I. THE EFFECT OF ELECTRONARCOSIS ON THE SECRETORY ACTIVITY OF THE PITUITARY GLAND.

II. STUDIES ON THE EXTENSOR MECHANISM OF THE SPIDER LEG.

III. A COMPARATIVE STUDY OF PERIPHERAL INHIBITION IN DECAPOD CRUSTACEANS.

Thesis by

Charles Herbert Ellis

In Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy

California Institute of Technology

1943

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Professors C. A. G. Wiersma and A. van Harreveld for their most helpful advice and cooperation throughout the course of these investigations.

PART ONE

THE EFFECT OF ELECTRONARCOSIS ON THE SECRETORY ACTIVITY OF THE PITUITARY GLAND.

INTRODUCTION

Since 1938 electroshock therapy has been rather widely used in certain mental diseases, more particularly in the involutional psychoses (see Wortis, 1941). This treatment consists of passing a current of rather high intensity through the brain for a very short time, usually 0.2 or 0.3 seconds. More recently the related procedure, electronarcosis, has been studied with a view toward determining its suitability and therapeutic value in the treatment of the schizophrenias (van Harreveld, Plesset and Wiersma, 1942; Frostig, et al, 1943; Globus, van Harreveld and Wiersma, 1943). Electronarcosis is not new. It was shown by Leduc (1902) that passage of an electric current through the brain produced a state of unconsciousness resembling sleep. Van Harreveld and Kok (1934 a, b) renewed investigations on the subject, but it has only been recently that the possible restorative action in mentally disturbed patients has been considered.

These studies have opened for investigation an experimental project of considerable magnitude, namely the careful examination of the physiological changes which occur when an electric current is passed through the head.

In view of the position of the pituitary gland with respect to the path of the current, it has seemed quite likely that a certain degree of hypophyseal hyperactivity might be expected subsequent to electronarcosis. It has been in an effort to determine whether or not such pituitary stimulation

actually occurs that the present investigation was undertaken.

METHODS

In view of the regulatory functions which the hypophyseal hormones exert over the majority of the endocrine glands, the relative secretory activity of the pituitary gland can most clearly be studied by investigating the changes occurring in the output of one or more of these hypophyseal "tropic" hormones. In studying the effects of electronarcosis on the activity of the pituitary gland, therefore, four of the pituitary hormones have been investigated. They have been, respectively, the thyrotropic, adrenocorticotropic, gonadotropic and specific metabolic hormones.

Experimental investigation has developed along two major lines. In one approach the electronarcotized animal has been used for its own assay, i.e., in the guinea pig experiments, for instance, the hormone levels were studied by comparison of the weights of the endocrine organs of the electronarcotized animals with those of the controls. The other approach involved the comparison of the hormone level in the blood of an experimental animal at various intervals with respect to electronarcosis by injecting serum collected at these intervals into groups of other animals.

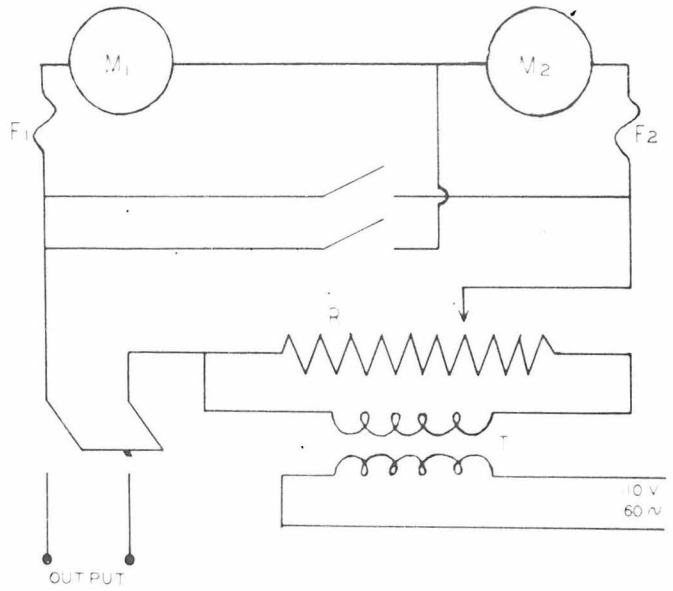
Electronarcosis was produced in both dogs and guinea pigs following the method of van Harreveld, Plesset and Wiersma (1942). In this procedure, a relatively high 60 cycle sinusoidal

current is applied for 30 seconds through electrodes placed in the temporal region; at the end of this period the current level is lowered to a point just low enough to permit a return of respiration. At this level the animal can be kept in a state of narcosis for prolonged periods. For the sake of routineness the guinea pigs were uniformly electronarcotized with an initial current of about 50 milliamperes and a maintenance current of 7 milliamperes. The corresponding levels for the dogs were of the order of 400 milliamperes and 70 milliamperes respectively. The circuit diagram of the apparatus used in producing the electronarcoses, was designed by Dr. A. van Harreveld, and is shown in Fig. 1.

In the guinea pig experiments the electrodes were 1 cm. brass disks; in the dog experiments the electrodes were somewhat larger, being either 2 cm. brass disks or large crescent-shaped strips of metal. In all cases the electrodes were padded with cotton and covered with gauze. Contact was assured in the guinea pig experiments by thoroughly wetting the electrodes and the skin of the animal with salt solution. In the dogs, an electrode jelly was used. In both dogs and guinea pigs the electrodes were held in position with bandages.

The methods of hormone assay were selected because of their sensitivity and their generally accepted usage in studies concerned with the pituitary hormones, and for the sake of clearness, discussion of the specific assay methods will be presented alongside of the respective results.

Figure 1. Diagram of Apparatus used for Producing Electronarcosis in Dogs and Guinea Pigs.



M-1 milliameter (0 - 50 ma.)

M-2 milliameter (0 - 250 ma.)

F-1 fuse ($\frac{1}{2}$ amp.)

F-2 fuse (1 amp.)

R 1.1 amp. 360 ohm wire wound variable resistance.

T isolation transformer.

RESULTS

Description of the Symptoms of Electronarcosis.

Dogs: The symptoms occurring when electronarcosis is applied to dogs have been comprehensively and adequately presented (van Harreveld, Plesset and Wiersma, 1942; Frostig et al, 1943), and since such a description is not wholly relevant to the present investigation no further description of these symptoms will be made.

Guinea pigs: The symptoms which occur when a guinea pig is electronarcotized, however, have not heretofore been mentioned. It should be of interest, therefore, to digress enough from the main problems of the present study to consider the most uniformly occurring reactions of this animal to the procedure. Immediately upon making the initial high current, the animal falls over on one side, and almost at once goes into a strong extensor spasm which usually is relaxed somewhat after about 25 seconds. Respiration, of course, is stopped during the entire 30 second application of high current, and does not usually return until about 15 seconds after the current has been reduced to the maintenance level. The high initial current produces ejaculation in male guinea pigs, which does not occur in either dogs or man. Upon dropping the current to about 7 milliamperes the remaining extensor tone disappears, with the accompaniment, as a rule, of a few clonic twitches. Thereafter the animal becomes quiet, shows usually no response to severe

pinching of the front feet, although in the hind feet the flexor reflex is frequently maintained, although at a low level. Pricking the skin over the body and head results in no response. Neither righting nor supporting reflexes are present. Considerable tone is found in nearly all guinea pigs, and frequently a more or less marked opisthotonic position is observed.

More like electronarcosis in humans than in dogs, the quiet picture of electronarcosis is gradually replaced by an increasingly noticable unrest or hyperkinesis on the part of the guinea pig. This can be suppressed to a greater or lesser extent by slightly raising the current very slowly. In the guinea pig, however, secondary raises in the narcotizing current are often accompanied by a new convulsion, arrest of respiration, and frequently by death of the animal.

Recovery following discontinuation of the current passage is usually immediate. The animal generally shakes its head a few times and then hops away in an apparently normal manner. In two animals a complete motor paralysis of the hind legs developed following a series of electronarcoses. Post mortem examination disclosed, however, that this condition was due to a trauma of the spinal cord resulting from compression by the vertebrae during the strong extensor spasm which occurred during the initial stages of the electronarcosis. Both of these accidents occurred when unusually high initial currents were used. In a number of experiments recovery has been somewhat

delayed, the animal having weak righting reflexes and little supporting tone for several minutes, after which time recovery was almost always complete.

Changes in the Output of Thyrotropic Hormone.

The fact that the function of the thyroid gland is under the control of a specific "tropic" hormone from the anterior pituitary was ~~first~~ demonstrated by treating hypophysectomized tadpoles with pituitary extracts (see Allen, 1938).

Methods utilized in studying the thyrotropic hormone must be based on a study of the activity of the thyroid gland. A number of criteria are available which indicate the degree to which the thyroid has been activated. These can be roughly grouped into two categories, those in which a gross or microscopic anatomical study of the thyroid gland has to be made, and those in which physiologically measurable changes in other parts of the body, correlated with an increased or decreased thyroid secretion, are investigated, e.g., basal metabolism.

Aron showed in 1929 that injection of an extract of pituitary glands containing the thyrotropic factor produced a marked increase in the weight of the thyroid glands in guinea pigs. This response has been used by many workers as a suitable indicator for assay of thyrotropic hormone (Aron, 1929, 1932, 1936; Aron and Klein, 1930; Rowlands and Parkes, 1934; Starr and Rawson, 1937; Junkman and Loeser, 1938).

Aron and his school (1936), Heyl and Laqueur (1935), Rawson and Starr (1938), Starr, et al, (1939), Bergman and Turner (1939) and others have shown that an even more sensitive criterion for thyroid hypertrophy is provided by the "micro-histometric" method which consists of making a careful histologic study of the thyroid tissue. The thyroid is composed of a rather large number of follicles or acini each of which is a secretory unit. These follicles are filled with a viscous substance known as "colloid". In the resting state the colloid in each follicle is surrounded by a single layer of epithelial cells which are flattened and almost squamous in type. In the immature guinea pig these cells measure about 3μ to 4μ in height. When activated by thyrotropic hormone, however, the cells thicken, becoming cuboidal or even columnar, and the degree to which this hypertrophic thickening occurs has been shown to be reasonably parallel to the amount of thyrotropic hormone injected.

Heyl and Laqueur (1935) showed, however, that certain pituitary extracts caused obvious histological signs of thyroid stimulation without producing any increase in the weight of the gland, while other preparations, although giving a marked increase in the thyroid weight, resulted in no noticable change in thyroid histology. They concluded, therefore, that there are two separate pituitary hormones responsible for the thyrotropic response, one resulting in increase in thyroid weight, the other, in the histologic signs of thyroid stimulation.

In the present investigation thyroid hypertrophy has been studied by both the gland weight method and the micro-histometric technique.

A number of guinea pigs were given a series of daily electronarcoses of 10 minutes duration. The initial current was 50 ma. for 30 seconds; the maintenance current was 7 ma. At the end of the series the animals were killed and their thyroid glands removed, weighed, and prepared for histological study[1]. A control animal was run simultaneously and parallel in all respects except for electronarcosis, and it and the electronarcotized animal were killed at the same time. In this way any extraneous thyroid stimulation was more or less nullified. The thyroids were found to be heavier by about 15% in the electronarcotized animals than in the controls (Table I). Histological study was made under oil immersion. The height of the average epithelial cell in 100 adjacent follicles was measured with a calibrated eyepiece micrometer, and the mean values showed an increase in height of the follicular epithelium from 5.5 μ to 8.4 μ (Table II). It is further significant that in those animals subjected to a series of electronarcoses of 10 minutes duration the average epithelial cell height was 8.9 μ, a value somewhat higher than that of the animals subjected to a series of 2 minute electro-narcoses in which a mean value of 8.2 μ was found (Table II).

T. The author wishes to express his thanks to Miss Ruth E. Estey for her assistance in preparing the slides for histologic study.

Table I. Effect of Electronarcosis on Thyroid, Adrenal and Ovary Weights in Guinea Pigs.

GROUP / ORGAN	CONTROL		TEST			
	NUMBER OF ANIMALS	GLAND WEIGHT MG. 100 GMS.	NUMBER OF ANIMALS	NUMBER OF ELECTRONARCOSES	GLAND WEIGHT MG. 100 GMS.	PERCENT DEVIATION $\left(\frac{W_T - W_C}{W_C} \times 100\right)$
THYROID	2	13.4	4	10 (2 MIN.)	15.4	14.9
ADRENAL	2	57.6	4	10 (2 MIN.)	82.2	42.8
	5	48.0	5	7-10 (10 MIN.)	74.4	54.6
OVARY	2	18.0	3	10 (2 MIN.)	25.0	39.0
	4	15.5	6	7-10 (10 MIN.)	20.3	31.0

Note. The duration of the daily electronarcoses is shown in parentheses in the column with the number of electronarcoses.

Table II. Effect of Electronarcosis on the Histology of the Thyroid Gland in Guinea Pigs.

DURATION OF ELECTRONARCOSIS	NUMBER OF ELECTRONARCOSES	HEIGHT OF THYROID EPITHELIUM ( ) $\mu\mu$	STANDARD ERROR $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$
CONTROL	0	5.98	2.060
	0	6.52	1.359
	0	5.13	1.531
	0	5.04	1.263
	0	5.03	0.438
	AVERAGE	5.54	
2 MINUTES	10	8.13	1.906
	10	7.71	2.148
	10	8.50	2.095
	10	8.25	1.525
	AVERAGE	8.15	
10 MINUTES	11	9.54	2.468
	7	8.30	.512
	AVERAGE	8.92	

The increased activity of the thyroid tissue is evidenced further by comparison of the general histological picture of the thyroid of the electronarcotized animal with that of the control. Not only is the acinar epithelium more cuboidal or even columnar than the squamous type of the controls, but there is a marked withdrawal of the margin of the colloid and conspicuous vacuolization in the colloid.

In an early series of dog experiments in which electro_ narcosis was given for other purposes, the thyroids were removed, placed in Bouin's fixative, sectioned at 8μ, stained with the Mallory azan stain of Heidenhain, and examined micro-histometrically. Two of these dogs had been subjected to a prolonged series of electronarcoses prior to the acute experiment, two had received but a single previous electronarcosis, and the other three had not had any electronarcoses. In Table III it will be noted that no significant difference in the height of the thyroid epithelium existed between the control animals and those which had received a single previous electronarcosis. Those which had received a prolonged series of electronarcoses, however, showed a slight but significant thickening of the follicular cells. These experiments were not controlled at all, but in the light of the guinea pig results it is significant to note that the height of the acinar epithelium had increased from 5.3μ in the so-called controls (which had not been subjected to previous electronarcosis) to a height of 6.6μ in the dogs which were subjected to a series of electronarcoses(Table III).

Table III. Effect of Electronarcosis on the Histology of the Thyroid Gland in Dogs.

DATE	ANIMAL	NUMBER OF ELECTRONARCOSES	BODY WEIGHT (KG)	THYROID WEIGHT (GM)	HEIGHT OF THYROID EPITHELIUM (100 FOLLICLES)	STANDARD ERROR $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$
12-16-41	32	1	11.6	0.972	5.22 μ	±0.902
12-20-41	35	1	11.0	1.227	4.96 μ	±0.712
12-27-41	36	0	8.5	1.263	4.82 μ	±0.619
12-31-41	19	0	- -	1.226	5.06 μ	±0.794
1-20-42	39	0	15.6	1.009	5.98 μ	±0.729
2- 6-42	34	11 (AVE. DURATION-1.8HRS)	16.3	1.288	6.77 μ	±1.165
2- 9-42	22	10 (AVE. DURATION-2.8HRS)	12.2	1.016	6.41 μ	±1.225
AVERAGE		0			5.29 μ	
		1			5.09 μ	
		10			6.59 μ	

In order to investigate more clearly whether this thyroid-stimulating effect is due to an increased release of thyrotropic hormone from the pituitary and not to a direct stimulatory effect on the thyroid, a series of determinations have been made of the relative levels of thyrotropic hormone in the blood serum of electronarcotized dogs. In these studies the method of assay selected was that reported by Smelser (1937), in which the material to be tested is injected intraperitoneally into day-old baby chicks. He showed that the weight of the chick thyroids increases almost linearly with the concentration of thyrotropic hormone injected. It was shown by Smelser (1937, 1938) and by Cope (1938) that day-old leghorn chicks are more satisfactory for thyrotropic hormone assays than guinea pigs, not only because of the negligible expense involved, but because they, especially cockerels, afford a more sensitive test. This method has also been used by Kabac and Liapin (1938), and has been compared with the guinea pig methods by Bergman and Turner (1939), and has become rather uniformly accepted as one of the most satisfactory methods for assay of thyrotropic hormone. The use of chicks has the added advantage that these animals are effected less by toxic substances in mammalian blood and urine than are guinea pigs and rats.

In studying the changes in the thyrotropic hormone concentration in the blood and urine of dogs following a series of electronarcoses, groups of day-old Austra-white cockerels[1]

---

1. The day-old cockerels were purchased from the C. J. Smith Hatchery, 1122 E. Huntington Drive, Arcadia, Calif.

were injected daily for 4 days, killed on the 5th day, and the thyroids were removed and placed in Bouin's fluid over night. The next morning the glands were blotted, weighed to the nearest 0.1 mg. and prepared for histological study. The thyroids in the chick are small, compact reddish organs almost spherical in shape, and are closely attached to the carotids not far from the origin of the subclavian arteries. After removal of the skin at the base of the neck, the crop is carefully pulled to one side, and in the groove formed by the pectoral girdle, the thyroids can be seen. They are easily distinguished from the pale parathyroids which lie both anteriorly and posteriorly, and from the large mass of the thymus which lies in close proximity to the trachea for some distance anteriorly.

Two dogs have been used. One sample of blood was taken from the dog at the time of the first electronarcosis and served as the control serum. Electronarcoses of 15 minutes duration were then given the dog, one each day, for 7 days in the case of dog No. 1, and for 5 days in the case of dog No. 2. After this time a second sample of blood was taken for the post-electronarcotic thyrotropic hormone assay.

The chick glands in the series injected with post-electronarcotic serum were heavier by 53% in one experiment and by 68% in another than the control series injected with the respective pre-electronarcotic sera (Table IV). The gland weights have been plotted in the form of frequency polygons (Fig. 2 c, 3 c), and examination of these figures shows clearly

Table IV. Effects of Electronarcosis on the Level of Thyrotropic, Adrenocorticotropic and gonadotropic Hormones in the Blood Serum of Dogs.

TABLE IV. EFFECT OF ELECTRONARCOSIS ON THE LEVEL OF THYROTROPIC, ADRENOCORTICOTROPIC AND GONADOTROPIC HORMONES IN THE BLOOD SERUM OF DOGS.

ORGAN	DOG NUMBER	NUMBER OF ELECTRO-NARCOSES	BEFORE ELECTRONARCOSIS		AFTER ELECTRONARCOSIS		PERCENT DEVIATION ($\% = \frac{W_A - W_B}{W_B} 100$)
			NUMBER OF CHICKS	GLAND WEIGHT MG.	NUMBER OF CHICKS	GLAND WEIGHT MG	
THYROID	1 ♂	7	17	3.28	26	5.02	53.0 %
	2 ♀	5	9	3.05	10	5.13	68.1 %
ADRENAL	1 ♂	7	17	9.43	26	14.30	52.0 %
	2 ♀	5	9	10.18	10	12.40	22.4 %
TESTIS	1 ♂	7	16	6.24	21	9.08	46.0 %
	2 ♀	5	9	7.56	9	10.23	35.3 %

Figure 2. Effect of Electronarcosis on the Level of the Pituitary Hormones in the Blood Serum of Dogs. Gland Weights of Chick Organs in Assays on Dog No. 1.

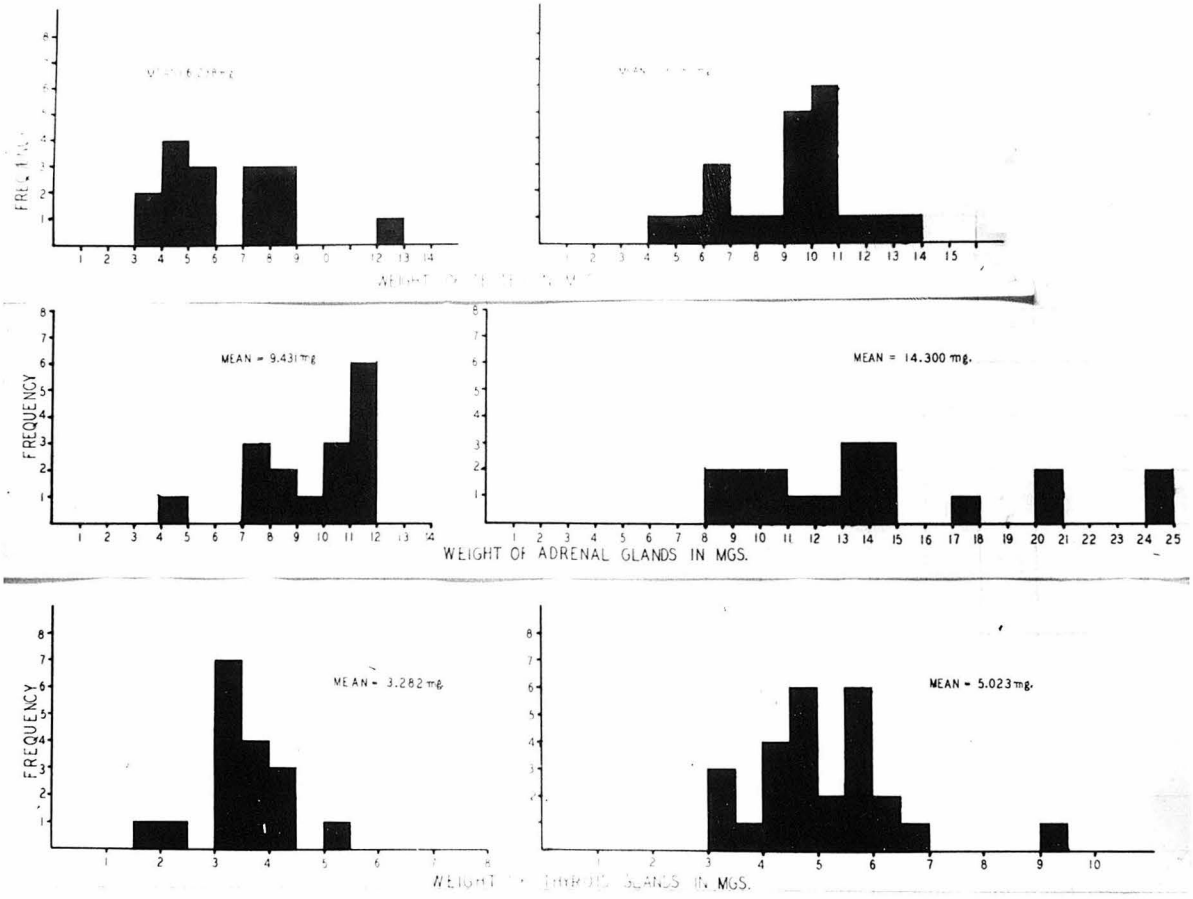
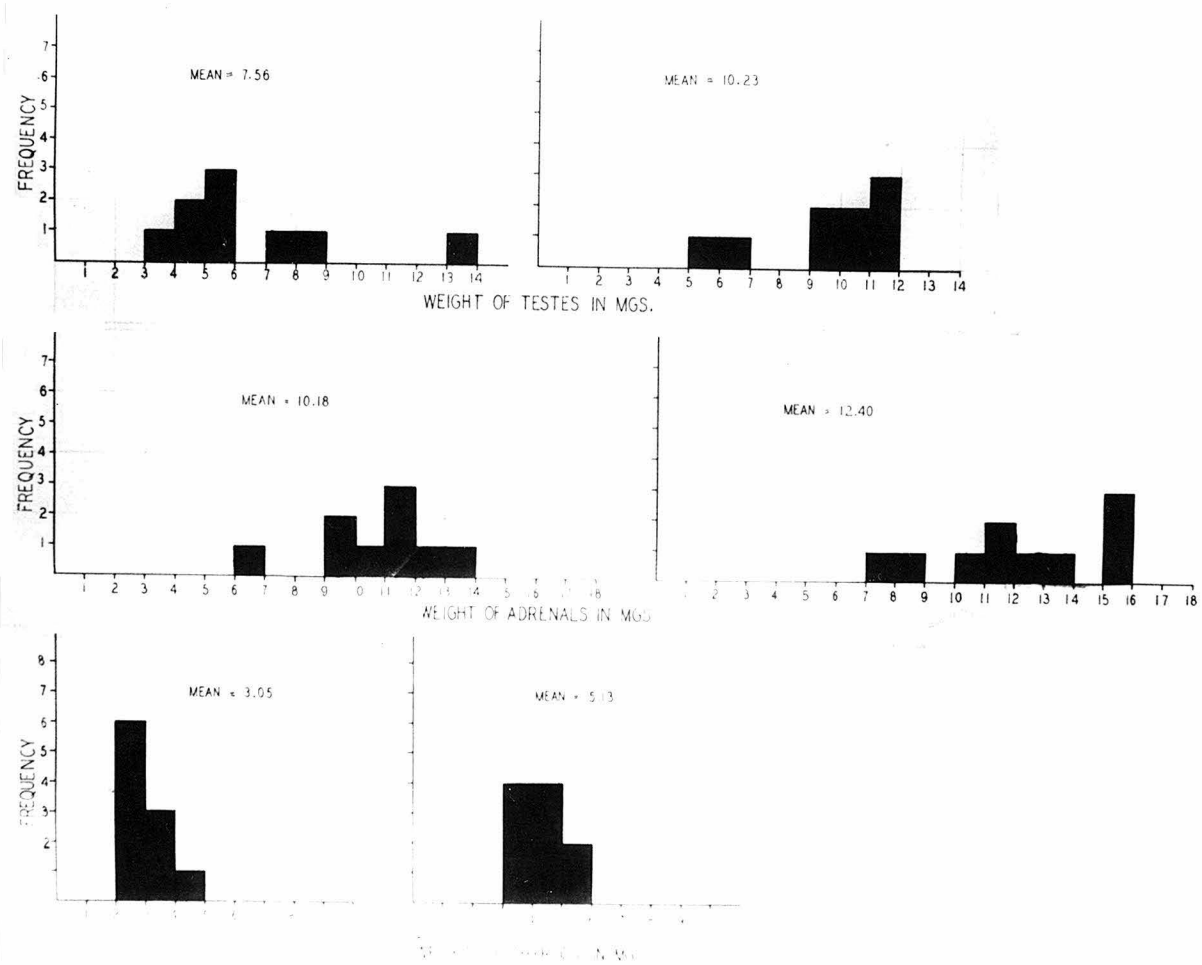


Figure 3. Effect of Electronarcosis on the Level of the Pituitary Hormones in the Blood Serum of Dogs. Gland Weights of Chick Organs in Assays on Dog No. 2.



the shift toward the right indicative of increased thyroid activity. The mean weights for the thyroids of the chicks receiving pre-electronarcotic serum were 3.2 mg. and 3.1 mg. respectively, and for those receiving post-electronarcotic serum, 5.0 mg. and 5.1 mg.

The epithelial cells of the chick thyroids show an increase in height of 2.1μ(from 4.0μ to 6.1μ) in one experiment and 1.5μ(3.6μ to 5.1μ) in the other (Table V). As Smelser (1938) has claimed that the colloid in chick thyroids normally shows little if any vacuolization, it has seemed probable that an increase in vacuolization could be used as another indicator of increased thyroid activity. The percentage of follicles showing vacuolization of the colloid is, to be sure, markedly higher in the glands from the chicks injected with post-electronarcotic serum. In dog No. 1, the approximate values were 20% before electronarcosis and 45% after 7 electronarcoses. In Dog No. 2, five electronarcoses increased the vacuolization of the chick thyroids from about 25% to 55% (Table V). It is also apparent from Table V that the urine and blood serum showed similar thyrotropic activity.

In order to see how permanent this increased thyrotropic output might be, electronarcosis was terminated in dog No. 2 after 5 successive days, and the tropic activity of the blood was followed for two weeks thereafter. The chicks for this series of determinations were divided into two groups, only one of which was injected with the sample of serum, the other serving as a control group. In this way certain unavoidable and unknown extraneous factors were obviated. The actual and

Table V. The Effect of Electronarcosis on the Level of Thyrotropic Hormone in the Blood and Urine of Dogs.

TABLE V: EFFECT OF ELECTRONARCOSIS ON THE THYROTROPIC HORMONE LEVEL IN THE BLOOD AND URINE OF DOGS

DOG		NUMBER OF ELECTRONARCOSES	PRENARCOTIC SAMPLE					POSTNARCOTIC SAMPLE					PERCENT DEVIATION		
NUMBER	SEX		NUMBER OF CHICKS	VOL. SERUM INJECTED	WEIGHT OF THYROIDS	HEIGHT OF EPITHELIUM	% FOLLICLES VACUOLATED	NUMBER OF CHICKS	VOL. SERUM INJECTED	WEIGHT OF THYROIDS	HEIGHT OF EPITHELIUM	% FOLLICLES VACUOLATED	GLAND WEIGHT	VACUOLIZATION	HEIGHT OF EPITHELIUM
1	♂	7	1	.1u	3.10 mg	3.65 u	17.4 %	6	.1u	4.90 mg	5.50 u	40.6 %	58%	50.6%	135 %
			5	.2u	3.72	4.85	22.4	6	.2u	4.67	7.05	61.7	26%	45.4%	175 %
			5	.1s	3.48	3.83	17.8	4	.1s	5.27	6.18	39.6	52%	61.4%	123%
			6	.2s	3.35	3.60	20.4	7	.2s	5.46	5.72	40.4	63%	59.0%	98%
		AVERAGE	17	- -	3.28	4.04	19.7	23	- -	5.02	6.14	45.9	53.0%	52.0%	133%
2	♀	5	9	.2s	3.05	3.63	24.8	10	.2s	5.13	5.04	53.9	58.1%	38.9%	117%

corrected values are shown in Table VI, and the corrected weights have been plotted in Fig. 4. In this figure it can be seen that whereas the blood sample obtained on the 7th day produced marked hypertrophy in the chick thyroids, the thyrotropic content of the serum had returned to the initial level by the end of the first week after the series of electronarcoses was discontinued, and had dropped even a bit lower by the end of the second week.

Changes in the Output of the Gonadotropic Hormones.

In the course of establishing electronarcosis as a method suitable for application to human beings, the observation was made that in the group of schizophrenic patients subjected to electronarcosis, two exhibited a resumption of menstruation following an amenorrhea of at least two years standing.

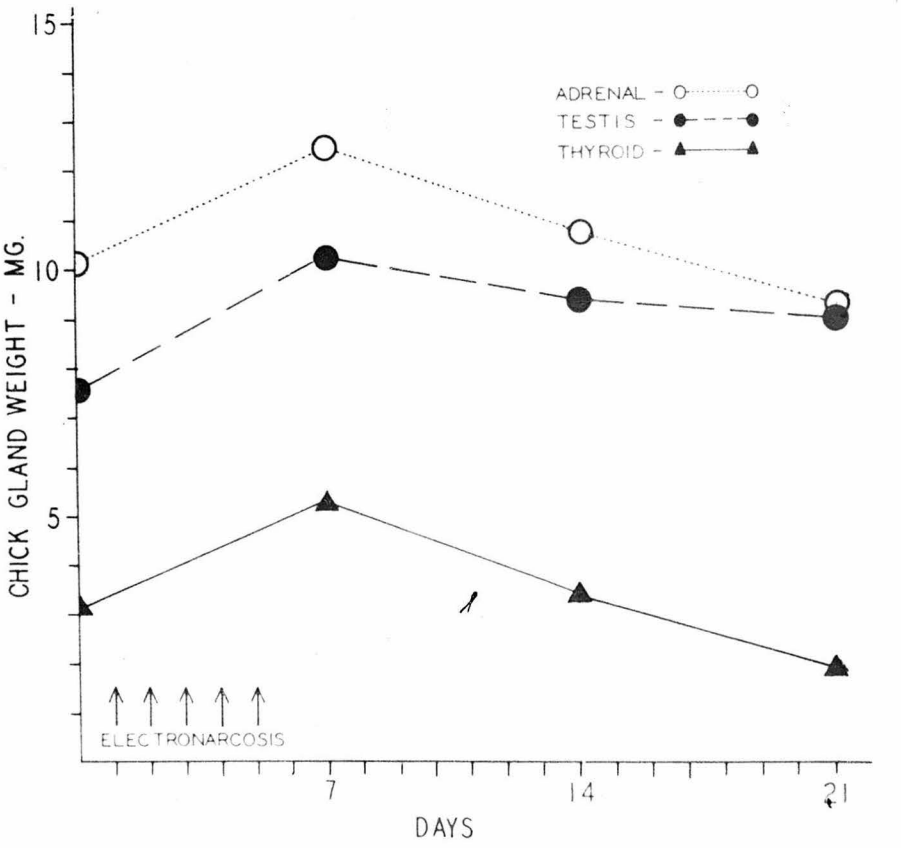
Investigation of the changes in the output of the gonadotropic hormones in laboratory animals following electronarcosis has followed the same lines as the study of the thyrotropic hormone. Young female guinea pigs have been used; as an indication of increased gonadotropic activity the weights of the ovaries in the electronarcotized guinea pigs were compared with those from the control animals which were kept in the same cage and under identical conditions. It will be seen in Table I that following a series of electronarcoses of 10 minutes duration the ovaries were about 30% heavier than those of the control animals. After a series of 10 electro-

Table VI. Effect of Electronarcosis on the Level of the Pituitary Hormones in the Blood Serum of Dogs. Electronarcosis was given once daily for 5 successive days between series 1 and 2, and was then discontinued.

TABLE 6 EFFECT OF ELECTRONARCOSIS ON THE LEVEL OF PITUITARY HORMONES IN THE BLOOD SERUM OF DOGS

GLAND	SERIES	CONTROL				INJECTED		
THYROIDS	1	13	2.88	- .12	-.040	10	2.93	3.05
	2	9	2.30	- .70	-.234	10	3.60	5.13
	3	12	2.48	- .52	-.156	8	2.94	3.40
	4	8	4.34	+1.34	+.446	10	3.51	1.94
			MEAN = 3.00					
TESTES	1	12	11.54	+ 2.09	+.224	9	9.74	7.56
	2	10	8.70	- .73	-.077	9	9.50	10.23
	3	13	9.07	- .36	-.038	8	8.95	9.29
	4	10	8.45	-.98	-.104	11	8.13	8.96
			MEAN 9.43					
ADRENALS	1	13	10.11	+.04	+.004	9	10.61	10.18
	2	10	8.47	- 1.60	-.188	10	10.44	12.40
	3	13	10.31	+.24	+.024	8	9.97	10.71
	4		[illegible]	[illegible]	[illegible]		[illegible]	9.21
			MEAN [illegible]					

Figure 4. Effect of Electronarcosis on the Level of the Pituitary Hormones in the Blood Serum of Dogs.



narcoses of 2 minutes duration the ovaries were likewise heavier than the controls by about 40%.

A series of 10 electronarcoses applied to a pregnant guinea pig resulted in resorption of the fetus, as was shown at autopsy by the marked vascular congestion of the uterine wall and the disintegrated character of the placenta and embryo.

Domm (1937) described the hypertrophy of both testes and ovaries in 18 day chick embryos when gonadotropic preparations were introduced into the egg. It was therefore considered practical to use the weight of the testes of the day-old cockerels described above as the indicator of changes in the gonadotropic hormone content in the blood of dogs subjected to electronarcosis. The removal of the testes consisted, after killing the animal with chloroform, of cutting through the pectoral girdle along the midventral line, and spreading the sides of the incision laterally and dorsally. The intestines and gizzard were pushed aside, whereupon the testes and adrenals were seen to lie in the midventral line in a readilly accessible position(Domm, 1937). The testes were removed with fine forceps, fixed in Bouin's fluid over night, and weighed to the nearest 0.1 mg. It was found that the chick testis weight response from the post-electronarcotic serum from dog No. 1 was 46% heavier than that from the control sample. The post-electronarcotic serum from Dog No. 2 produced a 35% increase in the chick testis weights (Table IV). These

weights have also been plotted in the frequency polygons in Figs. 2 and 3 respectively, in which the shift to the right is quite noticeable, the mean values showing an increase of almost 3 mg. in each case.

The gonadotropic hormone level apparently does not return to its initial value as soon as do either the thyrotropic or adrenocorticotropic hormones. It can be seen in Fig. 4 that while there is a tendency toward reversal of the stimulatory effect following cessation of the series of electronarcoses, the recovery is gradual, the values after 2 weeks still being noticeably higher than the initial value. As is shown in Table VI, the values have been corrected for extraneous influences by using a control series with each assay. The control values have been compared to the mean value, giving a correction factor for computing the corrected weights of the respective glands.

Changes in the Output of Adrenocorticotropic Hormone.

In examining the changes which occurred in the level of adrenocorticotropic substances in the blood following electronarcosis similar methods have been used. In the guinea pig experiments both gross adrenal weight and microscopic analysis of the sectioned glands have been used to indicate changes in adrenal cortical activity. The glands were dissected free with a pair of fine forceps, fixed in Bouin's fluid over night, blotted, and weighed to the nearest 0.1 mg. Table I

shows that the adrenal glands of guinea pigs subjected to a series of electronarcoses are considerably heavier than those of the control animals.

Histologically the adrenal cortex consists of four more or less well defined layers. From the medulla outward these are termed the zona reticulata, zona fasciculata, zona glomerulosa, and capsule. Examination of the stained sections of the adrenals of the above guinea pigs showed that the hypertrophy is most pronounced in the zona reticulata and zona glomerulosa, and relatively less apparent in the zona fasciculata (Table VII). It should be pointed out that these measurements refer to the radium, not to the total area of the layers of the adrenal cortex, so it is evident that if the area were considered, the hypertrophy of the zona glomerulosa, this layer being most peripherally located, would be relatively greater than the values indicate.

Changes in the level of adrenocorticotropic hormone in the blood serum of dogs was studied by injection into baby chicks. The adrenal response was measured by comparing the weights of the dissected chick adrenals from the series injected with post-electronarcotic serum with those from the series injected with pre-electronarcotic blood. In Figs. 2 and 3 it can be seen that as was shown in the thyroids and testes, the distribution of weights of the chick adrenals is shifted to the right. The serum from dog No. 1, following a series of 7 electronarcoses, resulted in an increase in the weights of the

Table VII. Effect of Electronarcosis on the Structure of the Adrenal Cortex in Guinea Pigs.

ANIMAL NUMBER	NUMBER OF ELECTRO-NARCOSES	BODY WEIGHT	ADRENAL WEIGHT MG/100 GM	THICKNESS OF ADRENAL CORTEX - MM.				
				TOTAL	CAPSULE	ZONA GLOMER.	ZONA FASCIC.	ZONA RETIC.
11	0	342	63.9	1.4756	.0124	.0992	1.1160	.2480
28	0	450	59.7	1.3353	.0305	.1208	.8906	.2934
33	0	556	55.5	1.0270	.0260	.0910	.7800	.1300
	AVERAGE	449	59.7	1.2793	.0229	.1037	.9289	.2238
12	11^{10}	296	52.0	1.0354	.0248	.0806	.6510	.2790
14	7^{10}	274	68.5	.9734	.0124	.0620	.7130	.1860
29	10^{2}	411	52.8	.8125	.0195	.0780	.5200	.1950
30	10^{2}	536	69.5	1.4755	.0455	.1300	.9750	.3250
31	10^{2}	362	55.5	.8645	.0195	.0975	.6175	.1300
32	10^{2}	341	151.2	1.6262	.0244	.3001	.7283	.5734
	AVERAGE	370	74.9	1.1312	.0243	.1247	.7008	.2814

	PERCENT OF TOTAL CORTEX THICKNESS				
CONTROL	[illegible]	[illegible]	8.4%	72.4%	17.5%
ELECTRONARCOTIZED	[illegible]	2.4%	11.%	62.0%	24.8%
RELATIVE CHANGE		+[illegible]%	+[illegible]%	-10.4%	+7.3%

chick adrenals from 9.4 mg. in the pre-electronarcotic series to 14.3 mg. in the post-electronarcotic series. This represents a weight increase of about 50%. The mean adrenal weights after injection with serum from dog No. 2 were 10.2 mg. for the pre-electronarcotic series and 12.4 mg. for the post-electronarcotic series, representing an increase of nearly 25% (Table IV).

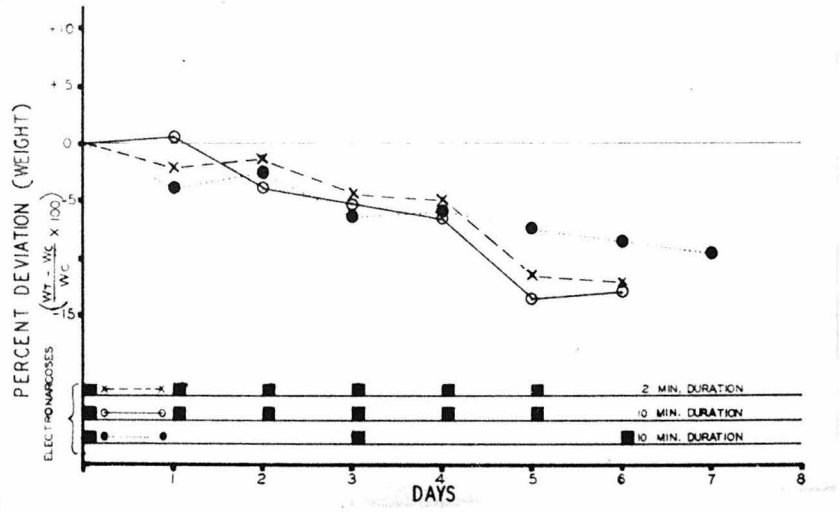
In following the changes in concentration of the adrenocorticotropic hormone in the serum of Dog No. 2 for two weeks after discontinuing the series of electronarcosis, it was found that the weights of the chick adrenals were still somewhat above the initial level by the end of the first week, but had returned to the original level by the end of the second week.

Effect of Electronarcosis on Body Weight.

It was observed early in the study of electronarcosis on guinea pigs that those animals which were treated daily soon began to be easily recognizable from the control animals because of their failure to add to their body weight. Careful weight records were kept, therefore, in nearly all of the guinea pig experiments. The summary of these observations is shown in Fig. 5. For easy visualization of the results the weight of the experimental animal is expressed as percent deviation from that of the control. In so far as was possible a control animal was used with each experimental animal in order to nullify extraneous environmental influences.

Following a daily electronarcosis of 10 minutes duration

Figure 5. Summary of Changes in Body Weight of Guinea Pigs After a Series of Electronarcoses.



Note: The values represented in the above graph are the averages from 4 electronarcotized and 4 control animals in each case.

the relative weight loss by the end of one week has been quite pronounced. The change, however, is almost identical with that obtained by giving a daily 2 minute electronarcosis. On the other hand, electronarcosis of 10 minutes duration given every third day results in a slower and less marked relative decrease in body weight. It should be pointed out with somewhat more emphasis that the electronarcotized guinea pigs as a rule did not actually lose weight, but merely failed to add to it at the rate which the control animals did.

Following thyroidectomy the weight falls off much in the same manner as in the intact animal (Fig. 6). It will be seen that for a period immediately following removal of the thyroid glands the body weight change was positive in direction, but as soon as electronarcosis was begun, the weight change became negative with respect to the control.

Effect of Electronarcosis on the Basal Metabolic Rate.

In guinea pigs a marked increase in the basal metabolic rate was found to occur following a series of daily electronarcoses. For routineness a duration of 10 minutes was used for each electronarcosis. The animals used in the basal metabolism determinations were, for the most part, the guinea pigs whose thyroids, ovaries and adrenals were subsequently removed and studied as heretofore described. A modified Benedict (1930) closed chamber respirometer was used, the details of which are shown in Fig. 7. The volume was corrected to standard temperature and pressure, and for changes which

Figure 6. The Effect of Electronarcosis on the Body Weight of Thyroidectomized Guinea Pigs.

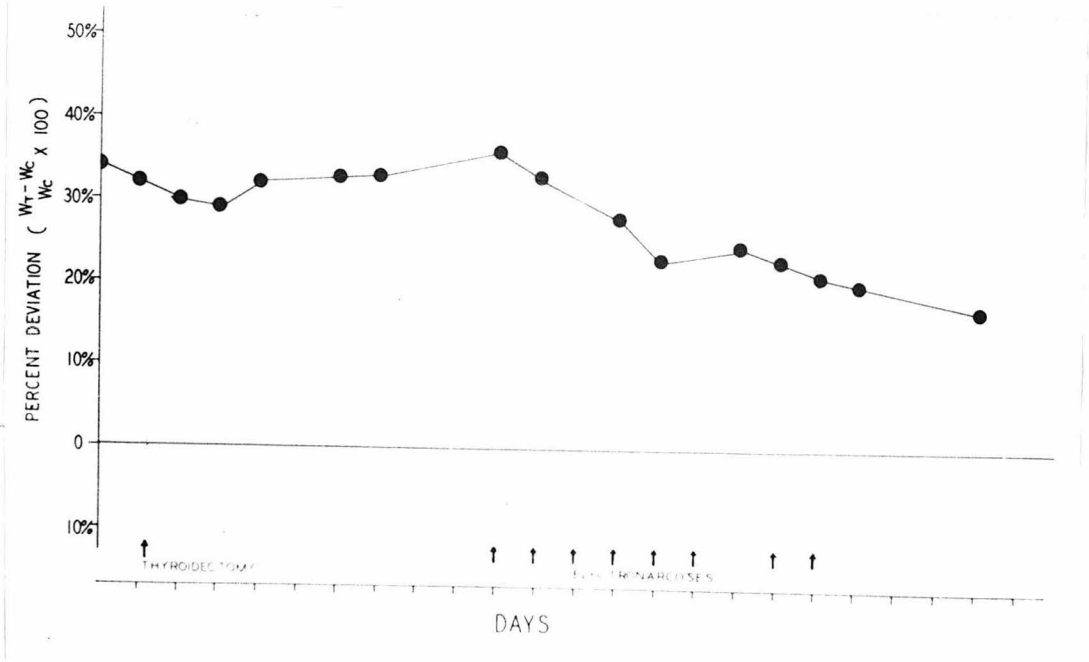
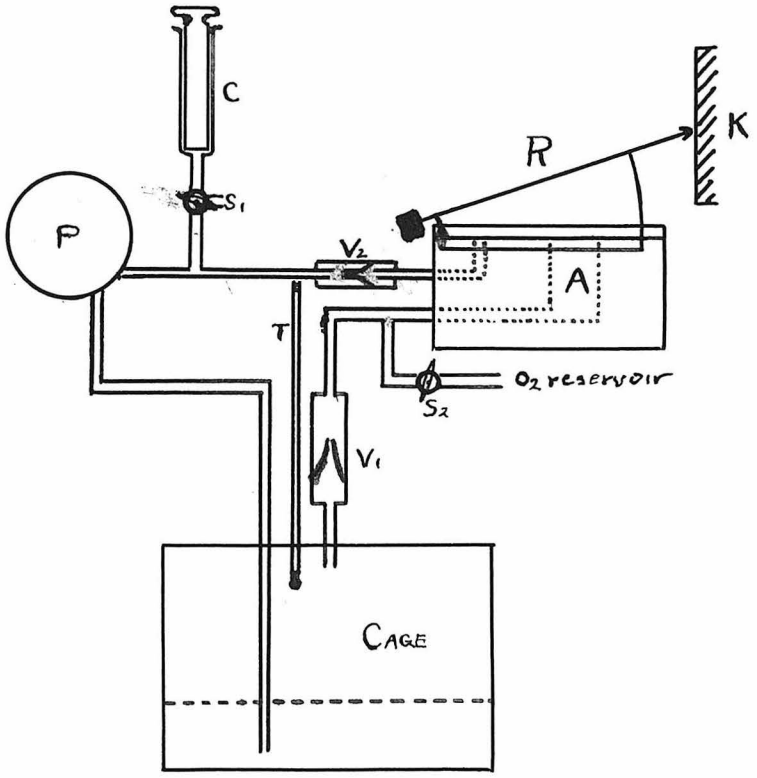


Figure 7. Diagram of Apparatus used in making Basal Metabolism Determinations of Guinea Pigs.



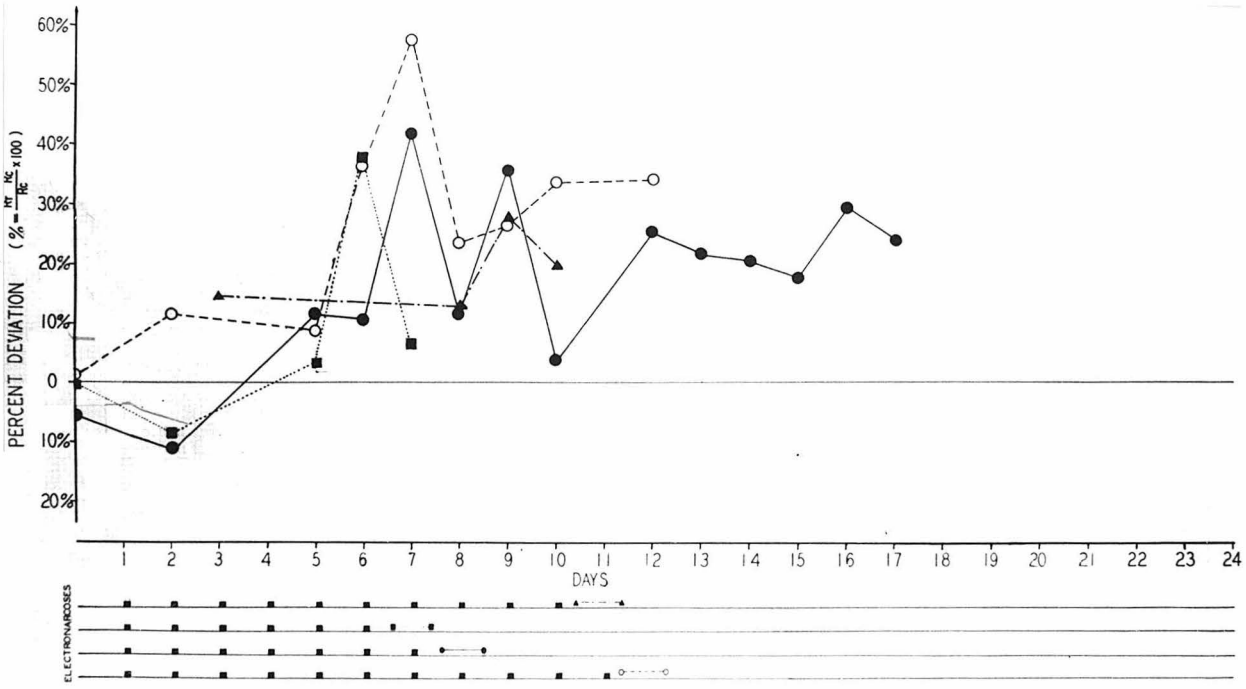
A = Soda lime CO_2 absorbent.
C = Calibration Syringe (50 ml. capacity).
K = Kymograph.
P = Pump for circulating air.
R = Krogh Respirometer.
S_1 and S_2 = stopcocks.
V_1 and V_2 = Valves to direct flow of air.

existed between the enclosed system and the external environment. The metabolism values were calculated on the basis of an assumed caloric conversion factor of 4.825 and the surface area was determined using the Rubner (1902, 1928) formula in which, in guinea pigs, $A = .085\sqrt[3]{W^2}$. The oxygen consumption was measured, as a rule, for a period of 20 minutes. The metabolic rate has been expressed as $C/M^2/hr$. This value has been corrected to 25°C by applying a correction factor calculated from the data of Verzár and Wahl (1931).

Determination of the respiratory quotient has been considered, but has not yet been made. In view of the demonstration by Anderson and Collip (1934) that it is not necessary to starve the guinea pigs before determining the basal metabolic rate, in the present experiments the animals have been supplied continuously with alfalfa pellets and water, and have been given fresh carrots once each week.

For ease of presentation the actual metabolism values have been converted into "percent deviation" from the control, an untreated animal having been used in each instance. The control values have been uniformly expressed, therefore, as unity, the values for the electronarcotized animals, as percent deviation from that level. These values have been plotted against the days following the beginning of the series of electronarcoses in Fig. 8. Electronarcosis has been indicated by a black square on the particular line at the bottom of the graph. A summary of the effects of daily electronarcosis on

Figure 8. The Effect of Daily Electronarcoses of 10 Minutes Duration on the Basal Metabolic Rate of Guinea Pigs.



the basal metabolic rate of these 4 test animals and their respective controls is given in Table VIII, and is presented graphically in Fig. 9.

It will be seen from these data that the highest rise in the metabolic rate occurred between the 6th and 9th day following the inauguration of the series of daily electronarcoses. It should be pointed out that one animal maintained an increased basal metabolic rate for at least 10 days after the series of electronarcoses was discontinued (Fig. 8), and it is to be regretted that the other animals were not followed for a longer time after the final electronarcosis, but as these animals were killed in order to remove their endocrine glands for weighing and histological study, this was not possible.

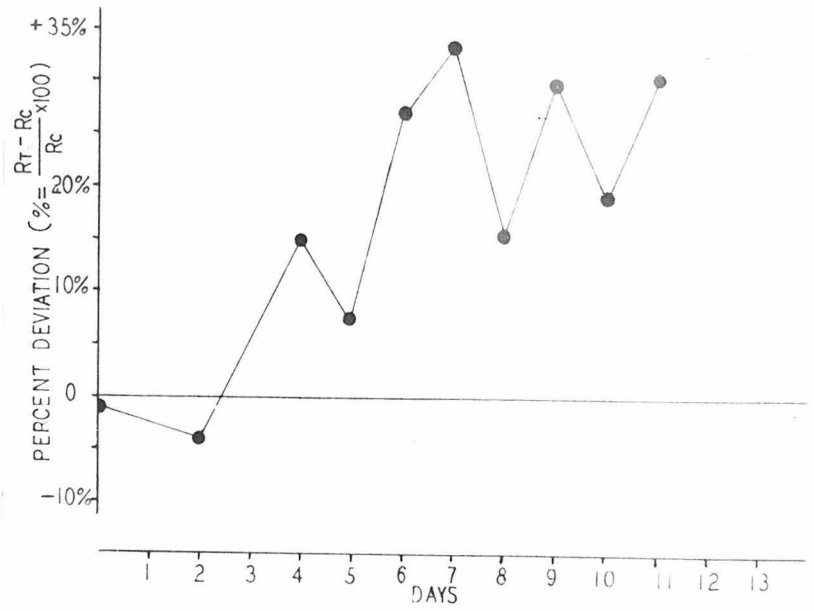
A parallel series of studies were made in which electronarcosis was administered on every third day. These determinations showed a similar, although less marked, increase in the basal metabolic rate, but instead of the metabolism remaining at a high level, it was restored to the normal level before the next electronarcosis was administered. In Fig. 10 the values for 2 test animals and their respective controls have been graphically presented, and the actual values have been included in Table IX. The significance of these changes cannot be predicted at the present time, as the data is too limited with such wide variation as was found to be present.

The short latency of the effect of electronarcosis on the basal metabolic rate has made it imperative to find just when the rise actually occurs. To this end a number of

Table VIII. Effect of Electronarcosis on the Basal Metabolic Rate of Guinea Pigs.

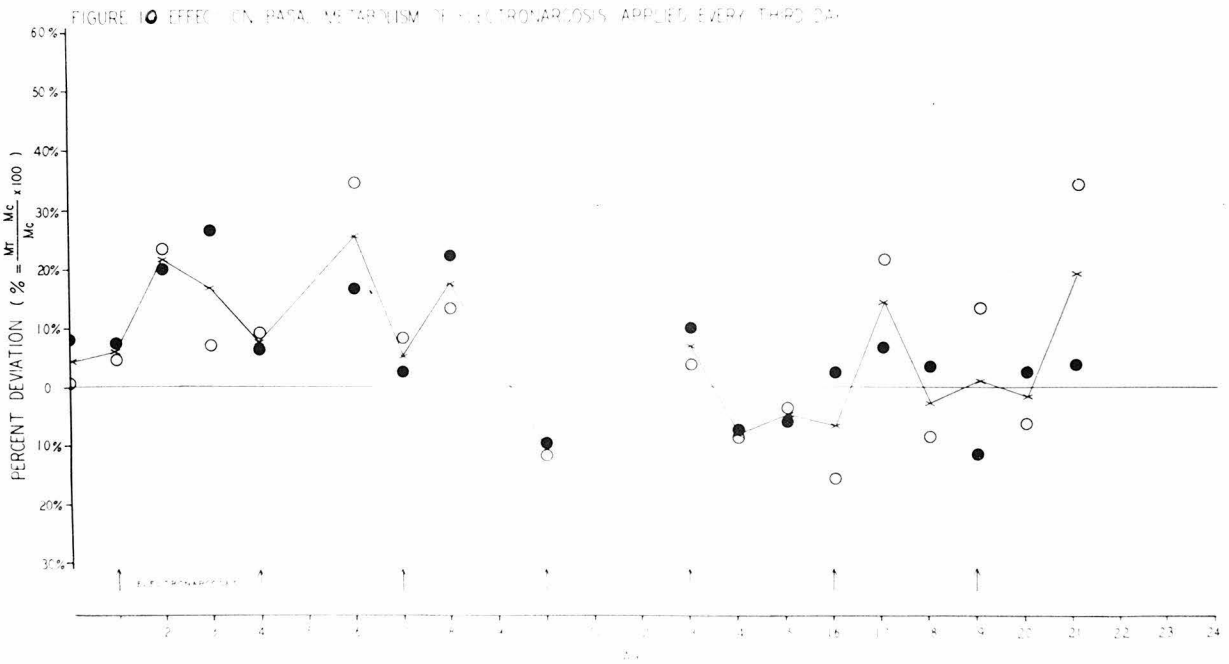
DAY	CONTROL		TEST		PERCENT DEVIATION $\% = \frac{M_T - M_C}{M_C} \cdot 100$
	C/M²/HR	STANDARD ERROR	C/M²/HR	STANDARD ERROR	
0	50.39	± 1.925	49.80	± 0.512	− 1.2%
1					
2	37.09	± 2.914	35.57	± 1.099	− 4.1%
3					
4	40.24		46.02		+14.7%
5	38.56	± 0.859	41.66	± 2.182	+ 8.1%
6	39.92	± 3.454	50.84	± 3.815	+27.3%
7	37.39	± 3.246	49.90	± 5.302	+33.6%
8	41.15	± 4.098	47.52	± 3.089	+15.4%
9	46.69	± 5.914	60.69	± 8.661	+29.8%
10	39.68	± 2.004	47.16	± 4.037	+18.8%
11	36.75	± 0.250	47.66	± 1.245	+29.9%

Figure 9. Effect of Electronarcosis on the Basal Metabolic Rate of Guinea Pigs.



Note: The values represent the averages of 4 test animals with their respective controls. Electronarcosis of 10 min. duration was given daily for the first 10 days.

Figure 10. Effect on Basal Metabolic Rate of Guinea Pigs of Electronarcosis Applied Every Third Day.



Note: The above values represent determinations on two test animals with their respective controls, one being represented by open circles, the other by closed circles. Electronarcosis was of 10 minutes duration in each instance. The solid line represents the average deviation.

Table IX. The Effect of Electronarcosis on the Basal Metabolic Rate in Guinea Pigs.

TABLE IX: EFFECT OF ELECTRONARCOSIS ON THE BASAL METABOLIC RATE IN GUINEA PIGS.

GROUP	NUMBER OF ELECTRO-NARCOSES	BASAL METABOLIC RATE C/M²/HR (CORRECTED TO 25°C) DAYS 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
DAILY ELECTRONARCOSIS	10				46.02					45.58	48.98	50.23											
	0				40.24					40.38	38.35	42.00											
	% DEV.				+14.4%					+12.9%	+27.8%	+19.6%											
	6	50.18		34.04			43.70	48.26	55.30	51.88	69.65	41.49		46.42	53.63	65.16	66.19	42.27	67.54				
	0	53.15		38.58			39.13	43.60	39.05	46.51	51.44	39.92		37.10	43.95	54.20	56.11	32.66	54.46				
	% DEV.	−5.6%		−11.8%			+11.7%	+10.7%	+41.6%	+11.6%	+34.4%	+3.9%		+25.2%	+21.6%	+20.2%	+17.9%	+29.4%	+24.0%				
	7	50.15		36.14			38.64	56.24	42.70														
	0	50.25		39.67			37.35	40.87	40.26														
	% DEV.	−0.2%		−8.9%			+3.5%	+37.6%	+6.3%														
	11	49.08		36.55			42.66	48.04	51.72	45.10	63.46	49.48		48.91									
	0	48.53		33.02			39.21	35.30	32.86	36.56	50.27	37.11		36.50									
	% DEV	+1.3%		+11.4%			+8.8%	+36.2%	+57.3%	+23.4%	+26.3%	+33.3%		+34.0%									
	AVE % DEV	−1.5%		−3.1%			+8.0%	+28.2%	+35.1%	+16.0%	+29.8%	+18.9%		+29.6%									
ELECTRONARCOSIS EVERY THIRD DAY	7	47.75	45.22	49.72	59.55	50.93		49.46	37.02	44.82		31.50			44.90	31.54	37.38	33.48	30.80	34.34	25.35	25.47	26.50
	0	44.26	42.04	41.40	47.01	47.91		42.30	36.13	36.66		34.97			40.74	33.95	39.76	32.67	28.31	33.18	28.67	24.90	25.48
	% DEV	+7.9%	+7.6%	+20.1%	+26.6%	+6.3%		+16.9%	+2.5%	+22.3%		−9.9%			+10.2%	−7.1%	−6.0%	+2.5%	+6.8%	+3.5%	−11.5%	+2.3%	+4.0%
	7	34.02	47.32	56.52	37.57	49.85		58.84	48.55	42.45		32.38			39.99	34.52	33.56	31.44	47.59	36.46	29.28	32.32	28.54
	0	33.92	45.66	45.75	35.07	45.70		43.74	44.90	37.40		36.55			38.47	37.56	34.82	37.28	39.06	39.92	25.75	34.42	21.25
	% DEV	+0.3%	+4.7%	+23.5%	+7.1%	+9.1%		+34.5%	+8.1%	+13.5%		−11.4%			+4.0%	−8.1%	−3.6%	−15.6%	+21.8%	−8.6%	+13.7%	−6.1%	+34.3%
	AVE % DEV.	+4.1%	+6.1%	+21.8%	+16.9%	+7.7%		+25.7%	+5.3%	+17.9%		−10.6%			+7.1%	−7.6%	−4.8%	−6.6%	+14.3%	−2.6%	+1.1%	−1.9%	+19.1%

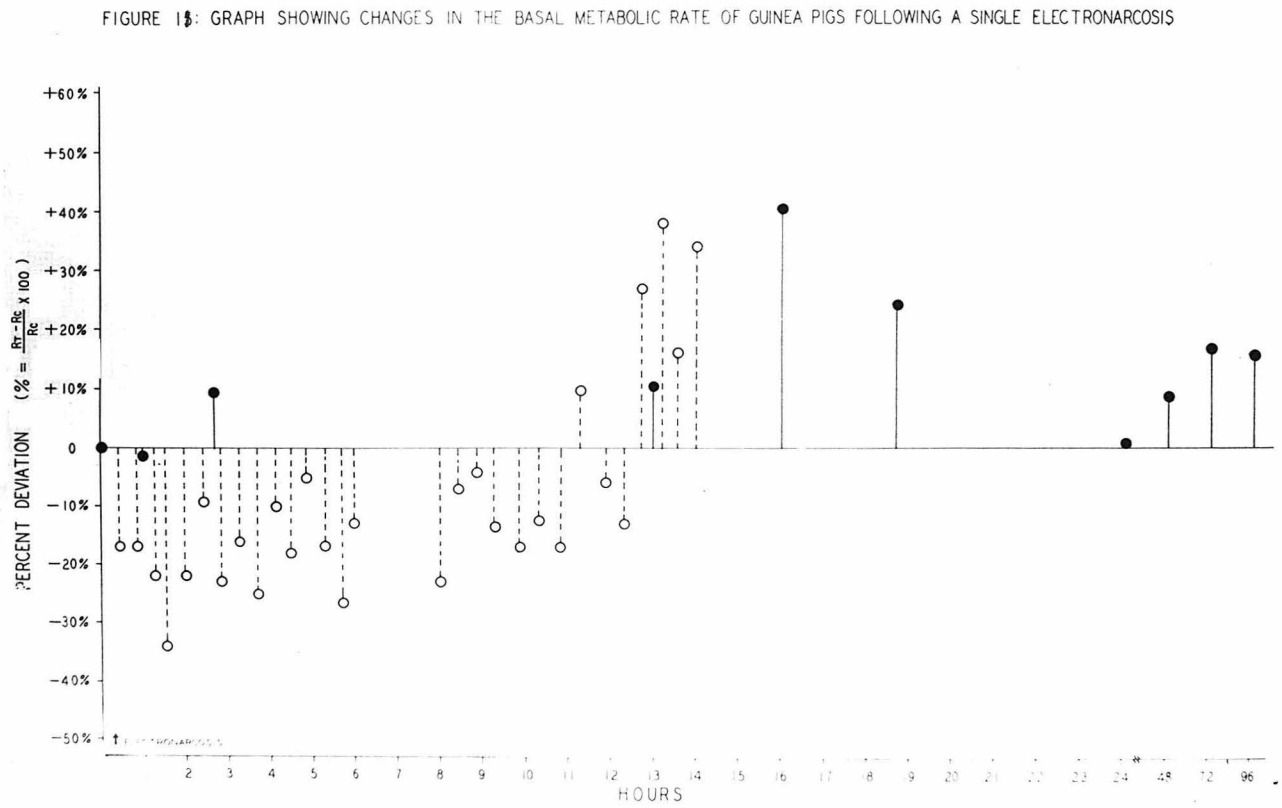
experiments have been carried out in which the basal metabolic rate of the animal has been taken at short intervals following a single electronarcosis. It will be seen in Fig. 11 that the rise occurs between 12 and 17 hours after the electronarcosis. Whether or not the early depression of metabolism seen in Fig. 11 is significant or not cannot be definitely stated because the animals in the series represented by the blacked-in circles showed considerable variability in this respect. It is clear, at any rate, that a single electronarcosis of 10 minutes duration produces a marked elevation in the basal metabolic rate reaching as much as 35% to 40% about 14 to 16 hours after the single electronarcosis.

DISCUSSION

Fugo and Gross (1942) have recently shown that increased amounts of pituitary gonadotropic hormones are secreted in response to continued injection of yohimbine hydrochloride. It is unfortunate that the other endocrine organs were not more thoroughly studied in the course of their investigation, as it would have been most interesting to find out whether only the gonadotropic secreting cells were stimulated or whether yohimbine produces a general pituitary stimulation.

That it is possible to produce an increased activity of the pituitary by electrical stimulation has been shown by Harris (1936, 1937), and has been indicated by the work of

Figure 11. Changes in Basal Metabolic Rate of Guinea Pigs following a Single Electronarcosis of 10 Minutes Duration.



Note: The open circles represent determinations on a single animal. The closed circles represent the average values from 4 test animals and 4 controls.

Hemphill (1943) in which it was shown that electroshock therapy results in an increased output of the 17-ketosteroids of the adrenal cortex corresponding quite closely to the increase in their output following injection of a pituitary extract containing the adrenocorticotropic hormone.

It is clear from the present investigation, moreover, that the pituitary is involved in the response of the various endocrine glands to electronarcosis. To be sure, it is conceivable that the general cortical and subcortical stimulation which results from passage of current through the brain might produce a direct nervous stimulation of the respective endocrine organs. It has, to be sure, been shown by a number of workers that, whereas direct stimulation of certain endocrine glands is possible (Uotila, 1939 a, b), the response to such nervous stimulation is of a much smaller order than that resulting from activation by the specific hypophyseal "tropic" hormone. That such direct stimulation of the thyroid, adrenal and testis has not been the mechanism involved in the response to electronarcosis has been clearly shown by injecting the blood serum of dogs into baby chicks. The fact that the chick organs showed greater hypertrophy when the chicks were injected with post-electronarcotic serum is conclusive proof that the endocrinotropic activity lies in the blood stream, not in the nervous system of the electronarcotized animal.

The question of the mechanism of this pituitary stimulation has not been answered. It is possible that passage

of the current through the brain produces a direct stimulation of the pituitary cells, but it is also quite possible that the general stimulation of all of the nervous elements of the brain may be involved, pituitary stimulation resulting from impulses relayed from the hypothalamus through the stalk of the pituitary gland. The latter view is held by Harris (1936, 1937) who was able to induce pseudo-pregnancy in rats by passing an electric current through the head. He also found that stimulation of the pituitary gland, hypothalamus, and tuber cinereum through needle electrodes caused ovulation in rabbits within about 40 hours. He was not able, however, to conclusively show that the stimulatory effect on the pituitary was mediated by nervous elements and not ~~to~~ by a spread of the current to the pituitary gland itself. It is likewise possible that a localized vasodilation might cause an increase in hormone secretion due to a temporary hyperemia of the pituitary gland. Further investigations toward determining which mechanism is involved are being considered for a future time.

That electronarcosis produces a stimulation of the entire pituitary gland is suggested by the increase not of just one or two of the hypophyseal hormones, but of at least 4 of them. They have been, however, with the possible exception of the specific metabolic hormone, hormones from the anterior lobe. Investigation of the effects of electronarcosis on the secretion of the pressor factor of the pars nervosa is planned for the near future in an attempt to find how general the stimulatory

action of electronarcosis may be.

At a future date it is likewise planned to further demonstrate that pituitary stimulation is actually responsible for the glandular responses to electronarcosis by applying the electrical stimulation to one of a pair of parabiotic animals. If the glands of the parabiotic mate also shows hypertrophy, it will certainly indicate the hormonal origin of the effects.

The question of whether the initial high current is responsible for the pituitary stimulation to an extent greater than the prolonged administration of the lower maintenance current cannot be definitely answered. The fact that the gland weights following a series of 2 minute electronarcoses show an increase of nearly as great a magnitude as those following a series of 10 minute electronarcoses would seem to indicate that the high initial current is the chief factor. It is planned in the near future to subject a series of guinea pigs to electroshock, in which a high current is applied for only a fraction of a second, to determine which portion of the current application is responsible to the greatest extent for the pituitary stimulation.

The observation that a marked increase in the basal metabolic rate follows electronarcosis is especially interesting because of the shortness of the latent period between the electronarcosis and the metabolism rise. Were this due to an increased thyroid output as a result of increased thyrotropic hormone secretion, one would expect a latent period of at least

2 or 3 weeks; in fact, it is well established that injection of thyroxine into normal animals is not usually accompanied by any great change in the basal metabolic rate for from 3 to 7 weeks. It seems much more likely, therefore, that the rise in basal metabolic rate which was found in the electronarcotized guinea pigs is due to a release of a substance such as the specific metabolic hormone first described by O'Donovan and Collip (1938). A survey of the literature concerning this factor shows that it is apparently widely distributed in all parts of the pituitary, and that when injected it can act as well in thyroidectomized, adrenalectomized, or even hypophysectomized animals. Injection of extracts rich in the principle have been reported to produce a rise in the basal metabolic rate of 30% within 4 hours (Billingsley, O'Donovan and Collip, 1939). (For confirmatory references see Teague, 1939, and Feinstein and Gordon, 1940).

It is obvious, however, that the data from the metabolic studies herein reported are much too few to permit a very critical analysis at the present time. The metabolic rise is uniformly present, but the mechanism is too obscure for presentation at this time.

Correlated rather closely with the changes in metabolic rate following electronarcosis are the changes observed on the body weight of the guinea pigs. With respect to a specific metabolic hormone it is especially significant that immediately after electronarcosis the thyroidectomized animal showed a

relative decrease in body weight as compared with the control animal. This would be expected if the metabolic rate were raised by the specific metabolic factor of the pituitary which is known to act independently from the thyroid gland.

Of considerable significance is the observation that the secretion of gonadotropic hormones is increased following electronarcosis. In the case of the pregnant guinea pig which resorbed her embryos it is likely that the follicle stimulating hormone of the pituitary was secreted in such amounts that the inhibition of theelin production during pregnancy was no longer maintained and the pseudo-abortion resulted. This may have resulted, however, from other disturbances of the endocrine secretions. In a previous investigation (unpublished) it has been noted that prolonged injection of thyrotropic hormone into pregnant guinea pigs frequently results in a similar resorption of the embryos. No adequate investigation of the mechanism involved has yet been made, but such a study should certainly prove most interesting.

In view of the intimate relationship existing between the gonadotropic hormones and the menstrual cycle it is particularly significant that a number of amenorrheic patients have resumed menstruation following a number of electronarcoses. This is especially interesting in respect to the observation by Ripley and Papanicolau (1941) that schizophrenics show a greater irregularity in their menstrual histories than do normal women. This has been shown also by Elliot (1941).

Ripley and Papanicolau (1941) showed that a correlation exists between the severity of the mental illness and the degree of abnormality of the menstrual cycle, and that improvement in the mental condition was usually accompanied by a more normal menstrual function. It becomes most enticing to consider the relation which may exist between the corrective effect of electronarcosis on the sex cycle and the possible therapeutic value of electronarcosis in schizophrenia or other mental disorders, or even the possibility of treating mentally disturbed patients for their hormone unbalance with the end toward improving their mental conditions.

The relationship between endocrinopathy and mentally disturbed states has been considered by several workers. Loehner (1938,1940) has used adrenal cortex extracts with a certain degree of success in treating a variety of psychotic conditions, as have Williams and Wright (1941). Liddell (1935) showed that adrenocortical extracts quieted sheep with experimental neuroses. In 1941 Sanchez-Calvo reported that examination of tissues from 40 cases of schizophrenia showed that "fast alle Hypophysen von 40 von uns untersuchten Fällen von Schizophrenie weisen deutlich nachweisbare funktionelle Veränderungen auf." Hemphill and Reiss (1943) have reported a beneficial effect in the treatment of involutional melancholia with a pituitary extract rich in adrenocorticotropic hormone. Hemphill and Reiss (1943) have advanced the idea that "the therapeutic effects of shock treatment in involutional melancholia may be due to an increase in anterior pituitary activity."

However, many causes and as many cures have been suggested for mentally disturbed conditions, and at present the data are insufficient to permit any definite conclusions in these respects. The facts show that the secretory activity of the pituitary is increased following electronarcosis, but the relationship between this finding and the clinical sequellae are most obscure.

SUMMARY

In studying the effect of electronarcosis on the secretory activity of the pituitary gland, electronarcosis has been applied to guinea pigs and dogs.

Increased secretion of the thyrotropic, adrenocorticotropic and gonadotropic hormones has been shown to result from this passage of electric current through the head of the animal. These increases have been shown to be reversible upon discontinuing the electronarcosis series.

The "tropic" activity following electronarcosis has been shown to be endocrine in nature by demonstrating that in dogs the blood serum shows a marked increase in its ability to produce hypertrophy in the thyroids, adrenals, and testes of day-old chicks.

In guinea pigs a marked elevation of the basal metabolic rate occurs subsequent to electronarcosis. In view of the short latency of this response it has been suggested that the increased B.M.R. results from an increased production

of a substance such as the specific metabolic principle described by O'Donovan and Collip.

The symptoms observed during electronarcosis in guinea pigs have been described.

LITERATURE CITED

Allen, B. M. 1938. The endocrine control of amphibian metamorphosis. Biol. Rev. 13:1-19.

Anderson, E. M., and Collip, J. B. 1934. Studies on the physiology of the thyreotropic hormone of the anterior pituitary. J. Physiol. 82:11-25.

Aron, M. 1929. Action de la préhypophyse sur la thyroïde chez le cobaye. Compt. rend. Soc. de Biol. 102:682-684.

Aron, M. 1932. Note de technique sur la mise en évidence et évaluation quantitative des faibles taux de "thyréo-stimuline" préhypophysaire présents dans le sang ou l'urine. Compt. rend. Soc. de Biol. 109:218-220.

Aron, M. 1936. Sur le titrage biologique de la thyréo-stimuline préhypophysaire: Le "seuil des mitoses" dans la thyroïde des cobayes traites. Compt. rend. Soc. de Biol. 123:250-253.

Aron, M., and Klein, M. 1930. Sur la présence, dans l'urine humaine, d'une substance douée de la même action sur la thyroïde que l'extrait préhypophysaire, et sur l'interprétation de la réaction de diagnostic de la grossesse. Compt. rend. Soc. de Biol. 103:702-704.

Benedict, F. G. 1930. A multiple-chamber respiration apparatus for rats and other small animals. Jour. Nutr. 3:161-176.

Bergman, A. J. and Turner, C. W. 1939. A comparison of the guinea pig and chick thyroid in the assay of the thyrotropic hormone. Endocrinology 24:656-664.

Billingsley, L. W., O'Donovan, D. K., and Collip, J. B. 1939. The specific metabolic principle of the pituitary. Endocrinology 24:63-68.

Collip, J. B. 1942. Corticotropic (adrenotropic) thyrotropic and parathyrotropic factors. Chapter III of "Glandular Physiology and Therapy" American Medical Assn. Chicago.

Cope, C. L. 1938. The young chick as a test for the thyrotropic hormone. J. Physiol. 94:358-364.

Domm, L. V. 1937. Observations concerning anterior pituitary-gonadal interrelations in the fowl. Cold Spring Harbor Symposia on Quant. Biol. 5:241-257.

Elliott, H. E. 1941. Comparison of non-psychotic women with schizophrenics with respect to body type, signs of autonomic imbalance and menstrual history. Psychiat. Quart. 15:17-22.

Feinstein, R. N., and Gordon, E. S. 1940. The specific metabolic principle of the pituitary. Endocrinology 27:592-596.

Frostig, J. P., Harreveld, A. van, Resnick, S., Tyler, D. B., and Wiersma, C. A. G. 1943. Electronarcosis in Animals and in man. (In preparation).

Fugo, N. W., and Gross, E. G. 1942. Anterior pituitary-stimulating action of yohimbine. Endocrinology 31:529-534.

Globus, J. H., Harreveld, A. van, and Wiersma, C. A. G. 1943. The influence of current passage on the brain. (In preparation).

Harreveld, A. van, and Kok, D. J. 1934a Über Elektronarkose mittels sinusoidalen Wechselstromes. Arch. néerl. Physiol. 19:24-57.

Harreveld, A. van, and Kok, D. J. 1934b Über experimentelle Katalepsie durch sinusoidalen Wechselstrom. Arch. néerl. Physiol. 19:265-289.

Harreveld, A. van, Plesset, M. S., and Wiersma, C. A. G. 1942. The relation between the physical properties of electric currents and their electronarcotic action. Am. J. Physiol. 137:39-46.

Harris, G. W. 1936. The induction of pseudo-pregnancy in the rat by electrical stimulation through the head. J. Physiol. 88:361-367.

Harris, G. W. 1937. The induction of ovulation in the rabbit, by electrical stimulation of the hypothalamo-hypophysial mechanism. Proc. Roy. Soc. London 122:374-394.

Hemphill, R. E., and Reiss, M. 1943. Corticotrophic hormone in the treatment of involutional melancholia with hypopituitarism and pituitary cachexia. Jour. Ment. Sci. 88:559-565.

Heyl, J. G. and Laqueur, E. 1935. Zur quantitativen Bestimmung der thyreotropen Wirkung von Hypophysen-vorderlappenpräparaten und die Einheit des thyreotropen Hormons. Arch. internat. de pharmacod. et de thérap. 49:338-354. (Cited by Collip, 1942).

Junkman, K., and Loeser, A. 1938. Die Wertbestimmung des thyreotropen Hormons der Hypophyse. Arch. f. exp. Path. u. Pharmakol. 188:474-488.

Kabac, J. M., and Liapin, N. I. 1938. A quantitative study of the thyrotropic action of extracts of anterior pituitary on chicks. Bull. de biol. et de méd. expér. de l'U. R. S. S. 5:334-338. (Cited by Collip, 1942).

Leduc, S. 1902. Production du sommeil et de l'anesthésie générale et locale par les courants électriques. Compt. rend. Acad. Sci. 135:199-200.

Liddell, H. S., Anderson, O. D., Kotyuka, E., and Hartman, F. A. 1935. Effect of extract of adrenal cortex on experimental neuroses in sheep. Arch. Neurol. and Psychiatry. 34:973-993.

Loehner, C. A. 1938. The therapeutic effect of adrenal cortex extract on the psychotic patient. Endocrinology 23:507.

Loehner, C. A. 1940. Further observations on the use of adrenal cortex extract in the psychotic and non-psychotic patient. Endocrinology. 27:378-380.

O'Donovan, D. K., and Collip, J. B. 1938. The specific metabolic principle of the pituitary and its relation to the melanophore hormone. Endocrinology. 23:718-734.

Rabinowitch, I. M., Mountford, M., O'Donovan, D. K., and Collip, J. B. 1939. Influence of a specific hormone of the pituitary on the basal metabolism in man. Can. Med. Assn. Jour. 40:105-107.

Rawson, R. W., and Starr, P. 1938. Direct measurement of height of thyroid epithelium: A method of assay of thyrotropic substance; clinical application. Arch. Int. Med. 61:726-738.

Ripley, H. S., and Papanicolaou, G. 1941. The menstrual cycle with vaginal smear studies in schizophrenia, depression, and elation. Am. J. Psych. 98:567-573.

Rowlands, I. W., and A. S. Parkes. 1934. Quantitative Study of the thyrotropic activity of anterior pituitary extracts. Biochem. J. 28:1829-1843.

Rubner, M. 1902. Die Gesetze des Energieverbrauchs bei der Ernährung. F. Deuticke, Leipzig and Wein. pp.280-281.

Rubner, M. 1928. Stoffwechsel bei verschiedenen Temperaturen. Beziehungen zur Grösze und Oberfläche. (In Bethe's Handbuch der Normalen und Pathologischen Physiologie. vol. 5. Pp. 154-166).

Sanchez-Calvo, R. 1941. Das Hypophyse-Thyroidea-System in der Schizophrenie. Acta Med. Scand. 108:332-350.

Smelser, G. K. 1937. Assay of thyrotropic hormone on day-old chicks. Proc. Soc. Exp. Biol. and Med. 37: 388-390.

Smelser, G. K. 1938. Chick thyroid responses as a basis for thyrotropic hormone assay. Endocrinology. 23:429-438.

Starr, P., and Rawson, R. W. 1937. Graphic representation of thyroid response to stimulation by thyrotropic hormone. Proc. Soc. Exp. Biol. and Med. 35:603-605.

Starr, P., Rawson, R. W., Smalley, R. E., Doty, E., and Patton, H. 1939. The microhistometric method applied to thyrotropic hormone assay. West. J. Surg. 47:65-75.

Teague, R. S. 1939. The relation of the melanophore hormone of the pituitary gland to oxygen consumption of the rat. Endocrinology. 25:953-961.

Uotila, U. U. 1939. The rôle of the cervical sympathetics in the regulation of thyroid and thyrotropic function. Endocrinology. 25:63-70.

Uotila, U. U. 1939b. The regulation of thyrotropic function by thyroxin after pituitary stalk section. Endocrinology. 26:129-135.

Verzár, F., and Wahl, V. 1931. Wirkung des Hypophysenvorderlappenshormons auf den O_2-Verbrauch von Meerschweinchen. Biochem. Zeit. 240:37-49.

Williams, E. H., and Wright, C. A. 1941. The adrenal cortex and psychotic states. Medical Record. 154:411-416.

PART TWO

STUDIES ON THE EXTENSOR MECHANISM OF THE SPIDER LEG.

INTRODUCTION

Most investigations dealing with the morphology and physiology of spiders have ignored the walking legs. These appendages, however, afford many interesting features which are apparently to be found only in this group of animals.

Because of this dearth in the literature covering the anatomy and physiology of the locomotor organs, and because of the lack of agreement on the part of the few previous investigators, a reinvestigation has been made of the structural relationships in the walking legs of a number of species representing the two most important suborders of the Araneae before making some physiological investigations into the several systems involved. The spiders which have been used in the present study include the tarantulas, Dugesiella californica, Delopelma helluo, and Aphonopelma cryptethus, representing the suborder Mygalomorphae; and the grass spider, Agelena naevia, the golden garden spider, Miranda aurantia, the crab spider, Misumena aleatoria, the black widow, Latrodectus mactans, the large orb weaver, Aranea cavicata, and the false "daddy long legs", Pholcus phalangioides representing the suborder Dipneumonomorphae. (For references used in the classification of the specimens see list at end of general bibliography).

In the present investigation no significant differences were found in any of the specimens studied in so far as either morphological or functional relationships were concerned.

MORPHOLOGICAL CONSIDERATIONS

A. SKELETON.

As in all arthropods, the skeleton of spiders is external. It is composed of two well defined layers, the cuticle and the underlying hypodermis. This condition maintains for the chitinous covering of the walking legs as well as for the covering of the rest of the body.

The walking legs are made up uniformly of seven segments which have been named differently by various workers. Milne-Edwards (1890) for instance, has named them proximo-distally coxopodite, basipodite, meropodite, carpopodite, propodite, and first and second dactylopodites. This system was also used by his pupil, Gaubert (1892). Most of the recent workers, however, have followed the nomenclature suggested by Schimkewitsch (1884) in which the sections of the leg from base to tip are called coxa, trochanter, femur, patella, tibia, metatarsus, and tarsus (Petrunkevitch, 1909; Comstock, 1913; Wood, 1926; Savory, 1931; Brown, 1939). It appears that for arthropods belonging to groups higher than the crustacea the nomenclature of Schimkewitsch is most commonly followed, and it will be used hereafter in this paper.

Because of their value as taxonomic characters, the external aspects of the legs, i.e., the placement of spines, hairs, etc., have been described frequently. Wood (1926) has described in considerable detail the skeleton of the three

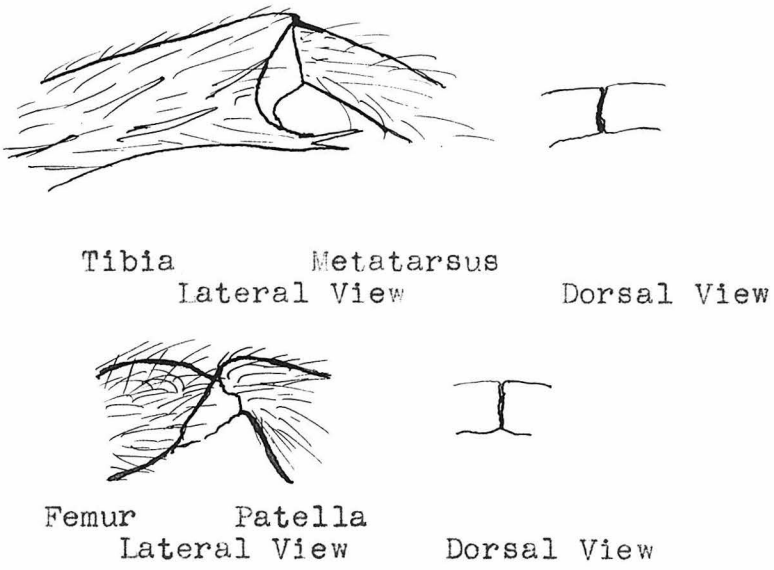
most proximal segments, i.e., the coxa, trochanter, and the proximal portion of the femur. Her work covered representative groups of arachnids including a dozen or more species of the Araneae. For the purposes of this investigation, however, the skeleton of the leg can be considered as a series of hollow cylinders, the only significant parts of which are the structures to be found at the respective joints. In this regard should be mentioned the thin flexible membrane on the ventral side of the leg at both the femoro-patellar and tibio-metatarsal joints.

The two above-mentioned joints are of especial significance because of the unique absence of extensor muscles. The articular union of the respective segments, i.e., the position of the fulcra of the joints, is, moreover, such as to make muscular extension impossible. Examination of Fig. 1 will show that in order to produce this movement it would be necessary for the tendon of the muscle to extend over the dorsal side of the articular membrane, in other words, outside the skeletal shell, a condition which is certainly not encountered.

B. TENDONS.

The tendons of the arachnid appendages have been briefly discussed by Gaubert (1892). He pointed out that "les tendons sont formes par des prolongements internes de la cuticule." The tendons are thus to be considered as cylindrical or oval invaginations of the cuticle, the surface of which is covered by a thin hypodermal membrane (continuous

Figure 1. Diagram of the Tibio-metatarsal and Femoro-Patellar Joints in the Tarantula.



with the skeletal hypodermis) which is "en rapport" with the muscular fibers.

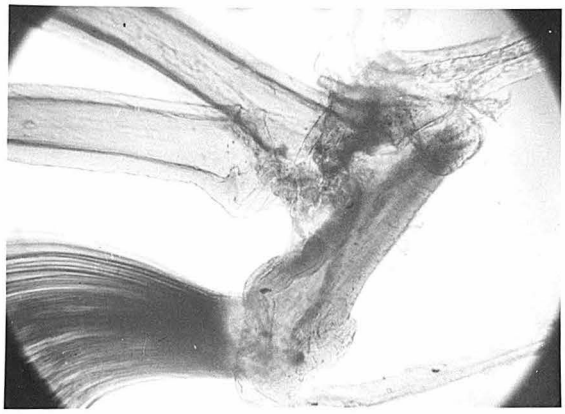
Woodworth (1908) has also emphasized the invaginatory origin of tendons in the legs of insects. It is significant that the tendons in the legs of insects and of spiders are not to be considered as extensions of the muscles, but as processes of the more distal segments upon which the respective muscles are inserted. The tendons of the other arthropods are likewise of this type, and are, therefore, not homologous with those of vertebrates in which the tendons are continuations of the sarcolemma of the muscle.

The cuticular nature of the tendons in spiders, as in insects, is shown at ecdysis by the fact that the tendons are shed at each molt. Upon examining a molt of Delopelma helluo the following tendons have been easily identified:

a). Tarsus and Metatarsus. In the tarsus are found two cylindrical bands of small diameter arising respectively from the upper and lower margins of the basal structure of the claws (see Fig. 2). Both of these tendons extend beyond the tarsus, the ventral one reaching its muscular component, the m. depressor unguium, in the extreme proximal portion of the metatarsus; the dorsal one uniting with the m. levator unguium which arises from the distal dorsal region of the tibia. (The names of the muscles are those of Petrunkevitch, 1909).

b). Tibia. From the ventral tibio-metatarsal inter-articular membrane arises a chitinous plate extending proximally

Figure 2. Photomicrograph of Dissected Claw Structures of a Tarantula, showing the Tendon Attachments.



for a short distance. This horseshoe-like plate is clearly an homologous structure with the plate found in the femoro-patellar joint. The latter structure was described by Gaubert (1892), but has been consistently ignored by subsequent investigators, and the presence of the structure in the tibia has, to my knowledge, never been reported. From the margin of this plate extend the tendonous strands to the m. flexor metatarsi bilobatus.

c). Patella. The only tendon observed in the patella of the molt appears to be the short radiating strands arising from the proximal ventral margin of the tibia. These strands provide the attachment for the large m. flexor tibiae. The other muscles of the patella apparently insert without tendons.

d). Femur. As in the tibio-metatarsal joint, the femoro-patellar joint is characterized by the presence of a conspicuous chitinous plate arising from the ventral interarticular membrane. The tendonous strands projecting from this plate make connection with the m. flexor patella longus in a manner wholly homologous with the aforementioned tibio-metatarsal joint. From the lower lateral margins of the proximal lip of the patella extend the short tendon fibers to which the m. flexor patellae bilobatus is attached.

e). Trochanter and Coxa. The tendons and the musculature of the trochanter and coxa have been adequately described by Wood (1926) and need no further discussion.

C. MUSCULATURE.

The musculature of the spider has been studied by a number of investigators (Treviranus, 1812; Lankester, 1885; Schimkewitsch, 1884, 1895; Petrunkevitch, 1909; Brown, 1939). The majority of these investigations have been limited to the musculature of the abdomen and endosternite; there have been, in fact, very few papers dealing with the musculature of the walking legs (Gaubert, 1892; Friedrich, 1906; Petrunkevitch, 1909; Wood, 1926). Petrunkevitch (1909) pointed out that most of these reports contained errors of a more or less serious nature. In a footnote he states, for instance, that

> "In the second volume of his textbook of experimental zoology which has just appeared Przibram reproduces Fredericq's diagram of the muscular system of a spider-leg. Evidently misled by Garber's old interpretation of the muscular system of appendages in Arthropods, he figures a flexor and an extensor in every segment of the limb. However, even a superficial examination of the articulations cannot fail to show that his supposed extensors are in reality flexors. If he had noticed that the axes of the patellar and metatarsal articulations lie not on the ventral side of the limb, but in its episynaxial (dorsal) surface, he would have escaped this error."

This concept that both flexor and extensor muscles occur in each segment of the leg is still widely accepted. Comstock (1913, 1941) reproduced the erroneous figure of Börner, and Savory (1931) presents a figure drawn by some of his students in which the same mistake occurs in spite of the fact that Petrunkevitch clearly stated in 1909, that no extensor muscles are to be found in the femoro-patellar and tibio-metatarsal

joints.

Because of the fragmentary nature of the literature concerning the structure and function of the leg muscles in the Araneae, it has been necessary to reinvestigate the entire matter.

Examination of the muscles of spiders shows a characteristic simplicity in the association of the component fibers, i.e., the muscle fibers are characterized by their loosely parallel arrangement, each fiber running the full length of the muscle. The fibers themselves are nearly transparent in the living animal, and are distinguishable only with difficulty from the nerve and blood vessel in the appendages. In stained preparations the fibers of the leg muscles are seen to be striated and multinucleate. The striation, moreover, shows the myo-fibrillar nature of the sarcomeric bands, and the typical divisions of the fiber into sarcomeres which are separated from one another by the characteristic "Z" disks (Krause's membrane). The darkly stained "Q" disk shows the lightly stained band in its mid-region known as Hensen's line (see Fig. 3 a, b).[1]

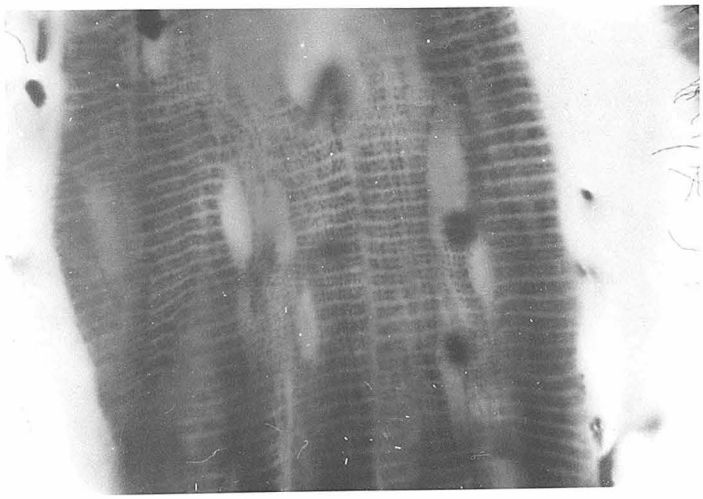
The histological study of spider muscles has been made from preparations of several types. 1) Teased fiber preparations have been made in which only a very few muscle

---

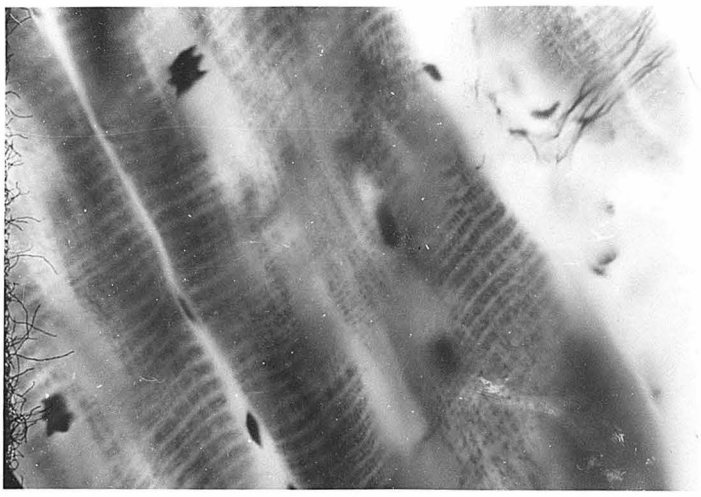
1. Careful examination of stained preparations show an apparent continuity of the striations from fiber to fiber. Whether or not this is real or merely an illusion is not clear, nor is a function for such a situation obvious.

Figure 3. Photomicrographs of Teased Muscle Fibers from the Leg Muscles of the Tarantula. (Stöhr Silver Nitrate-Sodium Hydroxide Method).

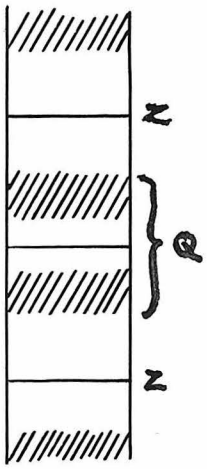
A



B



C

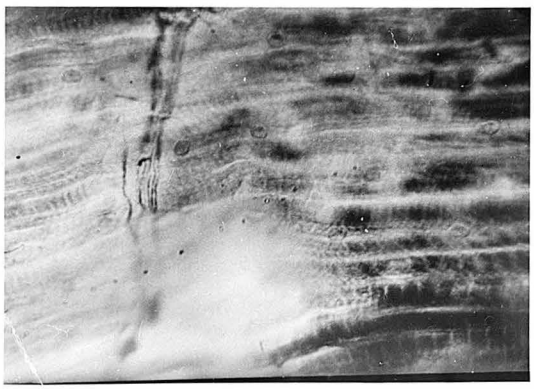


fibers from a given muscle were separated free from the rest, and stained with Stöhr's modification of the Schultze silver nitrate-NaOH technique (Stöhr, 1921). From these preparations it has been possible to observe the nature of the striations; the innervation, however, is not clearly shown by this method. 2) Sectioned preparations have been made using the paraffin method, and the sections have been stained with the Harris hematoxylin-eosinol method of Krajian (1940) and the protargol-gold chloride methods of Rogers (1931) and Bodian (1937). All of these methods have given fairly satisfactory preparations for studying the structure of the muscle fibers, but none of them has shown an adequate staining of the nerve fibers or their endings. A few preparations have shown isolated regions in which nerve fibers are visible (Fig. 4) but no satisfactory study of the innervation has been possible.

A survey of the disposition of the respective muscles in the legs shows that the musculature of the coxa and trochanter has apparently been quite adequately described by Petrunkevitch (1909), Wood (1926), and Brown (1939), and that the muscles of the metatarsus, tibia, and patella have, for the most part, been accurately described by Petrunkevitch (1909) and by Brown (1939). The musculature of the femur, however, and perhaps that of the tibia requires a more careful investigation as to the origin and insertion of the fibers, and the function of the respective muscles.

Concerning the musculature of the femur, a few

Figure 4. Photomicrographs of Isolated Regions in the Muscle Fibers of the Leg Muscles of the Tarantula Showing Nerve Fibers. (Silver Nitrate-Sodium Hydroxide Method of Stöhr).



interesting observations should be made. As can be seen in Fig. 5, there are four muscles in this segment of the leg. These form two pairs, one of which arises from the ventro-lateral lip of the trochanter and from the ventral portion of the femur and extends ventrally and medially the full length of the femur, inserting on the proximal margin of the chitinous horseshoe-shaped plate. These are the flexor patellae longi described by Petrunkevitch (1909) and, except for the insertion on the chitinous plate instead of on the proximal lip of the patella, have been accurately described. Whether or not they represent two bellies of a single muscle is not certain, but this may very well be the case.

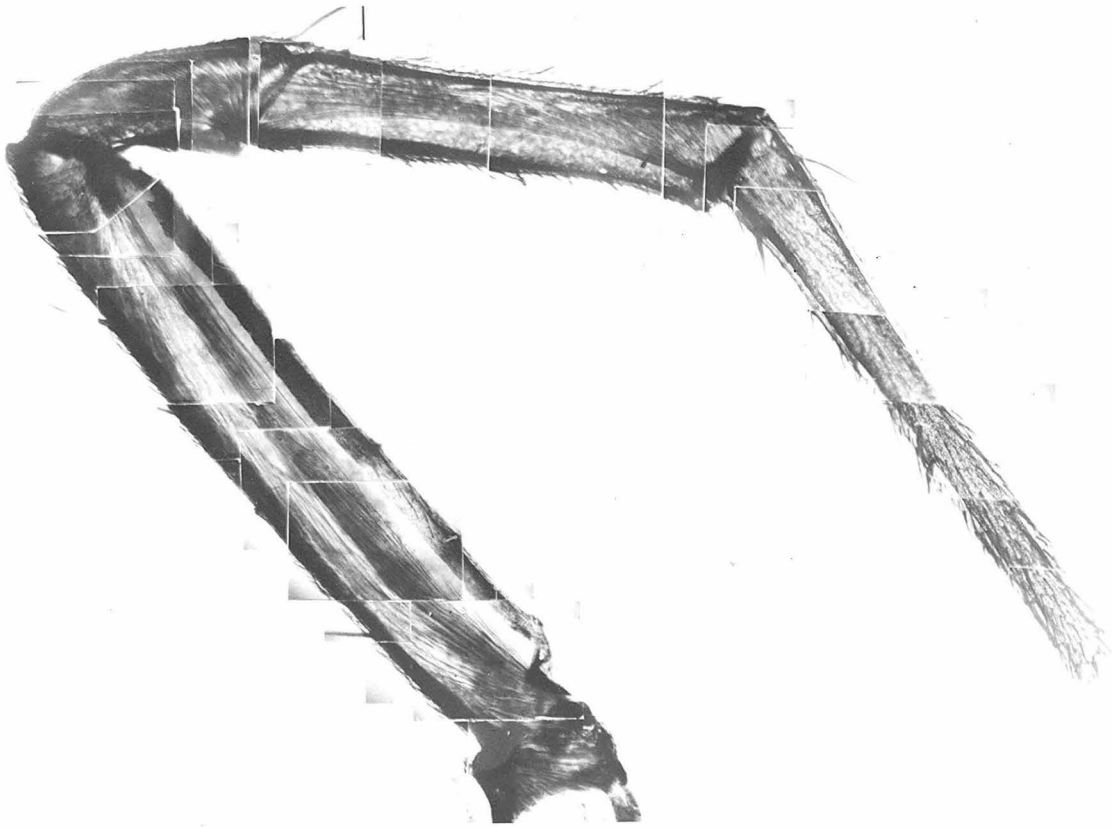
The other pair, lying laterally and dorsally, arise from the dorsal surface of the femur and insert directly on the proximal lip of the patella, as was described by Petrunkevitch (1909), who named them the flexor patellae bilobati.

A few isolated muscle fibers, arising from the distal dorsal wall of the femur and inserting on the dorsal side of the chitinous plate (Fig. 6) seem to be uniformly present in sagittally cut preparations.[2] It was at first thought that

---

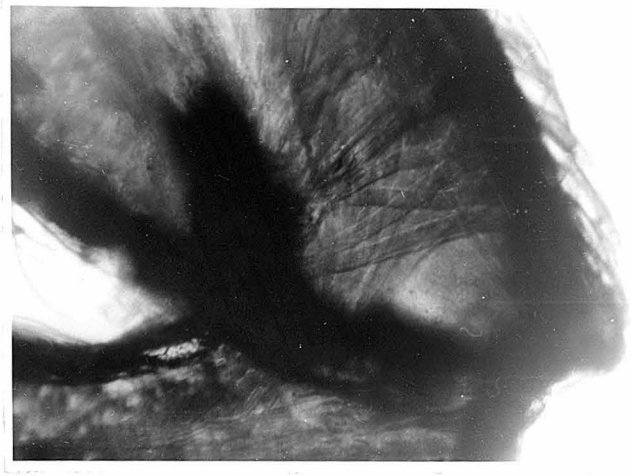
2. Method: The above sagittal preparations were made by first fixing the isolated leg in Petrunkevitch's (1933) fluid (Copper tri-nitro-phenol), dehydrating with graded alcohols, xylol, and imbedding in paraffin. The paraffin block was then trimmed under the dissecting microscope with a sharp scalpel until the mid-sagittal surface was obtained. The excess paraffin was dissolved away in xylol, the half-leg was washed through several fresh xylol baths, and was finally mounted in balsam.

Figure 5. Photomicrograph (composite) of Mid-sagittal Section Through Walking Leg of Miranda aurantia.



Note: The magnification is represented by the 1 mm. bar which was taken from a calibrated stage micrometer at the same magnification as the other photomicrographs.

Figure 6. Enlarged View of the Femoro-patellar Joint in Miranda aurantia, Showing the Fibers which Elevate the Chitinous Plate.



these fibers might be artifacts resulting from the cutting procedure, but care has been taken to make the cut from different directions with respect to the femoro-patellar joint, and in all cases these fibers were found to lie in almost identically the same position on the dorso-ventral median line of the femur. It appears, therefore, that these few fibers form an heretofore undescribed muscle, the function of which is the elevation of the chitinous plate.

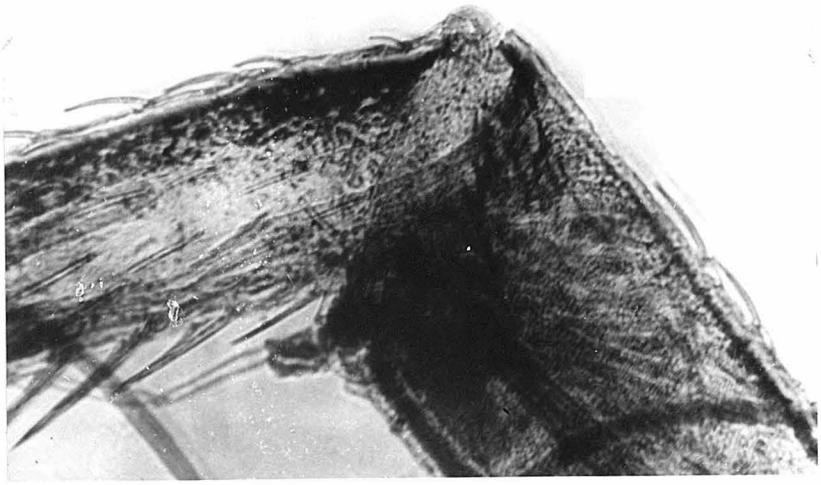
It is probable that this group of fibers has been overlooked because of the fact that they run in nearly the same direction as the more laterally located fibers of the m. flexor patellae bilobatus. Careful examination of Fig. 6 a, will show, however, that these large lateral muscles insert on the chitin ring of the patella, not on the plate.

It is not clear at present whether or not such a collection of fibers is present in the tibio-metatarsal joint, but in at least one preparation of a leg of the crab spider, Misumena aleatoria, it seems that such may actually be the case ~~(Fig. 7)~~ Fig. 7.

The observation has been made that all of the spiders studied show the same distribution of muscles in the legs. This survey has, moreover, included the very long legs of the false harvestman, Pholcus phalangioides.

A careful examination has been undertaken to demonstrate experimentally the function of each of the muscles in these segments of the appendages more distal than the trochanter.

Figure 7. Sagittal section through the tibio-metatarsal joint of Misumena aleatoria, showing the chitinous plate and possibly a few fibers inserting on the dorsal surface of this plate.



Most of the studies have been made on the walking legs of the tarantulas, Aphonopelma cryptethus and Dugesiella californica, although some observations have been made on the legs of the garden spider, Agelena naevia, the black widow, Latrodectus mactans, and the golden garden spider, Miranda aurantia. The leg was opened with a pair of small scissors and the chitin from the side away from the muscle to be studied was removed with a pair of fine forceps. Small platinum electrodes were then applied to the specific muscle, manipulations being carried out with the aid of Zeiss micro-manipulators and a dissecting microscope. Stimulation was applied using a Dubois-Reymond inductorium, the frequency generally being about 45 shocks per second. Contraction of the muscle was observed under the microscope to establish the selectivity of stimulation and movements of the parts was observed visually.

The muscles of the femur showed the following functions: 1) The lateral flexor patellae bilobati produced strong flexion of the femoro-patellar joint. 2) The more ventral flexor patellae longi also showed a flexor movement, but this was of a much weaker intensity.

The muscles of the tibia were found to show a similar situation, the strongest flexor movement having been obtained from stimulation of the dorso-lateral flexor metatarsi bilobati, while the flexor metatarsi longi, although giving extension, were much weaker. Stimulation of the muscle in the most distal

dorsal area of the tibia (in an attempt to find a levator for the plate in the tibio-metatarsal joint) produced flexion of the tarsus and drawing of the claws together and some flexion of the metatarsus.

Stimulation of the two muscles in the patella and the single flexor unguium in the metatarsus gave the expected movements, i.e., the respective lateral movements of the tibia and depression of the claw.

Selective stimulation of the few fibers which arise from the dorsal sagittal surface of the distal end of the femur and insert on the dorsal surface of the chitinous plate was not possible; every attempt at stimulation produced contraction of the flexor patellae bilobati. In a further attempt to determine the function of this small group of fibers a limited number of observations have been made in which electrodes have been inserted into the most distal area of the femur in the living intact animal. In no case was it possible to obtain any response other than flexion. In one such experiment, for instance, stimulation with a coil distance of more than 15 cm. gave no response whatsoever, and anything less than 15 cm. uniformly gave flexion of the joint. In the same preparation placement of the electrodes in the tibia gave similar results. It is doubtful, however, that these fibers exert any flexor activity in view of the fact that their force can be exerted only upward, lifting the chitinous plate. It should be emphasized that this plate is attached

at approximately its center to the proximal and distal portions of the thin flexible ventral membrane of the joint and can therefore, hardly be expected to have any marked flexor component to simple elevation of the plate.

D. CIRCULATION

The circulatory system of spiders has been studied by a number of workers, the most important of whom have been Blanchard (1853), Causard (1892, 1893, 1896), Willem (1918), and Petrunkevitch (1910, 1922). These investigators, however, like those who dealt with the musculature and the nervous system, have limited their investigations to the circulatory system of the cephalothorax and abdomen, passing over the circulation in the appendages in a most superficial manner. It is not the purpose of this paper to present any comprehensive survey of the circulatory system of spiders, but it is imperative that some understanding of the mechanisms of circulation be had in order to correctly interpret certain locomotor mechanisms which are to be discussed later.

As in other arthropods, the spider heart consists of a tubular muscular organ perforated by a variable number of ostia, extending anteriorly into the aorta. In spiders the heart lies entirely within the abdomen. The aorta traverses the pedicel whereupon it divides laterally, circumscribing the oesophagus, numerous branches arising from either side. Directly lateral to the oesophagus, the aortic forks send forth ventrally a thick branch which ends by forming a more

or less conspicuous bulbous sinus from which arise the arteries of the appendages (Petrunkevitch, 1910).

The further distribution of the arteries into the muscles and tissues of the leg has never been adequately described. Causard (1896) has figured these arteries as simple uninterrupted tubes (one to each leg) emptying into the cavity of the leg by a single perforation in each segment except the tarsus in which the artery ends as an open tube. Petrunkevitch (1910) states that it is possible to trace the pedal arteries as far as the claw, but says nothing concerning any branching within the leg. Branching occurs, however, and is present to an elaborate extent. By the simple expedient of injecting an animal with a colored latex medium[3] it has been possible to demonstrate a most profuse branching of the main pedal artery[4] (Fig. 8 a, b).

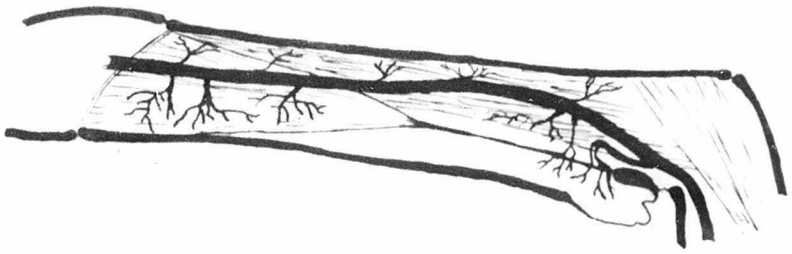
---

3. The author wishes to express his appreciation to Mr. C. Blair Coursen of the General Biological Supply House, Inc., who kindly made available a sufficient supply of the latex injection medium.

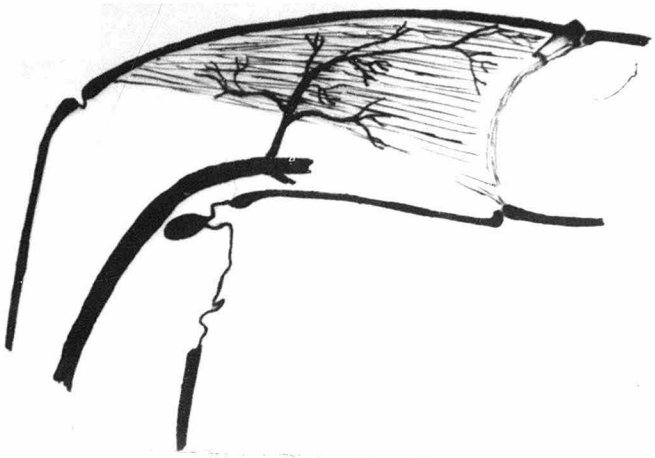
4. In order to obtain any degree of success with injection of the arterial system in the legs of spiders it was found necessary to remove the tips of the tarsi to provide an escape pathway for the increased pressure created by injection of the alkaline fluid into the abdomen. The method of injection, therefore, was as follows: The tarantula, or other spider, was first killed by placing it in a bottle containing a few drops of chloroform. The diluted alkaline latex solution was sucked up into the injection pipette, the tip of which had been drawn out into a rather fine capillary. The pipette was inserted through the dorsal wall of the abdomen, and the latex medium was forced into the animal with compressed air. Dilute HCl was injected by hypodermic syringe using a very small needle, forcing the acid into the body and appendages at various points thus setting the latex. It was found that when the legs were left intact no injection fluid was able to penetrate the vessels of the appendages, but when the tips of the legs were removed, a most satisfactory injection of even the finest branches was obtained.

Figure 8. The Distribution of Arterial Branches in the Tibia and Patella of the Tarantula.

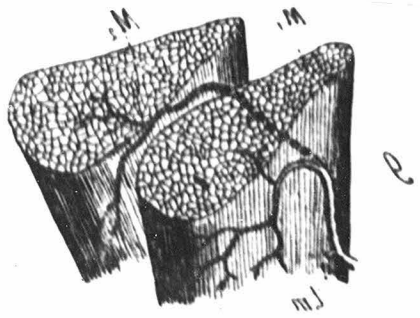
A.



B.



C. Drawing of Distribution of Arterial Branches in the Muscles of the Cephalothorax of Spiders (After Causard).



The branches arborize into the muscles in a manner quite similar to that described by Causard (1896) for the muscles of the cephalothorax (Fig. 8 c).

Being an open circulatory system, the venous arc is sinusoidal. Causard (1896) pointed out, contrary to Blanchard (1853) that no true membrane forming a distinct venous canal is present, but that the blood returns through the cavity of the appendage and freely bathes the surrounding tissues. A similar situation exists within the cavity of the body, the blood being carried by hydrostatic pressures into the pericardial sinus, from which it flows through the ostia to passively refill the heart during diastole.

These morphological details will be considered further in their relationship to the extensor mechanism in the legs of spiders.

THE MECHANISM OF EXTENSION

Petrunkevitch (1909) demonstrated that the muscular system of the legs of spiders is characterized by the absence of extensor muscles in most of the segments. This presents a problem of considerable importance in view of the alacrity of extensor movements in the femoro-patellar and tibio-metatarsal joints. He attributed to the "elastic properties of the interarticular membrane" the function of extension (1909, 1916, personal communication 1940). Gaubert (1892), on the other hand, gave the elasticity of the interarticular membrane the role of accessory flexion. It is my opinion that the interarticular membrane possesses little if any elasticity.

Were such a mechanism involved in extension it would seem imperative that the spider eventually assume a position of complete extension in death. This is not the customary position; spiders nearly always die with their legs completely flexed. In fact, so characteristic is this flexed position in death that some spiders assume the position when startled, as a protective reflex. This "sham death reflex" has been described and discussed by Robertson (1904).

This position taken in death would seem to be confirmatory to Gaubert's concept were it not for the fact that the membrane itself shows little if any elasticity. A rather large number of isolated legs from spiders of a number of different genera have been examined as follows: The leg was removed at the trochanter and the chitin covering the ventral half of the femur was removed, due care being taken to leave both the ventral and dorsal interarticular membranes intact. The muscles in the femur were carefully transected as distally as possible. The leg was then cut through the patella. The same procedure was carried out in the tibia, and in neither case did extension or flexion occur. In fact, the joint remained in precisely the position in which it was manually placed. This would certainly appear to render untenable any theory basing extension or flexion on an elasticity of the interarticular membrane.

How, then, does extension occur?

Osterloh (1922) has pointed out, concerning the

movements of the tarsal organs in male spiders,

> "Im männlichen Kopulationsapparat findet sich kein Muskel, welcher in der Lage wäre, diesen vom Ruhe in der Kopulazustand zu versetzen. Vertreten wird die Muskelwirkung durch die je nach Bedarf mehr oder weniger schwellbare Haematodocha. Und durch diese höchst einfache Lösung wird eine Vielgestaltigkeit der Bewegung erreicht, wie sie nur durch eine Unzahl möglich wäre."

An hydraulic mechanism appears to be present, also, to provide extension in the tibio-metatarsal and femoro-patellar joints of the walking legs.

It has been observed repeatedly that removal of the leg by cutting with scissors through the coxa or trochanter (or at any more distal point) is accompanied by an initial extensor movement of the distal joints. This can be seen even more markedly by taking the isolated leg between the thumb and finger and gently squeezing it. Gaubert (1892) reported the phenomenon and considered it as indicative of an extensor mechanism accessory to the "extensor muscle" which he erroneously considered to exist.

Injection of fluid by means of a hypodermic syringe into any part of the leg, either isolated or on the living or dead animal, is accompanied by extension of all parts distal and proximal to the point of injection. Injection into the abdomen or the cephalothorax also produces extension of the appendages. The pressure necessary for producing this effect is, moreover, small, a very light touch on the plunger of the syringe being sufficient to produce extension.

Petrunkevitch, although not mentioning the possibility of an hydraulic extensor mechanism, has observed the above phenomenon, having used it as an indication of the completeness of injection while studying the circulatory system (Petrunkevitch, 1910). He said, "Die Injektion musz so lange fortgesetzt werden, bis alle Beine straff auseinandergespreizt bleiben und die Stacheln an ihnen nahezu senkrecht abstehen." It should be pointed out in this connection that in injection experiments in which the circulatory system of the legs have been studied, extension still occurs during application of pressure to the syringe despite the cutting off of the claws to afford an escape of pressure, but on release of the applied pressure the legs quickly flex.

Spiders always appear to be quite turgid. This is seen on observing the great loss of blood following even a small perforation of the chitin of any living spider, whether the hole be in the abdomen, cephalothorax, or appendage. The pressure of this turgidity is considerable. On inserting a six inch capillary tube into the femur of a living tarantula, a column of blood was seen to climb higher and higher, pulsating with each heart beat, until it finally overflowed the tube. When the tube was tested, however, by immersing it in a beaker of salt solution, the rise due to capillarity was, at most, 1 cm. The same experiment was repeated with a 5 mm. layer of mercury included in the capillary, and in this experiment, also the fluid overflowed the tube.

As spiders become dehydrated they show, prior to death, a condition in which walking is not accompanied by extension of either the femoro-patellar or tibio-metatarsal joints. The spider walks with the legs in a more or less markedly flexed position. In such an animal (tarantula) injection of 2 ml. of a salt solution[5] was accompanied by a period in which the two above-mentioned joints were extended as readily as in a normal animal. It should be recalled, however, that any perforation is accompanied by a considerable loss of blood, and upon removal of the injection needle from the abdomen, the animal bled profusely and in a short time again lost the ability to extend the legs. A second injection of physiological solution resulted once more in a normal condition, and when the hole was closed with collodion the animal ran about his cage with more life than had been observed for several days. These observations were made on spiders which had been kept for several weeks without water to drink, a procedure which resulted in considerable dehydration of the animal. The blood pressure of the spider has also been lowered experimentally by withdrawing an

---

5. At first a salt solution known widely as "fly Ringers" was tried, but this proved to be quite toxic to the tarantula, resulting in death within a very few minutes. Regular "frog Ringers" solution also proved unsatisfactory. Since these solutions both have a molar concentration somewhat lower than that of the physiological salt solution for crayfish described by van Harreveld (1936), this latter fluid was tried and was found to be quite satisfactory. Use of this fluid kept the animal alive for somewhat longer than one hour following injection of the animal, so it was not considered necessary to determine the isotonicity of tarantula blood.

appreciable volume of blood via a small hypodermic needle, whereupon extension became markedly slower and less effective. Replacing the withdrawn blood with physiological solution gave a return of quick extensor movements of normal magnitude.

It has been pointed out that spiders characteristically die with the legs in a strongly flexed position. An interesting exception to this generalization was observed in the case of a male tarantula (Delopelma helluo) which fell into the water dish in its cage, and drowned. In this animal the legs were almost fully extended. Furthermore, the slightest pressure applied to the abdomen or to one of the legs was sufficient to not only extend the legs maximally, but to raise the spines as well. It is evident that the spider in this case had imbibed the water to such an extent that an abnormal turgidity occurred.

A corollary of this effect has been seen while attempting to make an histological preparation of spider legs in an extended position. The legs of a number of animals were removed and tied tightly around the femurs with thread. This sufficiently compressed the legs to cause extension of both distal joints. Dehydration with alcohol prior to imbedding in paraffin, however, was invariably accompanied by a loss of the extended position, the legs assuming a semi-flexed position.

In studying the effects of electrical stimulation of the leg nerve, it has been found that very strong stimulation

with electrodes inserted through the chitin of the femur resulted in extension of the two joints in question. This response, however, was considerably delayed. Following removal of the tip of the tarsus, such stimulation resulted in a much less pronounced extensor movement and was accompanied by the appearance of a growing droplet of blood at the severed end. Sealing the tip with plastacine resulted in extensor movements of the original height. This strong stimulation was of such an intensity (coil distance of 3 to 1½ cm.) that bubbles of hydrogen and oxygen appeared on the electrodes, and it is likely that the accumulation of gases was sufficient to produce the extensor phenomenon. With but few exceptions stimulation with weaker currents resulted in a flexor movement.

In two preparations stimulation of the nerve in the femur with coil distances of 11.0 and 12.0 respectively resulted in extensor movements of the tibio-metatarsal joint. This would seem to indicate that extension is under nervous control.[6]

In the intact animal cutting the tip of the leg produces a condition in which the leg in question is not extended upon stimulation of the animal in a manner which normally results in extension of the appendages, e.g., holding the animal in an inverted position. Sealing the tip restores the extensor

---

6. Rijlant (1932) has reported that in arachnids an electrical activity of a frequency of from 2 to 20 per second is present which is maintained only while the animal is on its legs. When the spider rests on its abdomen the activity becomes weaker and disappears. He states that passive elongation of the leg gives a reduction in extensor activity (0 to 5 per sec.) and an increase in flexor activity (30 to 70 per sec.).

mechanism. The same effect has been obtained in Miranda aurantia by opening the chitin of the femur on the intact animal. In such an animal pressure applied distal to the opening prior to sealing the hole results only in extension of the tibio-metatarsal joint, but pressure applied proximal to the opening results only in a marked effusion of fluid from the hole. Likewise, in an intact isolated leg pressure on the femur results in extension of both the tibio-metatarsal and femoro-patellar joints, but when the chitin of the femur is opened, pressing on the femur distally to the cut still gives extension of the two joints, but pressure applied proximally to the cut results only in loss of fluid from the aperture.

It is apparent, therefore, that the turgidity of the spider must be kept high if maximum extensor activity is to be maintained.

Ten Cate (1931) states that in Celaenia cutting off the abdomen results in stretching of the legs and an attempt to escape, but once the abdomen is removed, the animal falls down as in death, i.e., the legs are flexed and no extensor movements occur.

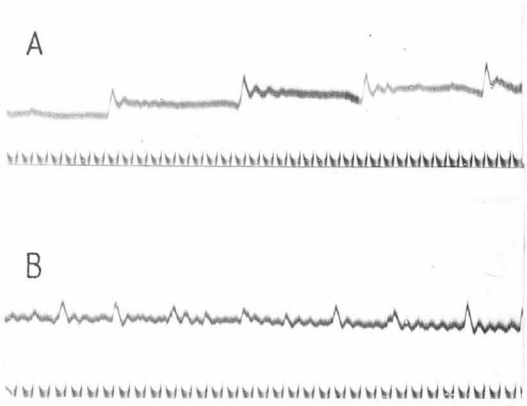
The observation was made by Plateau (1887) and by Gaubert (1892) that when spiders are suspended so that one or more of the legs are without a point of rest, those legs successively extend and flex through a small arc at a frequency identical to and synchronous with the systolic and diastolic

phases of the heart beat. Willem and Bastert (1918) and Willem (1920) made use of this movement of the tarsi to record changes in the cardiac rhythm when the animal was stimulated by various means, e.g., shining a bright light in the face of the spider. They were able to show that changes in position of the leg were more rapid during systolic extension than during diastolic flexion, and could thus be considered a true picture of the sequences of cardiac pulsation. It has been possible to confirm the synchronization of such movements with the beat of the heart in the crab spider, Misumena aleatoria, in which the movement of the dorsal chitin of the abdomen directly over the heart was most conspicuous. Moreover, it has been possible to record the electrocardiogram of the tarantula, Aphonopelma cryptethus, from electrodes inserted in the femur, as can be seen in Fig. 9. It should be pointed out, however, that the records were made from different individuals at different times so the amplitude and frequency of the two records are not comparable.

Steiniger (1934), observing these rhythmic movements of the appendages did not try to correlate them with the cardiac dycle, but attempted to explain them on the basis of a "Hemmungsreflex".

In Agelena naevia a number of observations indicate that only the femoro-patellar and tibio-metatarsal joints are involved in the above phenomenon. The ventral thin-walled

Figure 9. Electrocardiogram of Tarantula.



A = Electrocardiogram recorded with electrodes inserted into the abdominal cavity.

B = Electrocardiogram recorded with electrodes inserted into the femur of one of the walking legs.

sac at each of these joints showed balooning with the extensor phase of each such movement.

In order to further investigate the nature of the extensor system, an intact living specimen of Agelena naevia was lightly etherized and a hole was made in the ventral membrane of the femoro-patellar joint. The distal part of the leg barely showed perceptible extensor movement, but when the hole was closed with plastacine or collodion the leg became almost indistinguishable from the other unopened legs. In the same animal, complete transection of the metatarsus at approximately the mid-point did not stop extension in either the femoro-patellar or tibio-metatarsal joint. The extensor movements appeared to be weaker than normal, but were certainly present. Opening of the membrane at the tibio-metatarsal joint stopped extension at only that joint. Whether or not extension of this joint can occur when the femoro-patellar membrane is opened is not certain, but experiments on both Latrodectus mactans and Agelena naevia have shown that if the femoro-patellar membrane of a chloroformed spider (not dead) is opened, immediate strong flexion of that joint occurs without interfering with the extended position of the tibio-metatarsal joint of the same leg. In chloroform narcosis the legs are quite frequently kept in an extended position, the membranes showing considerable turgidity.

It appears to be more than coincidental that the only two joints devoid of extensor muscles and thus dependent on an

hydraulic extensor mechanism should likewise be the only joints in which are to be found the chitinous horseshoe-shaped plates described earlier in this paper. In fact, the inference is clear that this plate is in some way involved in the extensor mechanism. In order to examine the role which it may play in this function, a study of the movement of the plate during flexion and extension of the leg has been made. Such motion is more or less easily watched through the thin transparent membrane of the ventral side of the joint. The observation has been made that during flexion of the femoro-patellar joint the chitinous plate remains close to the surface. This is to be expected from the position of the attachments of the respective muscles, the flexor patellae bilobati attaching directly to the lip of the patella, whereas the flexor patellae longi, lying ventrally, is able to pull the plate in only a horizontal position. It is likewise obvious that during flexion the small group of fibers forming the levator of the plate can hardly be active, since the plate remains in a superficial position.

The pouch formed by the membrane on the one side, and the tendons and muscular components of the plate on the other, is balooned out by an inflow of blood with each extensor movement, hence during extension it was not possible to determine whether the plate is pulled deeper or whether this only appears to be so because of the balooning effect. At any rate, one loses sight of the plate during extensor movements.

In recapitulation, therefore, the absence of extensor muscles in the legs of spiders presents the problem of accounting for the quick extensor movements characteristic of these animals. The theory that the "elasticity" of the interarticular membrane might be the means whereby this is accomplished has been shown to be untenable. Experimental evidence has been presented showing that extension in intimately associated with the turgor of the spider leg, which is, in turn, associated with the blood pressure of the animal. In fact, the extensor phase of the slight movement of the legs in suspended spiders has been shown to coincide with the systolic phase of the cardiac cycle. It has been shown, moreover, that the three structures characteristic of the two joints devoid of extensor muscles are 1) a large sac-like space limited on the ventral side by the thin interarticular membrane and on the dorsal side by the muscle fibers and their tendonous insertions, and 2) a chitinous horseshoe-shaped plate to the dorsum of which is inserted 3) the small group of short fibers with the apparent function of lifting the plate.

It is my contention that elevation of this plate is in some way responsible for the extensor movements. The mechanism of this control, moreover, must be intimately associated with the movement of fluid in the leg. Two possible mechanisms would seem feasible; 1) if the venous return through the spaces in the leg were cut off by movement of the muscles, the pressure in the leg would increase and extension would

occur by the same principle of hydrostatics which produces extension of a coiled flexible tube upon passage of a stream of air through the tube, or 2) if the arterial pressure were diverted so as to increase the fluid in the membranous sac at the joint, the distal segment would be mechanically pushed into a position of extension.

A careful examination of the distribution of the main arterial trunk in the appendage shows that, although it runs somewhat dorsally through the major portion of the femur and tibia, on approaching the respective joints, it dips ventrally and passes in close proximity over the chitinous plate. In several preparations of the walking legs of tarantulas it appears that a rather large branch springs from the main artery just proximal to the plate, and appears to dip ventrally, arborizing laterally in the vicinity of the aforementioned membranous sac (Fig. 10).

This distribution strongly suggests that the mechanism involved in extension of the joint is simply that of partially closing off the main arterial flow, thus diverting a greater amount of the blood into the region of the membranous sac, balooning the latter structure and producing extension by mechanically pushing the distal segment. Such a mechanism has the distinct advantage of selectivity, either the tibio-metatarsal or femoro-patellar joint being able to extend independently.

It is likely, although not certain, that the claws

Figure 10. Branching of Artery in the Tibio-metatarsal Joint of the Tarantula.

are spread apart by an increase in hydrostatic pressure, and pulled together by muscular action. Elevation and depression of the claws, however, are clearly accomplished by muscular action.

SUMMARY

The musculature of the legs of spiders of several species has been studied. For the most part the work of Petrunkevitch has been confirmed by anatomical observation and physiological experimentation; however, one and possibly two heretofore undescribed muscles are reported. The function of these muscles has not been demonstrated physiologically, but from their structural arrangement they may have a role in raising the chitinous horseshoe-shaped plate in the femoro-patellar and tibio-metatarsal joints respectively.

An histological study of the leg muscles in spiders has shown them to consist of long, striated, multinucleate fibers loosely associated in parallel groups to form the respective muscles; that is to say, the fibers run parallel from origin to insertion for the full length of the muscle.

Distribution of the arterial supply in the legs has been found to be quite extensive. Branching and re-branching of the main artery results in an elaborate arborization intimately distributed throughout the muscles.

A discussion is presented which indicates that in those joints which characteristically lack extensor muscles extension is carried out by means of an hydraulic mechanism.

Two possible mechanisms are suggested; the one considered most probable involves partially closing off the main arterial stem, thereby diverting a greater amount of blood into the membranous pocket formed by the thin flexible interarticular membrane of the ventral surface of the joint. Extension is thus a purely mechanical result of the pressure exerted by the balooning-out of the membrane.

LITERATURE CITED

Blanchard, M. E. 1853. Sur les mouvements du fluide nourricier chez les Arachnides pulmonaires. Comptes Rendus Acad. des Sci. 36:1079-1081.

Bodian, D. 1937. The staining of paraffin sections of nervous tissue with activated protargol. The role of fixatives. Anat. Rec. 69:153-162.

Brown, R. B. 1939. The musculature of Agelena naevia. J. Morph. 64:115-166.

Cate, J. ten 1931. Physiologie der Gangliensysteme der Wirbellosen. Ergeb. Physiol. 33:137-336.

Causard, M. 1892. Sur la circulation du sang chez les jeunes Araignées. Comptes Rendus Acad. des Sci. 114:1035-1038.

Causard, M. 1893. Sur l'appareil circulatoire de la Mygale caementaria, Walck. Comptes Rendus Acad. des Sci. 116:828-830.

Causard, M. 1896. Recherches sur l'appareil circulatoire des Araignéides. Bull. sci. de la France et de la Belgique 29:1-109.

Comstock, J. H. 1913. The Spider Book. Doubleday, Page and Co. New York.

Comstock, J. H. 1940. The Spider Book. Revised Ed. Doubleday, Doran. New York.

Friedrich, P. 1906. Regeneration der Beine und Autotomie bei Spinnen. Arch. f. Entwick. d. Org. 20:469-506.

Gaubert, P. 1892. Recherches sur les organes des sens et sur les systemes tégumentaire, glandulaire et musculaire des appendices des arachnides. Ann. des sci. nat. Zool. 7e série 13:31-184.

Harreveld, A. van 1936. A physiological solution for fresh-water crustaceans. Proc. Soc. Exp. Biol. and Med. 34:428-432.

Krajian, A. A. 1939. Histological Techniques. C. V. Mosby St. Louis.

Lankester, E. R. 1884. On the skeletotrophic tissues and coxal glands of Limulus, Scorpio, and Mygale. Quart. J. Micr. Sci. N. S., 24:129-162.

Milne-Edwards, M. 1890. (Cited by Gaubert, 1892).

Osterloh, A. 1922. Beiträge zur Kenntnis des Kopulations-apparates einige Spinnen. Zeit. f. Wiss. Zool. 119:326-421.

Petrunkevitch, A. 1909. Contributions to our knowledge of the anatomy and relationships of spiders. Ann. Ent. Soc. Amer. 2:11-21.

Petrunkevitch, A. 1910. Über die Circulationsorgane von Lycosa carolinensis Walck. Zool. Jahrb. Abt. f. Anat. und Ont. der Tiere. 31:161-170.

Petrunkevitch, A. 1922. The circulatory system and segmentation in arachnida. J. Morph. 36:157-189.

Petrunkevitch, A. 1916. Morphology of Invertebrate Types. Macmillan New York.

Petrunkevitch, A. 1933. An inquiry into the natural classification of spiders, based on a study of their internal anatomy. Trans. Conn. Acad. of Arts and Sci. 31:299-389.

Rijlant, P. 1932. Les manifestations electriques du tonus et des contractions voluntaires et réflexes chez les arthropodes. Comptes Rendus de Soc. Biol. 111:631-635.

Robertson, T. B. 1904. On the "Sham Death" reflex in spiders. J. Physiol. 31:410-417.

Rogers, W. M. 1931. New silver methods for paraffin sections. Anat. Rec. 49:81-88.

Romeis, D. B. 1928. Taschenbuch der Mikroskopischen Technik. R. Oldenbourg München.

Savory, T. H. 1928. The biology of spiders. Macmillan New York.

Schimkewitsch, W. 1895. Über Bau und Entwicklung des Endosternits der Arachniden. Zool. Jahrb. Abt. f. Anat. 8:191-216.

Stöhr, Ph. d J. 1921. O. Schultze's Natronlauge-Silbermethode zur Darstellung der Achsenzylinder und Nervenzellen. An. An. 54:529-537 (Cited by Romeis, 1928, p 454).

Steiniger, F. 1934. Schutzstellung und akinetische Erscheinungen bei einigen Spinnen. Ein Beitrag zur Kenntnis visueller Anpassungserscheinungen. Zeit. wiss. Zool. 145:541-575.

Treviranus, G. R. 1812. Über den inneren Bau der Arachnider Zeit. f. Physiol. (Cited by Brown, 1939).

Willem, V. 1918. Observations sur la circulation sanguine et la respiration pulmonaire chez les Araignées. Arch. néerl. de Physiol. 1:226-256.

Willem, V. 1920. Les mouvements du coeur de la respiration chez deux espèces d'araignées. Arch. néerl. de Physiol. 4:452-453.

Willem, V., and C. Bastert 1918. Essais d'inscription des pulsations cardiaques chez une araignée. Arch. néerl. de Physiol. 2:285-289.

Wood, F. D. 1926. Autotomy in Arachnida. Jour. Morph. and Physiol. 42:143-195.

Woodworth, C. W. 1908. The leg tendons of insects. Am. Naturalist 42:452-456.

REFERENCES USED IN CLASSIFICATION

Chamberlin, R. V. 1940. New American Tarantulas of the family Avicularidae. Bull. Univ. Utah Biol. Series. Vol. 5:1-39.

Comstock, J. H. 1913. The Spider Book. Doubleday, Page New York.

Petrunkevitch, A. 1933. An inquiry into the natural classification of spiders, based on a study of their internal anatomy. Trans. Conn. Acad. Arts and Sci. 31:299-389.

Petrunkevitch, A. 1939. Catalogue of American Spiders. Trans. Conn. Acad. Arts and Sci. 33:133-338.

Petrunkevitch, A. 1939. The status of genus Eurypelma. Ann. and Mag. of Nat. History. Ser. 11, Vol. 4:561-568.

PART THREE

A COMPARATIVE STUDY OF PERIPHERAL INHIBITION IN DECAPOD CRUSTACEANS

REPRINTED FROM
THE JOURNAL OF EXPERIMENTAL BIOLOGY,
VOL. XVIII, No. 3, pp. 223–236, JANUARY 1942.

PRINTED IN GREAT BRITAIN

A COMPARATIVE STUDY OF PERIPHERAL INHIBITION IN DECAPOD CRUSTACEANS

BY C. A. G. WIERSMA AND C. H. ELLIS

William G. Kerckhoff Laboratories of the Biological Sciences,
California Institute of Technology, Pasadena, California

(*Received* 17 *June* 1941)

(With Two Text-figures)

INTRODUCTION

ALL the peripheral muscles of the decapod Crustacea receive, as far as is known, at least one inhibitory axon (Wiersma, 1941). A detailed study of the mechanism of this peripheral inhibition has been made for only a few muscles. Marmont & Wiersma (1938) examined especially the inhibition of the opener muscle of the crayfish, and Wiersma & Helfer (1941) the inhibition of the opener and closer muscles of the crab, *Cancer antennarius*. Although these studies were limited to so few muscles, significant differences in the nature of the inhibitory mechanisms of these preparations were found. The distribution of the inhibitory fibres has since been worked out for species of the *Astacura*, *Palinura*, and *Brachyura*, both for the anatomical relationships and the functional response to stimulation of the isolated inhibitory fibres (Wiersma, 1941). These recent additions to the knowledge of the distribution of inhibitory axons have made a more extensive comparative study of inhibitory mechanisms possible.

Patterns of innervation. In order to interpret the results of the present investigation it will be necessary to review briefly the distribution of efferent fibres for the muscles in the distal parts of the leg, as reported by Wiersma (1941). As can be seen in Fig. 1, innervation of these muscles in the three above-mentioned groups is identical with respect to the number and distribution of motor fibres. The inhibitory innervation consists always of three nerve fibres which show differences in distribution between the groups. These distribution patterns of the inhibitors may be briefly described as follows: In all three groups the *opener* muscle (abductor of dactylopodite) and the *main flexor* (of carpopodite)[1] are innervated by one inhibitory fibre (I). The second inhibitor (II) innervates the *stretcher* (extensor of propodite), and in *Panulirus* and the crabs runs only to this muscle. In *Cambarus*, however, the stretcher inhibitor also innervates the *closer* muscle (adductor of dactylopodite). The third inhibitor (III) shows great variation

[1] This has been established with certainty for *Panulirus* only, but is also very likely for *Cambarus* and the crabs.

in its distribution in the three groups. In *Cambarus* it innervates the *bender* (flexor of propodite) and the *extensor* (of carpopodite). In *Panulirus* it innervates the closer, the bender, the extensor, and the *accessory* flexor (of carpopodite).[1] In the crabs the third inhibitor is the 'common' inhibitor of Wiersma (1941), which innervates the opener, the closer, the stretcher, the bender, and the extensor, thus giving the opener and the stretcher a double inhibitory innervation consisting of the respective true inhibitors and the common inhibitor. In the paper mentioned, this way of innervation was studied only in *Cancer anthonyi*, but we found it to be exactly the same in the other species of crabs which have been used in this investigation, and in *Randallia ornata*. Since the latter species belongs to a different superfamily of the Brachyura, it is very likely that all true crabs show the same pattern.

In the following pages, in speaking of inhibition of the stretcher contraction of *Cambarus*, for instance, the term 'stretcher inhibitor-stretcher system' will be used, while in the tables roman numerals indicate the inhibitory fibres.

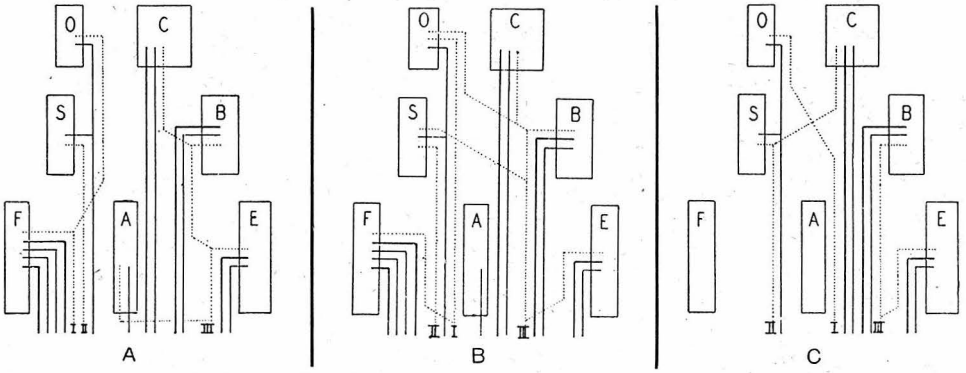


Fig. 1. Scheme of the innervation of the chelipeds and legs. A, *Panulirus*. B, crabs. C, *Cambarus*. Each line represents one axon. Full drawn lines = motor fibres, dotted lines = inhibitory fibres. Notice the similarity in number of the fibres in the different cases and the difference in distribution of the inhibitors. The muscles are represented by the letters: O = opener, C = closer, S = stretcher, B = bender, F = main flexor, A = accessory flexor, and E = extensor.

METHODS

The method of preparing single motor and inhibitory fibres which has been used throughout this investigation has been described in detail for the same preparations which were used here (Wiersma, 1941). In order to study the effectiveness of inhibition essentially the same method was used as that reported by Marmont & Wiersma (1938). In this method the lowest frequency of inhibition which will just suppress any shortening in the muscle when excitation and inhibition are started at the same time is determined for different frequencies of excitation. The necessary stimulating frequencies were obtained with two thyratron stimulators each of which had a range of frequencies more than ample to cover the physiological range. The stimulators were calibrated and were periodically checked as to frequency; in general only small and insignificant variations in the calibrations were found.

[1] This innervation of the accessory flexor was first demonstrated during the present investigation. In the crabs and the crayfish the innervation of this muscle has not yet been found.

The strength of stimulation was always kept low so as to obtain an impulse on each stimulus and yet avoid repetitive discharges. The completeness of the suppression of the contraction was determined by visual observation either with or without the aid of a binocular microscope, or by recording with an isotonic lever. Comparison of the values obtained by these methods showed no appreciable difference.

To study the effect of inhibition of the muscle action potentials an apparatus was used giving series of two shocks at various frequencies. (In the present investigation a frequency of 45 per sec. was usually employed.) The interval between the two shocks could be varied from 0 to 8 msec. This apparatus has been described by Keighley and was used for the study of the potentials of the opener and closer of *Cancer anthonyi* (Wiersma & Helfer, 1941).

The animals which were used in the present investigation were, for the Astacura, *Cambarus clarkii*; for the Palinura, *Panulirus interruptus*; and for the Brachyura, *Cancer anthonyi*, *C. antennarius*, *Pachygrapsus crassipes*, and *Loxorhynchus grandis*.

RESULTS

Determinations have been made for most of the inhibitory innervations as to (A) the effect of frequency of excitation on *Rc* ($Rc = Fi/Fe$: that is, the ratio between frequency of excitation, *Fe*, and frequency of inhibition, *Fi*, necessary just to suppress the contraction), (B) the *Rc* values of the different systems, and (C) the presence or absence of supplementary inhibition.

A. The effect of frequency of excitation on *Rc*

Marmont & Wiersma (1938) have shown that in the opener of *Cambarus Rc* is remarkably constant for different frequencies of excitation. In the slow closer system, on the other hand, they found that only at low frequencies of excitation could an *Rc* value be determined, and that at slightly higher *Fe* inhibition was always incomplete no matter how high *Fi* was made. Most of the systems which have been investigated are less easily inhibited than the opener but more easily inhibited than the slow closer of the crayfish. It was found that the majority of the preparations in every system showed a remarkable constancy of the ratio at different frequencies within a certain range. Several examples of this constancy have been listed in Table 1. It should be noted in this table that in the preparation of the stretcher system of *Panulirus* the ratio remains constant despite a tenfold increase in *Fe*.

In systems in which *Rc* is high it is invariably found that at higher *Fe* the system cannot be completely inhibited with any *Fi*. Inhibition thus becomes ineffective above a certain frequency of excitation. These systems thus resemble the slow closer of the crayfish, but none other shows such an extreme case. In these systems it is easier, therefore, to find how this change in *Rc* is established. Of the two possibilities, (*a*) a gradual increase of *Rc* with an increase in *Fe*, or (*b*) a sudden change to a state in which inhibition is only partial, the latter is the one which is found. Sometimes a small range of *Fe* in which an increase in *Rc* seems to be present is

Table 1. *Constancy of Rc with changes in frequency of excitation*

Animal	Inhibitor	Excitor	*Fi*	*Fe*	*Rc*
Cancer anthonyi	I and II	Stretcher-opener (both contractions)	12	20	0·60
			15	25	0·60
			18	30	0·60
			27	45	0·60
			37	60	0·62
			60	100	0·60
Panulirus	II	Stretcher	11	20	0·55
			16	30	0·53
			26	45	0·56
			35	60	0·58
			55	100	0·55
			105	200	0·53
	III	Fast bender	36	30	1·20
			55	45	1·22
			70	60	1·17
			119	100	1·19
Pachygrapsus	III	Slow bender	15	45	0·33
			20	60	0·33
			35	100	0·35
			40	120	0·33

Table 2. *Effect of different frequencies of excitation on Rc in cases in which inhibition becomes incomplete at high frequencies of excitation*

Animal	Inhibitor	Excitor	*Fi*	*Fe*	*Rc*
Pachygrapsus	III	Opener	55	45	1·22
			75	60	1·25
			—*	100	—
			75	60	1·25
Panulirus	III	Fast closer	60	45	1·33
			79	60	1·32
			—*	100	—
			80	60	1·33

* *Fi* 180 did not suppress *Fe* 100, but completely suppressed *Fe* 60.

Table 3. *Effect of different frequencies of excitation on Rc in cases in which Rc becomes lower at higher frequencies of excitation*

Animal	Inhibitor	Excitor	*Fi*	*Fe*	*Rc*
Cancer antennarius	I	Opener	17	30	0·57
			26	45	0·58
			35	60	0·58
			55	100	0·55
			62	120	0·51
			110	200	0·55
			60	300	0·20
Cancer antennarius	II	Stretcher	16	30	0·53
			25	45	0·57
			37	60	0·61
			50	100	0·50
			60	120	0·50
			100	200	0·50
			80	300	0·26

noticed, but this range is usually very limited in its extent. Thus, if *Fe* values are taken which are not too close together one will show the *Rc* which is constant, while the higher frequency cannot be inhibited (Table 2). The frequency of excitation at which inhibition in such systems becomes incomplete differs greatly. For example, the slow closer of *Cambarus* ceases to be completely inhibitable at a *Fe* of about 10 per sec.; for most of the fast closers the frequency is about 80–100; and in some preparations of slow systems the contractions fail to be completely suppressed at a *Fe* of about 200 per sec. That fatigue of inhibition can have an influence on these phenomena is shown by the fact that on repetition at one *Fe* a contraction may show at first the normal *Rc*, then show a state of only partial inhibitability, and, after rest, again show the normal ratio. At the higher *Fe*'s, however, even a completely fresh preparation will be only partially inhibitable. In these cases *Rc* cannot be applied to the incompletely inhibited states as it is by definition 'just complete' inhibition.

Some systems show an entirely different picture. In these, instead of being but partially inhibited at high *Fe*, *Rc* drops suddenly to a much lower value. This occurs especially in systems in which *Rc* is normally quite low (see Table 3). It should be noted that in both cases the *Fi* which completely suppresses the contraction at *Fe* 300 is even lower than the value which was necessary at *Fe* 200.

Whereas the *Rc* of the large majority of all the preparations of the different systems was quite constant with frequency, there were a very few scattered preparations in which a definite increase in ratio took place and in some others a decrease. It is apparent that these phenomena are associated with some particular property of the individual preparation and are not of general occurrence. We have therefore omitted values of this sort from further consideration, and whenever such a preparation was encountered the values were considered not trustworthy and were discarded.

It can thus be concluded that frequency has no influence on *Rc*. This does not mean, however, that *Rc* is constant in every system, but that such inconstancies as occur are caused by factors other than the frequency of excitation. In the determination of the *Rc* of different systems these factors form a disturbing influence, as will be clear from the following paragraphs.

B. The *Rc* values of the different systems

In respect to constancy of *Rc* in one preparation of one system and of the same system in different preparations, the systems fall into three groups: (*a*) In a number very little variation is found in different determinations. (*b*) The second group is composed of those which show a rather widespread variation both within the same preparation and between different preparations. (*c*) A third group shows two distinct ratios each of which is usually constant.

Group (*a*). In systems with a constant ratio the variations of *Rc* hardly ever surpass the limits set by the accuracy of the method, namely, about $\pm 8\,\%$. Examples

of this group are the opener of *Cambarus* and *Panulirus*, the stretcher of *Panulirus*, and several other systems, including the slow bender of *Pachygrapsus*, which is the most easily inhibited of any of the systems studied.

Group (*b*). Variable ratios are found most commonly in preparations which show a high ratio. It is likely that here the factors of facilitation and fatigue of the contraction and of the inhibition play a large part, which explains the difference in values often obtained in one preparation. It is possible, nevertheless, that there are here also real and rather large differences in the effectiveness of inhibition in different preparations. Though in this group the limits are much wider than in those in which the ratio is constant, it is quite possible to determine a mean value which in most cases does not differ from the extremes by more than $\pm 15\%$. Examples of this group are the closer inhibitor-fast closer systems of all the animals in which inhibition of this system is possible. In Table 5 the mean values alone are given for such variable systems and are marked with an asterisk.

Table 4. *Constancy of more than one ratio in the same system*

Animal	Inhibitor	Excitor	*Fi*	*Fe*	*Rc*	*Fi*	*Fe*	*Rc*
Loxorhynchus	I	Opener	13	30	0·43	17	30	0·57
			20	45	0·45	25	45	0·55
			27	60	0·45	34	60	0·57
			44	100	0·44	53	100	0·53
Loxorhynchus	II	Stretcher	12	30	0·40	14	20	0·70
			20	45	0·44	22	30	0·73
			27	60	0·45	33	45	0·73
			47	100	0·47	44	60	0·73
Panulirus	III	Slow extensor	26	45	0·58			
			35	60	0·58			
			63	100	0·63			
			11	25	0·44			
			13	30	0·43			
			22	45	0·49			
			26	60	0·44			
			47	100	0·47			

Group (*c*). The true opener inhibitors of the crabs are examples of systems which show two distinct values for *Rc*, each of which is remarkably constant. In this group one preparation may jump from one value to the other in two successive determinations, though most frequently the ratio in one preparation is constant at one value while that of another preparation (which may be of another leg of the same animal) is constant at the other. In Table 4 the values have been given for *Loxorhynchus* and for the slow extensor of *Panulirus*; in the two muscles of the crab the values are from different preparations, in the lobster the values were found in the same preparation.

The results of the determinations of *Rc* are given in Table 5 and Table 6. In Table 5 the ratios for the opener, closer, stretcher, and bender of all the animals are given, while Table 6 presents the results obtained from the extensor, flexor, and accessory flexor in addition to those from the four distal muscles of the lobster.

Effectiveness of inhibition in the opener muscle

The opener inhibitor of *Cambarus* has been previously reported to have a very effective inhibitory effect, giving an *Rc* of 0·41 (Marmont & Wiersma, 1938). In the present investigation in which more preparations have been used our mean value was also 0·41.

The opener inhibitor of *Panulirus* gives a ratio of 0·50, which indicates that exactly two excitatory impulses can be suppressed by a single inhibitory one.

The true opener inhibitor of the crabs shows, as has been pointed out, two distinct ratios. Both these ratios are about the same in the two species of *Cancer*, and are definitely higher than the corresponding ones in *Pachygrapsus* and *Loxorhynchus*, the lower ones in the *Cancers* being of the same order as the higher ones in the two other species.

The ratio for the common inhibitor in the crabs is always definitely higher than any of the ratios of the true opener inhibitor, and the values are in general much more variable. The ratios in the two species of *Cancer* for this inhibitor are, in contrast with the ones of the true opener inhibitor, lower than those of the other two genera.

Table 5. *Rc values for the inhibitory systems of the four most distal muscles*

Animal	Opener		Stretcher		Fast closer	Slow closer	Fast bender	Slow bender
	I	III	II	III	III	III	III	III
Panulirus	0·50	—	0·5*	—	1·25*	0·80	1·25	0·75
Cambarus clarkii	0·41	—	0·41 0·65	—	∞	5·*	1·25*	0·70
Cancer anthonyi	0·60 0·75	1·0*	0·5 0·6	1·0*	1·0*	0·75*	1·00	0·75 0·55*
Cancer antennarius	0·53 0·75	0·93	0·53 0·75	1·0*	1·3*	0·65* 0·95*	1·1*	0·50*
Loxorhynchus grandis	0·45 0·56	1·5*	0·45* 0·75	1·5*	1·5*	1·0*	1·4*	0·58
Pachygrapsus crassipes	0·40 0·50	1·40*	0·45* 0·65	1·25	∞	0·45* 0·6	∞	0·33

* Indicates that these systems show a rather wide variation.

Effectiveness of inhibition in the stretcher muscle

The ratio of the stretcher inhibitor system is in most cases very similar to that of the opener inhibitor system. In the majority of the preparations in all animals exactly the same ratio was found for the two systems when both inhibitors were stimulated simultaneously on the same electrodes.

Sometimes exceptions are encountered in certain preparations, although it is never found that a constant difference occurs in all preparations of a certain system. In some systems it is the opener, in others the stretcher, which is occasionally more difficult to inhibit.

In *Cambarus* the stretcher shows besides the same value as the opener a second value which is higher. In *Panulirus* the ratio is less constant than that of the opener, but does not show a definite second ratio.

The true stretcher inhibitor of the crabs shows two ratios which are not always exactly the same as those of the opener. Both lower and higher values are found, as can be seen from Table 5. In *Loxorhynchus* this is clearly demonstrated in Table 4, in which the lower values for the two muscles are about the same, but the higher values are different, that of the stretcher being noticeably higher.

The common inhibitor-stretcher systems of the different crabs give essentially the same ratios as those of the common inhibitor-opener systems of the same species.

Effectiveness of inhibition in the closer muscle

In all of the animals the slow closer contraction can be inhibited, and in most of them with relative ease. The most outstanding exception is the slow closer contraction of *Cambarus*, which, as was pointed out by Marmont & Wiersma (1938), can be inhibited only at low frequencies of excitation and at these only with difficulty. The very high *Rc* which they reported (6·2) has been confirmed by our observations, which gave ratios of the same order, approximately 5·0. The other slow closer systems show a range from about 1·0 in *Loxorhynchus* down to values as low as 0·4 in *Pachygrapsus*.

The fast closer contraction is not inhibitable in either *Cambarus* or *Pachygrapsus*. In both of these cases the muscle responds with a single twitch to a single impulse in the fast fibre, although this contraction in *Pachygrapsus* is relatively weak and in *Cambarus* is very strong. Not only was it impossible to inhibit the twitch contractions, but inhibition also was found to have no effect whatsoever on tetanic contractions. In the other animals where no visible contraction is obtained on a single impulse, there is definite inhibition although the ratios are either about 1·0 or well above it.

Effectiveness of inhibition in the bender muscle

As in the closers there are always two types of contraction in the bender. Again, the slow contraction is in every case easier to inhibit than the fast. In general the values for the fast contractions are about the same as those for the fast contractions of the closer muscle in the same species, but *Cambarus* presents a most interesting exception. In this animal both the slow and fast bender contractions are easily inhibited, the slow showing a ratio of about 0·7, the fast one of 1·2, values which check closely with those obtained by Marmont & Wiersma (1938), even though the latter determinations were made on a much smaller number of preparations. In *Pachygrapsus*, however, the fast bender is, as the closer, uninhibitable. In this animal, in contrast to *Cambarus*, a single impulse in the fibre for the fast bender gives a rather strong twitch contraction of the muscle. The fact that the slow bender contraction of *Pachygrapsus* shows a very low and constant value of 0·3, the lowest ratio obtained in any preparation, has already been mentioned. In general the slow bender contractions appear to show a definitely lower ratio than the corresponding slow closer contractions.

The effectiveness of inhibition in the muscles of the meropodite of Panulirus

As has been mentioned, in Table 6 are summarized the results from the muscles of the meropodite in the lobster. There are several points on which some comment should be made. The fast extensor contraction is here rather easily inhibited and yields the lowest value found for any fast contraction, namely, 0·80. It is interesting to note that this value for the *fast* extensor is much lower than that of the other fast systems innervated by the same inhibitor (closer and bender), and corresponds quite well with the values obtained for the similarly innervated *slow* bender and *slow* closer.

The four contractions of the main flexor show clearly that the 'slower' the system is the more easily it is inhibited. This enlarges the findings of van Harreveld & Wiersma in this muscle (1939). It may be noted that only the *slowest* flexor contraction is inhibited with about the same effectiveness as the opener which is inhibited by the same fibre. The fastest of the main flexor contractions is very difficult to inhibit, and the values tend to be quite inconstant, ranging from about 1·5 to 3·0.

Table 6. *Rc values for the inhibitory systems of* Panulirus interruptus

System	Opener I	Stretcher II	Closer III		Bender III	
			Fast	Slow	Fast	Slow
Rc	0·50	0·5*	1·25*	0·80	1·25	0·75

System	Extensor III		Accessory Flexor III	Flexor I			
	Fast	Slow		Fast	2nd fast	2nd slow	Slow
Rc	0·8	0·45 0·6	0·60	2·0*	0·80	0·70	0·52

* Indicates that these systems show a rather wide variation.

C. The presence or absence of supplementary inhibition

Marmont & Wiersma (1938) have shown that in the opener of *Cambarus* reduction of the muscle action potentials during inhibition is present only when the inhibitory impulses arrive within a restricted time limit before the excitatory ones. Inhibition in which the muscle action potentials are reduced has been called supplementary as against simple in which no reduction occurs. Wiersma & Helfer (1941) have shown that in certain inhibitor systems of *Cancer* no supplementary inhibition can be obtained no matter at what interval the excitatory and inhibitory impulses arrived at the muscle. These systems, therefore, show simple inhibition only. This was shown to be the case in the inhibition of the slow closer and of the opener by the common inhibitor. The true opener inhibitor of *Cancer* did, however, show supplementary inhibition which is in accord with the findings of Marmont & Wiersma (1938) on several other crabs. The present investigation concerns itself mainly with the determination of which systems are capable of giving supplementary inhibition and which ones are not. In contrast with the earlier papers the quantitative effects were treated more superficially.

In *Cambarus* it was found that besides the opener, the stretcher can be made to show reduction in muscle action potentials during inhibition. Marmont & Wiersma (1938) described the phenomenon for the opener of the crayfish thus: 'the action currents are reduced somewhat gradually at the onset of the supplemented inhibition but rise immediately (rebound) to their normal height when the inhibiting impulses are stopped.' In the present investigation the gradual onset and sudden rebound was also observed for the stretcher system (Fig. 2 A). No sign of reduction of the action potentials was found in the inhibition of the bender and closer contractions in the crayfish. It is of particular interest to note that the inhibitor which produces the phenomenon in the stretcher fails to do so in the closer.

It was found possible to elicit supplementary inhibition in both the true opener inhibitor and the true stretcher inhibitor system in all the crabs (Fig. 2 B). In all cases the supplementary inhibition shows the characteristics reported by Wiersma & Helfer (1941) for the opener system of *Cancer*, namely, the gradual depression at the onset of inhibition and the gradual growth upon release in contrast to the

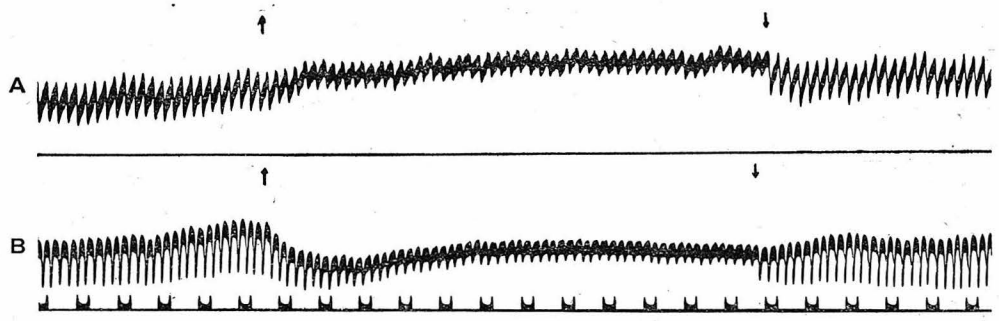


Fig. 2. Supplementary inhibition of action potentials of the stretcher muscle. A. *Cambarus*. Notice the immediate rebound to full height at the end of inhibition. B, *Cancer anthonyi*. At the end of inhibition the action potentials grow gradually to full height. ↑ = onset of inhibition. ↓ = release of inhibition. In both cases the inhibitory stimulus was given 2 msec. before the excitatory one. Frequency 45 per sec. Time 0·1 sec.

immediate rebound of the crayfish. In no case could supplementary inhibition be obtained with the common inhibitor. This enlarges the findings of Wiersma & Helfer (1941) with this inhibitor for the opener and the closer to include the other muscles which it innervates. Especial attention has been paid to the inhibition of the slow bender contraction of *Pachygrapsus* because it is the most easily inhibited contraction. Nevertheless, we have never obtained any signs of supplementary inhibition in this muscle. It should be pointed out in this regard that the slow closer and the slow bender contractions of *Pachygrapsus* do not lend themselves very well to this sort of investigation because of the small magnitude of the muscle-action potentials which they give (in sharp contrast to the very large magnitude of the fast-action potentials of these muscles). We have not paid especial attention to the presence or absence of the small depression of facilitation and the subsequent growth of the muscle-action potentials on release of simple inhibition which Wiersma & Helfer (1941) reported, but noted that it was present in several cases.

In *Panulirus* not one of the systems has ever shown any signs of supplementary inhibition. The four most distal muscles and the extensor have been carefully examined in this respect. As the complete absence of action-potential reduction in at least the opener and stretcher muscles was unexpected, a large number of preparations of the opener and of the stretcher have been made, but not one of these has given any indication of the phenomenon.

DISCUSSION

It is surprising that the ratio of the frequency of inhibitory impulses to the excitatory impulses for just complete inhibition is constant with changes in the frequencies of stimulation. Such constancy has been seen in nearly all of the preparations observed. The question arises how this constancy of *Rc* can be brought about. It is a well-established fact that in the muscles of decapod Crustacea the strength of a contraction depends on the excitatory frequency, and that with increase in frequency there is in these muscles not only an increase in contraction strength by algebraic summation but also by facilitation,[1] which results in successive nerve impulses becoming more and more effective. The effect increases the quicker the impulses follow each other. That this facilitation does not make contractions obtained on higher frequency stimulation relatively more difficult to inhibit than at low frequencies, which would result in an increase in *Rc*, can find its explanation only in a simultaneous increase in effectiveness of the inhibitory processes. It can be proven that this is indeed the case. If inhibition is started before excitation it is found that it can completely suppress a contraction caused by a frequency of excitation which it cannot suppress if both are started at the same time. Wiersma & van Harreveld (1934) found in *Eupagurus bernhardus* that if a frequency of inhibition is selected which cannot suppress but can only slightly reduce a test contraction when both are started at the same time, continued stimulation of the inhibitor suppresses successive test contractions more and more until fatigue of the inhibitory mechanism sets in and the contractions become again less completely suppressed. Under the same circumstances all inhibitory systems were observed to give similar results. The counterpart of this experiment, i.e. to test the influence of short inhibitions during continuous stimulation of the excitor, was performed with the following result: stimulating at frequencies near *Rc* values the effect of the inhibitions becomes gradually less and less, even during the time in which the contraction begins to diminish through fatigue. This shows that facilitation of the contraction makes it less inhibitable, even if the mechanical effect diminishes. It can thus be concluded that both excitation and inhibition undergo facilitation and that these facilitations balance each other at the *Rc* values.

Although the *Rc* value gives a measure of the relative rate of the facilitations of

[1] Facilitation of excitation has been shown to work at two places, for there is a facilitation of the action potential and of the contractile mechanism (Wiersma & van Harreveld, 1939). In the present discussion we have taken these two together, thus deliberately simplifying the picture. It seems possible that in the inhibitory process two similar facilitations are active, but at present it is not fruitful to discuss the theoretical consequences of such an arrangement.

excitation and inhibition of a system it is not possible to measure the absolute rates. However, the results obtained point to some conclusions. In a doubly motor innervated muscle inhibited by one fibre it is likely, for instance, that the two ratios for the two contractions can be taken as a measure of the relative strength of the excitatory facilitations. It thus becomes evident that in such cases as the fast closer contraction of *Cambarus* and the fast closer and fast bender contractions of *Pachygrapsus*, which are uninhibitable at any frequency, the ineffectiveness of inhibition seems to be directly associated with the excitatory process. In the case of the opener and stretcher, which usually show the same ratio, it is logical to conclude that the two facilitations, inhibitory and excitatory, of these muscles are the same. In the cases in which there is a difference in the ratios of these two muscles this will be most probably due to a difference in the inhibitory facilitation, since it seems unlikely that the excitatory facilitation of the same motor axon would change in one muscle and not in the other.

Since they share a single inhibitory fibre it might appear logical to consider the similarity of the *Rc* values for the fast extensor, the slow bender, and the slow closer systems of *Panulirus* as indicative of a similarity in the facilitation of the excitatory processes of these contractions. That such is not necessarily the case, however, is seen in *Cambarus*, in which the closer and stretcher are innervated by the same inhibitor, yet the stretcher contraction gives *Rc* values which are of the order of one-tenth those of the slow closer contraction. Any tenfold difference between the facilitation of these contractions is certainly not present.

It is clear that constant ratios depend on a great number of factors. If there is still facilitation of excitation or of inhibition from a preceding volley the ratio will shift to one side or the other. This will show at the same frequency on repetition. This factor can be excluded, however, by allowing sufficient time between stimulations and also by very short durations of the tests. Both have been utilized as much as practicable in the experiments. That the fast systems have, nevertheless, given rather inconstant ratios may well be due to the fact that these systems are generally rather inconstant. For instance, repetition of the same stimulation often results in contractions which are noticeably different, even though a prolonged rest period is given, which may well indicate variations in the facilitation of the excitation.

The appearance in certain preparations of two often well-defined ratios cannot be considered to be due to such an inconstancy. This type of variation is presumably due to a shift of one or the other of the facilitations to a different level which is the same in different preparations. This shift must be a very sudden one in those instances in which the second level was observed shortly after the first in the same preparation. The reasons for such a shift and for the constancy of the two levels are unknown.

The incomplete inhibitability of certain preparations at the higher frequencies of excitation which are completely inhibitable at lower frequencies is, however, presumably not due to any peculiarity of the facilitations. The most likely explanation is that the inhibitory mechanism is stimulated with too high a frequency,

and that certain of the impulses fail to have an effect. That this explanation is reasonable is shown by the fact that in preparations in which the ratio is favourable to the inhibitor, the excitor may fail first in a similar way. In such a case the ratio will be constant up to high values of excitation but will drop at still higher ones. It may be remarked that failure of excitation or inhibition does not influence the ratio of subsequent contractions at lower frequency levels, these being quite normal.

In crabs the opener and stretcher muscles are inhibited by two different fibres. The functional significance of this arrangement is unknown (see Wiersma, 1941), but it is of interest to note that the *Rc* values of the two inhibitors show a certain relation. In the two *Cancers*, where the true opener inhibitor is relatively ineffective, the common one is relatively effective, whereas in *Pachygrapsus* and *Loxorhynchus* a relatively effective true opener inhibitor is accompanied by a rather ineffective common inhibitor.

In previous papers it has been pointed out that the facilitation of the excitatory mechanism is most likely not due to spatial facilitation by the involvement of more and more muscle fibres but to a gradual increase of contraction strength in each muscle fibre. This means that the muscle fibre of the crustaceans does not contract in an all-or-none fashion. The results obtained with inhibition give additional support to this view. The absence of any effect on the muscle action potentials in most inhibitions shows that the inhibitory mechanism must be located after the process of this muscle action potential and since there is an innervation of each muscle fibre in many places the inhibition must be present at all these places. It is thus thought that inhibition like excitation is a gradual process in each muscle fibre, or better, at each nerve ending on each muscle fibre.

One of the most significant observations arising from the present investigation is the apparent lack of correlation between the ease with which an inhibitor is able to suppress the contraction of the muscle which it innervates and the presence of the phenomenon of supplementary inhibition. It would seem quite plausible that to obtain a maximum efficiency of the inhibitory process it would be necessary not only to block the transmission step between the muscle action potential and the contractile mechanism, but to suppress the earlier transmission step between the nerve action potential and the muscle action potential as well. This is, however, definitely not the means whereby certain of those systems showing a low *Rc* achieve their effectiveness, e.g. the opener system of *Panulirus* and the slow bender system of *Pachygrapsus*.

The presence of supplementary inhibition in both the opener and stretcher muscles of crayfish and crabs, muscles which have separate inhibitory axons, shows that the phenomenon is not limited to one inhibitor. At the same time the absence of the phenomenon in other muscles inhibited by these same inhibitors, for instance the closer of *Cambarus*, indicates clearly that the mechanism involved in supplementary inhibition is not to be found in the inhibitory innervation, but in the muscle itself. It is apparently an extra process whereby inhibition can be assured in those few systems in which the phenomenon can be demonstrated. *Panulirus* seems to be totally without this type of inhibition.

SUMMARY

The effectiveness with which different contractions in a number of muscles can be inhibited was investigated. As a measure of this effectiveness the frequency of inhibition which can just inhibit a contraction with a given frequency of excitation was determined. It was found that in all systems the ratio (*Rc*) of such inhibitory frequencies to that of the excitatory frequencies they can suppress was constant for a wide range of frequencies.

At high frequencies either the inhibition or the excitation may become less effective. This is explained by failure of the respective system to function normally at such a frequency.

The effectiveness of inhibition of different systems was determined. Some systems show a very constant *Rc* value; in a second group *Rc* varies within wider limits; and a third group shows two distinct *Rc*'s sometimes in the same preparation at different times.

Rc values have been found to vary widely. For instance, in the bender inhibitor-slow bender system of *Pachygrapsus* three excitatory impulses are suppressed by one inhibitory impulse; in the closer inhibitor-slow closer system of *Cambarus* one excitatory impulse needs five inhibitory impulses to counteract its effect. The fast closer contraction of *Cambarus* and the fast closer and fast bender contraction of *Pachygrapsus* were found to be uninhibitable, i.e. no effect of inhibition whatsoever was noticed on any of these contractions. All three systems are distinguished by giving a mechanical response to a single stimulus in contrast with all the inhibitable systems which do not respond to single impulses.

Reduction of the action potentials during inhibition is obtainable in only a few systems, namely, the opener inhibitor-opener and the stretcher inhibitor-stretcher systems of *Cambarus* and the crabs. (In the crabs this applies only to the 'true' inhibitors.) In all other systems, including every system of *Panulirus*, no reduction of the muscle action potential is obtained.

REFERENCES

VAN HARREVELD, A. & WIERSMA, C. A. G. (1939). *J. exp. Biol.* **16**, 121.
MARMONT, G. & WIERSMA, C. A. G. (1938). *J. Physiol.* **93**, 173.
WIERSMA, C. A. G. (1941). *J. comp. Neurol.* **74**, 63.
WIERSMA, C. A. G. & VAN HARREVELD, A. (1934). *Arch. Neérl. Physiol.* **19**, 458.
WIERSMA, C. A. G. & VAN HARREVELD, A. (1939). *Physiol. Zoöl.* **12**, 43.
WIERSMA, C. A. G. & HELFER, R. G. (1941). *Physiol. Zoöl.* **14**, 296.