A Method for Collecting the Blood from the Suprarenal Gland in the Dog, without Fastening, Narcotizing, Laparotomy or Provoking any Pain.§

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I.

The behavior of an organ in a naturally living animal—either under ordinary conditions or in an intentionally altered environment—can only be inferred with confidence from observations performed by means of a procedure which causes *per se* no alteration in that state of the animal or at the best the least. The less the alteration, the more honest the observation. The information of the behavior of an organ in the ordinary, daily, quiet state of an animal withal provides the proper background against which to test how the organ reacts to any special change in the animal body or in its surroundings.

In the previous studies^{1,2)} of the epinephrine output from the suprarenal glands of dogs we were able to obviate the use of a narcotic, which has an inhibiting action on the reflex discharge of epinephrine,²⁾ by the application of the de-afferentation of the abdominal wall and viscera to the cava-pocket method.

§) Read before the IV. Japanese Physiological Meeting at Mukden, 1925 (J. Biophysics, 1927, 2, XLII.), and demonstrated at the V. Jap. Physiol. Meeting at Tokyo, April 1926 (J. Biophysics, In the press.).

1) Sakuji Kodama, Tohoku J. Exp. Med., 1924, 4, 465.

2) Sakuji Kodama, Ibid., 1924, 4, 601; J. Biophysics, 19(23-) 24(-26), 1, 79; Tadashi Sugawara, Masa. Watanabé and Shizuka Saito, Ibid., 1926, 7, 1.

At all events, however, it is beyond dispute, that an animal in such a condition, viz. an animal provided with the cava pocket preparation, though no narcotic was resorted to, differs much from an animal in the normal state.

To make good this shortcoming in the experimental conditions inherent in the previous researches, the expedient was tried of collecting the blood from the suprarenal gland by preparing the lumbo-suprarenal vein through a lumbar incision in the non-fastened dog, the operation field of which had been previously de-afferented.

This procedure of collecting the suprarenal vein blood without opening the abdomen was indeed conducted in the *anaesthetized* dogs by Stewart and Rogoff some years ago.³⁾ Whether the blood from the suprarenal body is collected via the experitoneal route or by means of the cava pocket was found to elicit no distinguishable influence in the epinephrine output from the gland. The above italicized qualification, i. e. that the dogs in their essay were anaesthetized, is important in connection with certain evidence that will be brought out in the following pages.

Firstly we performed the operation and manipulation of collecting the suprarenal vein blood through the lumbar incision in dogs under ether anaesthesia. Then next some dogs, the dorsal roots from the IV thoracic to the II lumbar cord of which had been previously interferred with, came into application. Namely, the dog so de-afferented was fastened prone on the dog board without narcosis, the left suprarenal body was uncovered by the retroperitoneal route, and the blood samples from the suprarenal gland were secured. The dog, though not always, struggled somewhat from time to time. The breathing became markedly deep in every case, and continued so throughout the fastening, so that the gland moved backward and forward a great deal, thereby the operation as well as the collection of blood specimens was carried out with some difficulty. In three of the dogs the specimens were first drawn out from the suprarenal gland by the route of the lumbar incision, then the animal was turned to a supine position and the cava pocket was prepared, thus the effect upon the epinephrine output of the routine experimental factors involved in preparing the cava pocket were roughly evaluated for the unanaesthetized dog. (In the remaining cases other kinds of experiments were carried out).

Finally we found it not only quite feasible, but also rather distinctly easy in comparison with the fastened dog to practise the same procedures in the dog non-anaesthetized, non-fastened, but so de-afferented. In most

3) G. N. Stewart and J. M. Rogoff, Am. J. Physiol., 1921, 56, 213.

cases the animal quietly stood, sat, lay or even slept during the operation and also during the collecting of blood specimens. Certainly we have come across few dogs so vicious that we were unable to perform the experiment without first tying the mouth by means of a heavy string. Even such dogs breathed calmly, so that not the least difficulty was met with in operating and collecting the samples.

A description of this method will be now given :

Dogs of moderate size were found suitable for this experiment. In small dogs, such as those weighing under about seven kilos, the lumbar vein forming the distal part of the lumbo-suprarenal vein was often found too narrow for a cannula of the proper size ; if the diameter of the cannula is too small, the blood is apt to clot in it. Those weighing over twenty kilos, on the other hand, are not handy. In adult dogs this vein was found frequently somewhat larger in comparison with that in young ones of nearly equal weight.

At first the dorsal roots from the IX thoracic to the III lumbar cord were bilaterally extradurally sectioned, the Sherrington's procedure being applied. In fact the section down to the II lumbar roots is quite sufficient for the painless preparation in the vicinity of the suprarenal body, but it was occasionally found convenient for collecting the blood samples with ease to extend the lumbar incision a little further backwards ; for the de-afferentation of this region the III lumbar dorsal roots were moreover interfered with.

The section of the dorsal roots of eight pairs was performed at once, and when the animal had recovered the normal appearance and body weight—usually a matter of about one month—the actual experiment was then carried out.

In the actual experiment the dog so de-afferented was brought on the dog board shown in the accompanying photograph, and then allowed to behave itself unrestrictedly, except that the end of the long chain round the neck was fastened to the front edge of the cross-beam of the dog board. So, some dogs stood on their four legs throughout the experiment, as demonstrated in the photograph, some half-sat, the forelimbs being extended, some knelt and some slept though this was seldom. The half-sitting position is most convenient for the operation as well as the collection of blood samples. If the animal lies unevenly, but really on the side opposite to the operation, the operation field becomes narrow and deep. In such a case trials were made to obviate this by letting the animal stand or lie on its another side, but generally in vain ; the animal soon returned to its habitual posture.

Fig. 1.

The operation for the left gland will now be first described, and second some peculiar points in operating for the right body will be stated.

Clipping and shaving off the hair from the skin of the left loin and the desinfection were carried out as usual.

A straight, transverse incision, about 5 cms. long, was first made behind the last rib, the upward end of the incision lying about midway between the dorsal mid-line and the lateral edge of *M. latissimus dorsi* and about 1-2 cms. to the last rib. The incision was then extended 6-7 cms. longitudinally backward and somewhat downward, the posterior end of the incision lying at a distance of some centimeters—say, 3-5 cms.—from the crest of the ilium.

The lumbo-dorsal fascia was then cut in a manner similar to the skin incision, and in fact the thick portion of the fascia covering the *latissimus dorsi* was cut by a somewhat vertical incision and the far thinner portion over the boundary of the *latissimus dorsi* and *external oblique* by a horizontal incision. Through the *external oblique* now emerged nerves with accompanying blood vessels in parallel series. The most anterior of these nerves and vessels, which run along the dorsal wall of the retroperitoneal cavity, are the first lumbar nerve and vessels. This nerve was simply severed (as a matter of fact no painful manifestation was evoked because of the previous de-afferentation), and the vessels were doubly ligated and divided. The proximal end of this vein was now followed by means of

forceps in the direction of the ventral column, then it was readily seen that the vein passed transversally through the middle of the opaque buff-coloured body, the suprarenal gland. The vein was then lost sight of at the lateral and ventral edge of the gland, that is it turned its direction under the gland, where the gland and the vein adhere closely to each other, and the vein runs towards the inferior cava vein, receiving the blood from the suprarenal body through, say, some fensters and then forming the lumbo-suprarenal vein.

The opening was now extended a few centimeters backward, the muscles of the abdominal wall being cut near their origin by means of forceps, but not by a scarpel. The veins, various in number and size, but commonly very small and invariably distinctly many in comparison with the right side, entering the first lumbar vein at the interval of about 1.5 cms. from the lateral, ventral edge of the gland, were doubly ligated and divided. The small veins entering just or nearly at the lateral, ventral edge of the gland were only singly ligated and not divided. In cases where the artery accompanies the vein far proximal, that is very near to the lateral edge of the gland, the former should be carefully separated from the latter. This not infrequently occurred.

The lumbo-suprarenal vein was then searched for along the median edge of the gland by means of a small forceps. It seldom was discovered at a glance because of its being transparent the colour of the blood, but sometimes it was difficult to find. The vein is found between the upper two thirds and the under one third at the median margin of the gland, and really just cephalad of the ganglion in the suprarenal plexus. It is generally possible to reach the vein without giving any evident mechanical effect on the ganglion of nerve plexus. Not infrequently a lymph vessel was discovered lying closely with the lumbo-suprarenal vein. In making free the vein from its surroundings care should be exercised not to tear the lymph vessel. In general, in operating in the vicinity of the gland we have paid the utmost care to inflict as little mechanical effect upon the gland as possible. In a few cases we have come across a lymph gland of the size of a coffee-bean or a little larger at the median surface near the posterior end of the gland. It was removed. Following the isolation of the vein for a length of about a half cm. or more from the median margin of the gland, a long, somewhat strong silk thread was passed round the lumbo-suprarenal vein, as far proximal as may be, with a silver mounted bent needle. The two ends of the long silk thread were tied together in a knot close to their free ends. The portion of thread under the vein was pushed a little

ventrally, so as not to interfere in any way with the blood flow therein.

At the beginning of the experiments with this method a rubber thread was used in place of the silk, but it was afterwards given up since we were not able to note any special merit in it.

Returning now to the lumbal vein distal from the gland, the vein was doubly ligated at a distance of about 1.5 cms. from the lateral, ventral margin of the gland and cut off between the two ligatures. A small bulldog clamp, provided with a rubber tube on one limb, was placed on the vein, near the gland. A silk thread was passed round the vein and the first noose of a reef-knot made with it, leaving the noose drawn down to about 4 mms. diameter but untied. A snip was made somewhat obliquely half through the lumbal vein between the ligature at the distal end of the vein and the noose with fine sharp scissors and the nozzle of a glass cannula, as shown in the accompanying figure in its actual size, was inserted into the cut vein, the free end of the cannula being directed ventrally and somewhat backward, and the noose tied tight round the neck of the cannula, the reef-knot was then completed and firmly tied. The free ends of the reef-knot were left in

Fig. 2.
Cannulae for
collection of
suprarenal
vein blood.
(Natural size)

a length of about five cms. The neck of the nozzle was constructed as straight as possible, else the blood might not infrequently coagulate therein. The small portion of the distal end of the vein was cut off proximal from the ligature knot.

Now the operation was complete, but when the wound was found too narrow to admit the blood from the cannula by a graduated cylinder, the wound must be further extended backwards, the muscles of the abdominal wall being cut by means of a scarpel, but this has very seldom occurred in two years with this method.

Of the peculiar points in operating on the right side some words may be added. Firstly the vertical straight incisions through the skin and fascia are to be carried out quite closely to the last limb, secondly, the small veins entering the proximal portion of the first lumbal veins are much less in number than on the left side, and lastly the lateral surface of the gland stands nearly vertically and the lumbo-suprarenal vein forms the straight prolongation of the lumbal vein, so that it comes into sight without any preparation; the lumbo-suprarenal vein is quite short, accordingly the utmost care should be taken in isolating it.

The operation from the skin incision to the insertion of the cannula usually occupied forty to eighty minutes. Only seldom were we able to complete the operation in thirty minutes. The operation on the left side demonstrated before the Japanese Physiological Society at Tokyo last year was one of the shortest, and really the small veins entering the proximal portion of the first lumbar vein were few in that dog. The length of time needed for the operation mainly depends upon the number of these small veins and the readiness with which the lumbo-suprarenal vein is uncovered and separated.

During the operation as well as during the collection of blood specimens the dog was watched by a technical assistant sitting in front of the animal. Sometimes meat, milk, water or cake were given to the animal during the operation or at a pause of the experimentation.

In the early period of the experiments with this method the operation was carried out by the three of us, but in fact it is within the capacity of two workers.

For the collection of the suprarenal vein blood the loop round the lumbo-suprarenal vein is gently drawn out and the bull-dog clamp on the lumbar vein removed, the opening of a graduated cylinder being put under the free end of the cannula. The precautions taken for making collection of blood samples were as follows: Firstly a small portion of the round opening of the cylinder was nearly straightened in order to bring it in close contact with the wall of the end of cannula, and secondly the free end of the cannula was placed properly upon the edge of the opening of the cylinder by careful controlling the thread, about 5 cms. long, of the reef-knot firmly tied round the vein wall covering the neck of the cannula. The traction of the loop round the lumbar vein must also be carefully conducted, that is only gently and quite evenly, never strongly. These two kinds of manipulation were carried out by a worker who stood on the opposite side to the gland under experimentation, and the opening of the wound with the left index-finger and receiving the blood with the graduated cylinder were done by another worker sitting on the same side as the operation, as shown in the photograph.

When the collection was finished, the lumbo-suprarenal vein was left free by gently pushing the portion of the loop under the vein ventrally, and the bull-dog clamp was laid on the lumbar vein near the gland. The cannula and the portion of vein left between the nozzle of the cannula and the bull-dog clamp was rinsed by means of fat-free cotton attached to the end of a small cotton holder. The wound was also cleansed, and then

closed by means of haemostats.

When the blood samples from the gland had been collected from time to time and no other series of manipulation, as severe haemorrhage or a heavy poisoning with diphtheria toxine, had been conducted, the animal appeared quite normal at the end of the experiment which had extended over several hours. The blood flow as well as the epinephrine output for the quiet, physiological state of the animal was also found to continue at practically a similar rate, that is without suffering from any noticeable reduction, throughout the long experimental course.

In pause period intervening between the collections of samples in the studies on the effect of fastening and sensory stimulation, which will be presented in the forthcoming paper, for instance, the dogs ate and drank eagerly the food stuffs offered. During the operation it was also commonly so.

When the experiment came to a conclusion, the loop round the vein, the clamp and the cannula were removed, the lumbal vein tied near the gland, and the wound cleansed by the use of a warmed physiological saline solution and then sewed up.

After recovery of the animal the second gland was then utilized for research. The time interval necessary for the recovery depends partly or mainly upon the degree of damage to the animal body inflicted by the experiment with the first gland.

As is readily seen from the description above given, the state of the animal can remain practically normal through the whole course of the experiment of a long duration, if the blood samples of a small quantity, as practised commonly, is simply drawn from time to time, and if no special manipulation for evoking a marked alteration in the animal body or in its environment has been conducted.

Roughly speaking, by use of this method presented in this essay, true features of the epinephrine output under the daily, quiet life of the animal as well as under special circumstances can therefore be thus seen.

It is however beyond dispute that the pressure in the cannula in the lumbal vein cannot be taken as the true image of that existing in the lumbo-suprarenal vein in the quite intact animal. The blood flow through the gland in the quite normal viz. non-operated state is not reflected, stringently speaking, without some modification in the values obtainable by this method.

The establishing of the true features of the blood flow through the gland and consequently of the epinephrine output in the wholly normal state of animal must be looked on as impossible so long as a method has not been discovered for cutting off just a pound of flesh without shedding one drop of blood from the breast.

If the fact that the epinephrine output remains in the same rate under otherwise same conditions of the animal in spite of changes in the blood flow, the concentration of epinephrine being altered reciprocally, can be definitely and exactly established as holding true in every instance, this shortcoming in the present method would be covered. Data indicating such a relation have been repeatedly published by the Cleveland physiologists, and we also have been able to realize it occasionally. In the present state of our experience however, we believe, we are far from taking such a correlation as existing for every case.

As a matter of course it must be taken as different from the animal's natural daily life, to speak exactly, the dog is placed on the table in a laboratory room and surrounded by a certain number of experimentors wearing operating dress.

That this method cannot be applied to small animals as cats and rabbits because of the smallness of the lumbar vein is quite regrettable.

II.

A. The Spontaneous Liberation of Epinephrine in Dogs.

In the previous papers from our laboratory, the epinephrine output secured by means of the cava pocket method either in the normal, anaesthetized animals or in the non-anaesthetized, de-afferented dogs, when any manipulation other than fastening, preparation of the cava pocket was conducted, was spoken of as the so-called spontaneous liberation from the suprarenals. The word "so-called" is intentionally affixed, because the state of the animals in those experimental conditions must be undoubtedly regarded as distinctly abnormal as compared with their daily, quiet life.

By the present method, the blood from the suprarenal gland can be obtained from dogs, quietly standing, sitting or furthermore sleeping at will. That the rate of the blood flow through the cannula inserted in the lumbar vein can not be taken, quite strictly speaking, as the true image of that which would take place in the lumbo-suprarenal vein, provided this operation had not been conducted, is a shortcoming in this method, but it might by no means materially affect the results.

In the accompanying tables are displayed the rates of epinephrine output from the suprarenal body in the quiet state of the so de-afferented dogs, that is when nothing has been introduced into the animal's body nor any noticeable alteration in the environment has taken place, obtained in several kinds of experiments performed by us in the last one and half years, the essentials of the results of which will be given in the forthcoming communications. The values of the spontaneous epinephrine liberation yielded in the researches of Dr Saito in this laboratory, which will be published later, fall into line with those presented in this table.

In all the cases summarized in these tables the epinephrine content was estimated by means of the rabbit intestine segment method, the procedure of which as practised in this laboratory has been fully illustrated in a previous paper.⁴⁾ In a few cases the paradoxical pupil reaction, described in another previous essay,⁵⁾ has come into application for its determination, no reaction with 5–10 c.c. of the blood from the suprarenal gland being elicited, just as was predicted. The inferiority of the lowest limit detectable by means of the latter reaction against the rabbit intestine strip method is responsible for these negative results. (Of the comparison of the rabbit intestine strip method and the cat denervated eye reaction for assay of epinephrine in bloods or extracts and its significance will be further discussed on a later occasion).

TABLE I a.
*Spontaneous liberation of epinephrine from the both
suprarenals in one and the same dogs.*

Datum	Sex	Body weight (kilos.)	Side of suprarenals	Blood flow per kilo per minute (c.c.)	Quantity of epinephrine con- tained in 1 c.c. (mgrm.)	Epinephrine out at per kilo per minute (mgrm.)	Remarks
(26. VI. 1925	♂	12.8	l	0.23	0.00015	0.000034	Sensory & fastening experiment.
(26. VI. 1925		12.8	r	0.35	0.00015	0.000052	"
(10. XII. 1925	♂	14.9	l	0.12	0.000225	0.000027	"
(14. I. 1926		15.7	r	0.08	0.00045	0.00004	Haemorrhage experiment.
(17. XII. 1925	♂	25.0	l	0.22	0.000125	0.000027	Sensory & fastening experiment.
(27. I. 1926		26.0	r	0.38	0.00025	0.000095	Haemorrhage experiment.
(13. I. 1926	♀	21.5	l	0.22	0.000075	0.000017	Sensory & fastening experiment.
(28. I. 1926		21.5	r	0.26	0.00005	0.000013	"
(20. I. 1926	♂	14.1	l	0.28	0.00015	0.00004	Haemorrhage experiment.
(3. III. 1926		16.8	r	0.45	0.00015	0.000068	β-tetra experiment.
(26. II. 1926	♂	14.7	l	0.30	0.00005	0.000015	"
(11. III. 1926		14.9	r	0.44	0.00025	0.00012	Diphtheria poisoning experiment.
(16. IV. 1926	♂	12.5	l	0.26	0.0001	0.000026	Haemorrhage experiment.
(22. IV. 1926		12.7	r	0.24	0.000057	0.000014	Diphtheria poisoning experiment.
(23. IV. 1926	♂	20.3	l	0.23	0.00005	0.000015	β-tetra experiment.
(29. IV. 1926		19.0	r	0.26	0.0002	0.000054	Diphtheria poisoning experiment.
(24. IV. 1926	♂	24.6	l	0.33	0.0001	0.000033	Haemorrhage experiment.
(6. V. 1926		22.5	r	0.31	0.0001	0.000031	Coffein experiment.
(15. V. 1926	♂	10.0	l	0.37	0.0001	0.000036	Haemorrhage experiment.
(28. V. 1926		10.3	r	0.32	0.00015	0.000016	Adrenaline experiment.

4) Tadashi Sugawara, Masa. Watanabé and Shidzu. Saito, *Tohoku J. Exp. Med.*, 1926, 7, 6 ff.

5) Tadashi Sugawara, *Tohoku J. Exp. Med.*, 1927, 8, 357–370.

Datum	Sex	Body weight (kilos.)	Side of suprarenals	Blood flow per kilo per minute (c.c.)	Quantity of epinephrine con- tained in 1 c.c. (mgrm.)	Epinephrine output per kilo per minute (mgrm.)	Remarks
(20. V. 1926	♀	14.7	l	0.32	0.0001	0.000032	Coffein experiment.
(27. V. 1926		14.1	r	0.33	0.000075	0.000025	Diphtheria poisoning experiment.
(4. VI. 1926	♀	9.0	l	0.40	0.00005—	0.000020—	Adrenaline experiment.
(2. VII. 1926		8.9	r	0.53	0.0001	0.000053—	"
(12. VI. 1926	♂	16.7	l	0.3	0.0001	0.000027	Haemorrhage experiment.
(8. VII. 1926		17.5	r	0.27	0.0001	0.000026—	Adrenaline experiment.
(24. VI. 1926	♂	15.0	l	0.2	0.00005	0.000009	"
(16. IX. 1926		16.2	r	0.35	0.000037	0.000013	Peptone experiment.
(25. VI. 1926	♀	9.5	l	0.59	0.0002	0.000118	Adrenaline experiment.
(22. VII. 1926		8.5	r	0.72	0.00005	0.000036	Peptone experiment.
(1. VII. 1926	♀	15.0	l	0.21	0.0003	0.000063	Adrenaline experiment.
(15. VII. 1926		15.0	r	0.23	*—	—	"
(23. VII. 1926	♂	15.4	l	0.4	*—	—	"
(3. IX. 1926		15.2	r	0.24	0.00011	0.000026	Diphtheria poisoning experiment.
(2. IX. 1926	♂	17.3	l	0.25	0.00015	0.000042	β-tetra experiment.
(25. IX. 1926		16.0	r	0.27	0.00015	0.000041	Peptone experiment.
(1. X. 1926	♂	16.2	l	0.34	0.0001	0.000034—	Haemorrhage experiment.
(14. X. 1926		13.0	r	0.24	0.00025	0.00006	Peptone experiment.
(7. X. 1926	♂	13.5	l	0.25	0.0001	0.000025	"
(16. X. 1926		10.6	r	0.25	0.0002	0.000051—	Guanidine experiment.
(29. X. 1926	♂	19.8	l	0.56	0.0001	0.000056—	Haemorrhage experiment.
(25. XI. 1926		20.0	r	0.35	0.000087	0.000031	Diphtheria poisoning experiment.
(12. XI. 1926	♂	18.9	l	0.34	0.0001	0.000034	Haemorrhage experiment.
(26. XI. 1926		18.2	r	0.27	0.000075	0.000020	Diphtheria poisoning experiment.

"—" means "a little below than."

* Epinephrine content was assayed in these cases by means of denervated eye reaction of previously splanchnectomized cats. The same remark is used in Table 1. b. A.

	Blood flow per kilo per minute (c.c.)		Quantity of epinephrine contained in 1 c.c. (mgrm.)		Epinephrine output per kilo per minute (mgrm.)	
(Nos. of observation)	(on 22 dogs)		(on 20 dogs)		(on 20 dogs)	
Side of observation	l.	r.	l.	r.	l.	r.
Average	0.30	0.32	0.000108—	0.000141—	0.000031—	0.000040—
Maximum	0.59	0.72	0.000225	0.00045	0.000118	0.00012
Minimum	0.12	0.08	0.00005—	0.000037	0.000009	0.000013

In 22 dogs weighing 8.5–26 kilos, the operation field of which was previously desensitized and both the glands were experimented on at different times, listed in Table 1 a, the blood flow from the suprarenal gland was 0.59–0.12 c.c., mean 0.30 c.c. per kilo and per minute for the left gland, and 0.72–0.08 c.c., mean 0.32 c.c. for the right. Thus we find a surprising equality in the average of blood flow between both the side glands. Notwithstanding in individuals, however, such a close agreement

between the fellow glands could not be invariably detected, the variation was not so wide as for one to be double of the other. In 9 out of 22 dogs, the blood flows from the sister glands were wholly or nearly the same.

TABLE I b.
*Spontaneous liberation of epinephrine from one
suprarenal gland, left or right of dogs.*

Datum	Sex	Body weight (kilos.)	Side of suprarenal	Blood flow per kilo per minute (c.c.)	Quantity of epinephrine con- tained in 1 c.c. (mgrm.)	Epinephrine output per kilo per minute (mgrm.)	Remarks
<i>A. From the left suprarenal gland.</i>							
1. IV. 1925	♀	12.9	1	0.2	0.00015	0.000031	Fastening & sensory experiment.
9. IV. 1925	♀	10.0	1	0.28	0.00013	0.000038	"
16. IV. 1925	♂	11.0	1	0.06	0.000162	0.000009	"
15. V. 1925	♂	12.5	1	0.11	0.0001 —	0.000011	"
12. VI. 1925	♂	12.9	1	0.1	0.0002	0.000019	"
19. VI. 1925	♂	13.0	1	0.24	0.0001	0.000024	"
5. XI. 1925	♂	14.0	1	0.33	0.000075—	0.000026—	"
12. XI. 1925	♂	17.4	1	0.4	0.00005—	0.000019—	"
24. XII. 1925	♂	15.5	1	0.16	0.0001 —	0.000016—	"
10. III. 1926	♀	11.9	1	0.52	0.000075	0.000039	β-tetra experiment.
1. V. 1926	♂	10.7	1	0.26	0.00005—	0.000012—	Adrenaline experiment.
11. VI. 1926	♀	13.6	1	0.42	0.000137	0.000057	"
17. VI. 1926	♀	8.5	1	0.48	0.00015	0.000072	"
18. VI. 1926	♂	8.8	1	0.26	0.000112	0.000029	"
9. VII. 1926	♀	16.5	1	0.53	*	—	"
9. IX. 1926	♂	11.4	1	0.30	0.0001 —	0.000030—	Guanidine experiment.
2. X. 1926	♂	11.0	1	0.25	0.0001 —	0.000025—	"
23. X. 1926	♂	9.8	1	0.27	0.00015	0.000040	"
30. X. 1926	♂	11.9	1	0.33	0.0001 —	0.000033—	"
3. VI. 1926	♀	11.9	1	0.46	0.000075	0.000025	Peptone experiment.
31. VII. 1926	♂	15.0	1	0.20	0.00005	0.00001	"
29. VII. 1926	♂	13.1	1	0.49	0.000112	0.000055	"
30. IX. 1926	♂	18.1	1	0.40	0.0001	0.00004	"
24. VII. 1926	♂	11.0	1	0.19	0.00005	0.000009	"
8. X. 1926	♂	10.0	1	0.29	0.0001	0.000029	"
4. XI. 1926	♂	17.0	1	0.32	0.00005	0.000015	"
21. X. 1926	♂	14.0	1	0.3	0.0031	0.00003	"
17. IX. 1926	♂	12.2	1	0.34	0.00005	0.000017	"
20. XI. 1926	♂	17.0	1	0.27	0.00005	0.000013	Diphtheria experiment.
18. XI. 1926	♀	11.3	1	0.34	0.000075	0.000025	"
28. X. 1926	♀	17.4	1	0.37	0.00005	0.000018	"
6. XI. 1926	♂	18.0	1	0.37	0.0002	0.000079	"
17. IV. 1926	♂	20.1	1	0.14	0.0001	0.000014	Coffeine experiment.
13. V. 1926	♀	16.6	1	0.32	0.0001	0.000033	"
(Nos. of observ.)		(34)		(34)	(33)	(33)	
Average		13.4		0.30	0.0001 —	0.000028—	
Maximum		20.1		0.53	0.0002	0.000079	
Minimum		8.5		0.06	0.00005 —	0.00009	

Datum	Sex	Body weight (kilos.)	Side of suprarenal	Blood flow per kilo per minute (c.c.)	Quantity of epinephrine con- tained in 1 c.c. (mgrm.)	Epinephrine output per kilo per minute (mgrm.)	Remarks
<i>B. From the right suprarenal gland.</i>							
15. IV. 1926	♀	22.4	r	0.24	0.000075	0.000017	β-tetra experiment.
10. IX. 1926	♂	12.6	r	0.65	0.00005	0.000032	Peptone experiment.
26. IX. 1926	♀	15.0	r	0.51	0.0001	0.000051	Diphtheria experiment.
4. III. 1926	♂	15.0	r	0.24	0.000175	0.000042	"
10. VI. 1926	♀	13.2	r	0.46	0.0001	0.000045	"
11. XI. 1926	♂	13.7	r	0.16	0.000075	0.000011	"
26. XI. 1926	♂	18.2	r	0.27	0.000075	0.00002	"
Average		15.7		0.36	0.000092	0.000031	Averages of 7 observations.
Maximum		22.4		0.65	0.000175	0.000045	
Minimum		12.6		0.16	0.00005	0.000011	

TABLE I c.

Total averages of numbers listed in Table I a and I b.

	Blood flow per kilo per minute (c.c.)		Quantity of epinephrine contained in 1 c.c. (mgrm.)		Epinephrine output per kilo per minute (mgrm.)	
Side of observ.	l	r	l	r	l	r
(Nos. of observ.)	(56)	(29)	(53)	(27)	(53)	(27)
Average	0.3	0.33	0.0001—	0.00013—	0.000029—	0.000038—

In Table 1 b the data of the dogs in which only one side gland was utilized, 34 observations on the left gland and 7 on the right, are summarized as a supplement to Table I a, the values being found as nearly lying within the limits given in Table I a, and really as almost the same in the mean numbers compared with those displayed there.

Epinephrine content of the blood from the suprarenal was assayed by means of the intestine segment at 0.000108 mgrm. (0.000225–0.00005) in 1 c.c. blood for the left glands of 20 dogs, listed in Tab. 1 a and 0.000141 mgrm. (0.00045–0.000037) for the right, and 0.0001 mgrm. in 1 c.c. on an average for 53 left glands altogether (Tab. 1 c) and 0.00013 mgrm. for 27 right glands. Thus the concentration of epinephrine in the blood was almost similar on the both sides or it was very little higher in the right side, the averages being taken into account. In analogy with the blood flow, the concentration of epine-

phrine in the bloods from the fellow glands in individuals was in some cases wholly identical, while in the majority of cases there was some difference in both the sides, though not excessive. Only in two dogs the concentration in one side was respectively four or five folds that of the other side.

The same kind of value obtained by employment of the cava pocket on the de-afferented dogs was 0.00009–0.001, mean 0.00053 mgrm. in 1 c.c. blood in 11 experiments of Kodama¹⁾²⁾⁶⁾ and 0.0005–0.0015, mean 0.0008 mgrm. in 1 c.c. in 5 experiments (The value in Dog 20 on p. 68 is not here referred to, since epinephrine was there determined by the paradoxical pupil reaction in the cat.) in a previous paper of ours.⁴⁾

Owing to the low concentration of epinephrine, the epinephrine output from the suprarenal in our dogs, referred to in the above tables, with only the least deviation from the normal, daily, quiet life, is definitely remarkably smaller in comparison with the quantities arrived at by means of the cava pocket method, prepared either in the de-afferented dogs without receiving any kind of narcotic or in the normal, but etherized dogs. In reality, the former has a value of 0.00003 mgrm. (0.000118–0.000009) per kilo and per minute from the left gland of 20 dogs, and 0.00004 mgrm (0.00012–0.000013) from the right, accordingly 0.00007 mgrm. per kilo and per minute from both the glands, whereas the latter were calculated as 0.00005–0.0018, mean 0.00072 per kilo and per minute for non-anaesthetized dogs and 0.00022–0.0033, mean 0.00083 mgrm. per kilo and per minute for etherized dogs in the papers of Kodama,¹⁾⁶⁾²⁾⁷⁾ and 0.00038–0.00132, mean 0.00069 mgrm. per kilo and per minute and 0.00012–0.00083, mean 0.00048 mgrm. respectively in our paper.⁴⁾ The spontaneous liberation discovered by the use of the present method is one seventh to one eleventh of those yielded by means of the cava pocket in this laboratory and really one third of the values obtained by Stewart and Rogoff by the cava pocket and rabbit segment method in the dogs under ether or morphine plus ether.⁸⁾

In regard to the epinephrine output the right side gland seems to be a very little superior to the left, the average being taken into consideration. In the individuals there were cases, where some inequality was visible. In 4 out of 20 dogs the output from the one side gland was more than three times of the other.

In the nicotine experiment undertaken by one of us (Sugawara), the

6) Sakuji Kodama, *Tohoku J. Exp. Med.*, 1924, 4, 601.

7) Sakuji Kodama, *Tohoku J. Exp. Med.*, 1924, 5, 47, 149 & 157.

8) G. N. Stewart and J. M. Rogoff, *Am. J. Physiol.*, 1923, 66, 235.

initial cava pocket blood from some (5 out of 8) of the doubly splanchnectomized, anaesthetized cats was indistinguishable in respect to the epinephrine from the blood in the general circulation, while in the rest epinephrine was found in the cava pocket blood though far inferior to that in normal cats.⁹⁾ The epinephrine concentration and output determined with the initial cava pocket blood specimen in the latter group of cats are near or clearly above the lower limits found for the notfastened dogs, noted in Tables I a & I b.

The above comparison of the rate of epinephrine output observed by the present method and that by the cava pocket method in the de-afferented dogs is not based upon the data yielded from one and the same individuals, but it is really deduced from the vast number of experiments. Now we will place on record the events in three de-afferented dogs, in which the blood flowing from one (left) suprarenal gland was collected first by the lumbar route, and then the cava pocket was performed and the pocket bloods were secured. In these experiments the dogs were fastened on the table, prone respectively supine, throughout the experiment, accordingly the values in the epinephrine output gained through the retroperitoneal way must be considered as possibly due to the influence of fastening, this matter will be dealt with in our next communication. In fact these experiments were carried out prior to the first execution of the method fully commented on in the above section.

The results are epitomized in Table II, while one experiment is fully reproduced in order to illustrate the course of the experiment.

EXAMPLE I.

Dog 2. ♀.

26. IV. 1924. 9.5 kilos. L₂-D₉ dorsal spinal roots severed.
 27. V. 1924. 11.0 „ D₈-D₄ „ „ „ „
 15. I. 1925. 9.0 „ Hind legs normal.

Time	Room temperature (°C.)	Body temperature (°C.)	Rate of respiration per minute	Rate of heart beat per minute	Experimental process and others
10:20 A.M. 10:30	14.5	38	30	150	Fastend prone. Dog sometimes cried and struggled. Left lumbar incision started.

9) Tadashi Sugawara, Tohoku J. Exp. Med., 1925, 6, 430.

Time	Room temperature (°C.)	Body temperature (°C.)	Rate of respiration per minute	Rate of heart beat per minute	Experimental process and others
10:38 A.M.					Cannula inserted into the peripheral end of lumbo-suprarenal vein; completed the lumbar-route operation.
10:40 0:17 P.M.	15.0	37.9	30	78	Collected the left suprarenal vein blood specimens I and II, after discarded more than enough blood to empty the dead space. While collecting the samples dog was very quiet.
0:23 0:48	15.0	37.7	18	78	Collected the left suprarenal vein blood specimens III, IV and V from suprarenal vein successively. While collecting III and IV dog was quiet, but while collecting V cried and moved.
1:14 1:18 1:20 1:22	15.0	37.9	24	96	Sutured the lumbar incision.
1:30-1:57	15.0	37.9	24	180	Fastened on another table supine.
1:58 2:05½	16.5	37.1	24	186	Made cava pocket, by packing the viscera with moist gauze and ligating the abdominal aorta, dog cried and struggled violently.
2:10 2:20	16.5	37.5	18	168	Collected the specimen VI from cava pocket, after discarded 6.5 c.c. of cava blood, while the collection dog cried and struggled.
2:21 3:00 3:05	16.5 16.5	37.6 37.5	36 24	180 138	Collected the specimen VII from cava pocket, after discarded 6.0 c.c. of cava blood. While the collection of VII, dog was quiet.
					Final bleeding from abdominal aorta.

Epinephrine output:

Number of specimen	Blood flow (c.c.)				Epinephrine output (mgrm.)		
	Quantity	Duration of collection (sec.)	Quantity per min.		Quantity contained in 1 c.c.	Output per minute	
			per animal	per kilo		per animal	per kilo
(L.) I	2.6	40	3.9	0.43	0.00015	0.00058	0.000064
(L.) II	2.9	40	4.35	0.48	0.00015	0.00065	0.000072
(L.) III	1.5	40	2.25	0.25	0.00015	0.00034	0.000038
(L.) IV	3.5	60	3.5	0.39	0.00015	0.00052	0.000058
(L.) V	2.6	60	2.6	0.29	0.00015	0.00039	0.000043
(C.P.) VI	4.2	40	6.3	0.7	0.00175	0.01102	0.001224
(C.P.) VII	3.9	40	5.85	0.65	0.0015	0.00877	0.000974

(L.) Specimens from left suprarenal gland by lumbar route.

(C.P.) Specimens from both suprarenals by cava pocket method.

Fig. 3.* a. At 2 and 4 adrenaline blood solutions which contained 0.0002 mgrm. and 0.00005 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood were applied. At 3 I specimen solution was applied.

I: Decidedly weaker than 0.0002 mgrm.; a little stronger than 0.00005 mgrm.

Fig. 3. b. At 5 and 7 I and II specimen solutions were applied respectively. At 6 adrenaline blood solution which contained 0.0001 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood was applied.

I: A little stronger than 0.00005 mgrm. (Observation 4); a little weaker than 0.0001 mgrm. per 0.5 c.c.

II: A little weaker than 0.0001 mgrm.; somewhat weaker than 0.00015 mgrm. per 0.5 c.c. (Obs. 8).

* In all the intestine tracings, at the mark "x" atropin-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 to which a certain quantity of adrenaline chloride of Sankyo & Co. was added, or by the suprarenal vein blood solution. All the quantity of the bloods employed for one assay was 0.5 c.c.

In all tracings, time intervals 30 seconds.

Concerning preparing the adrenaline blood solution and of suprarenal vein blood solution see T. Sugawara, M. Watanabé and S. Saito, *Tohoku J. Exp. Med.*, 1926, VII, 15.

Fig. 3. c. At 8 and 10 adrenaline blood solutions which contained 0.00015 mgrm. and 0.0001 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood were applied respectively. At 9 and 11 II & III specimen solutions were applied respectively.

II: Weaker than 0.00015 mgrm. per 0.5 c.c.; a little weaker than 0.0001 mgrm. per 0.5 c.c.

III: Very little weaker than 0.0001 mgrm. per 0.5 c.c., as strong as 0.00005 mgrm. per 0.5 c.c. (Obs. 12).

Fig. 3. d. At 12 and 14 adrenaline blood solutions which contained 0.00005 mgrm. and 0.0001 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood were applied respectively. At 13 and 15 IV and V specimens solution were applied respectively.

IV: A little stronger than 0.00005 mgrm. per 0.5 c.c.; a little weaker than 0.0001 mgrm. per 0.5 c.c.

V: A little weaker than 0.0001 mgrm. per 0.5 c.c. or almost as strong as 0.0001 mgrm. per 0.5 c.c.

Fig. 3. e. At 16 and 18 adrenaline blood solutions which contained 0.001 mgrm. and 0.00075 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood were applied respectively. At 17 VI specimen solution was applied.

VI: Weaker than 0.001 mgrm. per 0.5 c.c.; stronger than 0.00075 mgrm. per 0.5 c.c.

Fig. 3. f. At 19 VII specimen solution was applied. At 20 adrenaline blood solution which contained 0.0005 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood was applied.

VII: Almost as strong as 0.00075 mgrm. per 0.5 c.c. (Obs. 18); decided stronger than 0.0005 mgrm. per 0.5 c.c.

The specimens were further assayed repeatedly (not reproduced) and were taken as follows:

- I: 0.00015 mgrm. in 1 c.c.
- II: 0.00015 mgrm. „
- III: 0.00015 mgrm. (or a little below than it) in 1 c.c.
- IV: 0.00015 mgrm. in 1 c.c.
- V: 0.00015 mgrm. (or a little stronger than it) in 1 c.c.
- VI: 0.00175 mgrm. in 1 c.c.
- VII: 0.0015 mgrm. „

TABLE II.

Comparison of the lumbar route method and the cava pocket method on one and the same, fastened, but non-anaesthetized dogs, D_4 - L_2 dorsal roots of which were previously severed.

No. of animal	Body weight (kilos)	Time	No. of specimen	Blood flow (c.c.)				Epinephrine output (nigrm.)			
				Quantity	Duration of collection (sec.)	Quantity per minute		Quantity contained in 1 c.c.	Output per minute		
						per animal	per kilo		per animal	per kilo	
Dog. I. ♂ 25. XII. 1925	14.0	{20. II. 1925. 9.0 kilos. L ₂ -D ₉ dorsal spinal roots severed. 29. IV. 1925. 12.0 kilos. D ₈ -D ₄ dorsal spinal roots severed. A.M. 10:15- -11:05 P.M. 12:41- 1:00- 1:24- Average	Fastend prone. Made left lumbar route operation.								
				I	2.6	60	2.6	0.18	0.0001	0.00026	0.000018
				II	1.3	60	1.3	0.19	0.0002	0.00026	0.000019
				III	1.5	60	1.5	0.11	0.0002	0.00030	0.000021
				Average	1.8	0.13	0.00017	0.00027	0.000019		

No. of animal	Body weight (kilos)	Time	No. of specimen	Blood flow (c.c.)				Epinephrine output (mgrm.)		
				Quantity	Duration of collection (sec.)	Quantity per minute		Quantity contained in 1 c.c.	Output per minute	
						per animal	per kilo		per animal	per kilo
Dog 2. ♀ 15. I. 1925	9.0	2:00- -2:35 2:44-	Dog fastened supine; cava pocket completed.							
		IV	3.0	30	6.0	0.43	0.00125	0.00750	0.000536	
		V. lumbosuprarenalis sinistra was very narrow and small blood flow from left lumbar route.								
		{26. IV. 1924. 9.5 kilos. L ₂ -D ₉ dorsal spinal roots severed.								
		{27. V. 1924. 11.0 kilos. D ₈ -D ₄ dorsal spinal roots severed.								
		A.M. 10:20	Fastened prone.							
		11:38	Made left lumbar route operation.							
		P.M. 12:17-	I	2.6	40	3.9	0.43	0.00015	0.00058	0.000064
		-12:18½	II	2.9	40	4.35	0.48	0.00015	0.00065	0.000072
		12:48-	III	1.5	40	2.25	0.25	0.00015	0.00034	0.000038
		12:58-	IV	3.5	60	3.5	0.39	0.00015	0.00052	0.000058
		1:12-	V	2.6	60	2.6	0.29	0.00015	0.00039	0.000043
			Average			3.32	0.37	0.00015	0.00050	0.000055
		1:20- -1:57	Fastened supine; cava pocket completed.							
		2:05-	VI	4.2	40	6.3	0.70	0.00175	0.01102	0.001224
		2:20-	VII	3.9	40	5.85	0.65	0.00150	0.00877	0.000974
			Average			6.1	0.67	0.00162	0.00989	0.001099
Dog 3. ♀ 4. II. 1925	20.0	{18. VI. 1924. 18.7 kilos. L ₂ -D ₁₀ dorsal spinal roots severed.								
		{19. I. 1925. 20.5 kilos. D ₉ -D ₄ dorsal spinal roots severed.								
		A.M. 10:55-	Fastened prone.							
		10:58-	Made left lumbar route operation.							
		12:00M.								
		P.M. 12:43-	I	3.5	30	7.0	0.35	0.0003	0.00210	0.000105
		12:57-	II	2.9	30	5.8	0.29	0.0003	0.00174	0.000087
			Average			6.4	0.32	0.0003	0.00192	0.000096
		1:13- -1:48	Fastened supine; cava pocket completed. Region of abdominal aorta and V. cava inferior below liver was somewhat sensitive.							
		1:57-	III	5.8	30	11.6	0.58	0.0004	0.00464	0.000232
		2:05-	IV	6.6	30	13.2	0.66	0.0006	0.00792	0.000396
			Average			12.4	0.62	0.0005	0.00628	0.000314
		2:14-	Abdominal wound sewed up, and the dog was fastened prone.							
			(Specimens were collected from left lumbar route.)							
		2:19-	V	4.3	30	8.6	0.43	0.0004	0.00344	0.000172
		2:31-	VI	4.5	30	7.0	0.35	0.0005	0.00350	0.000175
			Average			7.8	0.39	0.00045	0.00347	0.000173
		2:34-	Sewed the left lumbar incision wound.							
		2:40-	Dog was fastened supine.							
			(Specimens were collected from cava pocket.)							
		2:50-	VII	5.8	30	11.6	0.58	0.0015	0.0174	0.00087
		3:01-	VIII	3.6	30	7.2	0.36	0.00175	0.0126	0.00063
			Average			9.4	0.47	0.00162	0.0150	0.00075

In these dogs the de-afferentation was carried out for the sake of preparing the cava pocket, that is, the bilateral dorsal roots extending from the IV thoracic to the II lumbar cord were previously severed in two sections.

In the actual experiment the dog was first fastened prone on the plate dog board; Dog 2 sometimes yelled and struggled, whereas the remaining two behaved themselves fairly calmly. The lumbar route operation was then performed on the one (left) side. The fastening the dog on the table is definitely disadvantageous for this operation, since the breathing is apt to deepen considerably by this handling, thereby the suprarenal gland closely attached to the diaphragm moves backward and forward to a rather great extent, so that the operation can be performed only with some difficulty.

Some specimens were got successively or at certain intervals; during the collection the animals were pretty calm or a little excited from time to time. After the lumbar wound was sewed up, the dog was freed from fastening, but immediately followed by fastening supine on the board of another form, which is usually employed for experiments with the cava pocket in this laboratory, and then the cava pocket was equipped. Dog 2 yelled and struggled violently, while the rest struggled somewhat, but not so intensively as Dog 2.

That in the thus de-afferented dogs the isolating as well as ligating the large abdominal vessels produce signs of pain and moreover, though infrequently, violent reactive movements, has been repeatedly recorded in the previous papers from this laboratory.¹⁾²⁾¹⁰⁾ It was so even in the dogs, the uppermost three pairs of dorsal roots of which were previously sectioned besides the usual twelve pairs.¹¹⁾ As to the centripetal course of these remaining sensibilities we have been unable to draw any suggestion with confidence by a perusal of the literature on the subject. In this connection it may be recalled that recently Asai¹²⁾ was able to see in rabbits that the abdominal viscera and especially the stomach, liver, gall bladder, kidney, etc., etc. are provided with sensory fibres from the vagus in addition to the sympathetic fibres, namely to duplicate and extend the findings of Schiff¹³⁾ in dogs, that have been long while relegated to the background.

Collection of some specimens from the cannula inserted into the cava pocket followed. In Exp. 3 the abdominal cavity was then closed by ligating, the dog was transferred to the first dog board, and fastened

10) Yasu. Sataké and Iju. Fujii, *Tohoku J. Exp. Med.*, 1926, **7**, 522.

11) Sakuji Kodama, *J. Biophysics*, 19(23)24(-26), **1**, 79.

12) Shiro Asai, *Kyoto Igaku Zasshi*, 1926, **23**, 518, 559 & 1267.

13) M. Schiff, *Leçons sur la physiologie de la digestion*, II, 1867 Florence & Turin,

prone again, with the subsequent collections of blood through the cannula put into the proximal portion of the I lumbar vein. Afterwards the blood was collected once more from the cava pocket.

For, roughly speaking, no material difference has been established in the blood flow, between the fellow glands, and accordingly none in the epinephrine output, if the average be compared as above referred to, the rate of blood current from the left gland will be double and may be compared with that from the cava pocket, with the results displayed in the accompanying small table.

TABLE III.

	Blood flow (c.c.) per kilo & per minute by		Quantity of epinephrine contained in 1 c.c. blood by		Epinephrine output per kilo & per minute by	
	1. lumbar route	cava pocket	1. lumbar route	cava pocket	1. lumbar route	cava pocket
Dog 1 ♂	0.26	0.43	0.00017	0.00125	0.00004	0.00054
Dog 2 ♀	0.74	0.67	0.00015	0.00162	0.0001	0.0011
Dog 3 ♀	0.64	0.62	0.0003	0.0005	0.0002	0.0003

On consulting the data in this small table and also in Table II, it may be readily seen that the results presented in these tables harmonize well with the comparison made between the results obtained by the present method and those by the cava pocket method on the different, viz. not on one and the same, de-afferented dogs, provided the effect of fastening is taken into consideration.

The blood flow through the cannula in the lumbar vein in the two dogs, Dog 2 and Dog 3, was practically similar not only to the values obtained in one and the same dog with the cava pocket method, but also with the average value of the blood current determined by the present method on numerous de-afferented, non-fastened dogs, epitomized in Tables 1 a & 1 b. In Dog 1 the blood current measured with the new method was evidently smaller than that yielded in employing the cava pocket, making use of the same animal.

In working with anaesthetized dogs we have come across several instances of experiments with such a difference in the blood flow according to the methods applied. That all or nearly all small veins entering into the proximal portion of the lumbar vein are ligated in the present method, while in preparing the cava pocket they commonly remain without being tied, partly accounts for this smallness in the blood current estimated by the former method.

In a few examples, while we were exercising the lumbar route operation on anaesthetized dogs, it was tried to estimate the influence of ligating these small vessels upon the blood flow as well as the epinephrine concentration in the blood, with the complete realization of our expectations. The shutting out the veins entering into the main vein was followed by the corresponding increase in the blood flow and decrease in the epinephrine concentration, so that the epinephrine output remained practically unaltered.

In making the cava pocket on the other hand we do not, as a rule, sensibly search for the small veins pouring into the inferior cava vein under the liver, though it is a common occurrence and moreover a relatively strong vein has been found there, though seldom; this matter may be also held as responsible for the above mentioned inequality in the blood flow rates estimated with these two different methods. Although it is beyond dispute that these two points above related constitute sources of the dissimilarity in the flow rate assigned by these different methods, we do not believe that all the possibilities of the explanation of this dissimilarity have been exhausted by pointing them out. In preparing the cava pocket in dogs, for example, the coeliac and mesenteric arteries are also sometimes ligated for the sake of accelerating the flow through the cava pocket.

Turning now to the epinephrine concentration of blood it may be plainly noted that the blood collected through the retroperitoneal route contained a far smaller quantity of epinephrine in comparison with that by means of the cava pocket, really one seventh or one eleventh in Dog 1 or Dog 2 respectively, while it was merely about one half in Dog 3. The values (0.0005–0.0016 mgrm. in 1 c.c.) obtained with the cava pocket are similar to those arrived at previously by Kodama (0.00009–0.001, mean 0.0005 mgrm. in 1 c.c.) and also by us (0.0005–0.0015 mgrm., mean 0.0008 mgrm. in 1 c.c. blood), making use of the same procedure on the same kind of animal (*viz.* de-afferented dogs), while those with the lumbar route operation in the present communication range far above the average or near to the uppermost limit of those displayed in Tables I which are compiled from the data observed by the present method, namely from the freely standing, sitting or sleeping de-afferented dogs, or they exceed them by a little. The fastening of the animal should be probably held responsible for this slight greatness in the epinephrine concentration of blood in the present dogs; the effect of fastening upon epinephrine output will be dealt with in the next communication.

Owing to the smaller epinephrine concentration, the output of epinephrine estimated by the retroperitoneal route was also far smaller than that with the cava pocket; in Dogs 1 and 2 the former was one tenth of the latter or less, while in Dog 3 the difference was small. The quantities with the latter method differ by no means from those formerly obtained in this laboratory, under quite similar experimental conditions with the present (0.00005–0.0018, mean 0.0007 mgrm. per kilo and per min. of Ko-

dama, and 0.00038–0.00132, mean 0.00069 mgrm. of Sugawara, Watanabé and Saito.). The epinephrine discharge noted by the lumbar route operation in two out of the present three dogs is clearly large as compared with that obtained by the same operation, but from the non-fastened dogs. Dog 3 gave the upper limit value found for the non-fastened dogs.

After first collecting two samples from the cava pocket in Dog 3, the abdominal cavity was closed, the animal fastened prone again on another dog board, and the blood collected through the cannula put into the lumbar vein. The blood flow underwent no detectable change, but the epinephrine content was not the same as with the former collection through the same way, but quite agreed with that by way of the cava pocket. This is the result to be readily foreseen. The lumbar wound was then sewed up, and the abdominal cavity re-opened, the animal being bound supine on another table again. This time the epinephrine concentration increased in a marked way, namely, it trebled the former value, while the blood current was found reduced only slightly; accordingly the epinephrine output was calculated as more than double the former. That the re-opening of the abdominal cavity and handling anew of the viscera account for this occurrence may be probable.

Haemorrhage as called forth in this experiment which never much exceeded one thirtieth of the total blood amount of this dog is incapable of provoking any augmentation in the epinephrine discharge in the non-fastened dogs.¹⁴⁾

The results quoted in this section seem apparently to afford good evidence to justify the objection made to the cava pocket method, that the procedure itself, i.e. the necessary conditions of the observation (anaesthesia, unavoidable excitation of afferent nerves, opening of the abdominal cavity, etc.) already causes an increase of the epinephrine output, and consequently the effect of sensory stimulation, asphyxia, etc. is masked. In fact, however, it is not only definitely established by the evidences put forward from this laboratory, that the sole or at least main factor that masks the effect of sensory stimulation, etc. is really the use of anaesthetic, an inhibiting agent, but also the above conclusion is borne out from the experimentation on the non-anaesthetized dogs.

That the anaesthesia is capable of making invalid the unrestricted generalization of the above inference about the comparative study of both

14) Shidzuka Saito, presented before the V Jap. Physiological Meeting at Tokyo, 1926 (*J. Biophysics*, 1927, 2, In the press.).

methods, the lumbar route operation and the cava pocket, is conceivable from the following examples.

**B. Comparison of the Rate of Epinephrine Output
Estimated with the Retroperitoneal Route
Operation and the Cava Pocket Method
in the Anaesthetized Dogs.**

In all three instances of the non-anaesthetized, de-afferented dogs the influence of the cava pocket preparation upon the epinephrine discharge was plainly demonstrable. By reference to the inhibitory influence of anaesthesia upon the reflex output of epinephrine, clearly established in our previous communications, one may be justified in expecting a diminution or moreover a complete abolishment of the accelerating discharge of epinephrine inherent to the cava pocket preparing that takes place invariably when carried out on the non-anaesthetized animal.

In reality this anticipation was fully realized when we were working with dogs under anaesthesia. In the first place we may refer to the typical example of experiments in which both the methods yielded quite the same results in respect of the rate of blood flow as well as of the epinephrine output.

EXAMPLE II.
19. III. 1925. Dog ♀. 21 kilos.

Time	Room temperature (°C)	Anal temperature (°C)	Rate of respiration per minute	Rate of heart beat per minute	Corneal reflex	Muscle tone	Experimental processes and others
10:00 A.M. 10:02 10:10	16.0		96	144	+	+	Fastend prone. Narcotized by dropping ether and chloroform mix- ture on towel covering loose- ly mouth and nose of the animal, then put cannula into trachea and gave ether with Woulff's flask.
10:18 10:30-11:10	16.0	39.8	84	180			Completed left lumbar- route operation.
11:20 11:37	18.0	39.7	84	222	+	-	Collected specimen I & I' from l. suprarenal vein retro- peritoneally by lumbar way.

Time	Room temperature (°C)	Anal temperature (°C)	Rate of respiration per minute	Rate of heart beat per minute	Corneal reflex	Muscle tone	Experimental processes and others
11:54 12:10-12:45 P.M.	18.5	39.8	60	180	+	-	Made cava pocket, taking much care to ligate all veins entering into the cava pocket perfectly. A. coeliaca and a. mes. sup. were not tied.
12:55 1:12	19.0	(39.3)*	72	222	+	-	Collected specimens II and II' from both suprarenals by means of cava pocket method.
1:15 2:35	19.0		78	204	+	-	Final bleeding from abdominal aorta.

* Thermometer in peritoneal cavity.

Epinephrine output:

No. of specimen	Blood flow (c.c.)				Epinephrine output (mgrm.)		
	Quantity	Duration of collection (sec.)	Quantity per minute		Quantity contained in 1 c.c.	Output per minute	
			per animal	per kilo		per animal	per kilo
(L.) I	2.8	40	4.2	0.2	0.0005	0.0021	0.0001
(L.) I'	3.2	40	4.8	0.23	0.0005	0.0024	0.00011
		Average	4.5	0.21	0.0005	0.0022	0.0001
(C.P.) II	4.3	30	8.6	0.41	0.0005	0.0043	0.0002
(C.P.) II'	4.5	30	9.0	0.43	0.0005	0.0045	0.00021
		Average	8.8	0.42	0.0005	0.0044	0.0002

(L.): Specimens from left suprarenal gland by lumbar route.

(C.P.): Specimens from both suprarenals by cava pocket method.

Fig. 4. a. At 2 and 4 adrenaline blood solutions which contained 0.0002 mgrm. and 0.00025 mgrm. adrenaline hydrochloride solution per 0.5 c.c. were applied respectively. At 3 I specimen solution was applied.

Specimen I: Stronger than 0.0002 mgrm. per 0.5 c.c., and not much differ from 0.00025 mgrm. per 0.5 c.c.

Fig. 4. b. At 5 and 7 I and I' specimen solutions were applied respectively. At 6 adrenaline blood solution which contained 0.0002 mgrm. adrenaline hydrochloride per 0.5 c.c. was applied.

Specimen I: Stronger than 0.0002 mgrm. per 0.5 c.c. and as strong as or a little below than 0.00025 mgrm. per 0.5 c.c. (Obs. 4).

Specimen I': Stronger than 0.0002 mgrm. per 0.5 c.c. and almost as strong as 0.00025 mgrm. per 0.5 c.c. (Obs. 8).

Fig. 4. c. At 8 and 10 adrenaline blood solutions which contained 0.00025 mgrm. and 0.0004 mgrm. adrenaline hydrochloride per 0.5 c.c. were applied respectively. At 9 II' specimen solution was applied.

Specimen II': Below than both 0.0004 mgrm. per 0.5 c.c. and almost as strong as or a little stronger than 0.00025 mgrm. per 0.5 c.c.

Fig. 4. d. At 11 and 13 II and II' specimen solutions were applied respectively. At 12 adrenaline blood solution which contained 0.0005 mgrm. adrenaline hydrochloride per 0.5 c.c. was applied.

Specimen II: Below than 0.0005 mgrm. per 0.5 c.c.; almost as strong as II'.

Specimen II': Below than 0.0005 mgrm. per 0.5 c.c. and also a little below than 0.0003 mgrm. per 0.5 c.c. (Obs. 14.)

Fig. 4. e. At 14 & 16 adrenaline blood solutions which contained 0.0003 mgrm. & 0.0002 mgrm. adrenaline hydrochloride per 0.5 c.c. were applied respectively. At 15 II specimen solution was applied.

Specimen II: Below than 0.0003 mgrm. per 0.5 c.c. and stronger than 0.0002 mgrm. per 0.5 c.c.

To sum up:

Specimen I: Stronger than 0.0002 mgrm. (2 assays); not much differ from or as strong as 0.00025 mgrm., and it was assayed at 0.0005 mgrm. in 1 c.c.

Specimen I': Stronger than 0.0002 mgrm. and almost as strong as 0.00025 mgrm. It was taken as 0.0005 mgrm. in 1 c.c.

Specimen II: Below than 0.0005 mgrm.; almost as strong as II'; below than 0.0003 mgrm. and stronger than 0.0002 mgrm. It was taken as 0.0005 mgrm. in 1 c.c.

Specimen II': Below than 0.0004 mgrm., 0.0003 mgrm. and 0.0005 mgrm.; almost as strong as or a little stronger than 0.00025 mgrm.; almost as strong as II. It was assayed at 0.0005 mgrm. in 1 c.c.

The dog was fastened prone and then ether was given; it was dropped first on a towel in front of the animal's nose and then given by means of the tracheal cannula connected with the Woulff's flask containing ether.

The anaesthesia was kept pretty uniformly and really deep, so that the epinephrine output through the cava pocket was remarkably small. A comparison of the values in this dog with those of the same nature, epitomized in a previous communication⁴⁾ (Table VI on p. 73) will make it apparent.

The lumbar route experiment was first performed, and then the cava pocket. While the blood flow with the former was noticed as 0.2–0.23 c.c. per kilo and per minute, the latter method yielded 0.41–0.43 c.c., namely nearly the same velocity. The epinephrine content underwent practically no alteration, accordingly the rate of output remained completely unchanged, that is 0.0001 mgrm. per kilo and per minute for one (left) gland and 0.0002 mgrm. for the both glands. (Such a close agreement must be taken, as a matter of course, as accidental.)

Previously we were able sometimes to observe a very small initial epinephrine output and the inefficiency of sensory stimulation upon the output, when the animals were deeply anaesthetized (p. 74).⁴⁾ The present example agrees well with these previous findings. The experiments in

which Stewart and Rogoff were able to find the rate of epinephrine output as approximately the same whether it was estimated from suprarenal vein blood collected without opening the abdomen or after opening the abdomen,³⁾ were really done on anaesthetized dogs, one under morphine and ether, another under ether, and in fact their results fall into line with the present observation.

There are however other experimental data which do not point to the same conclusion as the Cleveland physiologists and the above example cited by us, a matter to be also anticipated. An example may now be placed on record.

EXAMPLE III.

24. XII. 1924. Dog ♂. 15 kilos.

Time	Room temperature (°C)	Anal temperature (°C)	Rate of respiration per minute	Rate of heart beat per minute	Corneal reflex	Experimental processes and others
9:50 A.M. 10:00-	14.0	38.8	36	120	+	Fastend prone. Etherized, dropping ether on towel covering loosely mouth and nose of the animal. Completed the left lumbar route.
10:12-10:52 10:53 11:22-	14.0	38.7	96	192	+	Collected the specimens I and I' from l. suprarenal vein retroperitoneally, after discarded blood more than the dead space.
11:30 11:36-	14.5	38.7	60	192	+	Dog quiet. Collected the specimens II and II' from l. suprarenal vein retroperitoneally.
11:40 12:50 P.M.-	14.5	38.5	84	240	+	Dog quiet. Collected the specimens III and III' from l. suprarenal vein retroperitoneally.
12:08 12:20-	15.0	38.6	60	180	+	Dog quiet. Fastend supine on another table, after sutured the lumbar incision.
12:35-12:55 12:57 1:10	16.0	38.0	60	198	+	Made cava pocket. Collected the specimen IV from cava pocket, after discarded 12 c.c. of cava blood.
1:11 1:22	16.0	38.0	72	300	+	Dog quiet. Collected the specimens V and V' from cava pocket, after discarded 11 c.c. of cava blood.
1:23 1:53	16.0	37.9	72	240	+	Dog quiet. Final bleeding from abdominal aorta.

Epinephrine output:

No. of specimen	Blood flow (c.c.)				Epinephrine output (mgrm.)		
	Quantity	Duration of collection (sec.)	Quantity per minute		Quantity contained in 1 c.c.	Output per minute	
			per animal	per kilo		per animal	per kilo
(L.) I	3.0	40	4.5	0.3	0.00025	0.00112	0.000075
(L.) I'	3.0	40	4.5	0.3	0.00025	0.00112	0.000075
(L.) II	2.6	40	3.9	0.26	—	—	—
(L.) II'	2.8	40	4.2	0.28	0.0004	0.00168	0.00011
(L.) III	2.5	40	3.75	0.25	—	—	—
(L.) III'	2.7	40	4.05	0.27	0.0003	0.00121	0.00008
(I (I'), II' & III')) Average			4.15	0.28	0.00032	0.00134	0.00009
(C.P.) IV	5.3	20	15.9	1.06	0.0007	0.01113	0.00074
(C.P.) V	4.0	20	12.0	0.8	0.00065	0.0078	0.00052
(C.P.) V'	4.1	20	12.3	0.82	0.00065	0.00799	0.00053
		Average	13.4	0.89	0.00067	0.00897	0.0006

(L.): Specimens from left suprarenal gland by lumbar route.

(C.P.): Specimens from both suprarenals by cava pocket method.

Fig. 5. a. At 4 and 5 adrenaline blood solutions which contained 0.0002 mgrm. and 0.00025 mgrm. of adrenaline hydrochloride per 0.5 c.c. of indifferent blood were applied respectively. At 6 I specimen solution was applied.

I: Weaker than 0.00025 mgrm. per 0.5 c.c.

Fig. 5. b. At 7 and 9 I' and I specimen solutions were applied respectively. At 8 adrenaline blood solution which contained 0.0001 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood.

I': About the same with I (Obs. 6); a little stronger than 0.0001 mgrm. per 0.5 c.c.

I: A little stronger than 0.0001 mgrm. per 0.5 c.c.; weaker than 0.00015 mgrm. per 0.5 c.c. (Obs. 10).

Fig. 5. c. At 10 adrenaline blood solution which contained 0.00015 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood was applied. At 11 and 12 I' and II' specimen solutions were applied respectively.

I': A little weaker than 0.00015 mgrm. per 0.5 c.c.; weaker than II'.

II': Stronger than I'; almost as strong as 0.0002 mgrm. per 0.5 c.c. (Obs. 13).

Fig. 5. d. At 13 and 15 adrenaline blood solutions which contained 0.0002 mgrm. and 0.00015 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood were applied respectively. At 14 III' specimen solution was applied.

III': Weaker than 0.0002 mgrm. per 0.5 c.c.; a little weaker than or almost as strong as 0.00015 mgrm. per 0.5 c.c.

Fig. 5. e. At 16 and 18 IV specimen solution was applied. At 17 adrenaline blood solution which contained 0.0003 mgrm. adrenaline hydrochloride per 0.5 c.c. indifferent blood was applied.

IV: Stronger than 0.0003 mgrm. per 0.5 c.c. (2 assays); weaker than 0.0004 mgrm. per 0.5 c.c. (Obs. 19).

Fig. 5. f. At 19 and 21 adrenaline blood solutions which contained 0.0004 mgrm. and 0.00035 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood were applied respectively. At 20 V specimen solution was applied.

V: Weaker than 0.0004 mgrm. per 0.5 c.c. and 0.00035 mgrm. per 0.5 c.c.

Fig. 5. g. At 22 and 24 V' and V specimen solutions were applied respectively. At 23 adrenaline blood solution which contained 0.0003 mgrm. adrenaline hydrochloride per 0.5 c.c. of indifferent blood was applied.

V': Weaker than 0.00035 mgrm. per 0.5 c.c. (Obs. 21); stronger than 0.0003 mgrm. per 0.5 c.c.

V: A little stronger than 0.0003 mgrm. per 0.5 c.c.

To sum up:

I: Weaker than 0.00025 mgrm.; about the same with I'; a little stronger than 0.0001 mgrm.; weaker than 0.00015 mgrm. It was taken as 0.00025 mgrm. in 1 c.c.

I': About the same with I; a little stronger than 0.0001 mgrm.; a little weaker than 0.00015 mgrm.; weaker than II'. It was taken as 0.00025 mgrm. in 1 c.c.

II': Stronger than I'; almost as strong as 0.0002 mgrm. It was assayed at 0.0004 mgrm. in 1 c.c.

III': Weaker than 0.0002 mgrm.; almost as strong as 0.00015 mgrm. It was assayed at 0.0003 mgrm. in 1 c.c.

IV: Stronger than 0.0003 mgrm. (2 assays); weaker than 0.0004 mgrm. It was taken as 0.0007 mgrm. in 1 c.c.

V: Weaker than 0.0004 mgrm.; weaker than 0.00035 mgrm.; a little stronger than 0.0003 mgrm. It was taken as 0.00065 mgrm. in 1 c.c.

V': Weaker than 0.00035 mgrm.; stronger than 0.0003 mgrm. It was assayed at 0.00065 mgrm. in 1 c.c.

The general course of experiment was similar to that above illustrated. In the lumbar route experiment the blood current was 0.28 c.c. per kilo and per minute and the concentration 0.00033 mgrm. in 1 c.c. blood, as a consequence the epinephrine output 0.00009 mgrm. per kilo and per minute, these figures being found for the subsequent cava pocket experiment as 0.89 c.c., 0.00067 mgrm. in 1 c.c., and 0.0006 mgrm. respectively. Namely preparing of the cava pocket was followed in this instance by an augmenting discharge of epinephrine, as the consequence of an unmistakable increase in the epinephrine content of blood and also a somewhat greater blood flow. This rate of epinephrine output ranges between the average and the highest value found for the initial output in the experiments of the same nature.⁴⁾

In the experiments under anaesthesia there were thus cases where some difference, though not so large, was definitely detectable between the rate of epinephrine output estimated by the lumbar route without opening the abdomen and that by the cava pocket method, besides instances in which both methods yielded practically the same results.

The differences found in this series of experiments on the anaesthetized dogs are however by no means of the same order with those detected in the non-anaesthetized, de-afferented dogs; the former are certainly insignificant when compared with the latter. This fact is quite readily comprehensible, if the action of anaesthesia upon the reflex output of epinephrine from the suprarenals be taken into account.

SUMMARY.

I. A method for collecting the blood from the suprarenal gland of the dog that is standing, sitting or sleeping at will is described in the text. Namely the blood is collected through the lumbar route operation in the dog, the operation field of which is beforehand desensitized by the interference of the corresponding dorsal roots, and really without fastening, narcotizing, laparotomy, or evoking any pain.

II. The spontaneous liberation of epinephrine in dogs estimated by the use of this method and the rabbit intestine segment method, that is the discharge when no manipulation other than practising this method itself is conducted and the animal is not excited, is determined for one gland as 0.00001–0.00012 mgrm., on an average 0.00003 (for the left)–0.00004 (for the right) mgrm. per kilo and per minute and 0.00007 mgrm. for the both glands together, the blood flow being assayed mean

0.3 c.c. for one gland per kilo and per minute, and the epinephrine concentration as mean 0.0001 mgrm. per 1 c.c. suprarenal vein blood. The epinephrine output determined by this new method is therefore really of a quite different order compared with the figures of the same nature obtained by the cava pocket method in the non-anaesthetized, de-afferented dogs as well as in the anaesthetized, normal dogs, presented in the previous papers from this laboratory. The striking smallness of the epinephrine liberation taken by this method is in virtue of the low concentration of epinephrine in the suprarenal vein blood obtained by it.

III. By some experiments with the non-anaesthetized, de-afferented, but fastend dogs, it was also definitely demonstrated that the preparing of the cava pocket induces a remarkable exaggeration of the epinephrine output, the results with the lumbar route operation in one and the same dog being brought forward for comparison.

By the use of anaesthesia this difference becomes much reduced, and finally it disappears completely when sufficiently deep anaesthesia is established.