Influence of Application of Cold or Heat to the Dog's Body upon the Epinephrine Output Rate.

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In cats, the iris of which has been completely denervated by removing the ciliary ganglion besides the superior cervical one, Hartman with his co-workers¹⁾ was able to bring about the dilatation of the denervated iris when the animal body was immersed in cold water and the rectal temperature began to fall. If the suprarenals were rendered inactive, cooling was proved to be incapable of causing the paradoxical pupil reaction. The results led them to conclude that stimulation by cold increases pronouncedly the epinephrine discharge; that this would, in turn, increase heat production if the amount of epinephrine were sufficient, thereby tending to compensate for the loss of heat, was their further suggestion. In the next investigations F. A. Hartman with W. B. Hartman²⁾ undertook to analyse the mechanism whereby the application of cold upon the animal body augments the epinephrine discharge, and came to witness that it is neither the excitement attendant upon the cooling nor the fall in general body temperature, but the peripheral stimulation of cooling which causes the release of epinephrine. Immersion of cats in water ranging from 39° to 45° did not appear to increase the epinephrine discharge. Almost all of the criticisms,³⁾ made by the Cleveland physiologists on the work of Hartman,¹⁾ are based on the findings in the hands of the latter investigators with the cava pocket method on anaesthetized animals.

1) F. A. Hartman, H. C. McCordock and M. M. Loder, *Am. J. Physiol.*, 1923, 64, 1.

2) F. A. Hartman and W. B. Hartman, *Ibid.*, 1923, 65, 612.

3) G. N. Stewart and J. M. Rogoff, *Ibid.*, 1923, 66, 257 ff.

The data which corroborate those of Hartman above quoted were published soon afterwards by Cannon.⁴⁾ The denervated heart in the animals surviving and living normally was taken as an indicator of epinephrine discharge. Taking the animal to a cold room, exposing to a cold draught from an open window or introducing cold water into the stomach called forth an acceleration of the denervated heart; when the suprarenals were rendered inactive these effects did not occur. He inferred that when the animal is exposed to cold, two calorigenic factors, epinephrine and shivering, are at work to protect the body against a fall of body temperature, and that when epinephrine is excluded from action the organism resorts to shivering as it does not when epinephrine is present.

Cannon⁵⁾ further noted that the denervated heart of cats responded with acceleration when fever was produced by intravenous or intramuscular injection of the suspension of dead typhoid bacilli. After injection cats with intact suprarenals showed first a rapid rise of temperature, the highest being reached in about 34 minutes and amounted to about 1°C., and then the temperature usually decreased, but began to rise again slowly and gradually. When the suprarenals were rendered inactive, injection of dead typhoid bacilli brought about only a second slow rise. The increase of denervated heart rate during the first elevation of temperature was too fast for taking the effect of a high body temperature upon the heart itself, on the base of Knowlton and Starling's study of the effect of temperature change on the isolated cat heart.

In this connection the previous findings of Kuno⁶⁾ may be recalled here: A bath of 40° to 45° accelerates considerably the rate of the denervated heart of rabbits (vagi and accelerantes were divided). An excess of 20 per minute in the bath of 40° and 44 to 45 in that of 45° was noted.

The conclusion made by Cannon on the effect of fever upon the epinephrine discharge from the suprarenals of cats seemingly contradicts that of Hartman from the experiments on cats in a warm bath from 39 to 45°, provided the acceleration of the denervated heart and the paradoxical dilatation of the denervated pupil be taken as an unmistakable indication of an increased epinephrine discharge, and further provided a certain portion of the acceleration of the denervated heart in Cannon's experiments be taken as the result of fever itself. In Cat 224 of Hartman a small increase in the denervated pupil was witnessed when immersed in water of 45°. The rectal temperature increased from 39.0° to 40.1°. Hartman would explain this increase in the pupil diameter by an increase in the tem-

4) W. B. Cannon and A. Querido, *Proc. Nat. Acad. Sci. U. S. A.*, 1924, **10**, 245; W. B. Cannon, A. Querido, S. W. Britton and E. M. Bright, *Am. J. Physiol.*, 19 (26-27), **79**, 466.

5) W. B. Cannon and J. R. Pereira, *Proc. Nat. Acad. Sci. U. S. A.*, 1924, **10**, 247.

6) Yas Kuno, *Pflügers Arch.*, 1914, **158**, 573.

perature of the iris. In passing it may be further related that in the previous publications⁷⁾ from this laboratory on the epinephrine liberation we found some agents which called forth some rise of the body temperature, but we are at present not able to say definitely the causal relationship between the body temperature rise itself and the augmentation of epinephrine discharge in such cases of toxic fever.

Cramer examined the suprarenal bodies of rats and mice which were exposed to cold, and detected some alterations in the medulla, as depletion of the load of epinephrine, when the body temperature decreased.⁸⁾ When mice were exposed to heat epinephrine disappeared sometimes from the periphery of the medulla.⁹⁾

Some years ago Nosaka¹⁰⁾ investigated the influence of the temperature of the environment upon the epinephrine store of suprarenals of rats. Suto and Inouye's method was applied. The epinephrine content of both the suprarenals was estimated on the mean as 0.00973 grm. *in toto* and 0.45 mgrm. per grm. of glands for 15 individuals in the room temperature (19–28°), 0.00725 grms. *in toto* and 0.33 mgrm. per grm. for 16 rats which were exposed to cold (5° in the mean) for about two weeks, and 0.01613 mgrm. respectively 0.75 mgrm. per grm. for 17 animals which were subjected to a higher temperature such as 35° for 7 to 9 days and nights. In the assay of the effects of fatigue and temperature on the suprarenals of rats, Vincent¹¹⁾ noted parenthetically that considerable reduction of the temperature of the surrounding medium may, independently of exercise, causes a reduction of the chromaphil reaction, if the animal's temperature becomes lowered; but this does not occur very frequently in the case of white rats.

Since it is alleged that the augmentation of epinephrine discharge and the increase of blood sugar content are closely related with each other, it may not be amiss here to quote the literature concerning the effect of the temperature of the surrounding medium upon the blood sugar content, but at present we may merely point out the papers loaded fully with such kinds of bibliography. A rather complete review of the researches concerning the influence of cooling of animal bodies on the metabolism of carbohydrate is presented in the paper of Morita,¹²⁾ which deals with the blood sugar content of cold-punctured rabbits. If a rabbit is punctured between the interbrain and midbrain along the middle line, the body temperature lowers progressively, but until it descends lower than about 30°, the blood sugar level remains unchanged. The previous publications on the effect of a higher surrounding temperature upon the blood sugar are given in the papers of Morita¹²⁾ and Fujii.¹³⁾ In the heat-punctured rabbits the blood sugar level remains unaffected.¹⁴⁾

The present writer carried out some investigations along these lines on non-anesthetized, non-fasted dogs, thereby somewhat different features were recorded; it must however be pointed out that from the previous writers

7) Tadashi Sugawara, Shidzuka Saito and Mamoru Nemoto, *Tohoku J. Exp. Med.*, 1927, **9**, 149; Sugawara and Hyozo Tada, *Ibid.*, 1927, **9**, 295; Sugawara, *Ibid.*, 1927, **9**, 368; Masa. Watanabé, *Ibid.*, 1927, **9**, 412; Watanabé, *Ibid.*, 1928, **10**, 29.

8) W. Cramer, *J. Physiol.*, 19(15-)16, **50**, *Proc. xxxviii*; *Ibid.*, 1918–19, **52**, *Proc. viii*; Sixth Scientific Report of the Imperial Cancer Research Fund, 1919 London, 1.

9) W. Cramer, *Brit. J. Exp. Path.*, 1926, **7**, 91.

10) Nosaka (野坂綱定), *Nihon Naikwagakukwai Zasshi*, 1924–25, **12**, 473 f.

11) S. Vincent, *Q. J. Exp. Physiol.*, 1925, **15**, 319.

12) Sachi. Morita, *Tohoku J. Exp. Med.*, 1921, **2**, 403.

13) Iju. Fujii, *Ibid.*, 1922, **3**, 88 f.

14) Sachi. Morita and Masaru Naito, *Ibid.*, 1922, **2**, 562.

the animals experimented on were of different kinds in both the series of experiments, the previous and the present, and that the severeness of cooling or heating has assuredly a direct bearing upon the results before us.

Methods.

Dogs, of which the dorsal roots from IX. thoracic to III. lumbar cord had been previously cut and which were found to have already recovered from the loss of body weight due to the operation, were exclusively used. The lumbar route preparation was carried out, and the blood from the suprarenal gland was assayed for the epinephrine content by means of the rabbit intestine segment method. All the procedures and techniques adopted in the present investigations were those given in the previous papers from this laboratory;¹⁵⁾ only the method of cooling or heating the dog remains to be mentioned. For applying an abnormal temperature to the body surface of the animal a double layered rubber coat was constructed in such a form as to enclose the whole body except the head. For collecting the suprarenal vein blood from both sides and measuring the anal temperature (and for tail) the coat was provided with suitable apertures. In order to let in water one opening near the bottom of every pouch for the limb and three pairs of openings at the dorsal edges of the coat on both sides were equipped with rubber tubes, which were usually closed by means of a screw-clamp. After putting the animal into the bag coat both the dorsal edges were closed on the dorsal middle line from the neck to the loin by means of cords. In running in water the rubber tube or tubes on the limb pouch were connected with the long limb of the syphon, the other limb of which was dipped in the water bath of a given temperature on a high table, by means of glass and rubber tubes, and the rubber tubes on the other limb pouches were opened, so that air could be got rid of; when the clamps on the rubber tubes connected with the syphon were lifted, water flowed into the double walled bag. In order to allow the water to flow out some rubber tubes on the dorsal edges of the bag and on the limb pouches were opened. The temperature of the water was measured by inserting the thermometer in one of the dorsal openings of the rubber coat.

Before putting the dog into the bag coat, the nails were cut and the paws bandaged in order to protect the rubber bag from scratching. When the bag coat was filled with water the dog became incapable of standing on its feet and lay down; therefore some water was driven out prior to the

15) Y. Sataké, T. Sugawara and M. Watanabé, *Tohoku J. Exp. Med.*, 1927, 8, 501; T. Sugawara, M. Watanabé and S. Saito, *Ibid.*, 1926, 7, 6 ff.

collection of the suprarenal vein blood samples, and they were taken in the proper posture of the animal. When the water in the bag was somewhat hotter, the dog struggled rather violently, so that it was necessary to hold it by the feet and back in order to prevent the bag from tearing, and when very hot water was let into the bag, the mouth of the dog was tightly bound, else the animal would have bitten the experimentors or the bag.

The use of this rubber bag enabled us to stimulate the animal with a temperature as high as one wishes, but it failed when wished to apply very severe cold; so in some cases a large hole, which corresponded to the form of the trunk of the animal, was made in a large block of ice, and cold was applied by putting the trunk of dog in this hole.

The experimental results are summarized in Tables I and II, the first table is loaded with the results of the application of cold and the second with those of heat. Of one example from each series of experiments the experimental course is given in full account with reproduction of the tracings, but the data of the estimations are omitted here, since they are included in the tables.

Results :

Cold stimulation was applied to five dogs. After completing the lumbar route preparation some samples of the suprarenal vein blood were collected, then the rubber coat was put on, and collection of some samples of the blood from the suprarenal capsule was made. The next procedure was the pouring of cold water into the coat. The temperature of the water in the bath was noted, and thereafter that in the coat was also measured from time to time. Some time, as ten to twenty minutes or more, elapsed until the next collection of the blood specimens was conducted, then the coat was taken off. Some minutes later the collection was again made. Such procedures were repeated, or they were followed by cooling of the dog by means of direct contact with the ice block in such a way as is described above. In Dog 5 they were conducted in the reverse order. In one dog, at the termination of the cold experiment, hot water (64°) was let into the coat, this resulted in an acceleration of the rate of epinephrine liberation. It is scarcely necessary to add here that the anal temperature, respiration and heart beat were measured from time to time.

The temperature of cold water in the bath was adjusted as 3° to 9° . The anal temperature decreased, but not greatly; in Dog 1 it descended from 40.4° to 39.2° , in Dog 2 from 39.0° to 38.5° , in Dog 3 from 38.9° to 37.8° (the dog in the coat was placed on an ice block), in Dog 4 from 39.2° to 37.8° and from 38.6° to 37.0° (when laid on ice) and in Dog 5

from 39.1° to 37.4° . On cooling the pulse rate invariably increased, while the rhythm of breathing underwent no definite variation, namely in most cases it became slower, but a somewhat faster rate was recorded during the cooling. The dogs shivered, and sometimes cried and struggled.

Now turning to the rate of epinephrine secretion, it may firstly be pointed out, that the epinephrine concentration in the suprarenal vein blood remained completely unaltered in the face of cold stimulation, which affected the body temperature though not so excessively, and secondly that the blood flow through the gland also remained unaffected or increased only a little. As a consequence the velocity with which epinephrine was liberated was quite or nearly equal during the rest spells and the cooling periods. Namely the cooling which was capable of affecting the rectal temperature to decrease to such an extent as 1.2° , 0.5° , 1.1° , 1.5° and 1.7° respectively, did not act to influence the rate of epinephrine discharge, contrary to our expectation by analogy of the experiments with cats, carried out by the previous investigators. It may possibly be justifiable to assume that this difference between the present investigations and the previous ones is explained by the difference of the kind of animal. Cats are in general highly sensitive to cold, whereas dogs are not. Working with dogs Araki¹⁶⁾ and Lépine¹⁷⁾ were able by cooling to cause glycosuria, appearance of lactic acid in the urine or hyperglycaemia. The body temperature of the dogs of the former investigator decreased remarkably, as to $28-23^{\circ}$ and 26° , while those of the latter writer had not so low a rectal temperature.

In conclusion of the cooling experiments, it must be added that in Dog 4 the application of hot water (64°) brought about a definite acceleration in the rate of epinephrine output, while the cooling in this dog was proved quite inactive, as in the other dogs.

The heating experiments, the data of which given concisely in Table II, will now be considered. They were carried out on seven dogs, six in Table II and Dog 4 in Table I.

When moderately hot water, as 55° to 60° , was poured into the coat, the animal looked quite comfortable though the breathing became faster and the conjunctiva hyperaemic. The pulse rate decreased invariably. The temperature of water in the coat was estimated as 43° , 45° and so forth. The anal temperature increased a little (in Dog 4 from 38.4° to 40.4° , in Dog 6 from 39.2° to 40.5 , in Dog 7 from 38.4° to 40.6° , in Dog 8 from 38.5° to 40.5° and in Dog 11 from 38.7° to 40.8°).

16) T. Araki, Hoppe-Seyler's Ztschr., 1892, **16**, 457 f.

17) R. Lépine, Le diabète sucré, Paris 1909, 192.

TABLE I.
Effect of cold application upon the rate of epinephrine output from the suprarenal gland of dogs.

No. of animal	Body weight (kilo)	Time of procedures (hour : min.)	No. of specimen	Blood flow (c.c.)			Epinephrine output (mgm.)		Room temperature (°C.)	Body temperature (°C.)	Rate of respiration per min.	Rate of heart beat per min.	Experimental procedure and others		
				Quantity of collection (sec.)	Duration of collection	Quantity per min. for ani- kilo	Quantity contained in 1 c.c.	Output per min.							
								for animal						per kilo	
Dog 1. 5. VI. 1926	11.0 ♂ left gland														
				I	3.0	40	4.5	0.41	0.00005	0.00022	22.0	39.9	36	120	After preparation of lumbar route Samples I and II were collect- ed, without coat. Samples III and IV were collect- ed after putting on the rubber coat. 12:20 p.m.—12:48 cold application. Samples V, VI, VII, and VIII were collected during the rubber coat was filled with cold water (6°C.). About 7 minutes after putting off rubber coat, Samples IX and X were collected. About 57 minutes after putting off rubber coat, Sample XI, XII, and XIII were collected.
			II	3.0	"	4.5	0.41								
		12:12 p.m.	III	2.1	"	3.1	0.28	0.00005	0.00015		22.0	40.4	36	126	
			IV	2.5	"	3.7	0.34								
		12:25	V	2.4	"	3.6	0.33	0.00005	0.00018	0.000016	22.0	39.8	30	156	
			VI	2.8	"	4.2	0.38								
		12:43	VII	2.4	"	3.6	0.33	0.00005	0.00018	0.000016	22.0	39.2	30	126	
			VIII	2.4	"	3.6	0.33								
		12:55	IX	2.2	"	3.3	0.30	0.00005	0.00016	0.000015	22.0	39.3	30	144	
			X	2.5	"	3.7	0.34								
		1:45	XI	1.1	"	1.6	0.14	0.00005	0.00009	0.000009	25.0	39.4	30	156	
			XII	1.3	"	1.9	0.17								
		XIII	2.0	"	3.0	0.27									
About 5 minutes after filling the rubber coat with 6°C. cold water Samples V and VI were collected. The dog was shivering, but quiet. After cooling the dog for about 23 min-utes Samples VII and VIII were collected. Then the rubber coat was put off and the dog was wiped up with a dry towel.															

After preparation of lumbar route Samples I and II were collected, without coat. Samples III and IV were collected after putting on the rubber coat.
 12:20 p.m.-12:48 cold application. Samples V, VI, VII, and VIII were collected during the rubber coat was filled with cold water (6°C.).
 About 7 minutes after putting off rubber coat, Samples IX and X were collected.
 About 57 minutes after putting off rubber coat, Sample XI, XII, and XIII were collected.

Dog 2.
5. XI.
192611.1
♂
left
gland
18. X. 1925. 10.8 kilos. L₃-D₃, dorsal spinal roots severed.
10:3-10:55 a.m. Lumbar route preparation.

left gland	11:21	I II III	1.6 1.6 1.5	3.2 3.2 3.0	0.29 0.29 0.27	a little below than 0.0001	a little below than 0.00032	a little below than 0.000029	16.0	24	150	Lumbar route was made, and Samples I, II and III were collected.	
	11:43	IV V	1.6 1.9	3.2 3.8	0.29 0.34	a little below than 0.0001	a little below than 0.00032	a little below than 0.000029	18.0	39.0	21	150	Samples IV and V were collected after putting on the rubber coat. 11:55 a.m.-12:20 p.m. cold ap- plication.
	12: 7 p.m.	VI VII	1.9 1.8	3.8 3.6	0.34 0.32	a little below than 0.0001	a little below than 0.00038	a little below than 0.000034	18.0	39.0	24	156	The rubber coat was filled with 5°C. cold water, and Samples VI and VII were collected. After collection the rubber coat was put off.
	1:11	VIII IX	1.8 1.9	3.6 3.8	0.32 0.34	a little below than 0.0001	a little below than 0.00036	a little below than 0.000032	18.0	39.0	18	156	Samples VIII and IX were col- lected after putting on the rubber coat.
	1:36	X XI	1.7 1.9	3.4 3.8	0.31 0.34	a little below than 0.0001	a little below than 0.00034	a little below than 0.000031	20.0	38.5	24	198	1:29 p.m.-2:42 cold application. After filling the rubber coat with 5°C. cold water. Samples X, XI, XII and XIII were collect- ed, and then the coat was put off.
	2:35	XII XIII	1.6 2.1	3.2 4.2	0.29 0.38	a little below than 0.0001	a little below than 0.00032	a little below than 0.000029	21.0	38.5	18	918	About 9 minutes after taking off the coat, Samples XIV and XV were collected.
	2:51	XIV XV	1.9 2.1	3.8 4.2	0.34 0.38	a little below than 0.0001	a little below than 0.00038	a little below than 0.000034	20.0	38.5	18	198	About 33 minutes after taking off the coat, Samples XVI and XVII were collected.
	3:15	XVI XVII	1.7 1.9	3.4 3.8	0.31 0.34	a little below than 0.0001	a little below than 0.00034	a little below than 0.000031	20.0	39.0	24	162	
	About 12 minutes after filling the rubber coat with 5°C. cold water, Samples VI and VII were collected. The dog was shivering. About 7 minutes after filling the coat again												

About 12 minutes after filling the rubber coat with 5°C. cold water, Samples VI and VII were collected. The dog was shivering. About 7 minutes after filling the coat again

Dog 4. 27. XI. 1926	8.3 ♂ left gland	10. XI. 1926. 9.0 kilos. L ₉ -D ₉ dorsal spinal roots severed. 10:25-11:5 a.m. Lumbar route preparation.																<p>Samples I and II were collected without coat. 11:30 a.m.-12:10 p.m. cold ap- plication. Dog was laid down on the large thick ice block. Samples III and IV were collected. The dog was then wiped up and warmed for 15 minutes by a steam heater. Samples V and VI were collected after putting on the rubber coat. 1:30 p.m.-2.5 cold application. The dog was cooled by filling the rubber coat with 5° cold water and laying down on the ice plate, then Samples VII, VIII and IX were collected. Coat was taken off, and dog wipe- d and warmed for 52 minutes by steam heater, X, XI and XII were collected. 3:4 p.m.-3:20 warm application. Dog was warmed by filling rubber coat with 64° hot water, XIII and XIV were collected. The coat was taken off, the dog returned to normal condition, then XV and XVI were collect- ed.</p>
		11:25 a.m.	I II	2.5 2.8	30 "	5.0 5.6	0.60 0.67	0.00015	0.00075	0.00009	17.6	39.2	24	150				
	12: 4 p.m.	III IV	3.2 3.7	" "	6.4 7.4	0.77 0.89	0.00015	0.00096	0.00011	19.0	37.8	15	270					
	1:20	V VI	3.2 3.2	40 "	4.8 4.8	0.58 0.58	0.0001	0.00048	0.00006	18.5	38.6	24	156					
	1:58	VII VIII IX	2.2 1.9 2.4	30 " "	4.4 3.8 4.8	0.53 0.46 0.58	0.00015	0.00072	0.00009	18.5	37.0	24	240					
	2:57	X XI XII	1.8 2.0 2.3	" " "	3.6 4.0 4.6	0.53 0.46 0.58	0.00015	0.00069	0.00008	19.0	38.4	24	192					
	3:18	XIII XIV	3.1 3.3	" "	6.2 6.6	0.75 0.79	0.00025	0.00165	0.00020	20.0	40.4	252	150					
	3:47	XV XVI	2.1 2.3	" "	4.2 4.6	0.51 0.55	0.00005	0.00021	0.00002	18.5	38.8	24	240					
	<p>Dog was laid down in hollow carved ice block, III and IV were collected after cooling in this manner for about 34 minutes. Dog was shivering violently and crying. Samples VII, VIII and IX were collected after cooling dog for about 23 minutes by filling rubber coat with 5° cold water and laying down on the ice block. Samples XIII and XIV were collected after warming dog for about 14 minutes by 64°</p>																	

No. of animal	Body weight (kilo)	Time of procedures (hour : min.)	No. of specimen	Blood flow (c.c.)			Epinephrine output (mgm.)	Room temperature (°C.)	Body temperature (°C.)	Rate of respiration per min.	Rate of heart beat per min.	Experimental procedure and others		
				Quantity of collection (sec.)	Duration of collection	Quantity per min. for ani- kilo								
													Quantity in I c.c.	Output per min.
							for animal	per kilo						
Dog 5. 4. XII. 1926	7.7 ♂ left gland	hot water. Dog very much struggled to get rid of, respiration became faster, tongue being hanged out of mouth.												
		8. XI. 1926. 8.5 kilos. L ₅ -D ₉ dorsal spinal roots severed.												
		11:20 a.m.-12:20 p.m. Lumbar route preparation.												
		1:21 p.m.	I	3.3	30	6.6	0.86	0.00015	0.00099	0.00013	20.0	38.5	24	120
		1:43	II	3.1	"	6.2	0.80	0.00015	0.00102	0.00013	20.0	38.5	24	138
			III	3.4	"	6.8	0.88							
			IV	4.1	"	8.2	1.06							
		2: 8	V	3.1	"	6.2	0.80	0.00015	0.00093	0.00012	21.5	38.4	12	288
			VI	3.2	"	6.4	0.83							
		3:17	VII	2.0	"	4.0	0.52	0.00015	0.00084	0.00011	22.0	39.1	30	120
			VIII	2.8	"	5.6	0.73							
			IX	2.2	"	4.4	0.57							
		4: 6	X	2.9	"	5.8	0.75	0.00015	0.00087	0.00011	20.0	37.4	24	270
			XI	2.7	"	5.4	0.70							
		5:14	XII	2.6	40	3.9	0.51	0.00015	0.00072	0.00009	16.0	38.7	30	150
			XIII	3.2	"	4.8	0.62							
Dog was laid down in the hollow carved on ice block; dog shivered violently. Samples III and IV were collected after cooling for 8 minutes, and Samples V and VI were collected after cooling for about 33 minutes. Samples XII and XIII were collected after cooling for about 31 minutes by 3° cold water filled into the coat. Dog was shivering, but not so violently as before.														

1:35 p.m.-2:10. Cold application. Dog was laid down in the hollow carved on the ice block. Samples III and IV were collected after cooling for 8 minutes, Samples V and VI were collected after cooling for 33 minutes. Dog was warmed by steam heater for about 1 hour, and then put on rubber coat, Sample VII, VIII and IX were collected. 3:35 p.m.-4:8. Cold application. The dog was cooled by filling the coat with 3° cold water. Samples X and XI were collected. After taking off coat and warming for 1 hour & 6 minutes, Samples XII & XIII were collected.

43°C. Dog entirely quiet, and seemed comfortable. Conjunctiva hyperaemic. After warming for about 26 minutes this hot water was replaced by 60° hot water. After warming with 60° hot water for more 12 minutes, Samples V, VI and VII were collected. Dog somewhat panting as in hot summer, tongue being stretched out.

22. XI. 1926. 10.0 kilos. L₇-D₉ dorsal spinal roots severed.
10:0-10:57 a.m. Lumbar route preparation.

11:39 a.m.	I	1.5	30	3.0	0.31	0.0001	0.0003	0.000031	16.5	38.5	18	168	After putting on rubber coat, Samples III, IV and V were collected. Warm application. 12:10 p.m.-12:36. Dog was warmed by 55° hot water; Samples VI and VII were collected. About 9 minutes after putting off coat, Samples VIII and IX were collected. About 1 hour after putting coat, Samples X and XI were collected.
	II	1.5	"	3.0	0.31								
	III	1.3	40	1.9	0.19								
	IV	1.4	"	2.1	0.21	0.0001	0.0002	0.000020					
12:33 p.m.	V	2.0	"	3.0	0.31				17.0	38.5	24	168	Dog was warmed by 55° hot water; Samples VI and VII were collected. About 9 minutes after putting off coat, Samples VIII and IX were collected. About 1 hour after putting coat, Samples X and XI were collected.
	VI	2.1	30	4.2	0.43	0.0001	0.00042	0.000043	18.0	40.5	414	90	
	VII	2.2	"	4.4	0.45								
12:45	VIII	1.3	"	2.6	0.26	0.0001	0.00026	0.000026	18.0	39.2	288	120	About 9 minutes after putting off coat, Samples VIII and IX were collected. About 1 hour after putting coat, Samples X and XI were collected.
	IX	1.4	"	2.8	0.28								
1:37	X	1.9	60	1.9	0.19	0.0001	0.00019	0.000019	18.0	38.4	24	174	About 1 hour after putting coat, Samples X and XI were collected.
	XI	2.2	"	2.2	0.22								

Dog was put on the coat and warmed for 23 minutes by 55° hot water filled into coat. Samples VI and VII were collected. Dog was very quiet and seemed to be comfortable, and afterward looked to be sleepy. Conjunctiva hyperaemic. Respiration became freer from time to time.

27. X. 1926. 13.7 kilos. L₇-D₁₀ dorsal spinal roots severed.
3. XII. 1916. 14.4 kilos. Experiment on haemorrhage (460 c.c. blood)
10:30 a.m.-12:10 p.m. Lumbar route preparation.

1: 1 p.m.	I	3.5	30	7.0	0.47	below than	below than	below than					After putting on rubber coat, Samples III and IV were collected. 3:5 p.m.-3:15 Warm application.
	II	3.6	"	7.2	0.48	0.0001	0.0007	0.000047	12.0	38.9	24	138	
	III	3.3	"	6.6	0.44	0.0001	0.00066	0.000044					
1:40	IV	3.7	"	7.4	0.49				19.0	38.9	45	120	After putting on rubber coat, Samples III and IV were collected. 3:5 p.m.-3:15 Warm application.

Dog 9.
13. I.
1927

Dog 8.
23. XII.
1926

9.8
♀
left
gland

No. of animal	Body weight (kilo)	Time of procedures (hour : min.)	No. of specimen	Blood flow (c.c.)			Epinephrine output (mgrm.)		Room temperature (°C.)	Body temperature (°C.)	Rate of respiration per min.	Rate of heart beat per min.	Experimental procedure and others		
				Quantity of collection (sec.)	Quan- tity per min.		Quantity contained in 1 c.c.	Output per min.							
					for ani- mal	per kilo		for animal						per kilo	
Dog 10. 14. I. 1927	11.1 ♀ right gland	3:15	V VI	3.7 4.1	7.4 8.2	0.49 0.55	0.00015	0.0011	0.00007	18.0	40.5	270	126	Dog was warmed by 65° hot water, then Samples V and VI were collected.	
		3:42	VII VIII	3.4 3.1	" "	6.8 6.2	0.45 0.41	below than 0.0001	below than 0.00068	below than 0.000045	18.0	39.2	42	168	About 26 minutes after taking off coat, Samples VIII and IX were collected.
		3:55	IX X	2.9 2.7	" "	5.8 5.4	0.39 0.36	below than 0.0001	below than 0.00058	below than 0.000039	18.0		30	210	About 39 minutes after taking off coat, Samples X and XI were collected.
		When coat was filled with hot water dog whined and struggled. Respiration frequent, conjunctiva hyperaemic, salivated much. Temperature of water in coat was 47°C.													
		24. XI. 1926.	10.0 kilos. L ₅ -D ₉ dorsal spinal roots severed.												
		21. XII. 1926.	10.8 kilos. Experiment on warmth.												
		10:15-11:25 a.m.	Lumbar route preparation.												
		11:45 a.m.	I	2.6 2.3	30 "	5.2 4.6	0.47 0.41	0.00052	0.000047	16.0	39.0	36	150	After putting on rubber coat, Sam- ples III and IV were collected.	
		12:25 p.m.	III VI	2.4 2.3	" "	4.8 4.6	0.43 0.41	0.00048	0.000043	17.5	39.0	48	180	12:51 p.m.-1:2. Warm application. Dog was warmed by 65° hot water, and Samples V and VI were collected.	
		1: 2	V VI	2.1 2.1	" "	4.2 4.2	0.38 0.38	0.0029	0.00026	18.0	41.3	300	120	About 12 minutes after putting off coat, Samples VII and VIII were collected.	
		1:15	VII VIII	1.6 1.5	" "	3.2 3.0	0.29 0.27	0.0019	0.00017	18.0	40.2	270	120	About 43 minutes after putting off coat, Samples VII and VIII were collected.	
		1:46	IX	2.1	"	4.2	0.38								

[illegible]

EXAMPLE I.

Dog 5. ♀

8. XI. 8.5 kilos. L₃-D₉ dorsal spinal roots severed.

4. XII. 7.7 kilos.

Time	Room temperature (°C)	Body temperature (°C)	Frequency of respiration (per min.)	Rate of heart beat (per min.)	Experimental process and others
10:40 a.m.					Dog was taken to operation room.
11:30	18.0	38.8	21	96	Lumbar route operation started.
12: 0 m.					Central end of l. suprarenal vein was prepared, and a somewhat thick and long thread passed under it.
12:20 p.m.	19.0	38.8	24	126	Small cannula was inserted into peripheral end of l. suprarenal vein.
1:21	20.0	38.5	24	120	Specimens I and II were collected.
1:35					Laid dog in a hole carved on a block of ice.
1:41					Dog shivered markedly.
1:43	20.0		24	138	Specimens III and IV were collected.
1:50					Dog was shivering more violently, whining.
1:53	21.0	38.2			
2: 5	21.0	38.2	15	198	
2: 8	21.5	38.4	12	288	Specimens V and VI were collected.
2:10					Dog was wiped up with a dry towel, and brought near steam heater.
3:10					Rubber coat was put on dog.
3:17	22.0	39.1	30	120	Specimens VII, VIII and IX were collected.
3:30					3°C cold water was flowed into coat and it was filled in 5 minutes.
					Dog was shivering, but not so violently as before. She was whining and growling operation table.
4: 0	20.0	37.4	24		
4: 6	20.0	37.4	24	270	Soon after drawing off coat Specimens X and XI were collected.
4: 8					Dog was wiped up carefully with dry towels.
5:14	16.0	38.7	30	150	Specimens XII and XIII were collected.
5:30					R. femoral artery was prepared, and about 100 c.c. blood was bled as a indifferent blood in epinephrine estimation.
5:45					Wounds sewed up.
6: 0					Dog was brought back to kennel.

Intestine tracings* (Reduced to $\frac{2}{3}$):—

Fig. 1. a. III: Decidedly stronger than 0.00005 mgrm.; far weaker than 0.0001 mgrm.

Fig. 1. b. V: Far weaker than 0.0001 mgrm. (Obs. 10); a little stronger than 0.00005 mgrm. I: Stronger than 0.00005 mgrm.

Fig. 1. c. VIII: Weaker than 0.0001 mgrm.; almost as strong as or a little stronger than 0.00005 mgrm. I: Very far weaker than 0.0001 mgrm. (Obs. 13).

Fig. 1. d. VIII: Stronger than 0.00005 mgrm. (Obs. 16); weaker than 0.0001 mgrm. X: Weaker than 0.0001 mgrm.

* In all the intestine tracings, at the mark "X" atropine-Tyrode's solution, in which the rabbit intestine segment was beating rhythmically, was replaced by indifferent blood solution, and at the "numeral" the indifferent blood solution was displaced by adrenaline blood solution, that is, the indifferent blood solution to which a certain quantity of adrenalin chloride of Sankyo Co. was added, or by the suprarenal vein blood specimen solution. All the blood solutions were prepared by diluting with 4 volumes of Tyrode's solution and the quantity of the bloods employed for one assay was 0.5 c.c. The numeral of suprarenal vein blood specimens and the quantity of adrenaline hydrochloride solution which was indicated by c.c. and concentration were noted on each tracing. For example "III" represents the III. suprarenal vein blood specimen, and "0.1 1/2000" shows "0.1 c.c. of adrenaline hydrochloride solution with a concentration of 1/2000 mgrm. in 1 c.c." i.e. 0.00005 mgrm.

In all tracings, time intervals 30 seconds.

Fig. 1. e. X : Clearly stronger than 0.00005 mgrm. (Obs. 19). XIII : Far stronger than 0.00005 mgrm.; weaker than 0.0001 mgrm.

Fig. 1. f. XIII : Far weaker than 0.0001 mgrm. (Obs. 22); decidedly stronger than 0.00005 mgrm.

To sum up :

Specimen I : Stronger than 0.00005 mgrm.; very far weaker than 0.0001 mgrm. per 0.5 c.c. It was assayed at 0.00015 mgrm. in 1 c.c.

Specimen III : Decidedly stronger than 0.00005 mgrm.; far weaker than 0.0001 mgrm. per 0.5 c.c. It was taken as 0.00015 mgrm. in 1 c.c.

Specimen V : A little stronger than 0.00005- mgrm.; far weaker than 0.0001 mgrm. per 0.5 c.c. It was taken as 0.00015 mgrm. in 1 c.c.

Specimen VIII : Stronger than 0.00005 mgrm.; almost as strong as or a little stronger than 0.00005 mgrm.; weaker than 0.0001 mgrm. (2 assays) per 0.5 c.c. It was taken as 0.00015- mgrm. in 1 c.c.

Specimen X : Clearly stronger than 0.00005 mgrm.; weaker than 0.0001 mgrm. per 0.5 c.c. It was assayed at 0.00015 mgrm. in 1 c.c.

Specimen XIII : Far stronger than 0.00005 mgrm.; decidedly stronger than 0.00005 mgrm.; weaker than 0.0001 mgrm.; far weaker than 0.0001 mgrm. per 0.5 c.c. It was taken as 0.00015 mgrm. in 1 c.c.

Blood flow, concentration of epinephrine and epinephrine output per min. per animal and per kilo of body weight are given in Table I.

EXAMPLE II.

Dog 11. ♀

22. XI. 1926. 10,0 kilos. L_5-D_9 dorsal spinal roots severed.

20. I. 1927. 11,8 kilos.

Time	Room temperature (°C)	Body temperature (°C)	Frequency of respiration (per min.)	Rate of heart beat (per min.)	Experimental process and others
9:30 a.m. 10: 0	13.0		18		Dog was taken to operation room. Lumbar route operation began.

Time	Room temperature (°C)	Body temperature (°C)	Frequency of respiration (per min.)	Rate of heart beat (per min.)	Experimental process and others
10:25					Central end of l. suprarenal vein was prepared, and then a thick and long thread was passed around it.
10:40	13.0	38.6	15	144	Small cannula was inserted into peripheral end of l. suprarenal vein.
10:50	13.0	38.6	15	144	Specimens I and II were collected.
11:40					Dog was put on rubber coat.
12:12 p.m.	14.0		18	144	Specimens III and IV were collected.
12:25					55° hot water was let into coat; it was filled in 9 minutes.
12:34					Temperature of water in coat 47°C. Dog was quiet and breathing frequently with tongue out of mouth. Conjunctiva hyperaemic.
12:38			96		
12:45					Temperature of water in coat 46°C.
12:46	15.0	40.8	330	96	Specimens V and VI were collected soon after drawing off water.
12:48					Rubber coat was taken off.
12:57			276	120	Specimens VII and VIII were collected.
1:42	15.0	39.5	18	144	Specimens IX, X and XI were collected after putting on rubber coat.
2: 9					65° hot water was flowed into rubber coat and it was filled in 7 minutes. During this procedure dog struggled to get rid off, cried. Conjunctiva hyperaemic. Respiration frequently, with tongue stretched out massive salivation.
2:21					Temperature of water in coat 51°.5C.
2:23	14.0	41.3	360		Hot water was drawn out from coat. Specimens XII, XIII and XIV were collected.
					Heart beat was hard to hear on account of gasping, and pulsation of femoral artery was also hard to touch.
2:39	14.0	40.4	42	132	Specimens XV and XVI were collected after putting off coat.
3: 0	13.5	40.0	18	210	Specimens XVII and XVIII were collected.
3:20					About 150 c.c. blood were bled from r. femoral artery as an indifferent blood for epinephrine estimations.
3:40					Wound sewed up.
3:55					Dog was brought back to kennel.

Intestine tracings* (Reduced to $\frac{2}{8}$):—

Fig. 2. a. I: Weaker than 0.00005 mgrm. III: Weaker than 0.00005 mgrm.

Fig. 2. b. III: Weaker than 0.00005 mgrm. (Obs. 11). V: A little weaker than 0.00005 mgrm.

Fig. 2. c. VII: A very little weaker than 0.00005 mgrm. (Obs. 14 and 16). X: Very much stronger than 0.00005 mgrm. XII: Very much far stronger than X.

Fig. 2. d. An other new intestine segment was used. X: Very much stronger than 0.00005 mgrm.; decidedly weaker than 0.0001 mgrm.

Fig. 2. e. XVII: Very far stronger than 0.0001 mgrm. (Obs. 25); a little stronger than or almost as strong as 0.0002 mgrm.

* See the foot-note on p. 561.

Fig. 2. f. XV : Very much stronger than 0.00015 mgrm.; far stronger than 0.00025 mgrm. XVII : Much stronger than 0.00015 mgrm. (Obs. 28).

Fig. 2. g. XV : Weaker than 0.0003 mgrm.

Fig. 2. h. XV : Stronger than 0.00025 mgrm. (Obs. 33 and 35); a little weaker than 0.0003 mgrm.

Fig. 2. i. XII : A little stronger than 0.0003 mgrm. (Obs. 36); stronger than 0.00035 mgrm.

Fig. 2. j. XIII : Stronger than 0.0004 mgrm. and 0.00045 mgrm.

Fig. 2. k. XIII : Stronger than 0.00045 mgrm. (Obs. 41); a little weaker than 0.0005 mgrm.

To sum up :

Specimen I: Weaker than 0.00005 mgrm. It was taken below than 0.0001 mgrm. in 1 c.c.

Specimen III: Weaker than 0.00005 mgrm. (2 assays). It was taken below than 0.0001 mgrm. in 1 c.c.

Specimen V: A little weaker than 0.00005 mgrm. It was assayed at weaker than 0.0001 mgrm. in 1 c.c.

Specimen VII: A very little weaker than 0.00005 mgrm. It was assayed at weaker than 0.0001 mgrm. in 1 c.c.

Specimen X: Very much stronger than 0.00005 mgrm. (2 assays); decidedly weaker than 0.0001 mgrm. It was taken as 0.00015 mgrm. in 1 c.c.

Specimen XIII: Stronger than 0.0004 mgrm. and 0.00045 mgrm. (2 assays); A little weaker than 0.0005 mgrm. It was assayed at 0.00095 mgrm. in 1 c.c.

Specimen XV: Very much stronger than 0.00015 mgrm.; far stronger than 0.00025 mgrm. (2 assays); weaker than 0.0003 mgrm.; a little weaker than 0.0003 mgrm. It was assayed at 0.00055 mgrm. in 1 c.c.

Specimen XVII: Very far stronger than 0.0001 mgrm.; much stronger than 0.00015 mgrm.; a little stronger than or almost as strong as 0.0002 mgrm. It was assayed at 0.0004 mgrm. in 1 c.c.

Blood flow, concentration of epinephrine and epinephrine output per minute per animal and per kilo of body weight are given in Table II.

But when the temperature of water in the bath went higher, as 65° , the temperature of water in the coat accordingly ascended to 50° or more and the rectal temperature fluctuated in Dog 9 from 38.9° to 40.5° , in Dog 10 from 39.0° to 41.3° and in Dog 11 from 39.5° to 41.3° ; namely in the last two dogs the body temperature became far higher than in the former series of heating experiments. In such cases the dog cried, struggled violently in order to get rid of the rubber coat, so that it was torn by scratching when the nails were not cut and the paws not bandaged. Faster breathing also occurred invariably, while the heart rate diminished a little. These findings on the pulse rate seem not to harmonize with those of various investigators, for example, those of Kuno on the urethanized rabbits.⁶⁾ Abundant salivation occurred also.

The influence of heating upon the epinephrine output corresponded with the intensity of heating. When the heating was not excessive, it remained quite unaffected as in Dog 7 and the first set of experiments in Dog 11, the blood flow rate and the epinephrine concentration during heating being found quite similar to those in the rest periods. In Dog 6, Dog 8 and Dog 9 the epinephrine secretion was calculated as augmented because of the increase of either epinephrine concentration or blood flow, but the augmentation was by no means significant. Contrary to these, a remarkable acceleration in the rate of epinephrine discharge was seen in Dog 10

and the last series of experiments on Dog 11 ; it resulted in a greater concentration of epinephrine. About seven times of the initial rate was noted. The subsequent samples, as specimens VII and VIII in Dog 10, which were collected 12 minutes after putting off the coat, and specimens XV-XVI and XVII-XVIII which were taken 16 and 40 minutes respectively after being freed from the coat, indicated that epinephrine continued still to be liberated at a somewhat faster rate, viz. a somewhat longer duration of after-action. The same is to be seen in Dog 6 (Specimens VII & VIII) also.

In the instances where a notable acceleration in the epinephrine secretion was thus recorded, the anal temperature also increased notably, that is 41° or more was measured, and the other reactions, as struggling, crying, etc., etc. were also excessive.

Summary :

The suprarenal vein bloods were collected from the non-anaesthetized, non-fasted dogs. The dorsal roots from IX. thoracic to III. lumbar cord were previously sectioned, and the lumbar route preparation was made. The epinephrine content was assayed by means of the rabbit intestine segment method.

1. Cooling of the dog was performed by means of a rubber coat, doubly layered, filled with cold water or by placing the animal's body in direct contact with an ice block. The anal temperature decreased 1.5° or 1.7° in some cases, the lowest temperature being estimated as 37° . No augmentation was noted in the rate of epinephrine discharge from the suprarenal gland.

2. Warming or heating of the animal was carried out by means of the rubber coat. When the temperature in the coat was not so high, the epinephrine secretion rate remained unaltered, but when the temperature of the water in the coat was increased, the anal temperature ascended more, and when the latter exceeded about 41°C the reactions of the animal became excessive, and at the same time the velocity with which epinephrine was liberated increased notably, such as about seven times that during the rest spells. The after-action was fairly long.