The ratio of $H^2/4\pi\rho$ to $\Omega_1^2 R_0 d$ is a measure of the relative importance of magnetic and inertia forces. The result (14) shows, therefore, that if this ratio exceeds the critical value of 0.1063, then the flow will always be stable.

I am indebted to Professor S. Chandrasekhar for some helpful comments. This work was supported by the Office of Naval Research.

[1] Chandrasekhar, S., "The Stability of Non-Dissipative Couette Flow in Hydromagnetics," these PROCEEDINGS, **46**, 253–257 (1960); see also Velikhov, E. P., "Stability of an ideally conducting liquid flowing between cylinders rotating in a magnetic field," *Soviet Phys.—JETP*, **36**, 995–998 (1959).

[2] Reid, W. H., "Inviscid modes of instability in Couette flow," *J. Math. Anal. and Applications* (to appear).

[3] Miller, J. C. P., *The Airy integral, giving tables of the solutions of the differential equation* $y'' = xy$, *British Association Mathematical Tables*, Part B (Cambridge: The University Press, 1946).

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*EXCESSIVE GROWTH OF THE SYMPATHETIC GANGLIA EVOKED BY A PROTEIN ISOLATED FROM MOUSE SALIVARY GLANDS**

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Communicated by Viktor Hamburger, January 4, 1960

The discovery that mouse sarcomas produce a diffusible agent which selectively promotes the growth of the sensory and sympathetic ganglia in the chick embryo,[1, 2] confronted us with three major problems: (*a*) the characterization of the growth agent, (*b*) the analysis of its mode of action on the embryonic nerve cells which were increased in number and in size under the impact of the agent to an extent unmatched by any previous experimental device, and (*c*) the question of the significance and distribution of nerve-growth agents in tumors and other tissues.

These problems were investigated using the method of explanting embryonic ganglia *in vitro* together with the tumor or other tissues to be tested. The finding that fragments of tumor explanted in proximity to the ganglia elicited exuberant nerve fiber outgrowth, and that this effect is apparent within 5–6 hours, greatly speeded up and simplified the bio-assaying procedures, thus making possible the biochemical approach to our problem.[3] The isolation of a nerve-growth promoting protein fraction from the neoplastic tissue was the first step in this direction.[4] In experiments aimed at the further characterization of the nature of the agent, Cohen made use of snake venom as a source of proteolytic enzymes. This led to the discovery that the snake venom also contains a nerve-growth promoting factor which replicates in all respects the effects of mouse sarcomas on the sensory and sympathetic ganglia of the chick embryo *in vitro* and *in vivo*.[5, 6] Since the snake venom is produced in a modified salivary gland, the mouse salivary glands were tested as another possible source of the nerve-growth promoting factor. The extract of the mouse submaxillary glands proved to contain a nerve-growth promoting agent even more potent than the two mentioned above.[7, 8] It was found that this agent likewise promotes the growth of the sensory and sympathetic embryonic ganglia of the chick

embryo, leaving other systems unaffected. The similarity extends also to other facets of the phenomenon such as the massive neurotization of the viscera of the chick embryo. The potency of this factor, its ready availability, its presence in various tissues of different vertebrates, suggested an investigation of its effects on a wider scale. In all previous experiments the chick embryo was the test object. We have now investigated the effects of the nerve-growth factor in mammals. The results of *in vitro* experiments on mouse and rat sensory and sympathetic ganglia were reported.[7, 8] We will report in this paper the results of *in vitro* effects on human fetal ganglia and of *in vivo* experiments on the sympathetic ganglia of newborn, young, and adult mice. We will also report on experiments designed to establish the relationship between salivary glands and nerve-growth agents. In a second paper, we will present evidence of the near-total disintegration of the sympathetic ganglia in mammals as a result of daily injections of the antiserum to the nerve-growth factor. The agents used in all these experiments were prepared by Dr. S. Cohen and they are described by him in another paper in this issue.

In vitro Effects on Sensory and Sympathetic Ganglia of Human Fetuses.—Four human fetuses, three about two-and-a-half months old and one about three-and-a-half months old, were obtained from the Medical School of Washington University. They resulted from hysterotomy for therapeutic abortion and were made available as soon as delivered.† The sensory and sympathetic ganglia of these fetuses were explanted in tissue culture: the same hanging drop technique was used as in all previous experiments.[3, 5, 7] The medium consisted of one part of chicken plasma, one part of synthetic medium, and one part of the purified salivary fraction. Control cultures were prepared using one drop of physiological solution instead of the salivary fraction. A total of forty experimental and forty control cultures were prepared; in each culture a large number of ganglia were explanted. The effects of the salivary extract on ganglia of the two-and-a-half month fetuses were similar in all respects to the effects elicited by mouse sarcomas, snake venom, and the mouse salivary extract on ganglia of chick embryos (Fig. 3), mouse, and rat embryos. A dense halo of nerve fibers surrounded the ganglia in the experimental cultures; it was already apparent 12 hours after the beginning of the experiment and increased in density and in size at the end of the first day (Fig. 5). A comparable, but somewhat slower, reaction was observed in ganglia explanted from the older fetus. A considerable degree of liquefaction of the cultural medium took place on the second and third day of culture; the experiments were then discontinued. Control ganglia showed few or no nerve fibers in the area surrounding the explant 24 hours after the preparation of the culture (Fig. 2).

In vivo Effects on the Sympathetic Ganglia of Newborn and Adult Mice.—(*a*) *Material and methods:* The effect of the salivary extract was tested on newborn and adult mice. Solutions of the active extract at different concentrations and different degree of purification were at first assayed in three groups of newborn mice and then on adult mice.

Group 1: Ten newborn mice were injected daily with the salivary extract referred to in Cohen's paper as fraction CM-1 (these PROCEEDINGS, this issue). This solution was injected subcutaneously in the amount of 0.05 ml per gm of body weight at a concentration of 1,500 units of biological activity per ml. One unit of biological activity is defined as that amount per ml required to elicit a 3+ response

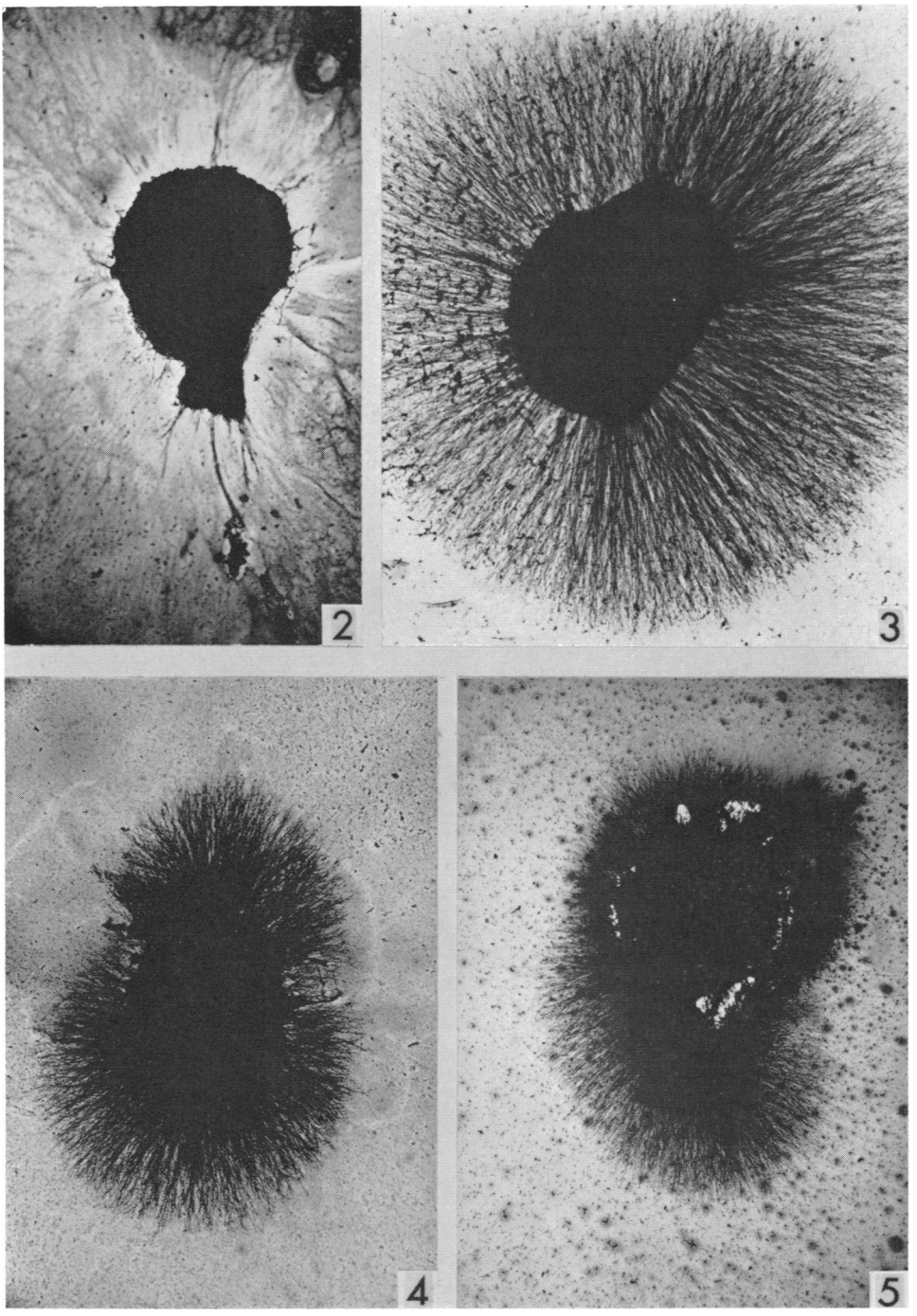

PLATE I.—Microphotographs of sensory ganglia after 24 hours *in vitro*. Silver impregnation. FIG. 2.—Ganglion of a $2^1/_2$ months human fetus in the standard control medium. FIGS. 3, 5.—Ganglia of a 7-day chick embryo (Fig. 3) and of a $2^1/_2$-month human fetus (Fig. 5) in a medium containing the purified salivary protein at a concentration of 1:18,000. FIG. 4.—Ganglion of a 7-day chick embryo in a medium containing mouse serum at a concentration of 1:30. Short and dense halo in Figs. 4 and 5 characteristic of strong and slightly inhibitory effects of the growth agent at high concentration.

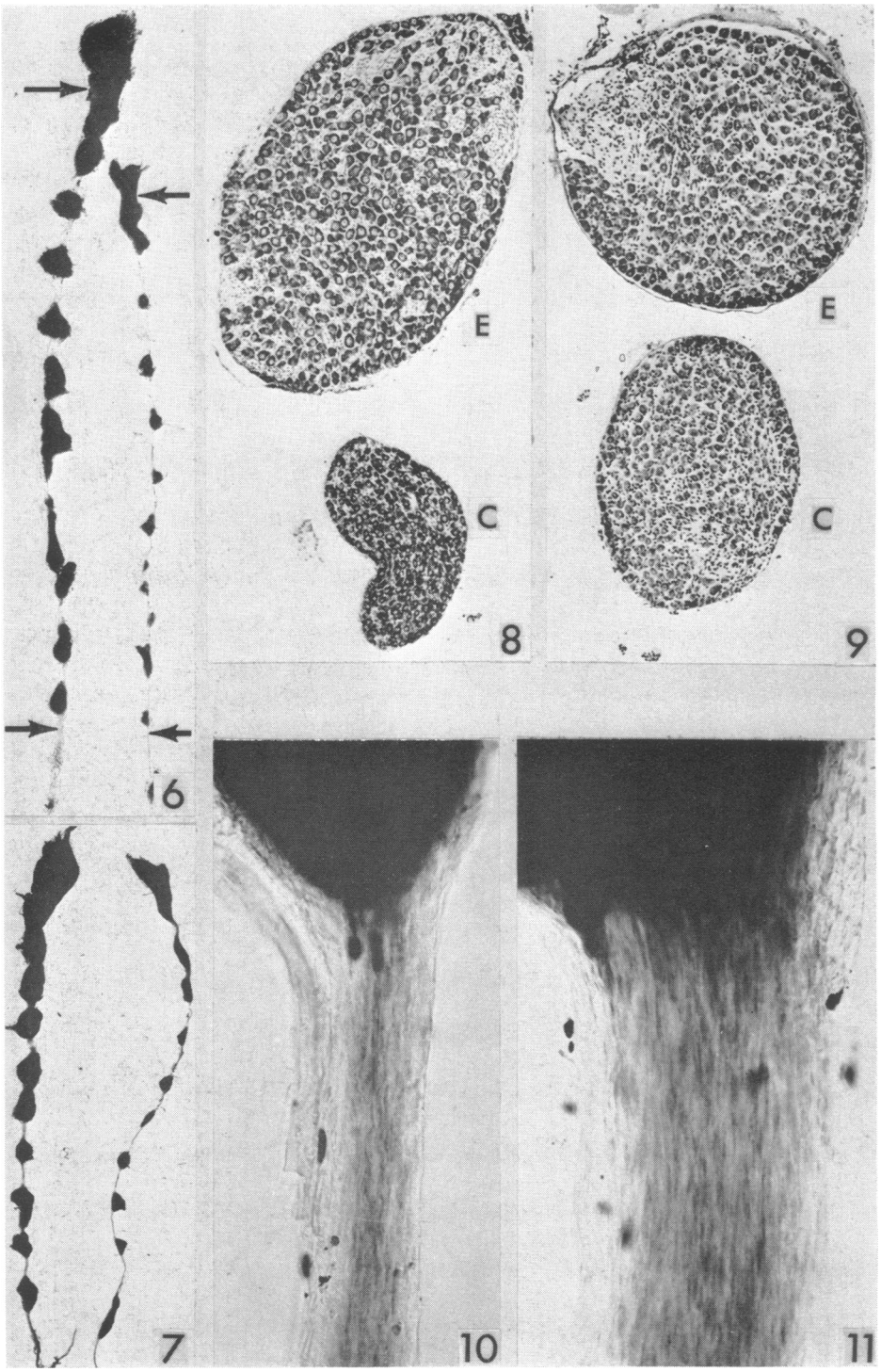

PLATE II.

FIGS 6, 7.—Whole mounts of the sympathetic thoracic chain ganglia of experimental (E) and control (C) mice 19 and 12-day old respectively. Experimental mice injected with the CM-3 salivary fraction since birth. FIG. 8.—Transverse section of stellate ganglia in experimental (E) and control (C) ganglia of Fig. 6. Sections through levels indicated by upper arrows in both chains of Fig. 6. FIG. 9.—Transverse section of superior cervical ganglia of two adult mice. E, mouse injected for 1 week with the CM-1 salivary fraction. C, control ganglion. FIGS. 10, 11.—Sympathetic nerve trunk of control and experimental chain ganglia of Fig. 6e at higher magnification. Low arrows in Fig. 6 indicate areas enlaged in Figs 10, 11.

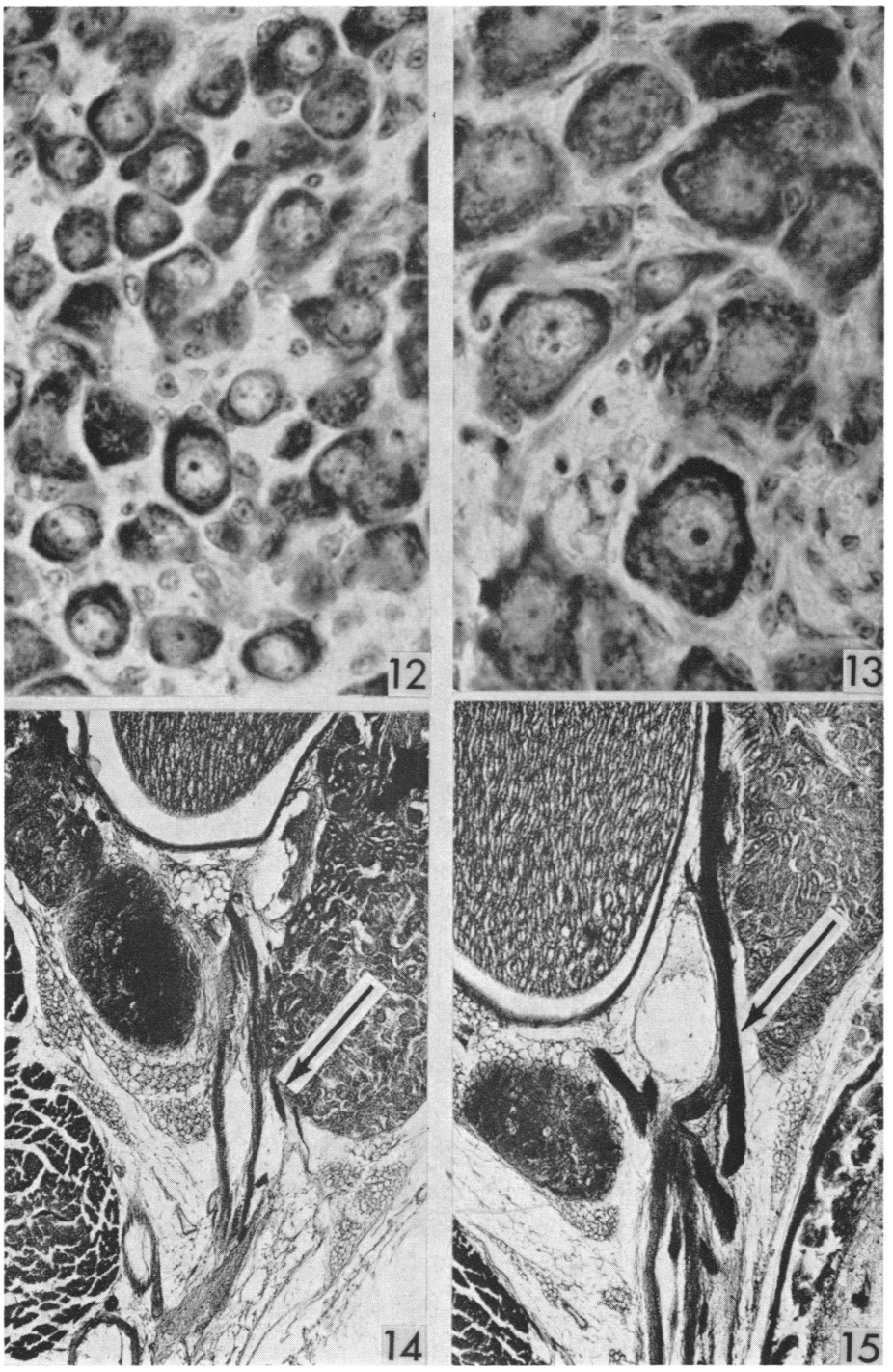

PLATE III.

FIGS. 12, 13.—Comparison of cell size in control and experimental stellate ganglia represented in Figs. 6, 8. Toluidin blue stain. FIGS. 14, 15.—Comparison of size of sympathetic nerve along the renal artery in control (Fig. 14) and experimental 19-day old mouse (Fig. 15) injected daily with the CM-3 salivary fraction since birth Arrows point to nerve in both figures. Cajal De Castro silver impregnation.

in tissue culture. The injected and control mice of the same litters were sacrificed between the 7th and the 14th day and examined for effects on the nervous system and other systems. When it was found that such treatment had consistently elicited a significant increase in the sympathetic ganglia, a second series of experiments was performed with a more concentrated solution.

Group 2: Thirty newborn mice were injected with the CM-1 fraction at a concentration of 6,000 units per ml. The solution was injected daily, in the same amount as used in previous experiments. The injected and untreated mice of the same litters were sacrificed every day or every other day between the second and the thirtieth day.

Group 3: Ten newborn mice were injected daily, from birth, with a more highly purified fraction referred to as CM-3 fraction in the paper by S. Cohen. The solution was injected in the same amount and in the same concentration as in the experiments of Group 2. The injected and control mice were sacrificed between 1 and 9 days and at 12 and 19 days respectively.

Group 4: Fifty adult mice were injected with the CM-1 fraction at the same concentration and in proportional doses per body weight as in groups 2 and 3. The injected and control mice were sacrificed in groups of 3 to 4 between the end of the first week and the fourth week and compared with controls.

The injected and control mice of all 4 groups were dissected and used for investigation of the sympathetic ganglia; other components of the nervous system were also examined. The sympathetic ganglia were either dissected out and stained with hematoxylin and toluidin blue for counts of mitotic figures and of nerve cells, or they were left *in situ* and studied after silver impregnation and sectioning of the whole organism. This material was used for the study of the neurofibrillar differentiation of nerve cells and of the peripheral distribution of sympathetic nerve fibers in injected and control animals.

Counts of mitotic figures were made in sympathetic ganglia of mice of the third group between 12 hours and 9 days. The mitoses were counted in each section of the superior or stellate ganglia in control and treated mice.

Area measurements were made in ganglia dissected from mice 5 to 27 day old, of groups 2 and 3. Each section was projected with the help of the camera lucida and the contour of the section was drawn on cardboard. The total number of sections was then weighed and the weight compared with that of the same control ganglion. Since the weight is proportional to the volume, the figures indicate a similar ratio between the volume of experimental and control ganglia. A total of 18 control and 18 experimental ganglia were measured and compared. The same technique was used on a small number of ganglia of the fourth group.

Cell counts were performed in sympathetic ganglia of groups 3 and 4, by inserting a micrometer disk in the ocular and counting all nerve cells in every other section of the experimental and control ganglia.

Two additional series of experiments were performed. In the first series we tested the serum of adult and weanling mice for the presence of the nerve-growth factor. A total of 150 adult mice of both sexes and of 30 weanling mice were tested. The blood was either collected from the blood vessels immediately after decapitation of mice in light chloroform anesthesia, or it was drained directly from the aorta and the heart in mice anesthetized with nembutal. The blood was

allowed to clot at room temperature and then stored for half an hour in the refrigerator. The serum was then collected in separated vials and each specimen tested on sensory ganglia of 8-day chick embryos explanted *in vitro* with the usual hanging drop technique. All sera were also tested at dilutions of 0.1, 0.01, and 0.001.

In the second series of experiments we extirpated the submaxillary and sublingual salivary glands in 25 adult and 5 weanling mice, and we inspected the sympathetic ganglia of the operated and control mice between 2 weeks and 6 months after the operations.‡

(*b*) *Effects of the purified salivary extract on newborn mice:* The newborn mice injected with the CM-1 fraction in the weak and even more in the strong concentration, exhibited side effects which will be mentioned but not described in detail. From the third day on, the growth rate dropped sharply and at the end of the first month, the mice were barely larger than at the end of the first week. The hair growth was severely impaired, the lids opened 6 to 7 days earlier than in controls and the cutting of the inferior and superior incisors and their calcification took place 5 days earlier than in controls. The animals recovered if the injections were discontinued at the end of the first month; dwarf mice resumed growth and three months later they did not differ from untreated controls at a macroscopical inspection. The effects mentioned above did not affect the vitality of the mice in the doses used. None of the forth injected mice died as a result of the treatment. No side effects were observed in newborn mice injected with the more purified fraction CM-3. They were healthy and vigorous as controls. Obviously, we are dealing with 2 factors, one of which was removed in the process of purification.

Both fractions CM-1 and CM-3 evoked a marked overgrowth of the sympathetic ganglia (Figs. 6, 7, 8, 9). In the following we will consider only the results of the injections of fractions CM-1 and CM-3 in the concentration of 6,000 units per ml (groups 2 and 3).

The average *volume increase* of the superior cervical ganglia in group 2, as determined by comparison of 13 experimental and 13 control ganglia was 3 : 1. The corresponding figures for the same ganglia of mice injected with fraction CM-3 and controls (group 3) were higher: measurements of 3 experimental and 3 control ganglia at 12 days gave a ratio of 4.1 : 1 and on 2 experimental and 2 control ganglia at 19 days gave 6.4 : 1. More long range experiments are planned to establish the ceiling of this effect.

The results of *cell counts* in 12 control and 12 experimental ganglia of mice injected since birth with the CM-3 fraction are given in Table 1. The increase in cell number in the injected mice over the controls averages 2.5 and 2.18 respectively in one 12- and one 19-day old mouse. The results of cell counts in adult mice, as given in the same Table for 5 controls and 2 experimental ganglia, show no increase in cell number in the injected adult mice over the controls. It is of interest to note that the total cell population in the normal stellate ganglia is 13,000 whereas the same ganglion in an experimental mouse has a population of over 30,000. The mechanism of this cell increase will be dealt with below.

The concomitant *increase in cell size* in injected animals is shown in Figures 8 and 9 for the stellate ganglia of the 19-day old mice and adult mice respectively. No size measurements were made. Since the volume increase in the ganglia was 4 to 6

times (see above) whereas the increase in cell number in the same ganglia was about twofold, we conclude that cellular hypertrophy has a greater share in the end effect than the increase in cell number.

Cytological examination showed that the hypertrophic neurons differ from controls also in the more intense basophilia and in the size of the nucleoi which are much larger than in controls (Figs. 12, 13). A parallel increase in neurofibrillar material in the hypertrophic nerve cells is apparent in the silver-stained ganglia. Observations of ganglia dissected from three-day old mice injected with the CM-1 or CM-3 fractions since birth, indicate that the size increase is already evident at that time.

It was of interest to decide whether the increase in cell number in the injected mice is due to an increase in *mitotic activity* or to other mechanisms such as the production of a larger number of sympathetic nerve cells at the expense of germinal or pluripotential cells present in the ganglia. The presence in the ganglia of a fairly

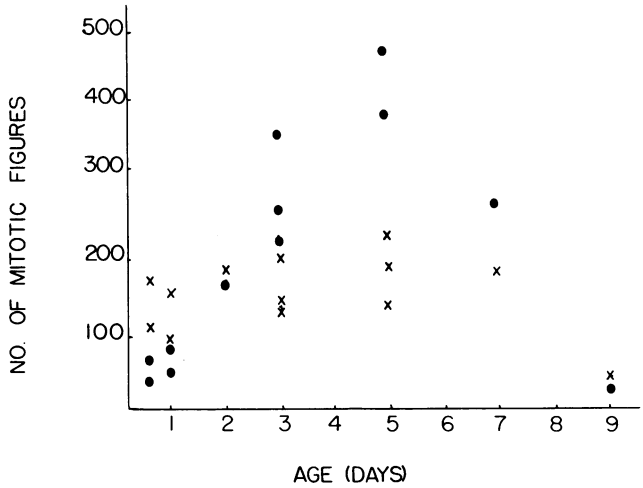

FIG. 1.—Effect of the nerve-growth factor on mitotic activity. Crosses and dots indicate number or mitoses per ganglion (superior cervical ganglion) in control and experimental mice respectively.

large number of small-sized cells, beside the satellite cells, could suggest this possibility. On the other hand, since the mitotic activity is still high at birth and comes to an end at 9 days, the injection of the nerve-growth factor in newborn mice could also affect this process. Counts of mitotic figures in control and experimental ganglia between 1 and 9 days showed a sharp increase in the mitotic activity of the experimental ganglia between 3 and 7 days with a peak at 5 days. These results, presented in Figure 1, favor the hypothesis that the increase in nerve cells in the injected mice is due to an increase in mitotic activity. Although there is no way of deciding how many of the dividing cells are neuroblasts, the results correlate well with the finding of an increase in cell number in later stages; they are also in agreement with observations on the effects of nerve-growth agents on mitotic activity in the spinal ganglia of the chick embryo.[9]

(*c*) *Peripheral distribution of sympathetic nerve fibers:* One would expect that the increase in cell number and cell size in sympathetic ganglia of the injected mice

would result in a parallel increase in the size of nerves emerging from the enlarged ganglia. This increase was in fact observed and it is documented in Figures 10 and 11. A comparison of the cephalic, thoraic, and abdominal regions of experimental and control mice shows an increased density and thickness of the nerve plexuses around the blood vessels of the injected animals. In the kidney, sympathetic nerve bundles were traced along the intrarenal blood vessels and also among the renal tubules, in much larger number in the experimental than in control animals. Figures 14 and 15 show the increase in thickness of the sympathetic nerve along the renal artery in a 19-day injected mouse compared to a control of the same age.

These effects, the increase in number and size of sympathetic nerve cells and the hyperneurotization of the viscera, are very similar to the effects called forth by mouse sarcomas, snake venom, and the salivary extract in the chick embryo.[1, 2, 5, 7] The two sets of experiments differ in two respects: (1) While in chick embryos, the sympathetic nerve fibers produced in excess follow in many instances anomalous routes and even force their way into the lumen of blood vessels, no such deviations were observed in mice injected with the salivary gland fractions. Thus the quantitative rather than the qualitative aspect of the distribution of sympathetic nerves seems to be affected in mammals in post-natal periods. (2) Whereas these agents evoke in the chick embryo a striking response also in the sensory ganglia,[2, 9] this effect is barely noticeable in newborn mice. Measurements of a number of sensory ganglia showed only a slight increase in the experimental material.

TABLE 1

TOTAL NERVE CELL NUMBERS IN SYMPATHETIC GANGLIA OF MICE INJECTED WITH SALIVARY GLAND FRACTIONS

Fraction	Age	Ganglia	Control	Experimental	Ratio Exp./Cont.
CM-3	12	Thoracic N. 6	1,365	3,212	...
		Thoracic N. 7	1,165	2,731	...
		Thoracic N. 8	1,155	4,422	...
		Thoracic N. 9	1,026	2,849	...
		Thoracic N. 10	1,465	2,567	...
			Total 6,176	Total 15,781	2.5
CM-3	19	Stellate			
		Thoracic N. 2	14,400	30,800	...
		Thoracic N. 3	1,644	3,684	...
		Thoracic N. 4	1,770	2,133	...
		Thoracic N. 5	1,094	3,144	...
		Thoracic N. 6	820	2,328	...
		Thoracic N. 7	1,362	2,758	...
		Thoracic N. 8	1,392	4,162	...
			Total 22,482	Total 49,009	2.18
CM-1	Adult	Stellate	14,000	13,689	...
		Stellate	12,000	...	...
			Average 13,000	13,689	1.05
	Adult	Sup. Cerv.	13,300	15,770	...
		Sup. Cerv.	16,415	...	...
		Sup. Cerv.	15,652	...	...
			Average 15,122	15,770	1.03

(*d*) *Effects of fraction CM-1 on adult mice:* The daily injection of 0.05 ml per body weight of fraction CM-1 (6,000 units per ml) was well tolerated and the injected

mice did not show any adverse effects. The sympathetic ganglia were considerably larger than in controls: the difference was evident at a macroscopic inspection of the 50 injected and 50 control mice. Area measurements of the ganglia in a few of cases gave a twofold enlargement, compared with controls. The size increase was due to cellular hypertrophy of the individual neurons (Fig. 9). The cell number was apparently not increased (see Table 1).

Nerve-Growth Effects of Blood Serum of Adult and Young Mice.—Previous experiments *in vitro* gave evidence of a mild nerve-growth effect elicited by embryonic mouse heart on sensory and sympathetic ganglia of chick embryo.[3] Traces of the same activity were then found in homogenates of striated muscle of adult mice. Bueker[10] detected activity in partially purified preparations of thymus, kidney, and muscle. We found in some instances evidence of activity in the urine of adult mice. The same activity was detected in mouse saliva. We then proceeded to test the serum of adult and weanling mice.

Observations to be reported in detail elsewhere disclosed a considerable sexual dimorphism in the sympathetic nerve cells of adult mice. In male mice, the sympathetic neurons are considereably and consistently larger and more intensely stained with basic dyes than in female mice of the same size. These results suggested tests of the blood serum of mice of the two sexes separately. The results are summarized in Table 2. A maximal effect was obtained from the serum of 15 male

TABLE 2

NERVE-GROWTH EFFECTS OF MOUSE SERUM *in vitro*

Age	Concentration	Response* ++++	++	+	±
Adult males	1/10	15			
	1	10	30	20	25
Adult females	1	1	7	10	32
Weanling	1		4		26

* ++++ to ± give degrees of effects from maximal to barely detectable.

mice and one female mouse. In 15 cases with a 4+ effect at a dilution of 1 : 10 (Fig. 4) an effect was still detectable at a dilution of 1 : 1000. The nerve-growth effect of the serum collected from adult females or from weanling mice of both sexes was consistently milder than that of the serum of adult males. These sex and age differences have a parallelism in the finding of Cohen (these PROCEEDINGS, this issue) of a higher specific activity in the submaxillary salivary gland of male than of female mice and of much lower activity in the same gland of weanling mice. These results indicate that the nerve-growth factor is present in higher concentration in the blood of adult male than female mice and that it is in even lower concentration in the blood of weanling mice. They also show a considerable variation among mice of the same group. Experiments in progress are expected to answer the question of whether such variations are correlated with physiological differences in the tested animals and if the stress resulting from the administration of the anesthetic may account for such variations.

Effects of the Extirpation of the Submaxillary and Sublingual Glands in Adult and Weanling Mice.—The operated mice were sacrificed between two weeks and six months after the operations and were compared with controls of the same size.

When mice in the weanling stage were operated, the controls were selected from the same litters.

In the first days after the operation, the animals showed signs of discomfort and the fur became ruffled. In the following days, the mice recovered, but in most instances the fur remained deranged. The operated adult males differed from controls also in another respect. They became much more tame than controls and easier to handle. The correlation between submaxillary salivary glands and other endocrine glands, in particular sex glands, have been investigated by many authors[11, 12] and will not be discussed here. They are mentioned, however, since they may have a bearing on the present results.

In all the experimental and control mice, the sympathetic chain ganglia were dissected out, stained with toluidin blue, and sectioned at 10 microns. Since the superior cervical ganglion might have been indirectly affected by the extirpation of part of its peripheral field of innervation, the salivary complex, we used instead the stellate ganglion which does not contribute to the innervation of the salivary glands.

Results.—Twenty operated adult males and twenty controls were examined between three weeks and two months after the operation. In five mice deprived of the salivary glands a slight decrease in size of the sympathetic nerve cells was detected. The nerve cells also stained less intensely than controls with toluidin blue and appeared similar to sympathetic nerve cells of female mice. No differences were noticeable in the other 15 operated mice. Five adult female mice were operated and compared with controls three weeks after the operation. No size differences were detected between these and control nerve cells. Equally negative were the results in 5 mice operated during the weanling stage. One of these was compared with a control of the same litter, six months after the operation, the other mice one month after the operation. In all instances, the sympathetic chain ganglia appeared of the same size as the controls and the histological examination of the stellate ganglia revealed no changes in cell size.

The serum of mice deprived of the salivary glands was also tested for the nerve-growth factor. In one mouse deprived of the glands two months earlier the serum evoked a 4+ effect *in vitro* as the serum of control mice. In the other specimen the effect varied from a 2+ to a barely detectable effect. These results indicate that the nerve-growth agent is present, even in the absence of the salivary glands.

Summary.—Previous work has provided evidence for the presence of nerve-growth promoting agents in a variety of biological materials: mouse sarcomas, snake venoms, and mouse submaxillary salivary glands. In the present investigation the effect of the active fraction isolated from the mouse salivary glands was tested *in vitro* on ganglia of human fetuses; it was found that it elicits the same effects as on ganglia of other species. The active fraction was then injected in newborn and in adult mice. In all instances the injection resulted in a marked increase of the sympathetic ganglia; the response varied with the age of the animal, the amount, and the purity of the fraction injected. In some instances a sixfold increase in size was observed. Slight nerve-growth promoting effects of different mouse tissues had been observed *in vitro* in previous experiments. We have now found evidence for the presence of the nerve-growth factor in the serum of adult and weanling mice. Maximal effects were obtained from the serum of adult male mice; the serum of female mice is less effective. This sex difference is paralleled by a sex difference in the size of adult sympathetic nerve cells.

* This work has been supported by a grant from the National Science Foundation and by a contribution from an Institutional grant of the American Cancer Society to Washington University.

† We wish to express our appreciation to Dr. Willard Allen of the Department of Gynecology and Obstetrics of the Washington School of Medicine who made this material available to us.

‡ 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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Communicated by Viktor Hamburger, January 4, 1960

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