15135

Prevention of Formation of End-Bulb Neuromata.*

EDGAR J. POTH, E. BRAVO FERNANDEZ, AND GLENN A. DRAGER.

From the Surgical Research Laboratory and the Department of Anatomy of The University of Texas Medical Branch, Galveston, Texas.

Painful amputation stumps become major problems during periods of extensive traumatic injuries such as result from modern warfare. Just why some amputation stumps become painful is not known. It has long been considered that end-bulb neuromas which form at the end of severed peripheral nerves may be the cause of this phenomenon, because these tumefactions may be painfully tender. All of these neuromas are not tender, and the excision of a tender neuroma does not always remedy a painful stump. The question has been raised as to whether the pain in an amputation stump is due entirely to the presence of a tender neuroma. If some procedure were devised by which neuroma formation could be prevented, the cause of painful amputation stumps could be more accurately determined. The complete elimination of neuroma formation in an amputation stump will be difficult because many small cutaneous nerves are divided and form neuromas, which may be potential sources of pain as proposed by Molotkoff.[1]

A recent review by Young[2] of the mechanism of regeneration of peripheral nerves, extensively studied by Cajal[3] and many others, indicates that the anatomico-biological characteristic of the peripheral nerve is one of unlimited capacity for growth of the regenerating axon so long as the central cell body of the neuron remains intact. This forward pressure of growth is maintained until the nerve fibers make functional connection with some type of end-organ, or under abnormal conditions, the fibers develop end-bulbs and Perroncito spirals,[3] making many types of peculiarly disorderly connections with the surrounding tissues typical of neuroma formation.[4] Recent evidence has shown the growth of the regenerating fibers under all conditions is guided by the "contact actions" of the surfaces along which they advance, thus returning in its essential implications to the "Mechanical Theory" of nervous regeneration originally propounded by Ranvier and Vanlair.[5] The growth, therefore, of nerve fibers to form a neuroma is a natural phenomenon. This growth is abnormal, because no sheath is present to prevent their irregular growth into scar tissue. The pain resulting from the manipulation of a neuroma may be due to the stimulation of these abnormal nerve endings by pressure or traction on the nerve fibers adherent to scar tissue. Boldrey[6] and Seddon[7] buried the nerve-end into bone and eliminated pain from the neuroma. Although a neuroma formed in the bone, it could not be stimulated mechanically.

Many methods have been devised in an attempt to prevent neuroma formation. These procedures can be listed under two groups: (1) those in which an attempt is made to kill or inhibit the growth of the nerve fibers by chemical and physical means, and (2) those which attempt to obstruct the pathway of the regenerating fibers mechanically. Recent reviews by Guttmann and Medawar[8] and Boldrey[6] discuss quite fully the different individual procedures. Huber and Lewis[4] originally suggested the injection of the stump

* Aided by a grant from Sharp and Dohme, Inc., Philadelphia.

1 Molotkoff, A. G., *J. Bone and Joint Surg.*, 1935, **17**, 419.

2 Young, J. Z., *Physiol. Rev.*, 1942, **22**, 318.

3 Cajal, S. R., *Degeneration and Regeneration of the Nervous System*, Oxford Univ. Press, 1928, **1**, 331 (Translated by R. M. May).

4 Huber, G. C., and Lewis, D., *Arch. Surg.*, 1920, **1**, 85.

5 Ranvier and Vanlair (cited by Cajal 3).

6 Boldrey, E., *Ann. Surg.*, 1943, **118**, 1052.

7 Seddon, H. J., 1941 personal communication, cited by Guttmann and Medawar 8.

8 Guttmann, L., and Medawar, P. B., *J. Neurol. and Psych.*, 1942, **5**, 130.

with 95% ethyl alcohol. Corner[9] advocated the excision of a wedge from the end of the nerve followed by suturing the two flaps, while Chapple[10] placed an epineural sleeve over the end of the nerve. Stookey[11] advocated a combination of the two procedures. More recently Kirk[12] and Bate[13] advocated electrodesiccation of the nerve stump. Following the simple ligation of the severed nerve with non-absorbable suture material sufficiently tightly to prevent regeneration of axones without rupturing the neurilemma sheath, Herrmann[14] reduced the occurrence of the phantom limb phenomenon to 5.8% in 120 patients subjected to amputation. Guttmann and Medawar[8] were unable to prevent consistently the regeneration of the axones of the sciatic nerve of the rabbit when using this procedure. In consideration of the mechanism of the regeneration of peripheral nerves, it becomes evident that no method which merely destroys an additional segment of nerve will result in prevention of neuroma formation. The neuroma will merely form at a higher level. Poth and Bravo Fernandez[15] in a preliminary report, discussed the implantation of the nerve stump into rigid tubes, closed at one end. This procedure quite effectively overcame the pressure growth of the nerve fibers and completely inhibited the formation of end-bulb neuromata. A detailed report of these observations will be the subject of this paper, because it is felt that if a proved successful method could be devised for the prevention of neuroma formation, a considerable clarification of the problem of the painful stump and phantom limb might be effected by the elimination of the neuroma as a causative factor.

Experimental. Methods and Results. The following fundamental procedure was carried out on mongrel dogs. The operations were performed under ether anesthesia and strict asepsis. The skin overlying the intermuscular groove on the lateral aspect of the thigh was incised. The sciatic nerve was exposed, dissected from its areolar tissue bed, and divided. The proximal stump was treated as indicated under the different subdivisions of this experiment. Thereafter, the skin was closed, using a continuous subcuticular suture of fine cotton. Whenever a wound infection occurred, the animal was dropped from the series. After varying periods, these animals were sacrificed and specimens of the nerve were taken for microscopic study. The specimens were fixed in Bodian's[16] No. 2 fixative and the sections were subsequently stained with Bodian's Activated Protargol Stain.[17]

Group I. Control Experiments. This group was comprised of 9 animals. The fundamental operation outlined above was performed, and a segment of the nerve 2 cm in length was excised. The proximal nerve stump was replaced in its bed without further treatment. These animals were sacrificed after a period varying from 30 to 90 days. In all instances typical neuroma formation had occurred (Table I and Fig. 1).

Group II. After performing the basic procedure, the proximal nerve stump was injected with absolute alcohol. This group was comprised of 2 animals; and in both instances, large end-bulb neuromata had formed at the end of 30 days.

Group III. Five animals comprised this group. After performing the basic operation, the proximal nerve stump was placed in a cellophane tube, the distal end of which was closed by a silk ligature. The results in this group are not entirely consistent. In all instances, there was considerable connective tissue reaction, and it was not always possible to identify the cellophane at the end of the experiment. In one instance the nerve, while still within the cellophane tube, showed an atypical diffuse enlargement. Some of the

9 Corner, E. M., *Proc. Roy. Soc. Med.*, 1918, **11**, 7.

10 Chapple, W. A., *Brit. Med. J.*, 1918, **1**, 399.

11 Stookey, B., *Surgical and Mechanical Treatment of Peripheral Nerves*, W. B. Saunders Co., Chap. 21, p. 461, 1922.

12 Kirk, N. T., *Amputations, Prac. of Surg.*, Dean Lewis, III, Chapter 10, 1943.

13 Bate, J. T., *Am. J. Surg.*, 1944, **64**, 373.

14 Herrmann, L. G., and Gibbs, E. W., *Am. J. Surg.*, 1945, **67**, 168.

15 Poth, E. J., and Fernandez, Bravo E., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 7.

16 Bodian, D., *Anat. Rec.*, 1937, **69**, 153.

17 Bodian, D., *Anat. Rec.*, 1936, **65**, 89.

TABLE I.
Experiments Grouped According to Treatment of Sciatic Nerve.

Group	Animal No.	Duration of experiment, days	Result
I. Control—2 cm of sciatic nerve resected	5	90	Large neuroma
	6	90	" "
	7	90	" "
	8	70	" "
	9	30	Small "
	10	45	Large "
II. Nerve stump injected with absolute alcohol	NA 53	15	Small "
	NA 54	30	Large "
III. End of nerve encased in cellophane	NA 12	120	Mass of scar tissue (Neuroma?)
	NA 13	20	No neuroma
	NA 17	200	Enlargement of nerve stump—no neuroma
	NA 20	120	Tapering nerve stump—no neuroma
	NA 23	160	Mass of scar tissue (Neuroma?)
IV. End of nerve placed in silver tube	NA 11	200	Out of tube. Large neuroma
	NA 16	200	Large neuroma
	NA 18	200	" "
	NA 19	200	No neuroma. Considerable scarring around tube
	NA 38	100	Out of tube. Large neuroma
V. End of nerve placed into arterial sleeve	NA 56	75	No neuroma
	NA 57	80	" "
VI. End of nerve placed into snugly fitting glass tube 2½ cm long	1	102	" "
	2	68	" "
	3	102	" "
	4	102	" "
	11	30	Enlarged—no neuroma
	12	65	Nerve out of tube. Moderate sized neuroma
	NA 1	230	No neuroma
	NA 2	230	" "
	NA 3	230	" "
	NA 4	230	" "
	NA 5	230	" "
	NA 7	120	" "
	NA 8	140	Nerve enlarged—out of tube
	NA 9	100	No neuroma
	NA 10	100	" "
VII. Intact sciatic nerve inj. with 10% tannic acid	I-1	8	Degeneration of nerve with fibrosis
	I-3	171	
VIII. Intact sciatic nerve inj. with 2% gentian violet	I-2	8	" " " " "
	I-4	171	
IX. Proximal end of nerve inj. with 10% tannic acid	NA 41	140	No neuroma
	NA 42	110	" "
	NA 43	140	" "
	NA 46	130	" "
	NA 47	15	" "
	NA 48	30	" "
	NA 49	130	" "
X. Proximal end of nerve inj. with 2% gentian violet	NA 28	120	" "
	NA 30	180	" "
	NA 31	180	" "
	NA 32	80	" "
	NA 35	180	" "
	NA 60	80	" "

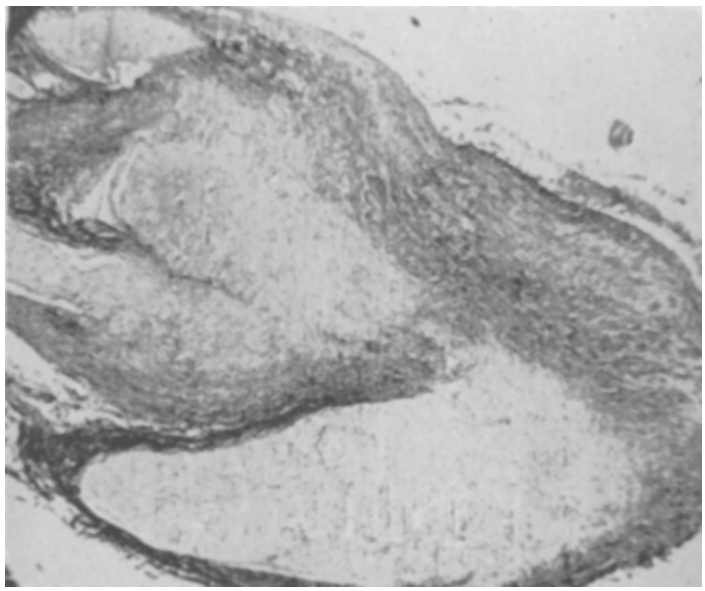

FIG. 1.
The usual neuroma formation 30 days after cutting the sciatic nerve of the dog. Stained with Bodian's Silver Stain. × 60.

fibers had grown beyond the tube and had reanastomosed with the distal segment. These cellophane tubes were thin-walled, non-rigid tubes.

Group IV. Following the basic procedure on the 5 animals comprising this group, the proximal nerve stump was placed within a silver tube, the end of which was closed and folded over on itself. The results in this group were relatively unsatisfactory because the nerves failed to be retained in the tube in all except one instance. Considerable scar tissue reaction occurred around the silver tube. In each instance, where the nerve had pulled out of the tube, a large neuroma had developed.

Group V. Two animals comprised this group. In addition to the basic procedure, the proximal stumps were placed in arterial sleeves. A segment of carotid artery was taken from the same individual animal and washed with heparin. The distal end of the artery was closed by a fine silk tie. Neither of these preparations showed the formation of a neuroma. The remains of the arterial sleeve were visible in the gross specimens.

Group VI. This group was comprised of 15 animals. Following the basic procedure, the proximal nerve stump was placed in a snugly fitting, glass tube about 2½ cm in length. In 2 instances the nerves had pulled out of the glass tubes and showed neuroma formation. In another instance, a large tube was employed, and here again, a neuroma had formed, filling and adapting itself to the confines of the tube. In one instance, where the glass tube had a small, pinpoint opening in the distal end, a fine strand of nerve fibers had grown through this opening and had reanastomosed with the distal segment. In the remaining 11 instances, where the nerves had remained within the snugly fitting glass tube, there was no evidence of neuroma formation. Five of the animals in this group were observed for as long as 230 days.

Group VII and VIII. In the foregoing experiments there is evidence that the formation of end-bulb neuromata can be inhibited by overcoming the growth pressure of nerve axones by fitting the end of a severed peripheral nerve into a rigid tube. The technical accomplishment of this maneuver is difficult and tedious and requires burying foreign substances in relatively large quanti-

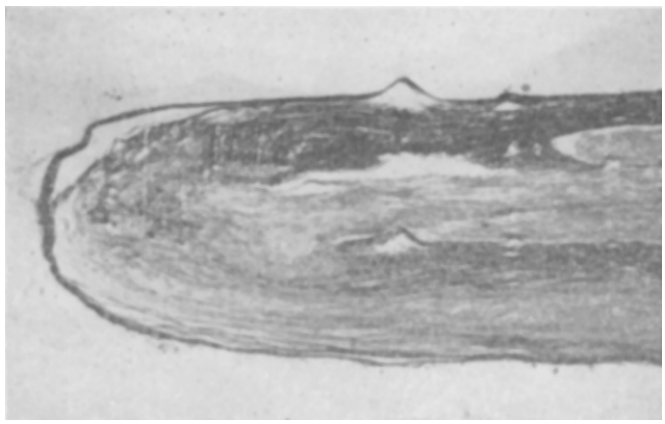

FIG. 2.

Showing the inhibition of the formation of a neuroma when the proximal stump of sciatic nerve had been encased in a snugly fitting glass tube for 230 days. The nerve fibers show little tortuous growth. Stained with Bodian's Silver Stain. × 60.

ties in the tissues. Not only would the procedure be impractical, but the end of the nerve frequently escapes from the encasing tube. Therefore, a search was made for some more practical method. The injection technic was reconsidered. The injection of alcohol does not prevent neuroma formation. The material injected must not cause massive, necrotic destruction of the substance of the nerve, because such a procedure would simulate the simple resection of the nerve at a higher level. The ideal substance for injection should possess some chemical inhibition for the nerve fiber and simultaneously fix or stimulate growth in the fibrous sheaths of the fibers thereby forming enclosing, rigid tubes whose lateral pressure would exert sufficient resistance to overcome the growth pressure of regenerating nerve fibers.

It was found that when tannic acid or gentian violet was injected into intact peripheral nerves, the nerve distal to the site of injection underwent complete degeneration. At the site of injection considerable proliferation of fibrous tissue occurred in what corresponded to the neurilemma. Progressing distally into this area the nerve fibers became more and more isolated and fewer in number until finally they disappeared completely, resulting in total interruption of the nerve pathways.

The sciatic nerve was isolated and a segment about 2 cm in length was injected either with ½% aqueous tannic acid or 2% aqueous gentian violet solution aseptically. These animals were sacrificed at 8- and 171-day intervals and specimens of the sciatic nerve proximal to, at, and distal to the site of injection were fixed in Bodian's No. 2 fixative and stained with Bodian's activated Protargol silver stain.[3]

The results of this study are shown in Fig. 3, 4, and 5.

Group IX. Seven animals comprised this group. Following the basic procedure, the proximal nerve stump was injected with not more than ½ cc of 10% aqueous tannic acid, containing a small amount of methylene blue, which served to stain the injected tissue and indicated the extent to which the nerve was infiltrated. These injections were made at multiple points in an attempt to inject the connective tissue around all fiber bundles. The purpose of this injection was not to destroy the nerve fibers, but rather to fix their surrounding fibrous sheaths to form what would essentially be enveloping, rigid tubes. In no instance could the development of a neuroma be demonstrated. In the case of one animal, however, one intact nerve bundle, which probably escaped injection, became reanastomosed

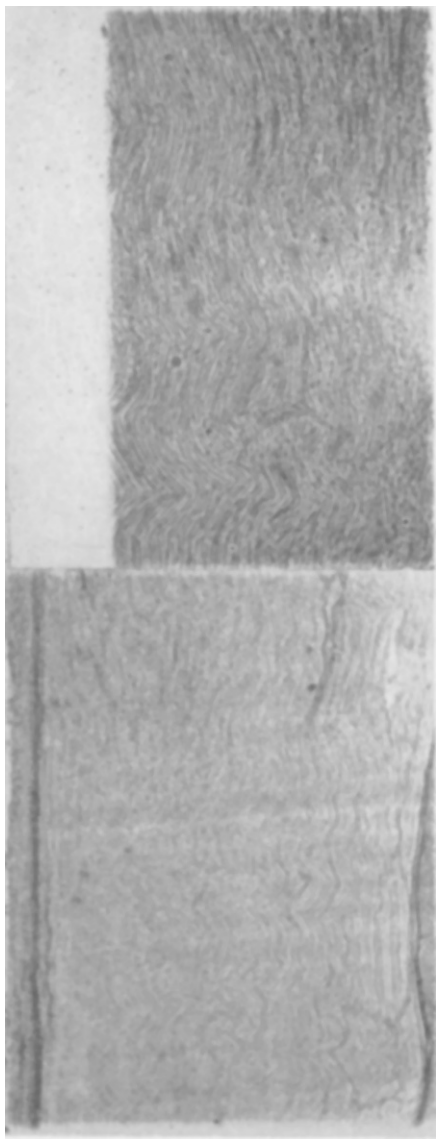

FIG. 3.

Showing the normal fibers of the sciatic nerve of the dog proximal to the site of injection of the nerve with 2% gentian violet 8 days previously. Stained with Bodian's Silver Stain. (*A*, bottom, × 85. *B*, top, × 120.)

with the distal segment.

Group X. Seven animals are included in this group. Following the basic procedure, the proximal nerve stump was injected with not more than ½ cc of aqueous 2% gentian violet, in the same manner as described in Group IX. In no instance was neuroma formation demonstrable. However, again, as in the previous group, a lateral strand of fibers, which had apparently escaped injection, had reanastomosed with the peripheral nerve segment.

A review of the results obtained in these studies indicates that when the end of a severed nerve is supported by a rigid tube, neuroma formation does not occur. Study of microscopic preparations of these nerves injected with gentian violet or tannic acid demonstrates that these substances do not kill all of the nerve fibers. Rather they induce both a chemical inhibition to growth and support the nerve end in a rigid, fixed, connective tissue sheath, which serves the same function as any other snugly fitting fixed support.

The Use of Gentian Violet for Injection of Peripheral Nerve Stump Following Amputation. After demonstrating the efficacy of gentian violet in the inhibition of neuroma formation, all of the severed nerves which could be identified were injected in 15 patients during amputation of the lower extremity. After freeing 2- or 3-inch lengths of the larger nerves, a tight rubber band or a small

FIG. 4.

Same experiment as shown in Fig. 3 and 5. Section at site of injection of 2% gentian violet into the intact sciatic nerve of the dog 8 days previously. Observe the early degeneration of the nerve fibers. Bodian's Silver Stain. × 120.

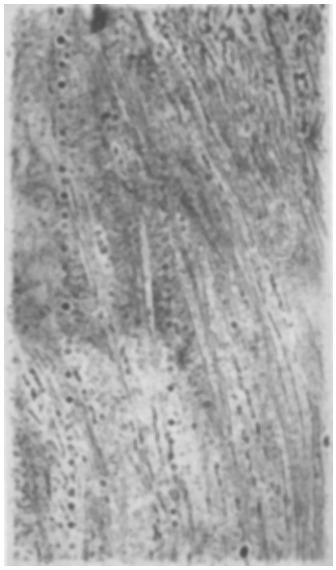

FIG. 5.

Same experiment as shown in Fig. 3 and 4. This section is taken distal to the site of injection of the intact sciatic nerve of the dog with 2% gentian violet 8 days previously. Note the coiled nerve fragments indicating the degeneration of the axones. Bodian's Silver Stain. × 120. Artifacts present are due to air bubbles in the balsam.

clamp was applied to partially compress the nerve to isolate the freed segment; and gauze was placed around the region during injection to protect the surrounding tissues from the dye, which was inadvertently spilled. The end of the nerve was ligated with fine silk so as not to cut through the sheath but still sufficiently firmly to interrupt the axones. Multiple injections were made into the isolated segment of the nerve to fully distend the sheath. The compressing clamp or rubber band was removed. In the larger nerves from 3 to 5 cc of 2% gentian violet was used. It is desirable always to use a fine caliber needle for the injection. While the number of cases is too small and the lapse of time too short to draw any conclusions as regards the formation of painful stump or tender neuroma, it can be reported that no patient suffered untoward pain because of the procedure, nor has healing been compromised in any way.

Discussion. The proximal end of a severed fiber of a peripheral nerve has the capacity for unlimited growth and will continue to grow until it reunites with an end organ or until its growth pressure is overcome by some other mechanism. The studies by Forssman[18] and Cajal[3] would indicate that a simple mechanical obstacle placed in their path is not sufficient to inhibit growth. Spurling[19,20] effected an orderly, parallel growth of nerve fibers by the use of tantalum foil to surround the site of anastomosis of a peripheral nerve. Weiss[21,22,23] studied the mechanism of nerve regeneration within arterial sleeves. Nerve fibers will grow into a rigid blind tube and completely fill the available space whereupon the fibers cease to grow without undergoing atrophy or degeneration. Likewise, the usual end-bulb neuroma finally ceases to grow when the lateral pressure of the surrounding scar tissue balances the growth pressure of the proliferating nerve fibers. It has been demonstrated again that the injection of ethyl alcohol into the stump of a severed peripheral nerve does not inhibit the formation of end-bulb neuromata.

The procedure of injecting gentian violet into the isolated segment of a severed nerve which has been ligated distally prevents the formation of end-bulb neuromata by both chemical inhibition and mechanical blocking of the pathway of the axones. It having been demonstrated that simple ligation of the central segment is not sufficient to prevent the proliferation of the axones, the additional precaution of injecting the stump with gentian violet is added to give the superimposed effect of chemical inhibitions of nerve regeneration.

Summary. Opposing the growth of nerve fibers by encasing the proximal stump of a severed peripheral nerve in a rigid tube will prevent the formation of an end-bulb neuroma. The injection of the nerve with tannic acid or gentian violet likewise inhibits neuroma

[18] Forssman, J. (Cited by Cajal [3]).

[19] Spurling, R. G., *Surg. Clin. North America*, 1943, **23**, 1491.

[20] Spurling, R. G., *Neurosurg.*, 1944, **1**, 133.

[21] Weiss, P., *Growth* (Supp.), 1941, **5**, 163.

[22] Weiss, P., and Taylor, A. C., *Arch. Surg.*, 1943, **47**, 419.

[23] Weiss, P., and Taylor, A. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 77.

formation. The mechanisms probably are not identical. In addition to exerting lateral pressure and limiting the blood supply, tannic acid and gentian violet exert chemical inhibition to the growth of the nerve for a varying period following the injection which further favors the encasement of the resting nerve fibers by a connective tissue envelope.

This method of preventing the formation of end-bulb neuromata may aid in solving the problem of painful amputation stumps and the phenomenon of the phantom limb. No contraindications to injecting the nerve ends in amputation stumps have been observed.

15136

Vaginal Response to Adrenal Stimulation.*

JOSEPH SCHILLER.† (Introduced by F. H. Pratt.)

From the Department of Physiology, Boston University School of Medicine.

The participation of the adrenal cortex in the estrous cycle, established by Wyman,[1] has been confirmed since under various experimental and natural conditions. However, the results of those experiments do not necessarily imply that the adrenal cortex influences the estrous cycle through the release of sex hormones rather than through hormones acting on the general metabolism. The isolation of both estrone (Beall[2]) and of progesterone (Beall and Reichstein[3]) from the adrenal cortex points to the former possibility as a primary factor. In spite of the chemical findings the physiological evidence is scant, and limited to the experiments of Moon,[4] Corey and Britton,[5] Nelson,[6] and Bourne and Zuckerman.[7] The availability of adrenocorticotrophic hormone furnishes an additional technic for this type of investigation.

Material and Methods. Young female rats of the Long-Evans strain were injected daily for 5 days (unless otherwise specified) with adrenocorticotrophic hormone (ACTH).‡ Hepatectomy was performed by making an incision parallel to the ribs and extirpating the median and lateral left lobes. Mortality was 5%. Grafting of the adrenals was done by suturing both glands to the spleen and the "takes" were practically 100%. All sections were stained with hematoxylin-eosin and vaginal smears were taken by the lavage method.

Results. The administration of ACTH to normal females gave inconclusive results. The estrous phase seemed in some instances to be prolonged beyond normal; but since prolonged estrus may occur spontaneously, and since the diestrous phase was unaffected by ACTH, it was concluded that ACTH was ineffective in normal female rats. (Table I.)

On the assumption that the liver might inactivate part or all of the estrogenic substances released by the adrenal cortex

* Aided with a grant by the American Academy of Arts and Sciences from the Permanent Science Fund.

† Present address: Carnegie Institution, Department of Embryology, Baltimore, Md.

1 Wyman, L. C., *Am. J. Physiol.*, 1928, **85**, 414.

2 Beall, D., *J. Endocrinol.*, 1940, **2**, 81.

3 Beall, D., and Reichstein, T., *Nature*, 1938, **142**, 479.

4 Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 36.

5 Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1931, **99**, 33.

6 Nelson, W. O., *Anat. Rec.*, Suppl., 1941, **81**, 97.

7 Bourne, G., and Zuckerman, S., *J. Endocrinol.*, 1941, **2**, 268.

‡ The ACTH was obtained through the courtesy of Dr. E. B. Astwood, and was prepared according to the technic described by R. Tyslowitz (*Science*, 1943, **98**, 225). In all probability the product contained some lactogenic hormone. However, ACTH prepared by us from hog pituitaries according to the technic of G. Sayers, A. White, and C. N. H. Long (*J. Biol. Chem.*, 1943, **149**, 425), which behaves like a pure protein, gave identical results in our hands.