the preparation of white matter from the spinal cord: (D) cross-section of one-half the cord after the latter has been split in the median plane; (E) the same after the grey matter has been wiped out. The pial membrane (*p*) has caused the flat ribbon to curl backwards during embedding. The posterior column is cut off along the dotted line. Paraffin-embedded; stained with van Gieson's stain.

F and G. Cross-sections of a sciatic preparation, (F) midway along the sciatic trunk, and (G) along the tibial nerve 35 mm. below. The nerve was not incubated. Fixation in 1 per cent osmic acid; paraffin-embedded.

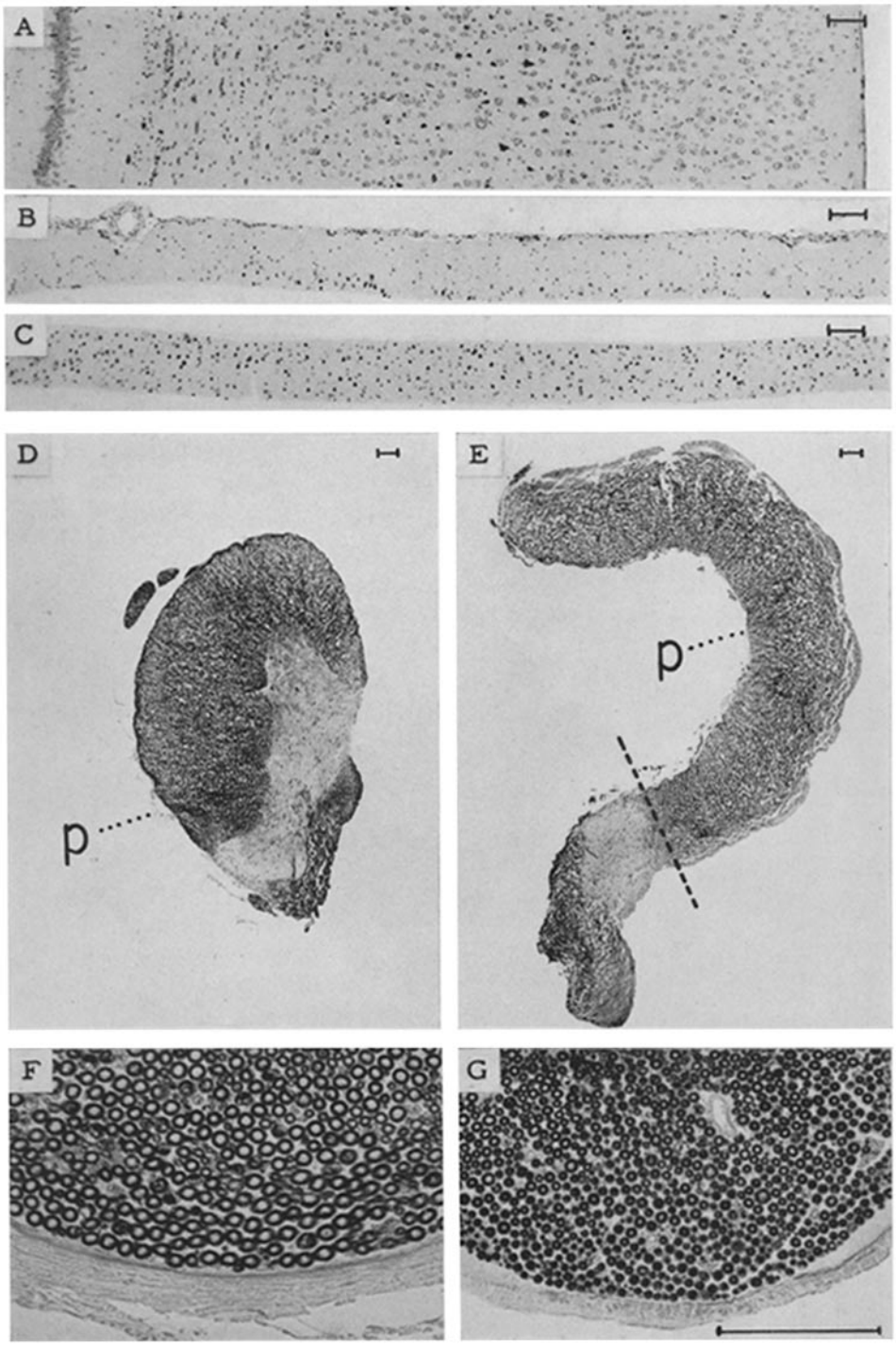

FIG. 1

(Majno and Karnovsky: Lipogenesis in nervous tissue)

PLATE 43

FIG. 2. Histology of rat sciatic nerves at various ages. Scale, 100 micra. Left column: stained for myelin (osmic acid method, (15)); right column: stained for axis cylinders (protargol method (15)). The figures indicate the age in days. The first myelinated fibres appear, in this nerve, during the 3rd day.

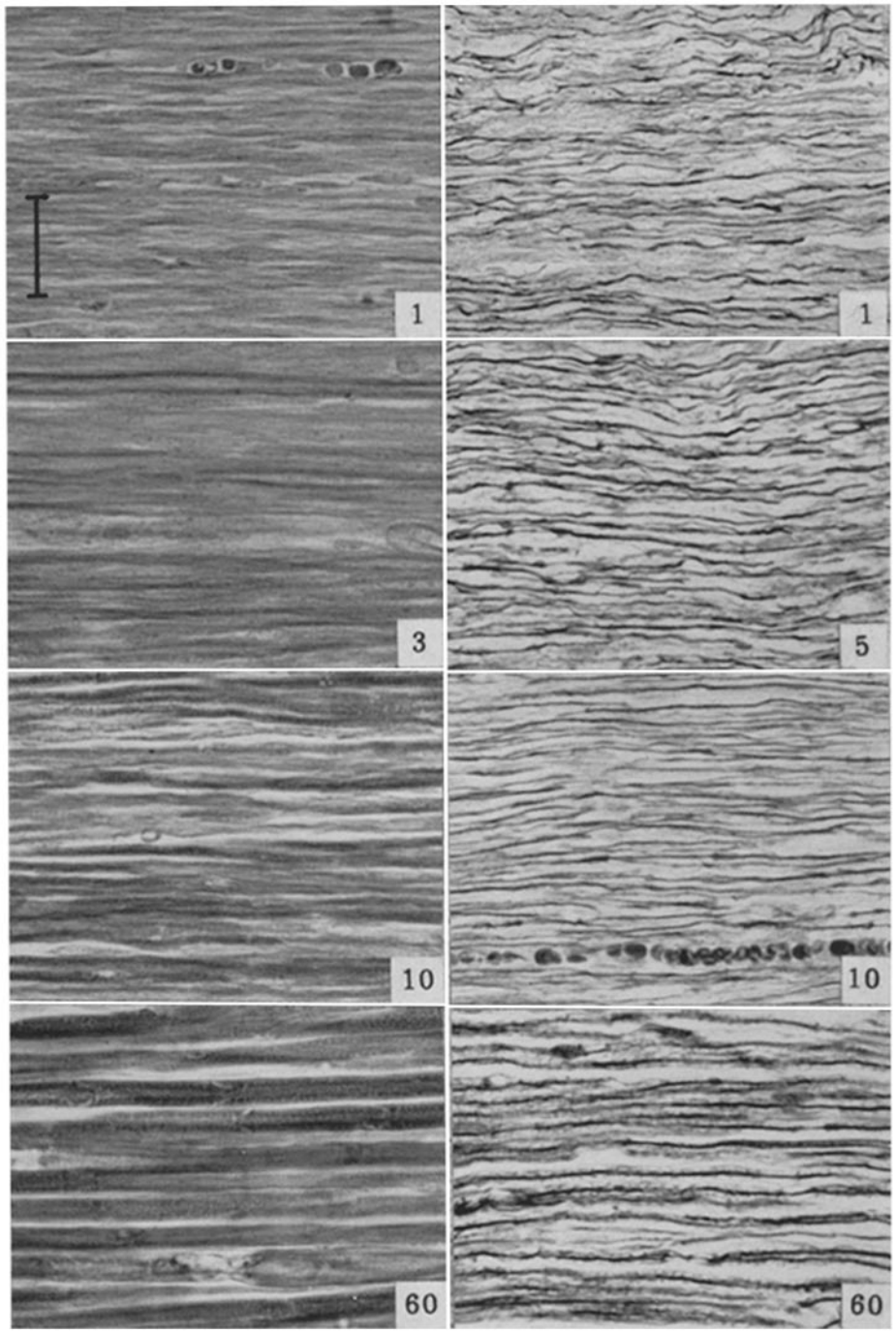

FIG. 2

(Majno and Karnovsky: Lipogenesis in nervous tissue)

PLATE 44

FIG. 3. Histology of rat sciatic nerves at various ages. Scale, 100 micra.

Formalin fixation; hematoxylin and eosin. The figures indicate the age in days. Note the decreasing number of nuclei, and compare with Text-fig. 9.

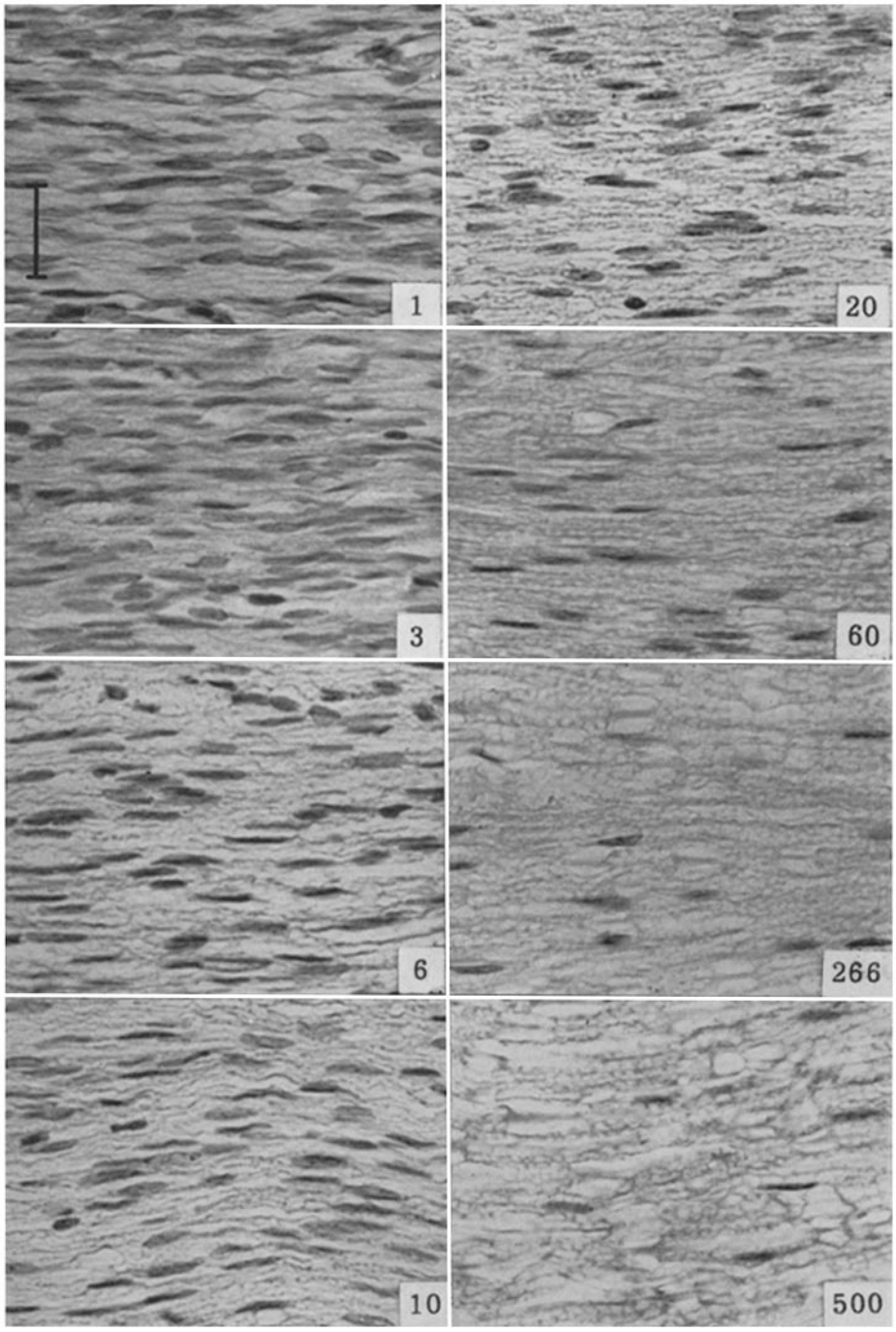

FIG. 3

(Majno and Karnovsky: Lipogenesis in nervous tissue)