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‡ The microphotographs in this and in the following paper were made by Mr. Cramer Lewis of the Department of Illustration of the Washington University School of Medicine.

[1] Levi-Montalcini, R., *Ann. N. Y. Acad. Sci.*, 330–343 (1952).

[2] Levi-Montalcini, R., and V. Hamburger, *J. Exptl. Socl.*, **123**, 233–288 (1953).

[3] Levi-Montalcini, R., H. Meyer, and V. Hamburger, *Cancer Research*, **14**, 49–57 (1954).

[4] Cohen, S., R. Levi-Montalcini, and V. Hamburger, these PROCEEDINGS, **40**, 1014 (1954).

[5] Levi-Montalcini, R., and S. Cohen, these PROCEEDINGS, **42**, 571–574 (1956)

[6] Cohen, S , and R. Levi-Montalcini, these PROCEEDINGS, **42**, 695–699 (1956).

[7] Levi-Montalcini, R., in *The Chemical Basis of Development*, (Baltimore: The John Hopkins Press, 1958), pp. 645–664.

[8] Cohen, S., in *The Chemical Basis of Development*, (Baltimore: The John Hopkins Press, 1958), pp. 665–676.

[9] Levi-Montalcini, R., and V. Hamburger, *J. Expl. Zool.*, **116**, 321–362 (1951).

[10] Bueker, E. D., I. Schenklein, and J. Bane, *Anat. Record*, **133**, 256 (1959).

[11] Lacassagne, A., *Compt. Rend. Soc. Biol.*, **133**, 280–281 (1940).

[12] Junqueira, L. C. U., A. Faier, M. Rabinovitch, and L. Frankenthal, *J. Cell. and Comp. Physiol.*, **34**, 129–158 (1949).

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*DESTRUCTION OF THE SYMPATHETIC GANGLIA IN MAMMALS BY AN ANTISERUM TO A NERVE-GROWTH PROTEIN**

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The observation by S. Cohen that a rabbit antiserum against a protein fraction of the salivary gland inactivates the *in vitro* nerve-growth effects of this protein (pp. 302–311, these PROCEEDINGS) suggested to him to test the effects of the antiserum on newborn mice. The finding of a remarkable decrease in size of the sympathetic ganglia of the injected mice prompted an extensive investigation of the effects of the antiserum on the sympathetic ganglia of mice and other mammals. The results of this study are reported in the following pages.

Materials and Methods.—Newborn mice were injected daily with 0.05 ml of the rabbit antiserum per 1.5 gm of body weight.† Controls of the same litters were either injected with serum of a normal rabbit, or they were not treated at all. Twenty experimental and twenty control mice were sacrificed between the 12th hour after the first injection and the 25th day. Two groups of newborn mice, injected for 8 and 20 days respectively after birth, were sacrificed three and four months after the termination of the treatment. An equal number of untreated mice of the same litters were available for control. The same techniques as used in the previous experiments (pages 373–384) were used for area measurements, cell and mitotic counts, and for histological examination.

The effect of the rabbit antiserum was then tested on newborn rats, rabbits, and one pair of kittens. The amount injected was in the same proportion to the body

weight as in the experiments with newborn mice. A limited number of experiments were also performed on adult mice and on one pair of young adult squirrel monkeys.

Effects of the Antiserum on Newborn Mice.—The injection of the rabbit antiserum in newborn mice did not affect their development in any way. Mice injected daily for a period of 25 days did not differ from mice injected with normal rabbit antiserum or from untreated controls. Since mice injected with the normal serum were identical in all respects with untreated controls, including the development of the sympathetic ganglia, only untreated animals were used for comparison in most experiments.

The effects of the antiserum on the development and growth of the sympathetic ganglia were dramatic in their sudden outburst, in their severity, and in the end effects. An inspection of sympathetic ganglia at different trunk and head regions in the same animals showed that all ganglia were affected in the same way; therefore, the data presented here on the superior cervical ganglia can be considered as representative of the degree of reduction which took place in all sympathetic ganglia.

A decrease in mitotic figures is already noticeable 12 hours after the first injection; the decrease becomes more pronounced at the end of the first day. Between the second and the third day, the mitotic activity is sharply reduced and the number of degenerating cells is greatly increased. The neuroblasts which are present are considerably smaller than the control cells. As a result, the volume of the ganglia is reduced to approximately $^1/_6$ of the controls on the fourth day. No cell counts were performed at this stage since most of the neuroblasts are still small and not easily distinguishable from satellite cells.

Between the fourth and the ninth day, the process of cell atrophy and death continues at a somewhat slower pace; the disintegrative processes of preceding stages have in fact so much reduced the population of nerve cells as to set limits to the process itself.

The effects of the daily injections of antiserum in a 9-day mouse are illustrated in Figure 5. The products of disintegrating cells which in earlier stages encumbered the ganglia have now disappeared. A few nerve cells, smaller than controls but otherwise in apparently normal condition, are scattered among a uniform population of satellite cells. The latter do not seem to be affected by the antiserum at this stage. A reduction in their number takes place in more advanced stages when only a few satellites are still present in each section of the diminutive ganglia (Fig. 8).

At 20 days, counts of nerve cells in the superior cervical ganglia of experimental and control mice show that the sympathetic nerve cells are reduced in one instance to less than 1 per cent of the control (Fig. 1, Table 1). The reduction of volume of the same ganglion was to 4.3 per cent of the control. The process seems to have reached its peak. Counts of cells in another pair of experimental and control ganglia of a 25-day-old mouse give slightly higher figures: 1.7 per cent of the cell population of the control are present in the experimental ganglion. Such differences may well be accounted for as individual variations. It should be noted that a considerable variation in the cell population was also observed in ganglia of untreated mice (see Table 1). The results, seem, however, to indicate that the process has not

further advanced, and that the few cells which escaped death in earlier stages may now be able to survive.

It was of interest, in this connection, to examine the injected mice some months after the termination of the treatment. Seven newborn mice of two litters were injected daily with the antiserum until the eighth and the twentieth day respectively. Experimental and untreated mice of the same litters were examined three to four months later. The treated mice did not differ from controls in their general appearance. The lack of adverse effects on the visceral functions of the injected mice was illustrated by the fact that one of these mice delivered a normal litter.

Two experimental and two control mice of each group were sacrificed at the end of the third month and the sympathetic chain ganglia were dissected out and compared. Two more pairs of mice injected for 8 days were sacrificed at the end of the fourth month (Figs. 2, 8). The results are given in Table 1. The reduction in cell number in the superior cervical ganglia fluctuates between 2.56 per cent and 0.84

TABLE 1

EFFECT OF ANTISERUM INJECTIONS ON THE VOLUME AND CELL NUMBER OF THE SYMPATHETIC GANGLIA IN MAMMALS*

Mammal	Age, Days	No. of Inject.†	Ganglion	Volume Ratio, Exp./Cont.	Number of Cells Cont.	Number of Cells Exp.	Cell Ratio, Exp./Cont.
Mouse	2	2	Sup. Cerv.	0.37:1			
Mouse	4	4	Sup. Cerv.	0.16:1			
Mouse	9	9	Stellate	0.13:1			
Mouse	20	20	Sup. Cerv.	0.04:1	13,300	91	0.70:100
Mouse	25	25	Sup. Cerv.	..	16,415	279	1.70:100
Mouse	90	8	Sup. Cerv.	..	16,447	421	2.56:100
Mouse	120	8	Sup. Cerv.	..	14,800	140	0.94:100
Mouse	120	20	Sup. Cerv.	..	13,000	110	0.84:100
Mouse	Adult	20	Sup. Cerv.	..	15,652	5,328	34.00:100
Rat	4	4	Sup. Cerv.	6.64			
Rat	7	7	Sup. Cerv.	10.54	32,000	2,310	7.00:100
Rabbit	3½	3	Sup. Cerv.		66,300	6,200	9.00:100
Rabbit	5	5	Sup. Cerv.		67,000	9,300	14.00:100
Rabbit	7	7	Sup. Cerv.		68,000	11,050	16,00:100
Cat	7	7	Sup. Cerv.		114,000	8,600	7.70:100
Monkey	7	7	Sup. Cerv.	1.65			

* Details of experiments in text.
† Beginning at birth, except for adult mice.

per cent of the corresponding control ganglia. In all instances, the residual cells were much smaller and did not stain with basic dyes as deeply as the controls. The function of the surviving cells will be tested in other mice of the same litters which are still alive. The 20-day treatment, as well as the 8-day treatment, is therefore sufficient to destroy 97 per cent to 99 per cent of the sympathetic nerve cells. Since the mice were examined some months after the end of the treatment, it is proved that the damage inflicted to nerve cells is irreversible. Experiments with higher amounts of antiserum and possibly an even more potent antiserum will show whether it may be possible to attain a total extermination of the sympathetic nerve cells.

Effects of the Antiserum in Other Newborn Mammals.—The few experiments performed up to now on newborn rats, rabbits, and kittens are not sufficient to give more than a very incomplete idea of the effects of the antiserum in these species.

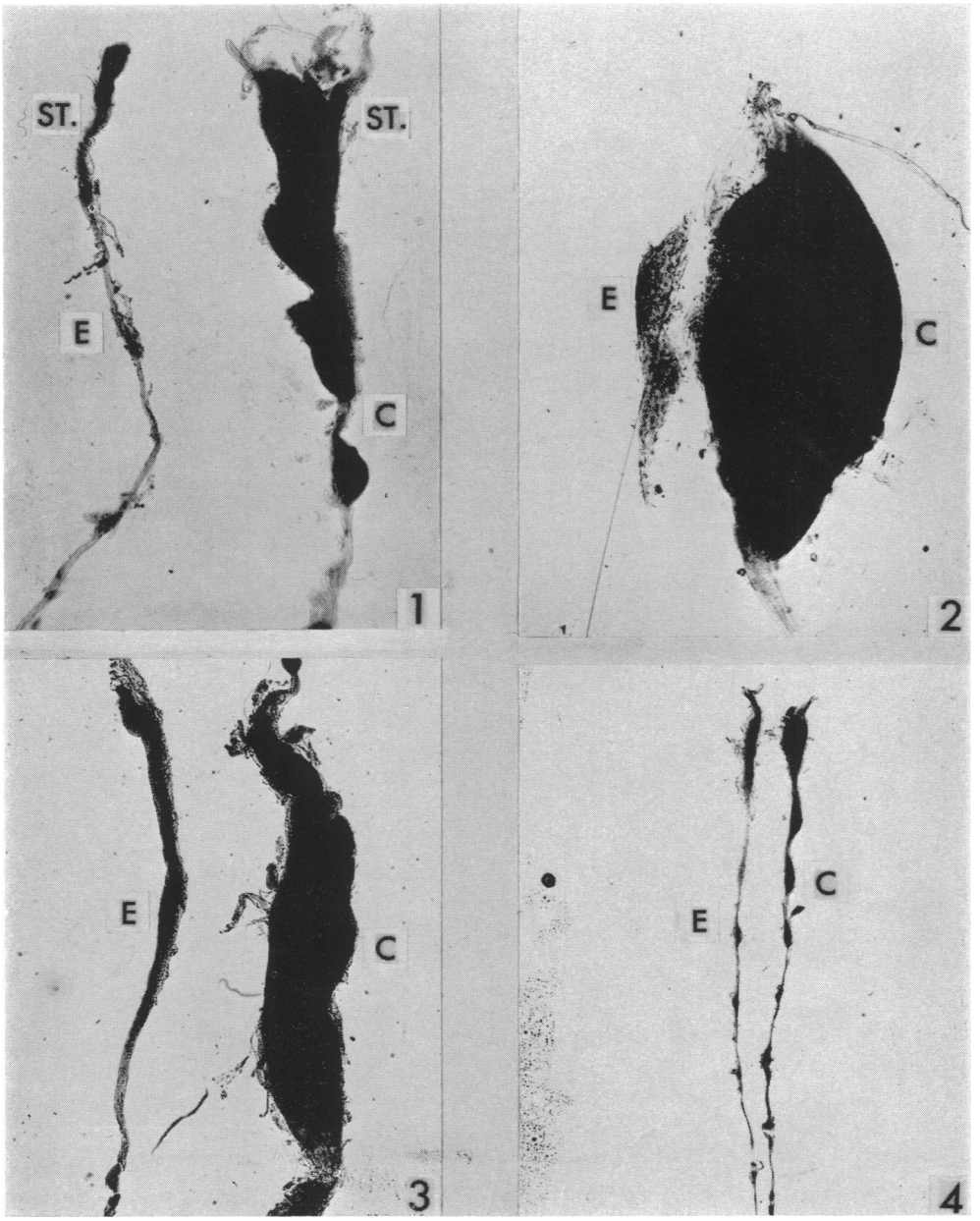

PLATE I.—Effects of rabbit antiserum against the purified protein of mouse salivary gland.

FIG. 1.—Stellate (St.) and first ganglia of sympathetic thoracic chains in control (C) and experimental (E) 20-day old mice. Experimental mouse injected daily since birth with the antiserum to the mouse salivary protein. 30×. FIG. 2.—Superior cervical ganglia in control (C) and experimental (E) 4-month old mice. Experimental mouse injected daily from birth to the 8th day with the antiserum to the salivary protein. 33×. FIG. 3, 4.—Effects of the antiserum in a 7-day old rat (FIG. 3) and in a $3\frac{1}{2}$-day old rabbit (FIG. 4); both injected daily since birth. FIG. 3, stellate ganglia in control (C) and experimental rat (E). 30×. FIG. 4, stellate and sympathetic thoracic chains in control (C) and experimental (E) rabbit. 4×.

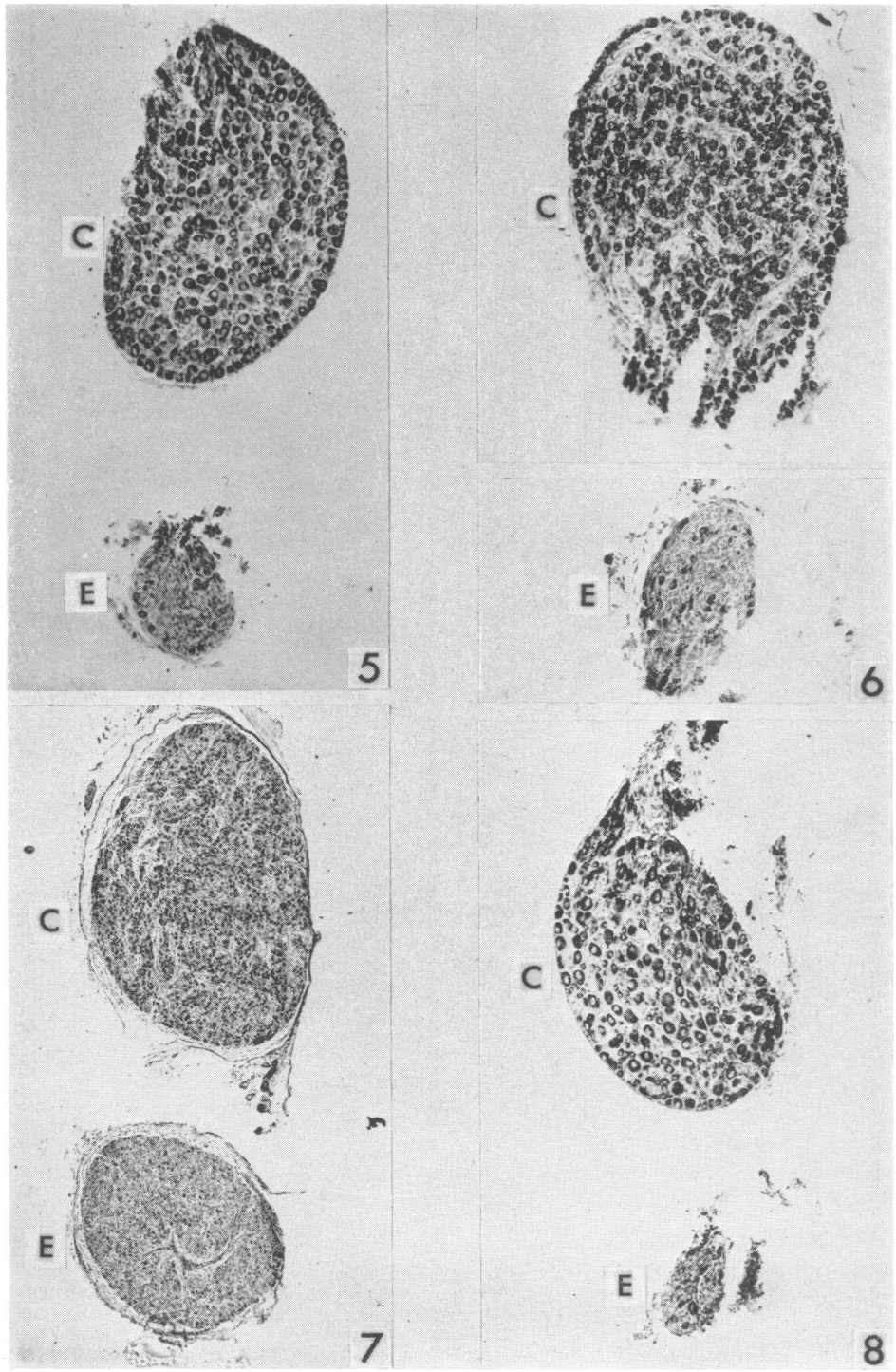

PLATE II.—Transverse sections of superior cervical ganglia in control (C) and experimental (E) animals, injected with rabbit antiserum to the salivary protein.

FIG. 5.—9-day old mice; E, injected daily since birth. 90 ×. FIG. 6.—7-day old rats; E, injected daily since birth. 90×. FIG. 7.—7-day old kittens; E, injected daily since birth; the ganglion consists of satellite cells and few nerve cells. 30 ×. FIG. 8.—4-month old mice; E, injected from birth to the 8th day. 60×.

The results obtained are, however, so impressive as to justify the contention that the antiserum affects the sympathetic ganglia of these mammals in a similar way as described above for newborn mice (Figs. 3, 4, 6, 7).

Two newborn rats were injected with the antiserum (0.05 ml per 1.5 gm of body weight). The experimental and control animals were sacrificed respectively at 5 and 7 days. The results observed are so similar to the ones described in mice at the same developmental stages as to make a description unnecessary. The number of cells in the superior cervical ganglion of one injected rat at 7 days was reduced to 7 per cent of the control (Table 1). The surviving cells are reduced in size when compared to controls but otherwise apparently in good condition (Figs. 3, 6).

Three newborn rabbits were injected with the rabbit antiserum and sacrificed at $3^1/_2$, 5, and 7 days. Since the ganglia are rather large in this species and the nerve cells easily distinguishable from satellites even in earlier stages, cell counts were feasible in three-and-a-half day old ganglia. A high reduction in the number of nerve cells was observed: only 9 per cent of the control population is present in the injected rabbit at this stage (Fig. 4). The two rabbits of the same litter, injected and sacrificed two and four days later respectively, showed a somewhat less severe atrophy of 14 per cent and 16 per cent respectively. Furthermore, the surviving cells were not reduced in size as in the younger animal. Investigation of animals injected for a longer period will answer the question whether rabbits develop a resistance to the antiserum which could account for such results.

The effects of the antiserum in one adult mouse, one young squirrel monkey, and a newborn kitten are shown in Table 1.

Discussion.—The results reported in this and in the previous paper bring to light the remarkable capacity of sympathetic nerve cells of newly born mammals to increase in size and number when injected with a mouse salivary gland protein, as well as their vulnerability to an antiserum to this protein.

The possibility that the sympathetic ganglia in man may be likewise affected by the nerve-growth factor is suggested by the *in vitro* experiments reported in the preceding paper.

The salient aspects of these phenomena are: their universality, their target specificity, and the unsuspected growth potentialities of young and adult nerve cells which materialize under the impact of nerve-growth promoting agents.

Of particular significance is the new finding that the antiserum against the salivary gland protein not only inactivates this protein, but, if injected alone into a newborn mammal, destroys its sympathetic cells almost totally in a remarkably short time. No other organs and tissues appear to be affected. On the other hand, normal serum leaves the sympathetic system as well as all other systems intact.

The question arises whether the near-total destruction of the sympathetic nerve cells is a direct or an indirect effect of the antiserum. It is conceivable that in the normal animal a factor circulates which is necessary for growth and maintenance of the sympathetic system and that the antiserum inactivates this factor. This hypothesis is supported by the detection of the nerve-growth agent in the blood of young and adult mice. Alternatively, the antiserum may exert a cytotoxic affect by combining with the antigen in or on the surface of the nerve cells. The same questions are raised in other investigations of the cytotoxic effects of the antisera.[1] Immuno-

chemical techniques, such as the detection of antibodies labeled with a radioactive isotope or with a fluorescent dye, may show if there is localization of the antiserum on the sympathetic nerve cells and if so, provide evidence in favor of the second alternative. These experiments will be performed in the near future.

The results reported in these and in previous papers[2-5] give evidence for the existence of closely related nerve-growth promoting proteins in two seemingly unrelated sources: mouse sarcomas and salivary glands. One may wonder whether both structures produce these proteins. We have positive evidence that the mouse sarcomas manufacture the nerve-growth agent even when they grow in the chick embryo.[6,7] Corresponding experiments in which the salivary glands were transplanted in the chick embryo were negative so far. Furthermore the nerve-growth agent was detected in serum of adult mice which had been deprived of their salivary glands two months earlier. The sympathetic ganglia of these mice did not show appreciable differences from control mice. Since the salivary glands and their homologue, the snake venom glands, contain the agent in high concentration, the only alternative would be that these glands store, but do not produce, the growth agent. If this suggestion would prove to be correct, we would still be faced with the problem of identifying the source of the nerve-growth agent in the organism. The possibility ought to be considered that the nerve-growth agent is not manufactured in any particular organ or organs, but in the mesenchymal tissue. The only evidence in favor of this hypothesis comes from the *in vivo* and *in vitro* experiments with mouse sarcomas and from *in vitro* experiments with explants of embryonic mouse heart.[8] Epithelial structures like carcinomas proved to be completely deprived of the property of stimulating nerve growth *in vivo*[9] as well as *in vitro*.[8] Research in progress is now aimed at the investigation of this problem.

Finally, we should like to call attention to other aspects of these experiments. They gave evidence of the high tolerance of the organism for such deviations from normality as a sixfold increase in volume of the sympathetic ganglia, or their near-total extinction. The injected animals apparently did not differ from controls when raised and observed in the sheltered conditions of the laboratory. Physiological tests are expected to reveal these differences and will therefore be applied to the treated and control animals.

Summary.—We have reported the effects of an antiserum to the nerve-growth factor which selectively destroys most of the sympathetic nerve cells in a variety of newborn mammals. Daily injections for a period of 8 days resulted in the disappearance of up to 99 per cent of the sympathetic nerve cells. This process is irreversible. It is suggested that the salivary glands do not produce the nerve-growth agent but merely accumulate and store it. The mode of action of the antiserum and the possible role of the nerve-growth agent as a maintenance factor for sympathetic neurons are under investigation.

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† For preparation of the antiserum see paper by Cohen, S., 302–311, these PROCEEDINGS.

[1] Edds, M. V. (Ed.), *Conference on Immunology and Development* (Chicago: The University of Chicago Press, 1956).

[2] Cohen, S., and R. Levi-Montalcini, these PROCEEDINGS, **42**, 695–699 (1956).

[3] Levi-Montalcini, R., and S. Cohen, these PROCEEDINGS, 571–574 (1956).

[4] Levi-Montalcini, R., in *The Chemical Basis of Development* (Baltimore: The John Hopkins Press, 1958), pp. 645–664.

[5] Cohen, S., in *The Chemical Basis of Development* (Baltimore: The John Jopkins Press, 1948) pp. 665–676.

[6] Levi-Montalcini, R., *Ann. N. Y. Acad. Sci.*, 330–343 (1952).

[7] Levi-Montalcini R., and V. Hamburger, *J. Exptl. Zool.*, **123**, 233–288 (1953).

[8] Levi-Montalcini, R., H. Meyer, and V. Hamburger, *Cancer Research*, **14**, 49–57 (1954).

[9] Bueker, E. D., *Cancer Research*, **17**, 190–199 (1957).