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Studies in Demyelination, II. Enzymatic Patterns in Central Nervous System and Sciatic Nerve in Experimental "Allergic" Encephalomyelitis. (29203)

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We have studied the enzymatic patterns of the sciatic nerve in Wallerian degeneration (1), which was considered to be an experimental condition suitable for study of multiple sclerosis and for the demyelination process in general. We then turned our attention to experimental allergic encephalomyelitis, since demyelination produced by resection of the sciatic nerve cannot be strictly compared to demyelination produced by infections and metabolic disturbances. Many authors(2,3,4) consider experimental allergic encephalomyelitis to be a better experimental model for multiple sclerosis. The clinical picture and the occurrence of remissions and relapses in some of the animals show a striking analogy to multiple sclerosis. We, therefore, studied the changes in the enzymatic patterns of the central nervous system as well as those of the sciatic nerve and looked for an analogy between the changes in the enzymatic patterns observed in Wallerian degeneration(1, 5) with those occurring in the demyelination due to experimental allergic encephalomyelitis.

The enzymatic pattern in Wallerian degeneration showed an increase in the acid and alkaline phosphatases, in adenosinase and in β-glucuronidase(1,5). Roizin(6) tried to correlate the demyelination with the activity of oxidases, peroxidase and acid phosphatase of white matter in some multiple sclerosis cases and in experimental allergic encephalomyelitis in *Macacus rhesus* monkeys by histochemical techniques. He found increased enzyme activity in the demyelinated areas.

A 10-fold increase of β-glucuronidase has been found in Wallerian degeneration by determination of the enzymatic activities of the homogenized sciatic nerve(1). This high β-glucuronidase activity was also observed in the sciaticus of the rat by histochemical methods in Wallerian degeneration(7). Ten days after transection there was an increase in β-glucuronidase in the myelin sheath, but none in the nuclei of the axis cylinder cells or the connective tissue(7).

No alkaline phosphatase, adenosinase nor β-glucuronidase determinations have been reported in experimental allergic encephalomyelitis.

The encephalitogenic activity of nervous tissue is mainly exerted by preparations rich in white matter(8,9) and is absent from fetal or unmyelinated brain(9,10). Experimental allergic encephalomyelitis (EAE) can be produced by nervous tissue of foreign species as well as by homologous tissue(9-13), and the antigenicity is considered to be organ-specific rather than species-specific. It is for this reason that we decided to study the enzymatic patterns of the spinal cord in EAE. We divided the spinal cord into 4 main sec-

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TABLE I. Enzymes in Nerve Tissue of Normal Rabbits and of Rabbits with Wallerian Degeneration and with Experimental Allergic Encephalomyelitis.

	Normal (13)*	WD (8)*	EAE (10)*
	β-Glucuronidase		
Brain	6.2 (± 1.48)	5.8 (± .62)	6.8 (± 1.00)
Cerebellum	6.8 (± 1.46)	6.8 (± .59)	8.9 (± 1.87)
Spinal cord: Cervical	5.3 (± 1.00)	5.2 (± .76)	13.0 (± 7.07)
Thoracic	4.9 (± .97)	4.7 (± .75)	17.5 (± 9.72)
Lumbar	4.5 (± .93)	4.6 (± .68)	20.0 (±10.60)
Sacral	5.7 (± 1.02)	5.4 (± .77)	24.5 (±11.72)
Sciatic nerve	5.8 (± 1.30)	43.0 (±18.60)	6.6 (± 1.50)
	Acid phosphatase		
Brain	3.5 (± .90)	3.1 (± .53)	3.0 (± .55)
Cerebellum	4.0 (± .95)	3.8 (± .88)	3.7 (± .70)
Spinal cord: Cervical	2.2 (± .54)	2.1 (± .55)	2.1 (± .48)
Thoracic	1.8 (± .47)	1.6 (± .38)	1.8 (± .45)
Lumbar	1.8 (± .36)	1.5 (± .40)	2.0 (± .50)
Sacral	2.5 (± .60)	2.4 (± .55)	3.1 (±1.12)
Sciatic nerve	.7 (± .33)	3.1 (± .11)	1.2 (± .42)
	Adenosinase		
Brain	.48 (± .17)	.44 (± .15)	.45 (± .19)
Cerebellum	.46 (± .13)	.44 (± .06)	.44 (± .08)
Spinal cord: Cervical	.25 (± .06)	.22 (± .05)	.25 (± .08)
Thoracic	.21 (± .04)	.18 (± .04)	.26 (± .09)
Lumbar	.20 (± .05)	.18 (± .06)	.26 (± .07)
Sacral	.26 (± .06)	.22 (± .06)	.37 (± .12)
Sciatic nerve	.11 (± .08)	.45 (± .25)	.19 (± .07)
	Alkaline phosphatase		
Brain	3.9 (±1.17)	4.0 (±1.10)	3.8 (±1.18)
Cerebellum	4.0 (±1.16)	3.8 (± .92)	3.7 (± .62)
Spinal cord: Cervical	2.2 (± .77)	2.2 (± .71)	2.1 (± .85)
Thoracic	1.4 (± .59)	1.3 (± .39)	1.2 (± .29)
Lumbar	1.3 (± .50)	1.3 (± .54)	1.3 (± .43)
Sacral	2.4 (±1.12)	2.0 (± .69)	2.0 (±1.17)
Sciatic nerve	.7 (± .36)	.7 (± .54)	.8 (± .45)

The β-glucuronidase unit is the number of μg of phenolphthalein liberated by 100 mg wet tissue during 1 hr at 37.5° in acetate buffer 4.5.

Both phosphatases are determined under conditions similar to those of King and Armstrong (glycine buffer of pH 10 was used for the alkaline phosphatase). However, one unit refers to number of mg phenol liberated by 100 mg wet tissue in 24 hr at 37.5°. Adenosinase units are number of mg phosphorus, liberated by 100 mg wet tissue in 24 hr at 37.5° in acetate buffer 4.8.

Values are the arithmetic mean with standard deviation in parentheses.

* No. of rabbits.

tions: cervical, thoracic, lumbar and sacral. The brain, cerebellum, and sciaticus were also studied. The enzymatic patterns of the central nervous system and the sciatic nerve of normal rabbits and rabbits with Wallerian degeneration were used as controls for comparison with that of EAE.

Methods. Experimental allergic encephalomyelitis was produced in rabbits 16 to 18 weeks old (2.5-3.0 kg) by injecting intradermally into the foot pads of each paw a bovine spinal cord homogenate containing Freund's adjuvant and prepared according to a modified method of Kabat *et al*(9,14). Four grams of fresh bovine spinal cord were first homogenized with 4 ml physiological saline, then with 20 mg dry *Mycobacterium butyricum* (Difco) and 1 ml heavy mineral oil. Four-tenths ml of this mixture, corresponding to about 250 mg of fresh tissue, was divided in 4 portions and injected as described above. Approximately 2 to 3 weeks following inoculation some of the animals developed ataxia and incontinence of urine and feces. The animals were sacrificed by injection of Nembutal-Na (Veterinary, Abbott), when complete paralysis of the lower extremities had developed. The brain, cerebellum, spinal cord, and sciatic nerve were dissected and cleaned.

TABLE II. Lipid Phosphorus Content (mg) in 100 g Dry Tissue.

Segment of spinal cord	Normal rabbits	EAE rabbits
Cervical	850	950
Thoracic	890	1000
Lumbar	900	960
Sacral	820	920
Sciatic nerve	620	280

The nervous tissues were homogenized with water to a final concentration of 1% wet weight and the following enzymes were determined: acid and alkaline phosphatases, adenosinase and β-glucuronidase. The methods have been described(1) except for the alkaline phosphatase, which was determined according to King-Armstrong's method, using glycine buffer of pH 10.

Results. High β glucuronidase activity had been observed in the sciatic nerve during Wallerian degeneration. In EAE rabbits this enzyme was found to be substantially increased in the spinal cord. A slight increase of adenosinase in the lower parts of the spinal cord was also found in rabbits with EAE. The β-glucuronidase activity in these animals gradually increased from the cerebellum down to the sacral section of the spinal cord; but it is normal in the sciatic nerve of these animals in contrast to those with Wallerian degeneration (Table I).

The lipid phosphorus of the sciatic nerve was decreased by 50 to 60% in Wallerian degeneration. No significant differences were found in the lipid phosphorus content of the spinal cord between EAE and normal animals, whereas the lipid phosphorus was lowered in the sciatic nerve of EAE rabbits (Table II).

Summary. Acid phosphatase, alkaline phosphatase, adenosinase and β-glucuronidase were determined in the central nervous system and sciatic nerve of rabbits with experimental allergic encephalomyelitis and the enzymatic pattern compared with that of normal rabbits and rabbits with Wallerian degeneration. The only enzymatic change common to both Wallerian degeneration and EAE was an increase of β-glucuronidase, occurring at the respective site of demyelination. A 10-fold increase was observed in the resected sciatic nerve in Wallerian degeneration. In EAE the β-glucuronidase activity gradually increased from the cervical part of the spinal cord down to the sacral section, where the enzyme activity was about 5-fold the normal value.

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