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ARMY MEDICAL RESEARCH LAB., FORT KNOX, KY. (REPORT NO. 65)

RETENTION OF THE EOSINOPENIC RESPONSE AFTER BILATERAL SYMPATHECTOMY IN THE DOG

NELSON, WILLIAM J.; LYNCH, JUANITA R.; KELLER, ALLEN D.
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Report No. 65

RETENTION OF THE EOSINOPENIC RESPONSE AFTER BILATERAL SYMPATHECTOMY IN THE DOG*

by

William J. Nelson, Biologist, Juanita R. Lynch, Biologist, and
Allen D. Keller, Physiologist

from

Neuro-Endocrine Section, Physiology Branch
Army Medical Research Laboratory
Fort Knox, Kentucky
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ABSTRACT

RETENTION OF THE EOSINOPENIC RESPONSE AFTER BILATERAL SYMPATHECTOMY IN THE DOG

OBJECT

To determine if removal of the thoracic and abdominal sympathetic chains eliminates or alters the eosinopenic response to a group of stimuli which are known or believed to increase adrenal cortical activity. This experiment was designed to elucidate the currently suspected association of endogenously secreted epinephrine with adrenal cortical activity.

RESULTS

Bilateral removal of the thoracic and abdominal sympathetic chains in the dog does not prevent the marked decrease in the circulating eosinophils from occurring as a result of surgical trauma, insulin hypoglycemia, a bout of rage or exposure to cold.

Surgical trauma was outstandingly more reliable and predictable as an eosinopenic stimulus than was hypoglycemia, rage or cold.

CONCLUSIONS

The functional maintenance and activation of the adrenal cortices, assayed by the eosinopenic response, is not dependent upon nerve impulses exiting from the central nervous system over the thoracolumbar outflow of the autonomous nervous system.

There is no definite indication that adrenal cortical activity is in any way dependent upon endogenously secreted epinephrine.

RECOMMENDATIONS

There remains the remote possibility that the adenohypophysial elaboration of ACTH is dependent upon a highly localized release of epinephrine or sympathin in the immediate environs of the adenohypophysis through a mechanism other than by nerve impulses exiting from the central nervous system over the thoracolumbar outflow. This possibility should be specifically investigated. To this end the

present type of assay-study should be extended to hypothalamectomized-sympathectomized dogs in which the superior cervical sympathetic ganglia are removed in addition to the thoracic and abdominal chains.

Submitted by:

William J. Nelson, Biologist
Juanita R. Lynch, Biologist
Allen D. Keller, Physiologist

Approved: Ray G. Daggs
RAY G. DAGGS
Director of Research

Approved: Carl F. Tessmer
CARL F. TESSMER
Lt. Colonel, MC
Commanding

RETENTION OF THE EOSINOPENIC RESPONSE AFTER BILATERAL SYMPATHECTOMY IN THE DOG

I. INTRODUCTION

For several years investigative and conjectural activity has been centered around the possible role of endogenously secreted epinephrine in the adenohypophysial maintenance and activation of the adrenal cortices. Emphasis was early placed on the concept that endogenous epinephrine was specifically essential to trigger the release of adrenocorticotrophic hormones into the blood stream. This was based on the fact that the injection of pharmaceutical epinephrine (adrenalin) into animals elicited adrenal cortical activity as evidenced by a decrease in the cholesterol and ascorbic acid content of the adrenal cortices (1) and a precipitous fall in circulating eosinophils (2). More recently, less emphasis is being placed on the essentiality and/or specificity of epinephrine in this response. It is now realized that many other chemicals when injected into animals produce the same end result as adrenalin. In addition, Gordon (3) has shown that demedullation of the adrenals in the rat does not eliminate or lessen the response of the adrenal cortices to appropriate stimuli. Similarly, Recant, Hume, Forsham and Thorn (4) have shown that sympathectomy in the dog does not eliminate the eosinopenic response following intramuscular injection of formalin, and Long and associates (5) have demonstrated that high cord section in the rat does not eliminate adrenal cortical responses to a variety of stimuli.

It is interesting that the concept of epinephrine being essential ever arose, and particularly that it flourished, in view of the long-standing knowledge that bilateral removal of the sympathetic chains in laboratory animals does not precipitate critical metabolic deficits (6). For instance, if epinephrine were essential to adrenal cortical maintenance and activation, bilateral sympathectomy, which removes all known neurogenic elaboration of epinephrine or sympathin (7), would precipitate an adrenal insufficiency equal in magnitude to that produced by adrenalectomy!

The foregoing analysis is perhaps an oversimplification of the epinephrine concept, nevertheless, it illustrates the desirability of attempting to determine in a crucial manner if endogenously secreted epinephrine is, under any physiological circumstances, associated with adrenal cortical activity. The experiments described herein were designed with this objective in mind.

The eosinopenic response was used as the index of adrenal cortical activity, because of its convenience as well as its current acceptance as a reliable criterion in this connection (2, 8). The overall procedure was to determine the response of circulating eosinophils to three stimuli (rage, hypoglycemic crisis, and exposure to cold) which are known or believed to cause the liberation of epinephrine and/or sympathin into the circulating blood and a stimulus (surgical trauma) which is known to excite the adrenal cortices (9) but which has not

necessarily been thought to cause liberation of epinephrine as a moderating substance. The stimuli were applied both before and after removal of the thoracic and abdominal sympathetic chains.

II. EXPERIMENTAL PROCEDURES

Animals

Female dogs were used ranging in weight from 6 to 14 kilograms. They were housed in temperature controlled rooms and were conditioned to cage and laboratory routines. The daily ration was constant and consisted of 10 grams of frozen horse meat and 10 grams of Purina Laboratory Chow per kilogram of body weight. Water was available at all times.

Hematological Determinations

Thorn's (10) modification of the technique described by Dunger (11) in 1910 was used for eosinophil counting. The diluting fluid was made by mixing 5 cc. of a 2% aqueous solution of eosin Y and 5 cc. of acetone. This mixture was diluted to 100 cc. with distilled water and stored under refrigeration until needed. The fluid was filtered before use and kept in an ice bath while counts were being made. A standard white cell pipette was used for dilution. Counts were made in a Fuchs-Rosenthal type chamber, having a depth of 0.2 mm. and a total volume of 3.2 mm^3. Counts were made on venous blood which was prevented from coagulating by an evaporated mixture of 2 parts potassium oxalate and 3 parts ammonium oxalate.

All tests were begun as soon as possible after entering the laboratory in the morning with an interval of at least 2 weeks between tests. Tests were begun uniformly 24 hours after the previous meal and food was not given during the test.

Administration of Pharmacological Adrenalin

Adrenalin was administered subcutaneously over the dorsum of the neck. The dosage adopted for routine use was 0.05 mg./kg. body weight. Eosinophil counts were made at 2-hour intervals for 8 hours.

A Bout of Rage

Three dogs were used in which a marked (pathological) increase in susceptibility to rage was precipitated by the placing of destructive lesions in the hypothalamus. Very slight stimulation, such as merely approaching, touching or handling the animals, caused them to go into paroxysms of snapping, barking and growling. During a test the animal was teased with a stick or glove for 30 minutes, allowed to rest for 30 minutes, then stimulated for a second 30 minutes. Eosinophil counts were made at 1, 2, 3, 4 and 6 hours subsequent to the beginning of the initial bout of teasing.

Hypoglycemic Crisis Induced by Insulin

Insulin was administered subcutaneously over the dorsum of the neck. Doses of 1½ to 3 units per kg. of body weight were used in the unoperated dogs. In the sympathectomized dogs a smaller dose was used, 1/10 to ½ unit per kg. body weight, because of the increased sensitivity to insulin which obtains in such animals. Glucose and eosinophil determinations were made on the initial blood sample and subsequently at 3, 5 and 7 hours. Blood sugar determinations (method of Somogyi modified by Nelson (12)) were made primarily to verify absorption of insulin from the subcutaneous depot. The clinical condition of the animal was always used as the principal criterion for estimating the magnitude and severity of the hypoglycemic crisis.

Exposure to Cold

The dogs were abruptly removed from their regular room, 23-25°C, and placed into a cage in a room maintained at 3-5°C. Unoperated dogs were exposed to this temperature for 3 to 5 days. After sympathectomy the exposure was reduced to 8 hours. Eosinophil counts were made at various intervals depending upon the duration of the exposures and other circumstances. Rectal temperatures and the presence of shivering, with an estimation of its magnitude, were recorded simultaneously.

Sympathectomy

These operations were carried out under nembutal anesthesia with endotracheal intubation to maintain respiration during the thoracic portion of the operation.

The right sympathetic chain was removed during the first operation and the left chain during a second operation performed 2 weeks later. Each operation involved 3 incisions as illustrated in the photograph of a dog showing the 3 scars two weeks after operation (Figure 1). The first incision, parallel to the vertebral column on the side of the abdomen, allowed the lumbar chain to be freed from its grey and white rami and severed at or below the level of the 4th lumbar vertebra. Through the second incision in the 9th or 10th interspace the lower thoracic rami communicantes were broken and the abdominal chain pulled beneath the diaphragm into the thorax. The third incision in the 3rd or 4th interspace allowed the remaining rami to be broken and the cervical continuation of the chain to be severed above the stellate ganglion. Thus, under ideal conditions the ganglionated chains were removed in one piece from below the level of the 4th lumbar vertebra to above the stellate ganglion. The mounted chains removed from Dog 144 are shown in Figure 2.

Eosinophil counts were done on blood samples taken before and approximately 4 to 6 hours following the initiation of surgery. Additional counts were done at 0800 hours for 2 days postoperatively and again at 6 to 8 days.

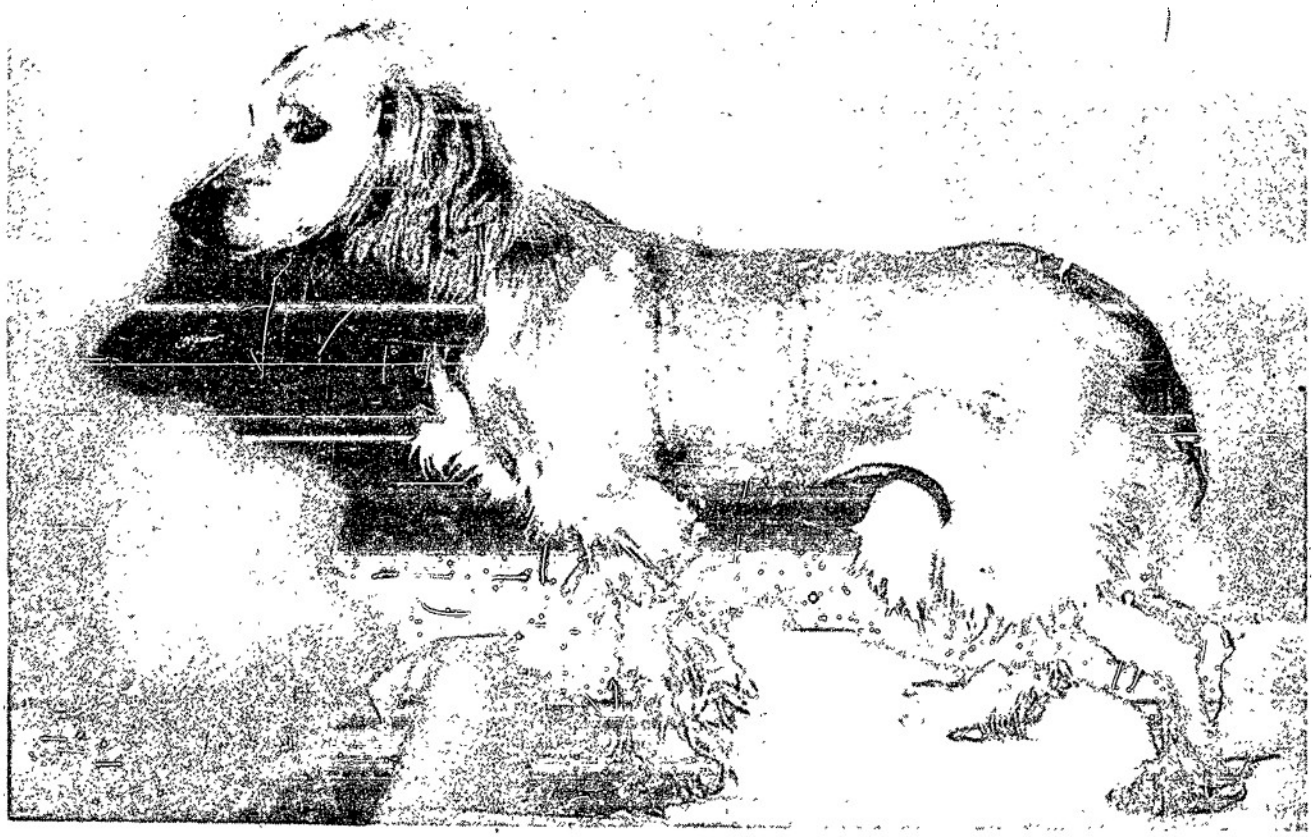

FIG. 1. PHOTOGRAPH OF DOG 62 SHOWING SCARS TWO WEEKS AFTER LEFT SYMPATHECTOMY.

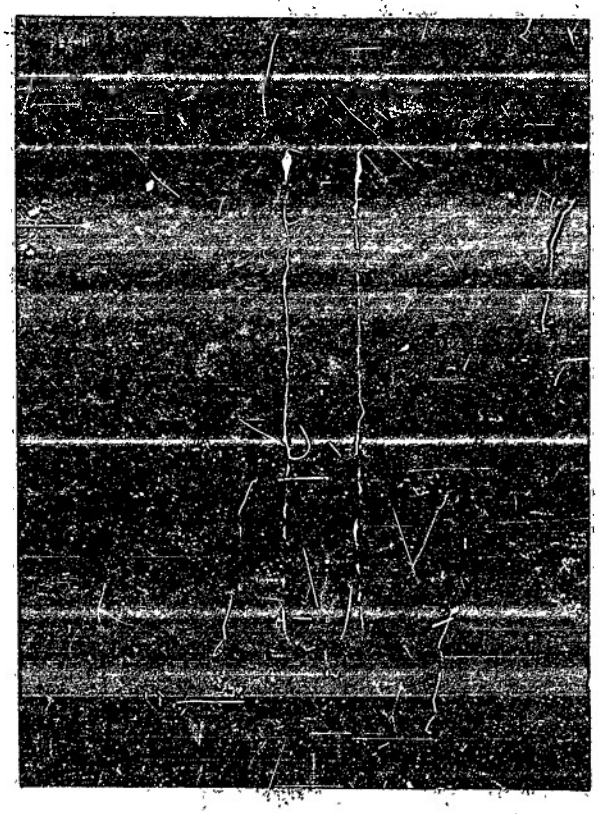

FIG. 2. PHOTOGRAPH OF FORMALIN-FIXED SYMPATHETIC CHAINS REMOVED FROM DOG 144.

Laparotomy Subsequent to Bilateral Sympathectomy

Under nembutal anesthesia, a midline incision was made in the abdomen. Through this incision the small intestines were brought to the surface and spread on the drapes. Subsequently for a 5 to 15 minute period they were manipulated by hand and allowed to dry. In addition, in some of the animals, one uterine horn and ovary were also removed. Eosinophil counts were done at the same time intervals as stated for the sympathectomy procedure.

III. RESULTS

The variation in the daily circulating eosinophil level from one animal to another was extremely wide, ranging from 30 to 2200 per mm^3 (see tables). For convenience in analyzing the data per cent change has been used as recommended by Thorn (10). This was calculated according to the formula:

$$\left(\frac{\text{Number of Cells at Each Successive Count}}{\text{Number of Cells at Zero Count}} - 1\right) \times 100$$

Response to Injected Adrenalin

A decrease in eosinophils from 50 to 60 per cent usually occurred after a subcutaneous dose of 0.05 mg. adrenalin injected as a 1:1000 aqueous solution. The time at which the maximum decrease was obtained varied from 1 to 8 hours, occurring most frequently at 6 hours. Increasing the dose to 0.1 mg./kg. usually produced only a slightly greater response but occasionally caused a 70 to 80 per cent decrease. Intramuscular or intravenous administration did not elicit a response which varied significantly from that obtained with subcutaneous injections. Table 1 summarizes the data and Figure 3 gives individual experiments in graphic form.

TABLE 1

EOSINOPHIL RESPONSE TO ADRENALIN IN NORMAL DOGS

Dog	Stimulus	Eosinophil Cell Number					Per Cent Change			
		0 Hr	2 Hr	4 Hr	6 Hr	8 Hr	2 Hr	4 Hr	6 Hr	8 Hr
46	.05 mg./kg.	511	711	244	178	267	+ 39	- 52	- 65	- 48
49	0.1 mg./kg.	1422	400	667	578	178	- 72	- 53	- 59	- 87
62	.05 mg./kg.	1568	2238	1512	1094	612	+ 43	- 4	- 30	- 61
64	.05 mg./kg.	2237	1862	1712	731	988	- 17	- 23	- 67	- 56

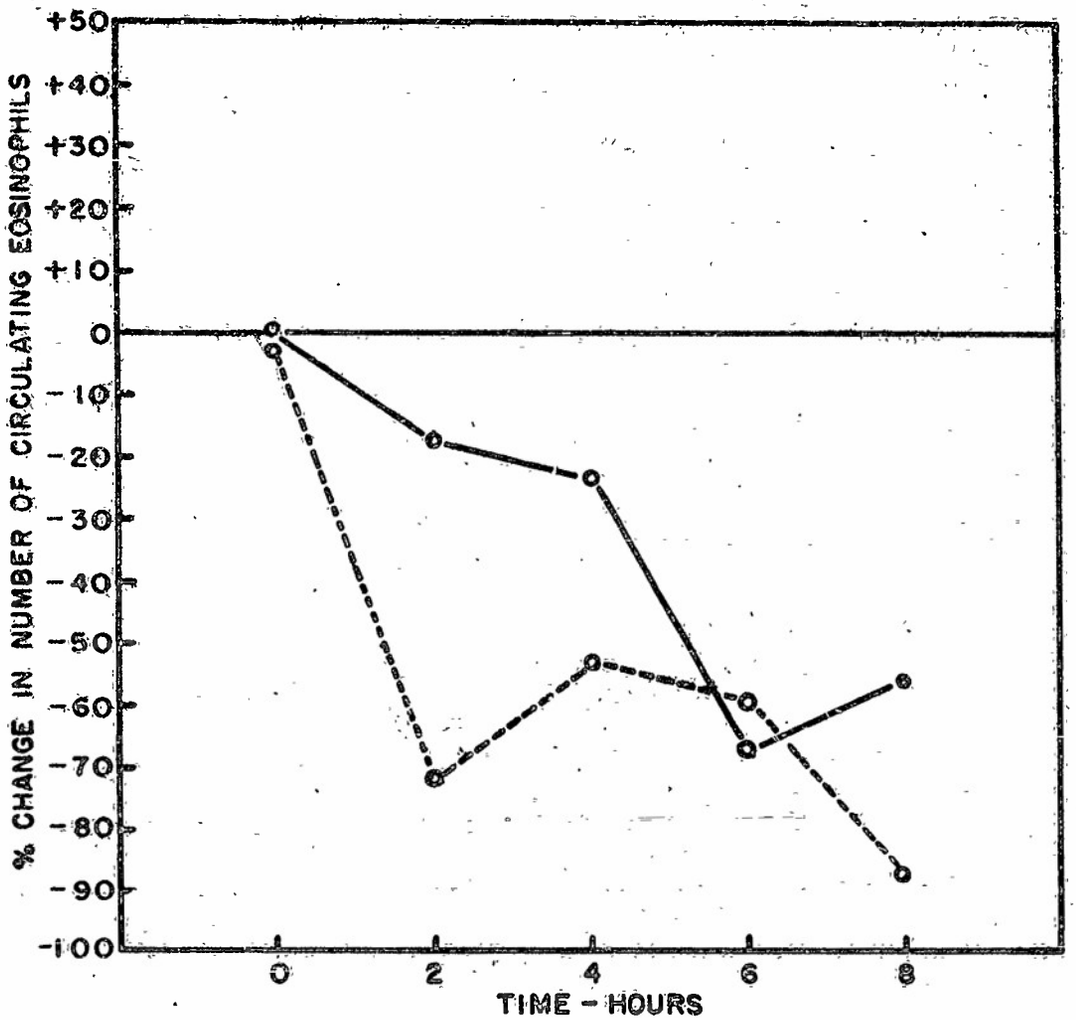

FIG. 3. EOSINOPHIL RESPONSE TO ADRENALIN

Solid line represents response following subcutaneous administration of 0.05 mg. of adrenalin per kilogram of body weight in Dog 64.

Dash line represents response following subcutaneous administration of 0.1 mg. of adrenalin per kilogram of body weight in Dog 49.

Response Following a Bout of Rage

In the animals exhibiting a hypersensitivity to rage, a rapid decrease in the eosinophil level occurred following a period of intentional teasing. This decrease ranged near 60 per cent at 1 hour and in some cases continued through the second and third hours.

TABLE 2

EOSINOPHIL RESPONSE TO A BOUT OF RAGE BEFORE AND AFTER SYMPATHECTOMY

Dog	Eosinophil Counts						Per cent Change				
	0 Hr	1 Hr	2 Hr	3 Hr	4-5 Hr	6-7 Hr	1 Hr	2 Hr	3 Hr	4-5 Hr	6-7 Hr
28	900	460	500	355	377	333	- 49	- 44	- 61	- 58	- 63
	544	600	519	319	250	431	+ 10	- 5	- 41	- 54	- 21
	638	300	437	506	337	650	- 53	- 31	- 20	- 47	+ 1
35	333	155	133	177	488	577	- 53	- 60	- 47	+ 47	- 73
	319	456	175	219	219	181	+ 43	- 45	- 31	- 31	- 43
36	600	244	288	311	288	288	- 59	- 52	- 48	- 52	- 52
	506	512	481	356	181	175	+ 1	- 5	- 30	- 64	- 65

First line in each case represents response before sympathectomy, second and third lines represent response after sympathectomy.

After these preparations were sympathectomized, an eosinopenic response still occurred which was essentially equal in magnitude to that before sympathectomy. However, the characteristics of the response changed in most instances such that the decrease developed more slowly. Only one dog in one test showed a decrease at 1 hour. In the majority of the tests some decrease was seen at 2 hours and the maximum decrease was usually attained at 3 hours. Although the magnitude of the decrease was generally less after sympathectomy than before, the difference probably was not great enough to be significant. In this connection attention is called to the fact that there was no way of quantitating the magnitude of the rage reaction. Table 2 summarizes these data and Figure 4 illustrates individual experiments in a graphic manner.

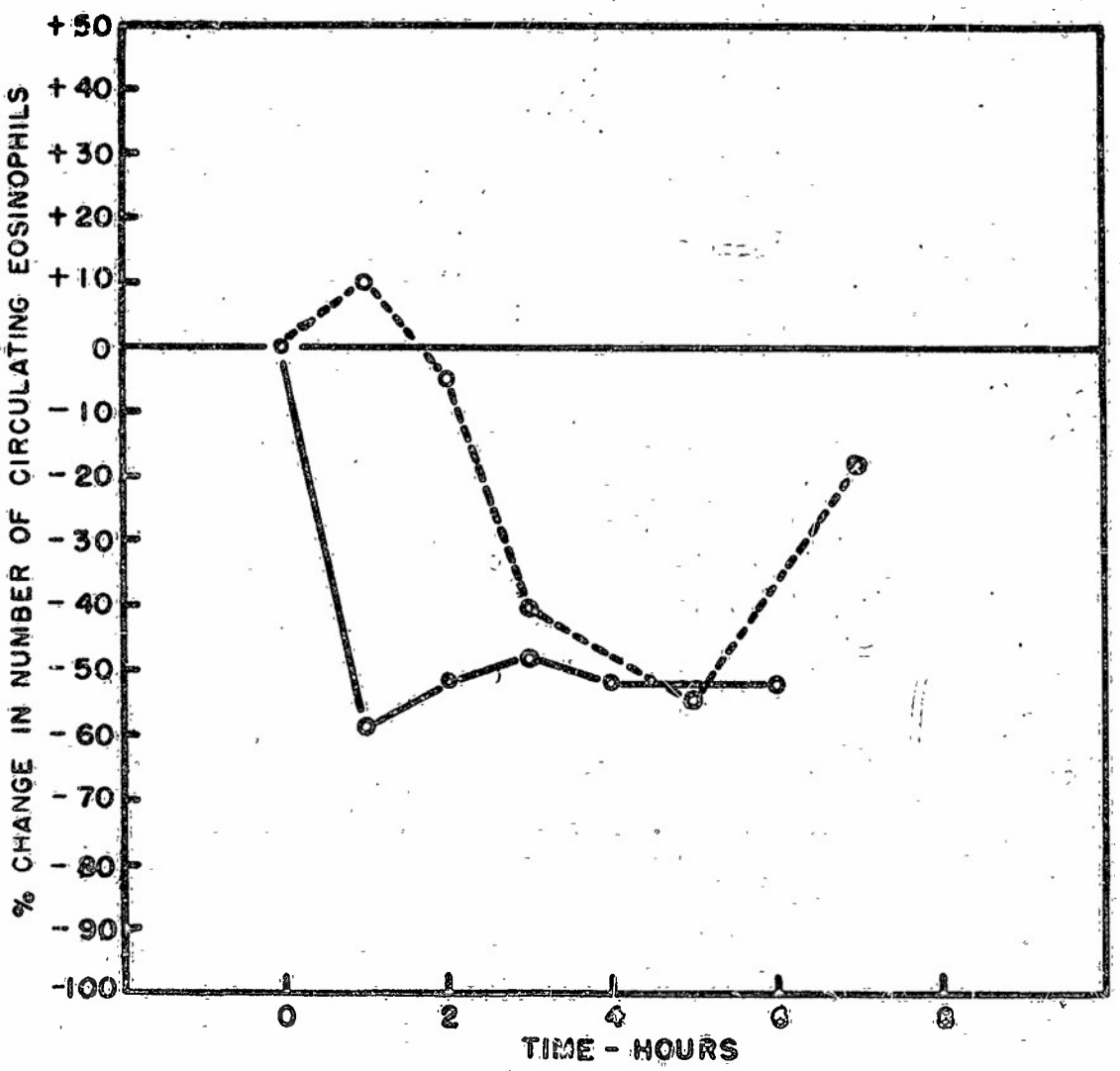

FIG. 4. RESPONSE TO A BOUT OF RAGE

Solid line represents response elicited from Dog 36 before sympathectomy.

Dash line is response exhibited by Dog 28 after sympathectomy.

Response to Hypoglycemic Crisis Induced by Insulin

In unoperated animals hypoglycemic crises produced a decrease in circulating eosinophils in the neighborhood of 50 to 60 per cent with the maximum decrease occurring at 5 to 7 hours. This response compares rather closely both in magnitude and time relations to that obtained from adrenalin injections.

TABLE 3

EOSINOPHIL RESPONSE TO INSULIN-HYPOGLYCEMIA IN NORMAL DOGS

Dog	Units of Insulin per Kg. Body Wt.	Eosinophil Cell Number						
		0 Hr	1 Hr	2 Hr	3 Hr	5 Hr	7 Hr	24 Hr
46	$1\frac{1}{2}$	1111	600	1144	800	533	422	1422
	3	800	600	400	288	533	444	355
49	$1\frac{1}{2}$	755	622	444	511	333	200	488
	3	400	355	111	400	200	22	777

Following bilateral sympathectomy a decrease in circulating eosinophils ranging from 50 to 70 per cent occurred, with the time of maximum decrease usually at 7 hours but sometimes as late as 24 hours. Thus again the response appears to occur somewhat more slowly after sympathectomy than in the unoperated animal. These data are given in Tables 3 and 4, and representative responses are illustrated in Figure 5.

TABLE 4

RESPONSE TO INSULIN-HYPOGLYCEMIA AFTER SYMPATHECTOMY

Dog	Units of Insulin per Kg. Body Wt.	Eosinophil Cell Number						
		0 Hr	1 Hr	2 Hr	3 Hr	5 Hr	7 Hr	24 Hr
28	$\frac{1}{4}$	369	438	294	200	125	94	444
	1/10	538	419	500	588	394	331	312
35	$\frac{1}{4}$	319	369	288	313	175	188	144
	$\frac{1}{2}$	275	219	225	181	394	150	287
36	$\frac{1}{4}$	506	512	481	356	181	175	818
	$\frac{1}{2}$	794	718	682	526	250	331	719
46	$\frac{1}{2}$	661	744	494	462	313	194	844

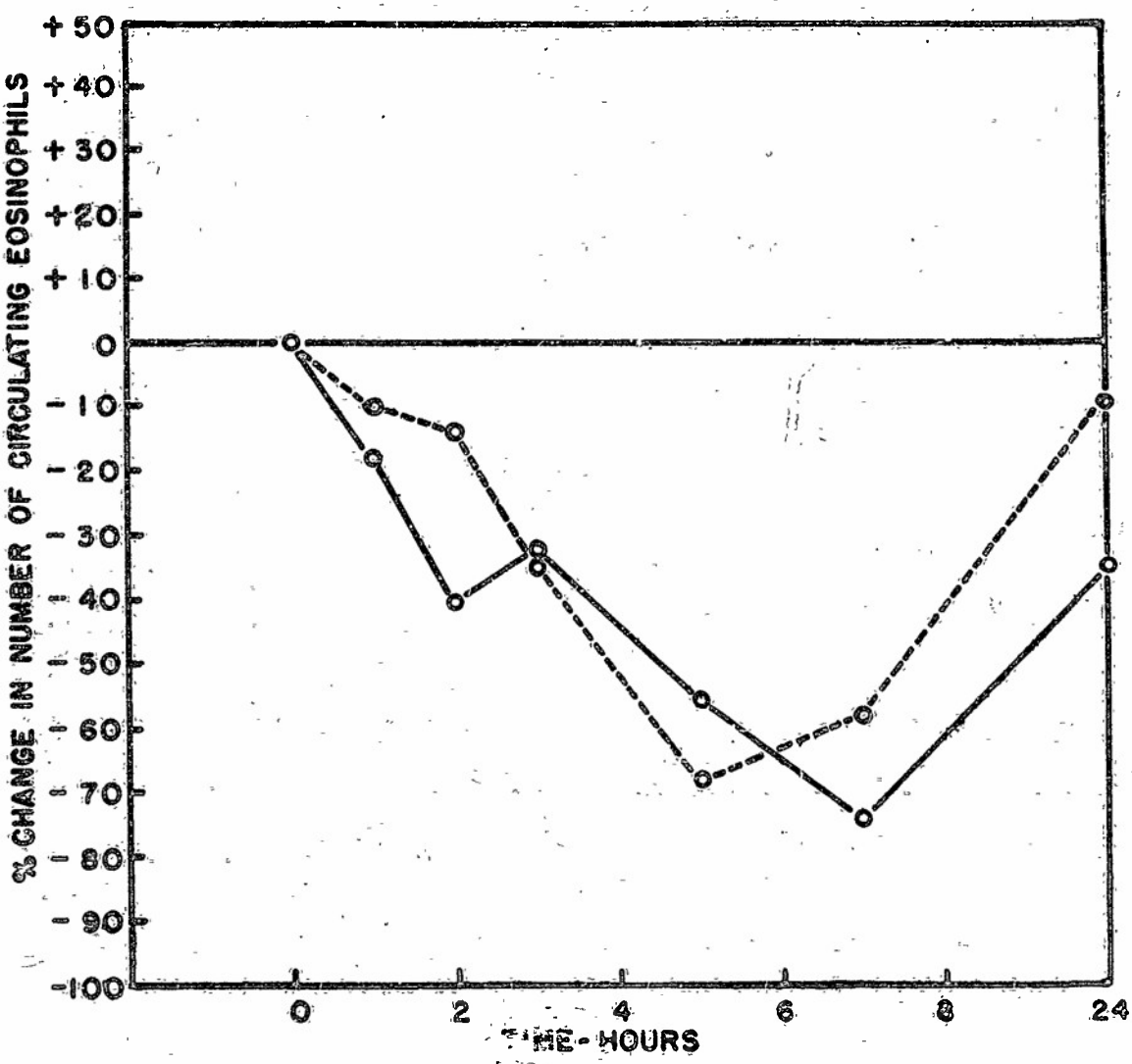

FIG. 5. EOSINOPHIL RESPONSE TO INSULIN HYPOGLYCEMIA.

Solid line is the response exhibited by Dog 49 to 1-1/2 units per kilogram of body weight.

Dash line is the response elicited after sympathectomy in Dog 36 by 1/2 unit per kilogram of body weight.

Exposure to Cold

Exposure of unoperated dogs to a temperature of 3-5°C. for a period up to 5 days produced an eosinopenic response only occasionally. Thus, such a stimulus in most dogs proves to be inadequate to excite the adrenal cortices.

TABLE 5

EOSINOPHIL RESPONSE TO COLD EXPOSURE

Dog	Relation to Sympathectomy		Eosinophil Cell Number (1) Rectal Temperature (2)					
			0 Hr	3 Hr	6 Hr	9 Hr	1 Day	2 Days
46	Before	(1)	533	444	466		622	
		(2)	38.2	37.8	38.0		38.2	
	1 month after	(1)	294	294	119			431
		(2)	38.0	35.6	35.2		35.4	38.6
	1 year after	(1)	175	469	25		31	106
		(2)	38.7	38.3	38.8		38.8	38.2
135	Before	(1)	81		81		75	
		(2)	37.9		37.8		38.0	37.8
	2 weeks after	(1)	31	25	13	19	13	19
		(2)	38.0	36.8	34.1	38.6	38.9	
137	Before	(1)	75		81		38	
		(2)	38.4		37.5		38.7	
	2 weeks after	(1)	94	119	13	63	19	100
		(2)	38.1	37.0	36.5	38.7	38.2	
144	Before	(1)	325		288		950	
		(2)	38.9		38.4		38.7	37.3
	2 weeks after	(1)	319	625	63	13	400	394
		(2)	39.0	38.0	35.0	38.8	38.9	

A matter of weeks after bilateral sympathectomy, exposure to 3-5°C. for 8 hours caused a considerable lowering in body core temperature in all animals except one. Concomitantly in these animals a marked eosinopenia amounting to 60-90 per cent resulted. The dogs in which a fall in body temperature occurred were lean and were conspicuous for the lack of insulating subcutaneous fat. In one animal (not included in the table) which was pathologically obese (hypothalamic puncture), body temperature did not fall to a comparable degree during the exposure period and no eosinopenia developed. In Dog 46 an eosinopenia occurred in response to cold exposure 1 year after sympathectomy, although at this

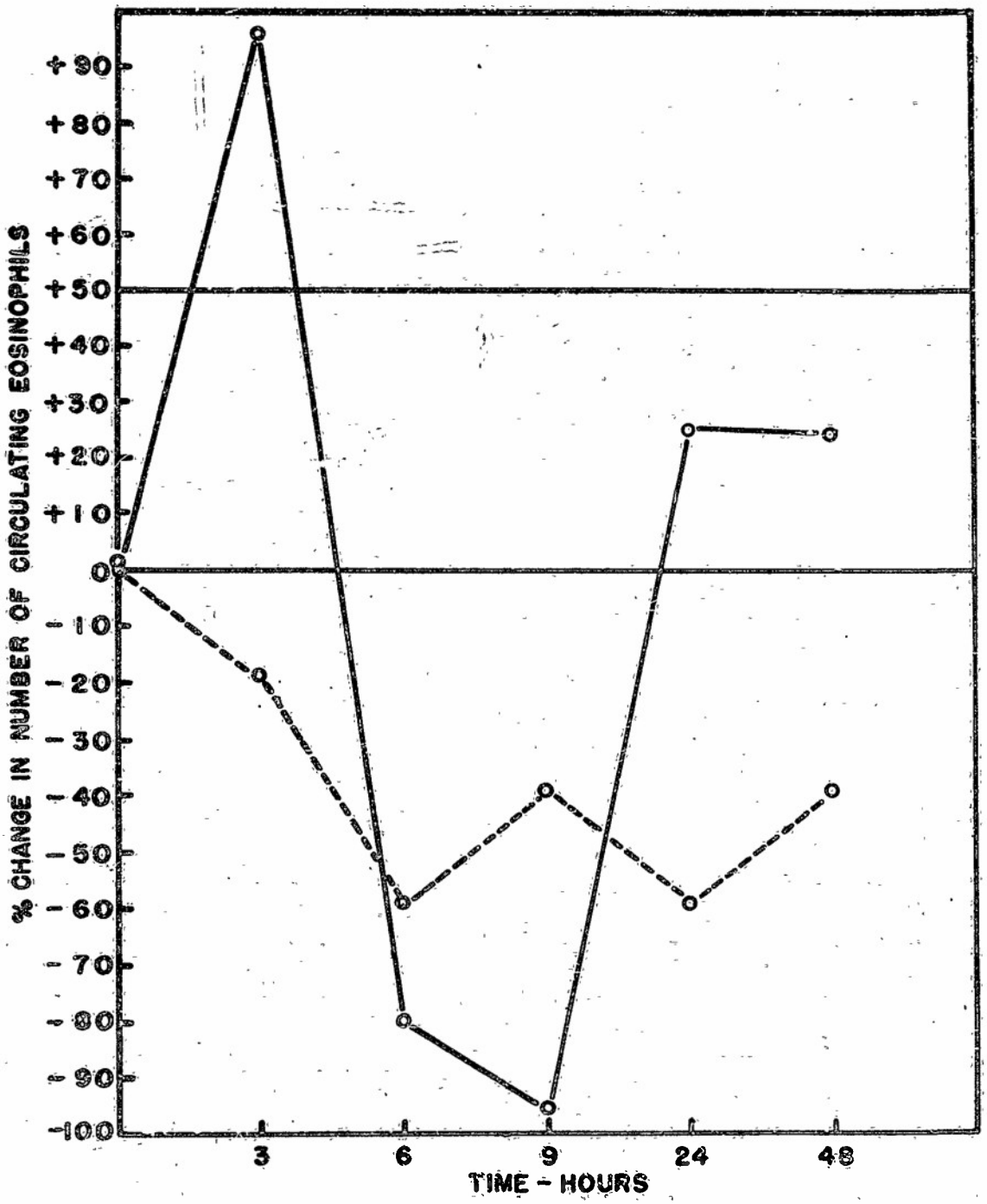

FIG. 6. EOSINOPHIL RESPONSE TO COLD EXPOSURE FOLLOWING SYMPATHECTOMY.

Solid line represents response exhibited by Dog 144 exposed to a temperature of 3-5°C for 8 hours with a fall in rectal temperature from 39.0°C to 35.0°C.

Dash line represents response exhibited by Dog 135 whose body temperature fell from 38.0°C to 34.1°C under same conditions.

time there was no appreciable fall in body temperature during the test period. Table 5 summarizes this data and Figure 6 gives individual responses in graphic form.

Surgical Trauma

Surgical trauma produced an eosinopenic response far greater in magnitude and duration than any of the other test stimuli applied in this series. The decrease in circulating eosinophils was usually greatest on the morning following surgery. The fall ranged from 80 to 100 per cent, the mean being greater than 90 per cent.

After removal of the abdominal and thoracic chains, the eosinopenic response to surgical trauma was not appreciably different from the above, as is illustrated in Table 6 and Figure 7.

TABLE 6

EOSINOPHIL RESPONSE TO SURGICAL TRAUMA

Dog	Absolute Eosinophil Cell Number					Per Cent Change			
	Before Surgery	4-6 hrs	1 day	2 days	6-8 days	4-6 hrs	1 day	2 days	6-8 days
46	578	44	0	133	467	- 92	-100	- 77	- 19
	500	69	0	388	269	- 86	-100	- 22	- 46
62	1443	369	19	1438	612	- 74	- 99	- 1	- 58
	1100	1446	25	1556	519	+ 31	- 98	+ 41	- 53
135	63	0	0	0	75	-100	-100	-100	+ 19
	31	37	0	31	44	+ 19	-100	0	+ 42
137	87	0	0	87	125	-100	-100	0	+ 44
	37	0	0	44	69	-100	-100	+ 19	+ 86
144	931	431	125	675	494	- 54	- 87	- 27	- 47
	621	37	13	294	250	- 94	- 98	- 52	- 59

First line in each case represents the response to removal of the left sympathetic chain, the right having been removed 2 weeks previously.

Second line represents the response to laparotomy with manipulation of the intestines 2-4 weeks after bilateral sympathectomy.

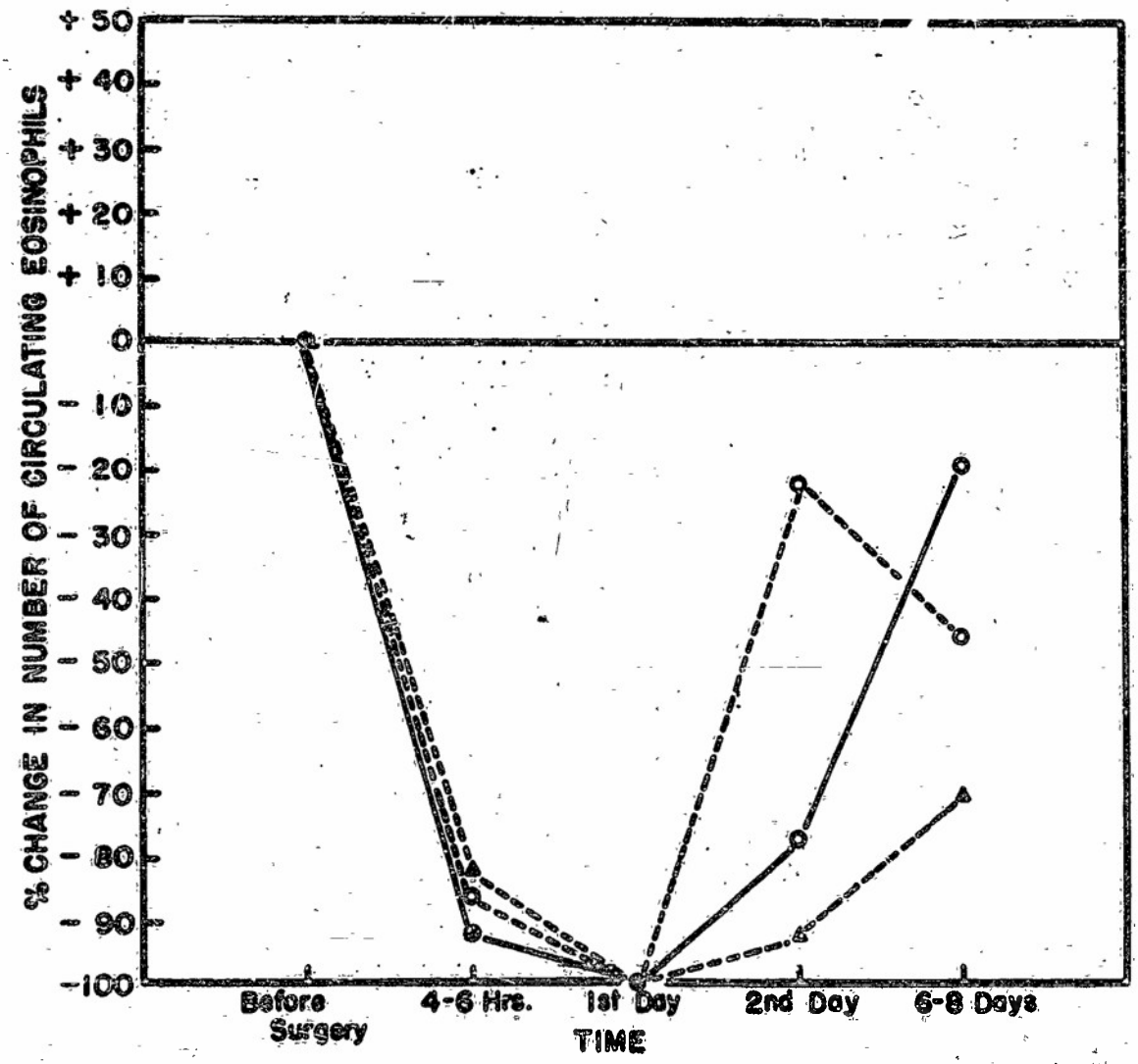

FIG. 7. EOSINOPHIL RESPONSE TO SURGICAL TRAUMA.

Solid line is the eosinophil response exhibited by Dog 46 to removal of the left sympathetic chain (May 1950), the right chain having been removed 2 weeks previously.

Dash line connecting circles is the response to laparotomy, with manipulation of the intestines, 3 months later (August 1950).

Dash line connecting triangle is the response to laparotomy, with manipulation of the intestines, one year later (August 1951).

IV. DISCUSSION

The foregoing data clearly demonstrate that removal of the thoracic and lumbar sympathetic chains does not eliminate the pronounced decrease in the number of circulating eosinophils which occurs characteristically after a bout of rage, a hypoglycemic crisis, or a major surgical procedure and which occurs on occasion in the unoperated dog as a result of exposure to cold. If it is assumed that bilateral sympathectomy removes completely the elaboration of sympathin and epinephrine, the data also demonstrate that adrenal cortical activity is not dependent on being triggered by these substances. Accordingly, mechanisms other than epinephrine must exist for activating the adrenal cortex when the need arises. Indeed, one might readily raise the question as to whether epinephrine (endogenous or otherwise) ever is the direct activator of the adenohypophysis. Pharmacological adrenalin has powerful and multitudinous pharmacodynamic properties and, accordingly, the release of adrenocorticotrophic hormones into the blood following its administration could well be an indirect response. In fact, the slowness and relative mildness of the response to adrenalin rather suggests that this is actually the case.

Although it has been generally accepted that the elaboration of epinephrine and sympathin is dependent upon nerve impulses arising within the central nervous system and exiting to the effector organs by way of the thoracolumbar outflow of the autonomic system, it is requisite to point out that this conclusion should not necessarily be considered as absolute. In this connection, the following facts must be kept in mind: (1) After removal of the thoracic and lumbar portions of the sympathetic chains, the epinephrine elaborating cells of the adrenal medulla and the post-ganglionic cells and fibers of the superior cervical and prevertebral sympathetic ganglia, do not degenerate. The possibility exists, therefore, that blood changes or other physiological processes might act as stimuli to these isolated ganglion cells causing a liberation of adrenalin or sympathin in the absence of direct central nervous system influence. (2) Adrenalin or an adrenalin-like substance might be liberated into the blood stream at nerve endings or by nerve cells located within the central nervous system. For example, Markee, Sawyer and Hollingshead (13) believe that the anterior pituitary is under adrenergic control by way of adrenergic cells located in the hypothalamus which liberate epinephrine into the blood of a local hypophysial portal system. (3) It is also conceivable that a limited number of adrenergic fibers might exit from the central nervous system by way of the craniosacral outflow of the autonomic system.

Even granting the possibility that other adrenergic sources remain following bilateral sympathectomy, it should be stressed that the "en masse" liberation of adrenalin and sympathin is definitely eliminated by this surgical procedure (7). Therefore, if the activity of the adrenal cortex is in any way dependent upon being triggered by an adrenergic mechanism it would, of necessity, be limited to a localized liberation of epinephrine either within the adenohypophysis or in its immediate environs as outlined above.

The predominant change in the rate of decrease of the circulating eosinophils which occurred in response to rage and occasionally following cold exposure and hypoglycemia after sympathectomy is the only indication afforded by the present data that adrenal cortical activity is in any way affected by autogenic adrenergic influences. It is reasonable to suspect that this delay in the eosinophil response could be due to the absence of epinephrine from the adrenal medulla as suggested by Long and associates (5), nevertheless, caution should be exercised in accepting this interpretation since the evidence is not conclusive. In this connection, speculatively, it is of some interest to note that the eosinopenic response was much more rapid following rage than the other stimuli in the presympathectomized dogs. This correlates well with the conventional association of epinephrine liberation during rage. One would expect a response of such rapidity if endogenous epinephrine were actually the direct activator of the adenohypophysis.

It is of considerable interest to note that a major surgical procedure proved to be outstandingly more reliable as an eosinopenic stimulus than was adrenalin injection, hypoglycemia, rage or cold. This is of practical significance. For instance, if one were limited to the use of a single criterion for the experimental assay of the functional status of the adrenal cortices, surgical trauma would definitely be the stimulus of choice (14). On the basis of percentage fall and particularly the duration of the eosinopenic response adrenalin injection, rage, hypoglycemia and cold exposure proved to be relatively mild stimuli compared to surgical trauma. This occurred in spite of the fact that the animals exhibited no adverse clinical symptomatology following the surgical procedures.

V. CONCLUSIONS

The functional maintenance and activation of the adrenal cortices, as assayed by the eosinopenic response, are not dependent upon nerve impulses exiting from the central nervous system over the thoracolumbar outflow.

There is no definite indication that adrenal cortical activity is in any way dependent upon endogenously secreted epinephrine.

A major surgical procedure is outstandingly a stronger eosinopenic stimulus than is the subcutaneous injection of adrenalin, hypoglycemia induced by insulin, a bout of rage or a bout of cold exposure.

VI. RECOMMENDATIONS

There remains the remote possibility that the adenohypophysial elaboration of ACTH is dependent upon a highly localized release of epinephrine or sympathin in the immediate environs of the adenohypophysis through a mechanism other than by nerve impulses exiting from the central nervous system over the thoracolumbar outflow. This possibility should be specifically investigated. To this end the present type of assay-study should be extended to hypothalamectomized-sympathectomized dogs in which the superior cervical sympathetic ganglia are removed in addition to the thoracic and abdominal chains.

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