PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 337–338 (1975)

Effect of Pinealectomy on Rat Brain Myelin (38534)

RICHARD RELKIN[1] AND LARRY SCHNECK
(With the technical assistance of Wai Y. Fok)

Division of Endocrinology and Metabolism, Department of Medicine; Department of Neurology; Isaac Albert Research Institute, Kingsbrook Jewish Medical Center, Brooklyn, New York 11203

We recently reported (1) that pinealectomy performed on rat pups of both sexes yielded results on lipid analysis of whole brains at age 40 days which indicated a delay in brain maturation; the data allowed the inference that this delay probably involved myelin formation. To definitively establish whether myelin formation actually is dedecreased by neonatal pinealectomy, the following study was performed on purified myelin.

Materials and Methods. Forty inbred rat pups of the Wistar strain were randomly chosen from litters of approximately equal size (i.e., 9–10). These animals, comprised of even-numbered groups of littermates, were exposed daily to an environment of 14 hr of absolute darkness and 10 hr of overhead lighting, yielding 100–150 ft-c at the cage surface. The spectrum of emission of this light source was 3500–30,000 Å, with a peak at 10,000 Å. On the fourth day of life alternate members underwent either a pinealectomy, according to a technique described previously (2), or a sham-pinealectomy. Operations were performed using ether anesthesia. Coding for designation was achieved by injecting India ink under the skin on the lateral aspect of one or more legs. Throughout the experiment water was available *ad libitum* and a standard comprehensive diet (Purina laboratory chow) was used. The temperature in the room was controlled and kept at approximately 23° (range 21–25°).

Litters were weaned at age 24 days. As the rate of myelination reaches a plateau at approximately 45 days of age (3), all rats were weighed at age 40 days, killed with ether, the skulls opened, and the presence or absence of the pineal assessed by means of a dissecting microscope (15x). The brains were removed *in toto* and immediately frozen.

Myelin was isolated using sucrose gradient centrifugation (4). The purity of the myelin fraction was confirmed by electron microscopy.[2] The dried myelin fraction was extracted with chloroform–methanol 2:1, and total cholesterol, total phospholipids, and sphingomyelin were determined as described previously (5). The chloroform–methanol soluble fraction was partitioned according to Folch-Pi (6). The lower phase was dried under nitrogen, dissolved in chloroform, and fractionated on silicic acid columns by the method of Vance and Sweeley (7). The glycolipids were eluted with acetone–methanol 9:1, and the concentration of the glycolipids was determined by the orcinol method of Svennerholm (8). Sulfatide was determined according to the method of Kean (9), and the cerebroside was estimated by subtracting the concentration of sulfatide from the total orcinol-positive material.

Results. The myelin analyses (Table I) for the male and female groups were separately subjected to t tests ($\alpha = 0.01$). The significantly different values noted in the pinealectomized versus sham-operated males and females respectively were those for myelin, cerebroside, sulfatide and sphingomyelin.

Total brain wet weights ± SE of the male pinealectomized and sham-operated rats were 1.36 ± 0.02 and 1.55 ± 0.02 g, respectively. These same respective values in the females were 1.31 ± 0.02 and 1.48 ± 0.03 g ($P < 0.01$ in each sex). The water content ± SE of these brains was 81.22 ± 1.96 and 78.50 ± 1.44% in the pinealectomized and sham-operated males, respectively, and 81.73 ± 1.23 and 78.06 ± 1.09% in the pinealectomized and sham-operated females, respectively ($P < 0.01$ in each sex).

In the male groups the average body weights ± SE of the pinealectomized and

[1] Present address: Easton Hospital, Easton, Pa. 18042.

[2] Kindly performed by Dr. Masazumi Adachi.

TABLE I. EFFECT OF PINEALECTOMY ON RAT BRAIN MYELIN[a]

Myelin and components	Male		Female	
	Pinealectomized (8)[b]	Sham (8)	Pinealectomized (8)	Sham (8)
Myelin[c]	4.96 ± 0.15[d]	7.34 ± 0.19	4.65 ± 0.18[d]	6.65 ± 0.21
% dry wt myelin				
Phospholipid	34.24 ± 1.01	32.50 ± 0.89	33.62 ± 1.10	34.68 ± 1.23
Cholesterol	11.84 ± 0.26	11.02 ± 0.33	12.28 ± 0.30	11.84 ± 0.38
Cerebroside	8.54 ± 0.22[d]	10.57 ± 0.20	8.13 ± 0.24[d]	10.40 ± 0.25
Sulfatide	1.42 ± 0.02[d]	1.90 ± 0.03	1.40 ± 0.02[d]	1.79 ± 0.02
Sphingomyelin	0.85 ± 0.02[d]	1.31 ± 0.03	1.17 ± 0.03[d]	1.55 ± 0.02

[a] Results are expressed as mean ± SE.
[b] Number of animals.
[c] Expressed as mg/g wet weight of brain.
[d] For this sex, pinealectomy vs sham $P < 0.01$.

sham-operated rats at 40 days of age were 126.4 ± 5.0 and 131.7 ± 4.1 g, respectively. These same respective values in the females were 129.2 ± 4.5 and 125.8 ± 5.2 g ($P > 0.7$ in each sex).

Examination of the brains of all rats completing the experiment confirmed the absence of pineal tissue in all pinealectomized animals, and intact pineal glands in all sham-pinealectomized animals.

Discussion. Since myelin and such commonly accepted myelin markers as cerebroside and sulfatide were lower in the pinealectomized animals of both sexes, it can be concluded that neonatal pinealectomy results in decreased myelin formation. With respect to myelin this places the pinealectomized rats at an earlier developmental age. Consistent with this interpretation of the data are the findings, confirmatory of our previous study (1), that the brains of the pinealectomized rats weighed less and had a greater water content than those of the sham-operated rats. In order to determine whether the observed decrease in myelin formation is also associated with abnormal myelin composition, we are currently performing fatty acid analyses of myelin lipids.

Summary. Analysis of purified myelin from the respective brains of both sexes of 40-day-old rats revealed that neonatal pinealectomy results in decreased myelin formation.

1. Relkin, R., Fok, W. Y., and Schneck, L., Endocrinology **92,** 1426 (1973).
2. Relkin, R., Amer. J. Physiol. **218,** 328 (1970).
3. Davison, A. N., *in* "Applied Neurochemistry" (A. N. Davison and J. Dobbing, eds), p. 178, F. A. Davis Co., Philadelphia (1968).
4. Cuzner, M. L., and Davison, A. N., Biochem. J. **106,** 29 (1968).
5. Schneck, L., Adachi, M., and Volk, B. W., Pediatrics **49,** 342 (1972).
6. Folch-Pi, J., Lees, M. B., and Sloane-Stanley, G. M., J. Biol. Chem. **226,** 497 (1957).
7. Vance, D. E., and Sweeley, C. C., J. Lipid Res. **8,** 621 (1967).
8. Svennerholm, L., J. Neurochem. **1,** 42 (1956).
9. Kean, E. L., J. Lipid Res. **9,** 319 (1968).

Received July 29, 1974. P.S.E.B.M. 1975, Vol. 148.