and probably essential to, the induction of dependent thyrotropic tumors. The possibility that the cells capable of thyrotropic tumor formation did not survive the transplantation seems much less likely. The transformation of a normal thyrotropic cell into a dependent tumor cell thus appears to involve a change in the requirement for, or response to, hypothalamic factors. The role of hypophyseal irradiation in induction of thyrotropic piutitary tumors by radiothyroidectomy with I^{131} was not further clarified.

The author is indebted to Miss Lois Jenkins for excellent technical assistance.

1. Clifton, K. H., *Cancer Res.,* 1959, v19, 2.
2. ———, *Acta Union Internationale Contre le Cancer,* 1962, v18, 293.
3. Gorbman, A., *Cancer Res.,* 1956, v16, 99.
4. Furth, J., *ibid.,* 1957, v17, 454.
5. Clifton, K. H., Furth, J., *ibid.,* 1961, v21, 913.
6. Bardin, C. W., Liebelt, A. G., Lipscomb, H. S., Mountcastle, W., Liebelt, R. A., *Proc. Am. Assn. Cancer Res.,* 1962, v3, 302.
7. Rennels, Edward G., *Anat. Rec.,* 1962, v142, 271.
8. Lundin, P. M., Schelin, U., *Acta Path. et Microbiol. Scand.,* 1962, v54, 66.
9. Greer, M. A., Yamada, T., Iino, S., *Ann. N. Y. Acad. Sci.,* 1960, v86, 667.
10. Blanquet, P., *Adv. Biol. and Med. Physics,* Vol. 8, pp. 225-314, (Ed. C. A. Tobias & J. H. Lawrence) 1962.
11. Mühlbock, O., Boot, L. M., *Cancer Res.,* 1959, v19, 402.
12. Liebelt, A. G., Liebelt, R. A., *ibid.,* 1961, v21, 86.
13. Furth, J., Clifton, K. H., *Cancer,* 1957, v10, 842.

Received September 3, 1963. P.S.E.B.M., 1963, v114.

Electrical Activity in Sympathetic Nerves of Immunologically Sympathectomized Rats.* (28732)

MICHAEL J. BRODY (Introduced by J. P. Long)
Department of Pharmacology, College of Medicine, State University of Iowa, Iowa City

Mouse salivary gland contains a factor which promotes the growth of the sympathetic nervous system(1). The antiserum to this nerve growth factor produces extensive and permanent destruction of sympathetic ganglion cells when the material is administered to newborn animals(2,3). Although the damage to the sympathetic nerves produced by the antiserum in rats is virtually complete, a small number of distinct ganglion cells and fibers remain. A recent study in this laboratory(4) demonstrated that in rats treated with the anti-nerve growth factor the residual sympathetic fibers were not functional. This was shown in the perfused hindquarters of treated rats where both direct and reflex activation of the sympathetic chains failed to elicit a vasoconstrictor response. The purpose of the present investigation was to determine if immunization with the anti-nerve growth factor alters the nature of the electrical activity emanating from the sympathetic tracts.

Methods. Experiments were performed on 4 control and 4 immunized rats drawn from litters raised in the laboratory. The immunization was carried out by treating newborn rats with daily subcutaneous injections of a bovine antiserum developed to mouse salivary gland nerve growth factor.† The antiserum with a concentration of 68,000 units/ml was administered subcutaneously for 14 days at a dose of 1% of body weight. Nerve potentials were recorded from the sympathetic chains when the animals were 2 months old.

Preparation of the rats was performed under deep ether anesthesia. The animal was tracheotomized and a catheter was inserted

* This investigation was carried out under a Grant-in-Aid from Am. Heart Assn., and supported in part by the Scott Co. Heart Assn. (Iowa).

† Antiserum was graciously supplied by Dr. R. K. Richards, Abbott Laboratories, North Chicago, Ill.

into a jugular vein. The sympathetic chains were exposed in the lumbar region through an abdominal midline incision. A small (2 mm wide at the tip) shielded stainless steel bipolar electrode was placed on one of the chains which was sectioned distal to the electrode placement. The area surrounding the electrode was covered with light mineral oil. The animals were immobilized with gallamine triethiodide and ventilated with a positive pressure pump. Experiments were carried out under light ether anesthesia.

Potentials were amplified by a Tektronix Inc. type FM 122 low-level preamplifier and displayed on an oscilloscope. The electrical activity was recorded with a Polaroid camera utilizing Polaroid film (3000 speed/type 47). Amplification was set at 20 μV/cm in all experiments.

Spontaneous discharge, discharge during asphyxia, and the noise level in the system following death of the animal were recorded. Asphyxia was induced by turning off the respirator. Photographs were made during the peak of the response which usually occurred 30 seconds after the pump was turned off. The animals were sacrificed by a lethal intravenous dose of sodium pentobarbital at the end of the experiment.

Results and discussion. Tracings obtained from one control and one immunized animal are shown in Fig. 1. The results illustrated are identical in all respects to those obtained in the other experiments. A considerable degree of spontaneous discharge was recorded from the sympathetic nerves of control animals. By comparison the potentials obtained from the nerves of the treated animals, although greater than background noise, were of a low order of magnitude. A dramatic increase in electrical activity was observed in the control rats during asphyxia; increments in both frequency and magnitude of discharge were elicited. Conversely, in the immunized rats asphyxia evoked only a very small increase in electrical activity.

These electroneurographic findings are entirely compatible with other histological and physiological observations which have demonstrated the effectiveness of the antiserum

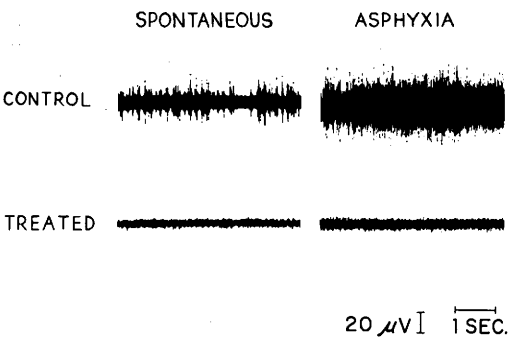

FIG. 1. Electrical activity recorded from the lumbar sympathetic nerve of a control and a treated rat. See text for description.

to the sympathetic nerve growth factor(3,4). It appears that destruction of the sympathetic ganglion cells markedly reduces the capacity of the sympathetic axons to transmit action potentials. It is of interest that discharge was obtained from the nerves of the treated rats. On the basis of observations that reflex and electrical activation of sympathetic fibers in immunized rats failed to evoke vasoconstriction(4), it was expected that no activity would be recorded from these fibers. The presence of a low order of discharge may be explained in several ways. The residual fibers in the treated group may be of a different type than those from which potentials were obtained in the control group, or they may be the same fibers except for an impaired capacity to develop action potentials of full magnitude.

The possibility that the potentials recorded from the immunized rats arise from different fiber types is suggested by the fact that the discharge height is lower and the frequency of the spikes higher than those obtained from lumbar chains of control animals. The discharge emanating from these fibers in the control rats would be masked by the larger discharge from other fibers in the chain. If the fibers which discharge at low potential subserved a function other than the release of norepinephrine, this would explain why electrical stimulation of the sympathetic nerves in treated rats failed to elicit vasoconstriction.

The residual ganglion cell population in rats which have received the antiserum is not

normal; the surviving cells are reduced in size(3). It is entirely conceivable that the residual fibers are the same type as those found in control animals except that the antiserum treatment has in some way reduced the ability of the nerve to develop an electrical potential. If this were the case the failure of electrical stimulation of these nerves to produce vasoconstriction could be explained on the basis that a threshold potential necessary for release of norepinephrine (from the nerve terminals) was not reached.

The present results do not allow for a conclusion as to which factor is responsible for the type of activity recorded from the sympathetic fibers of rats treated with the anti-nerve growth factor. It may be that neither of these factors ultimately contributes to the finding that the sympathetic nerves in the immunized animals fail to exhibit adrenergic function. The significant loss of norepinephrine content in structures innervated by the sympathetic nervous system following immunological sympathectomy(5) must also be taken into consideration.

Summary. Electrical activity was recorded from the lumbar sympathetic chains of control rats and rats treated with an antiserum developed to the sympathetic nerve growth factor derived from mouse salivary glands. The magnitude of the potentials recorded from the nerves of the immunized animals was considerably smaller than the magnitude of discharge seen in control animals. These observations provide further evidence for the effectiveness with which the anti-nerve growth factor damages the sympathetic nervous system. Several possible explanations for the type of activity recorded from the nerves of immunized animals were considered.

The valuable technical assistance of Richard A. Shaffer is gratefully acknowledged.

1. Levi-Montalcini, R., Angeletti, P. U., *Quart. Rev. Biol.,* 1961, v36, 99.

2. Cohen, S., *Proc. Nat. Acad. Sci.,* Wash., 1960, v46, 302.

3. Levi-Montalcini, R., Booker, B., *ibid.,* 1960, v46, 384.

4. Brody, M. J., *Circulation,* 1963, v28, 695.

5. Levi-Montalcini, R., Angeletti, P. U., *Int. J. Neuropharmacol.,* 1962, v1, 161.

Received September 23, 1963. P.S.E.B.M., 1963, v114.

Species-Specificity of Endogenous Pyrogen in Serum.* (28733)

LON R. WHITE AND ROBERT G. PETERSDORF

Department of Medicine, University of Washington and King County Hospital, Seattle

Intravenous administration of bacterial endotoxin into rabbits and dogs is followed by the appearance in the serum of a secondary fever-producing substance, endogenous pyrogen (EP), which differs in its biological properties from the endotoxin originally administered(1). EP is presumably derived from granulocytes injured by the endotoxin. These cells are the only tissues consistently pyrogenic when injected into animals of the homologous species(2). Furthermore, animals made leukopenic with nitrogen mustard produce less endogenous pyrogen(3) and have less fever(4) than controls.

* Supported by grants from Nat. Inst. of Allergy and Infect. Dis., U.S.P.H.S.

Endogenous pyrogen (EP) in the serum of febrile animals and pyrogen derived from polymorphonuclear leukocytes (LP) have been presumed to be biologically equivalent. In ordinary dosage both produce monophasic febrile responses occurring after a brief latent period, both are active in animals made tolerant to bacterial endotoxin and both are inactivated by heating at 90°C for 30 minutes(5). However, there is at least one difference in the biological properties of these two pyrogens. Whereas LP is pyrogenic only in species from which it is derived(6), preliminary experiments have demonstrated that serum EP from rabbits given typhoid vaccine is pyrogenic in dogs and vice versa(7).