On the Stability of Adrenaline Hydrochloride and Epinephrine
Extracted from Bovine Suprarenal Medulla by Folin
in Various Media and of Epinephrine Liberated
from Suprarenal Glands of Dogs and
Cats in Defibrinated Blood.§

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The relation of the media in which the adrenaline is dissolved to the
velocity with which it deteriorates has been frequently investigated by
several writers; the investigations seem however by no means exhaustive,
but rather of an incidental nature. In order to make good this shortcoming in our present knowledge of the stability of adrenaline or epinephrine
the experiments as shown in the heading have been undertaken by the pre-
sent writer.

Adrenalin chloride of Sankyo Co. and the bovine medullary extract
by Folin, Cannon and Denis were diluted in various kinds of vehicles,

§ The term "epinephrine" is used to indicate the active principle of the suprarenal
medulla, and "adrenaline" the well-known commercial preparation of the active substance,
to avoid confusion.
that is, re-distilled water, 0.85% NaCl, Ringer-Locke and Tyrode solution and defibrinated dog blood. They were prepared in the strength of 1:1,000,000 and 1:200,000 in each of the diluents. The defibrinated suprarenal vein blood of dogs and cats was also diluted to a similar strength as above with the defibrinated dog blood.

In the majority of cases they were preserved at the body temperature, that is at 37.5 to 39.5°, and in a few cases the aeration was further jointed, while in other supplementary cases, few in number, the effect of preserving the samples on ice was tried.

At the very beginning of every experiment the actual content of adrenaline in the commercial preparation and epinephrine in the extract was estimated by means of Kodama's modification of the method of Folin, Cannon and Denis, and subsequent estimations were then made by the rabbit intestine segment method, the adrenaline chloride solution being used as the standard. The epinephrine in the suprarenal vein blood was assayed also by the latter method.

The process of the deterioration was followed at somewhat regular and short intervals for an ample length of time.

I.
Adrenalin Chloride of Sankyo Co. in Various Solutions.

The adrenalin chloride solution of Sankyo Co. was determined soon after uncorking on its acidity, 1/50 n. NaOH solution and 1% phenolphthalein solution being used. The actual adrenaline content of the solutions employed in the present investigations, 5 bottles in toto, was 76.6 to 87.0 per cent, mean 81.1 per cent of the prescribed value (1:1,000), and the quantity of 1/50 n. NaOH solution required to neutralize each 5.0 c.c. of the adrenalin chloride solution was 1.85 to 2.0 c.c., on an average 1.92 c.c. (In this connection it may be added that Sankyo Co. improved the bottle for the adrenalin solution in the beginning of 1927, so that the preparation is estimated to contain 90% or more nowadays). All the contents of the five bottles were mixed together for the sake of avoiding any variation in the acidity in each sample taken, and preserved carefully in a coloured bottle in the ice chamber.

For diluting the adrenalin chloride solution with water only re-dis-
Stability of Adrenaline and Epinephrine in Various Media

Tilled water was used, pH of which was potentiometrically determined such as 7.02 by Dr. Mikami in this laboratory with chinhydron electrode. The adrenaline salt, Locke or Tyrode solution was prepared in the following manner: To prepare, for example, the adrenaline salt solution of a strength of 1:1,000,000 or 1/1,000 mgrm. in 1 c.c. 5.0 c.c. double salt solution is joined to 15.0 c.c. of the salt solution and 5.0 c.c. of the adrenaline solution prepared with re-distilled water in the strength of 1/200 mgrm. in 1 c.c. The salt solution containing adrenaline in the strength of 1:200,000 or 1/200 mgrm. in 1 c.c. is introduced by diluting the adrenaline solution of 1/400 mgrm. in re-distilled water in a way similar to that described above.

The defibrinated blood containing adrenaline in a strength of 1:200,000 or 1/200 mgrm. in 1 c.c. is prepared by adding the non-diluted adrenalin chloride solution of Sankyo Co. to the defibrinated blood, and that of 1:1,000,000 was introduced from that of 1:200,000 by further adding the defibrinated blood.

In diluting the adrenaline solution, non-diluted or that diluted with re-distilled water, with several kinds of vehicles, the adrenaline solution is joined in conclusion. Before the mixing all the fluids are kept cold on ice. Solutions thus prepared were preserved in brown bottles.

The estimation of adrenaline or epinephrine was made by means of the rabbit intestine strip method. Just before applying the adrenaline mixture to the intestine segment every kind of mixture was made to the adrenaline-Tyrode-blood mixture. This was done by adding the compensative solution, properly prescribed. The composition of the vehicles and of the compensation solution for each vehicle for converting it into Tyrode are as follows: Tyrode: NaCl 0.8, KCl 0.02, MgCl₂ 0.01, NaH₂PO₄ 0.005, CaCl₂ 0.02, NaHCO₃ 0.1, Aq. dest. to 100.0; Locke: NaCl 0.9, KCl 0.042, CaCl₂ 0.024, NaHCO₃ 0.01, Aq. dest. to 100.0; compensation solution for Locke: NaCl 0.7, MgCl₂ 0.02, NaH₂PO₄ 0.01, CaCl₂ 0.016, NaHCO₃ 0.19, Aq. dest. to 100.0 and that for the normal saline solution (0.85%): NaCl 0.75, KCl 0.04, MgCl₂ 0.02; NaH₂PO₄ 0.01; CaCl₂ 0.04, NaHCO₃ 0.2, Aq. dest. to 100.0.

The initial adrenaline concentrations in the diluted solutions were repeatedly assayed, while they were kept cold. Then the specimens and indifferent blood were warmed in parallel. When cold specimens were tested the cold indifferent blood was used.

For warming the solutions and blood at 38 to 39.5° an incubator or the water bath in the arrangement for estimating epinephrine by the rabbit intestine strip was used. When aerated, the air was sent from a gasometer under a certain pressure of water through a glass tube, the apex of which is provided with some holes and immersed near the bottom of the intestine chamber. It is, further, needless to say that every possible care was taken to avoid any variation in the reaction of the fluids.

Nearly all the results are given diagrammatically in the following pages. In preparing the diagrams the initial content in each solution was always taken as 1.0, regardless of its absolute value. When no trace of inhibition of intestinal movement by applying the test object at a given time was found, the mark on the line of 0.0% is plotted on the chart at that given time, though the time at which all the active principle disappeared might be in reality earlier than that time.

(a) In re-distilled water.

14 samples with the adrenaline solution 1 : 1,000,000 (1/1,000 mgrm. in 1 c.c.), prepared with re-distilled water, were warmed to 38.5 to 39.5° without aerating. As is readily seen from Chart 1 the activity remained for 3 to 3½ hours without being affected, then it set in to reduce but very slowly. 80% of the activity was yet manifested at the end of 4 hours, and 70% at the end of 6 hours. Aeration much accelerated the deterioration, as the other two examples show, a matter well known. At the end of 1 hour 10% or more was found to be lost, and half an hour later a further 10% was lost. At the end of 2 hours 60 to 70% was detected, and about the half of the initial activity was manifested at the end of 3 hours, a further evanescence being established with certainty after the lapse of half an hour.

The adrenaline in the strength of 1 : 200,000 was tested in two experiments without aerating, the results of which are not reproduced graphically. The temperature of the water bath was 39°. In one experiment the full strength was found at the end of 6 hours, then the experiment was discontinued; and in another a small reduction as 0.06% of the initial value was detected first at the end of 27 hours.

In our laboratory the adrenaline solution for matching in the assay of the epinephrine in the blood from the suprarenal gland or in the suprarenal extract is always diluted first only with re-distilled water, and kept on ice till the time of making the adrenaline Tyrode blood solution. Neither the normal salt solution, Locke nor Tyrode has been used for this purpose.
Employing his frog vessel preparation Läwen\textsuperscript{3} observed no vaso-constrictory action of the suprarenine borate solution in a concentration of 2 : 10,000,000, preserved for one and a half months in a corked flask or for 14 days in a bottle stoppered with cotton. Harada\textsuperscript{4}, who made use of a method similar to that of Läwen, noted no destruction of the adrenaline chloride solution of Sankyo Co., diluted to a strength of 1 : 10,000,000 by distilled water, namely ten times more diluted than in the present samples, and kept at 18° for one hour. When a solution with the same intensity was kept at 40° and supplied with oxygen gas, only a little loss was discovered in the vaso-constrictory power at the end of half an hour.

In the very beginning of the present researches, the adrenaline solution was diluted with the usual distilled water and preserved firmly stoppered in an ice chamber. That in the

\textsuperscript{3} A. Läwen, Arch. f. exp. Path. u. Pharm., 1904, \textit{51}, 422.

\textsuperscript{4} Yutaka Harada, Mitt. med. Fak. K. Universität Tokyo, 1925, \textit{32}, 423 ff.
strength of 1/40 mgrm. in 1 c.c. remained unaltered at the end of 2 weeks, and lost the half of its activity after the lapse of 98 days. That in the strength of 1/200 mgrm. in 1 c.c. no decrease of the inhibitory power on the intestine movement was observed at the end of one week, but 80% of the initial strength was found at the end of two weeks and only one third after a lapse of 81 days. The adrenaline solution in a strength of 1/1,000 mgrm. in 1 c.c. was found unchanged at the end of 4 days, but at the end of one week a small deterioration occurred (7% was lost). Keeping for two weeks occasioned a great loss such as two thirds of the initial value, and at the end of 58 days no action on the intestine strip was witnessed.

(b) In the 0.85% NaCl Solution.

That adrenaline is more stable when it is diluted with the normal saline solution than with Locke etc. is pointed out by several investigators. Swetschnikow,5) who made use of the rabbit ear vessels in testing adrenaline crystallisatum Takamine and adrenaline hydrochlorium Takamine, stated that neither standing at the room temperature for 3 to 5 hours nor warming to 40° for a few minutes gives rise to any appreciable loss of the activity. The adrenaline solution of 1:100,000, prepared by diluting with 0.9% NaCl showed in the bands of Vander Hoof and Haskell6) little or no loss of pressor action, and it became inert only after fifty nine days. Adrenaline was assayed by the blood pressure of the dog. Harada,4) who employed Lüwen-Trendelenburg's method, came to witness a rapid damage of adrenaline in the normal saline solution. No material

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5) W. A. Swetschnikow, Pflügers Arch., 1914, 157, 475.
difference was found in this respect between the normal salt solution and the Ringer. Adrenalin chloride of 1 : 10,000,000 in the salt solution was destroyed at 18° in a marked way in only ten minutes and became quite inert at the end of 15 minutes. When, warmed to 40° under the oxygen supply only 3 minutes were enough to render it totally inactive.

With the normal saline fluid 6 experiments were performed in my hands. The strength of adrenaline was just or nearly 1 : 1,000,000 and kept at 39 to 39.5°. At the end of one hour no trace of deterioration was disclosed. At the end of two hours 65 to 85% of the initial value was found remaining, and one hour later further decomposition was found, but only to a small extent. 60% or more was found as the mean at the end of three hours, and 50% or more one hour later.

So the adrenaline is destroyed in the normal saline solution somewhat more quickly than in distilled water, but remarkably slower than in the Locke or Tyrode as is given in the following chapter.

(c) In the Locke and Tyrode solution.

That adrenaline becomes inert far more rapidly in the Ringer-Locke fluid compared with the distilled water or acid medium is the one definite fact acceptable to all investigators.

In a communication of Ogawa7) reference was made to the fact that adrenaline in strength of 1 : 10,000,000 in Ringer loses its action within one hour when kept at the body temperature under oxygen supply. In the experiments of Swetschnikow,5) in which deterioration of adrenaline in Locke solution without warming or oxygen supply was followed by means of the rabbit ear vessels, adrenalinum crystallisatum Takamine in strength of 1 : 2,000,000 held its activity of 98% of the initial at the end of 15 minutes, of 96% at 46 minutes and of 30% after a lapse of 86 minutes; at the end of 143 minutes the perfusion resulted in the dilatation of the blood vessels. Adrenalinum hydrochloricum Takamine in a concentration of 1 : 5,000,000 was reduced to 97%, 94%, 41%, 37% and 11% at the end of 22, 45, 84, 102 and 145 minutes respectively. At the end of 3 hours the solution gave rise to the dilatation of the blood vessels. When kept at the body temperature it became inactive within only 10 to 15 minutes.

When exposed at 37° for 5 hours, in the hands of Abderhalden and Gellhorn5) who tested adrenaline by means of the frog heart strip, d-l-

8) E. Abderhalden and E. Gellhorn, Pflügers Arch., 1923, 199, 452 ff.
adrenaline hydrochloride in Ringer solution containing \( \text{H}_2\text{O}_2 \) in a strength of 1:5,000 remained unchanged, that of 1:500,000 was still effective and that of 1:2,000,000 became inactive. If a solution of adrenaline of 1:20,000 in Ringer is kept at the room temperature with aeration the initial strength was found unaltered at the end of 2 hours, notwithstanding the solution was coloured red, and at the end of 8.5 hours only a small decrease was discovered.

Harada\(^4\) who tested a very weak solution as 1:10,000,000 came to see a rapid disappearance of the vasoconstrictory action of the adrenaline in Ringer's fluid. Under 18° almost no reaction was obtainable at the end of 15 minutes and a double length of time was sufficient to render it totally inert. At 40° under oxygen supply, the solution was seen to be quite inactive at the end of 3 minutes.

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**Chart 3. Adrenalin chloride of Sankyo Co. in Locke's solution.**

- ○... 7. X. '24: 1:1,000,000; 39.0°C.
- ●... 5. IV. '26: 1:4,000; 0.0007 mgm. in 1 c.c.) 39.5°C.
- □... 6. VI. '26: 1:1,000,000; 39.0°C.
- ▲... 19. V. '24: 1:100,000; 39.5°C.
- △... 15. VI. '29: 1:100,000; 39.5°C.
- ▼... 21. IX. '24: 1:200,000; 39.0°C.
- △... 29. III. '26: 1:100,000; 38.5°C.
- ○... 6. IV. '29: 1:100,000; 39.0°C.
Groër and Matula\textsuperscript{9}) reported an augmenting effect of alkali upon the adrenaline in comparison to water. Maiweg\textsuperscript{10}) failed however to ascertain this, but progressive deterioration of adrenaline in a weak alkaline solution was perceived by him. He also pointed out that the pressoric activity fainted away more rapidly than the chromatogenic affinity to the Folin. Vander Hoof and Haskell\textsuperscript{6}) also related a rapid loss of adrenaline in alkaline vehicles. On perfusing Locke solution containing adrenaline through the isolated kidney or ear of a rabbit, Kudrjawzew\textsuperscript{11}) obtained the fluid with the vasoconstrictory power, stronger, more durable and more stable than before.

With the Ringer-Locke five samples of the adrenaline solution in strength of 1,000,000, one of 1:1,400,000 and five of 1:200,000 were prepared and preserved at 38.5 to 39.5°. At the end of a half hour the dilute solution lost about half of the intestino-inhibitory power, and a half hour later only one third or less of the initial strength was noted. With the stronger solution (1:200,000) half the value of the initial was found at the end of one and a half hours, and a half hour later only two fifths or one fifth was found yet being retained. At the end of three hours only one tenth was found remaining.

A similar number of experiments as with the Ringer-Locke was run with the Tyrode; four with 1:1,000,000 adrenaline and four with 1:200,000, the temperature applied being 38 to 39.5°. The deterioration progressed more rapidly than in the Ringer-Locke. Fifteen minutes warming was enough to reduce the activity of the diluted adrenaline Tyrode solution to half or far less. The activity completely disappeared or nearly so from the solution at the end of a half hour. For halving the potency of the strong solution (1:200,
it was not necessary to wait thirty minutes, and for totally nullifying sixty minutes were proved already too long.

Contrary to some views above referred to, no increase of the adrenaline action in both kinds of fluid was observed, and stability such as that an adrenaline Ringer of a strength 1:500,000 remains still effective in spite of exposing it at 37° for 5 hours was not yielded in the present investigations.

As was to be anticipated, the adrenaline-Tyrode was proved considerably more stable when it was kept cold. Two samples of the solution in a strength of 1:1,000,000 were tested at the end of 1 hour 31 minutes and 2 hours 48 minutes respectively and there was no trace of reduction.

The rabbit intestine segment is suspended in the Locke's fluid in the Cushing laboratory and in the Tyrode in this, so we carried out a comparison experiment with a view to seeing whether or not this difference may give rise to some inequality in the epinephrine content determinable by the segment method for the cava pocket blood, etc. Some days the cava pocket blood specimens from dogs, for example a dog weighing 18.6 kilos on 12 December 1924, were estimated by the present writer by using the Locke and by Dr. Watanabe simultaneously by using the Tyrode. We were never able to note any difference in the values obtained by us both for one and the same specimen. This fact is parenthetically reported in a previous paper of ours (p. 15).2)

(d) In the defibrinated blood of the dog.

As cited elsewhere, abundant evidence has been recently accumulated for the fact that blood plasma, serum, organ extracts, optones (Abderhalden), biogenic amines, aminoacids, etc. operate to increase the activity of adrenaline upon the movement and tonus of various tissues.

The stability of adrenaline in the defibrinated blood or serum was investigated by some investigators. Embden and Fürth observed the pressoric effect of adrenaline hydrochloride in defibrinated bovine blood in a strength of 0.05 mgrm. in 1 c.c. under aeration at 38–40°. The pressure rise was measured as: 66 mms. Hg. immediately after the preparation, 58 mms. a half hour later, 30 mms. at the end of one and a half hours and 18 mms. at that of two hours. In the strength of 1.6 mgrms. in 1 c.c. of defibrinated dog blood there was no destruction of adrenaline at the end of two hours when preserved at 40° under aeration.13) In a paper of Elliott it was mentioned that after standing one hour in well aerated blood at a temperature of 39° adrenaline suffers but little loss, even in so weak a solution as that of 0.06 mgrm. in 10 c.c. of blood.14) When an adrenaline solution in serum (1:500,000) was kept for six hours at the body temperature under

14) T. R. Elliott, J. Physiol., 1905, 32, 446.
oxygen supplying any effect upon the frog leg vessels was no longer visible in an experiment of O'Connor. Adrenaline chloride in a strength of 0.2 mgrm. in 2.0 c.c. of rabbit serum gave the typical blood pressure rise after 1 hour's preservation at room temperature or 2 hours' at 37° in the experiments of Mifuji. Harada was able to see only a little loss of adrenaline of a concentration of 1:3,300,000 in 0.5% per cent citrate blood within 10 minutes when kept at 40° under aeration. In a paper of Tanaka a somewhat different effect of serum upon the adrenaline action was noted. The influence of the latter upon the rabbit intestine segment was inhibited, though not excessively, by serum of ox or rabbit.

With the dog defibrinated blood the solution of adrenalin chloride of Sankyo Co. of a concentration of about 1:1,000,000 (1/1,000 mgrm. in 1 c.c.) was prepared and kept at 37.5–39.5°. The intestino-inhibitory ability diminished step by step; the velocity with which the reduction progressed was evidently smaller than in the Tyrode, but nearly equal to that in the Locke. The reduction took place a little more rapidly than a stronger...
solution (1 : 200,000) in the Locke. At the end of a half hour 20–30 per cent of the activity was lost, and half of the initial strength or more was found at the end of one hour. Between one and a half hours and two one third or two fifths of the initial value was detected, and then no further diminution was observed to occur, a noteworthy matter to which we are able to find no previous reference. The longest of the observations extended to 5 hours, but no more.

When kept on ice, a specimen of the adrenaline blood of 1 : 1,000,000 and a specimen of 1 : 200,000 was found wholly active as the initial when tested at the end of 30 hours.

II.

Bovine Medullary Extract by Folin, Cannon and Denis in Various Solutions.

That the suprarenal vein blood and the suprarenal extracts exercise somewhat dissimilar influence compared with adrenalin chloride of Sankyo Co. upon the different tissues or test objects, as the rabbit intestine segment, the cat paradoxical pupil, the Folin's test, etc. has been established in this laboratory.18) Accordingly it may be tentatively assumed that the active principle in the suprarenal medulla can not be meant simply the adrenaline alone. In connection with the investigations in the above chapter, therefore, the Folin's extracts of the bovine suprarenal glands were tested in the like manner.

The removal of the glands, separation of the medullary tissue and its extraction by Folin, Cannon and Denis were carried out as described in a previous paper of the present writer.12) The extraction was done as a rule on the day of the removal and separation. Only in one instance it was done on the next morning and in another two days later. In all 15 extracts were made from 15 oxen. The stability was examined on the day of extraction, or on the next day or some days later. The extracts were preserved in the ice box.

The quantity of 1/50 n. NaOH solution required to neutralize each 10 c.c. of the original extracts, from which the diluted specimens for further dilution with various fluids were prepared, varied between 4.4 to 6.8 c.c., 5.77 c.c. being the mean. From one grm. of the medullary tissue are prepared 50 c.c. of the original extract. In the investigations in the above

18) Sugawara, Tohoku J. Exp. Med., 1927, 8, 355; (12); Watanabe and Sato, Ibid., 1928, 11, 433.
chapter and also in practising the rabbit intestine segment method in usual, adrenalin chloride solution was first taken as 0.1 c.c. in the strength of from 1:1,000,000 (or sometimes 0.05 c.c. in a strength of 1:2,000,000) to 0.5 c.c. of that of 1:200,000 (or sometimes 0.2 c.c. of the strength of 1:40,000) and in the cases of medullary extract in the dose of 0.1 to 1.0 c.c. of 10,000 times diluted solution from the medullary tissue. So the amounts of normal NaOH solution required to neutralise them are calculated as ranging from 0.000024 (or sometimes 0.000048) to 0.00000096 c.c. (or sometimes 0.00000024) for the former and from 0.0000577 to 0.00000577 c.c. for the latter. And when they were finally applied to the rabbit intestine segment in the intestine chamber these acidities were further lessened with the alkaline Tyrode fluid of from 25 times to 2.5 times. In assaying one and the same sample of the suprarenal vein bloods, we sometimes applied it to the intestine in both forms, non-diluted and diluted, simultaneously, accordingly the adrenalin chloride solution used for matching varied in the strength of adrenaline and at the same time in acidity. In such a case no difference was witnessed in the results; namely such a small difference in the acidity of the solutions has no influence thereupon. In fact the minimum effective dose of hydrochloric acid upon the rabbit intestine segment, determined by some observers,\(^1\) is plainly greater than these under present question. No organ extract was added to the control adrenalin chloride solution.

The results with the re-distilled water will be mentioned first. The extract fluid was diluted with the re-distilled water to the strength, expressed in the term of adrenalin chloride of Sankyo Co., of 1:1,000,000, and kept at 38.5–39.5°, without aeration. When it was tested at the end of 10 to 20 hours after the dilution, there was no trace of diminution in the intestino-inhibitory ability; a tendency to evanescence became manifest there after. In one case 60 per cent of the initial value was found at the end of 27 hours, and in another case no inhibitory action was observed at the end of 56 hours. When it was left on ice, the evanescence took place far later, that is the solution in a strength of 1:1,000,000 held its full ability for 9 days at least, and at the end of 13 or 14 days a small weakening of the potency was visible, and in one instance the solution was not capable of inhibiting the intestine when it was applied at the end of 30 days.

Schkawera and Kusnezow\(^2\) were able to find the Locke's fluid


to contain the adrenaline like substance, incomparably more stable than adrenaline, after perfusing it through the bovine suprarenal medulla. This view was seconded by an observer\(^{21}\) who worked with rabbits, while another writer\(^{22}\) was unable to find any superiority in the stability of the perfusion fluid over that of adrenaline.

With 0.85% NaCl solution seven experiments were made. The concentration of the active principle, determined by the rabbit intestine and expressed in the term of adrenalin chloride of Sankyo Co., was 1:1,000,000 or a little less. The results are given in Chart 6. As was foreseen, it was not so stable as in the re-distilled water, but much more stable than adrenalin chloride of Sankyo Co. in the same kind of vehicle, that is in 0.85% NaCl fluid. While the latter commenced to lose its activity not long after one hour's keeping at 39-39.5°C, the extract saline fluid remained wholly unaffected till the end of three and half hours at 39.5°C. Then the evanescence set in and progressed but slowly. At the end of 6 or 7 hours the inhibitory effect of more than the half of the initial was still manifest.

With Locke's fluid five ex-

\(^{21}\) Naito, Chosen Igakukwai Zasshi, 1925, 735.

\(^{22}\) Kazu. Takenaga, Pfliegers Arch., 1924, 205, 289 f.
experiments were done, one of which was dosaged to contain as much epinephrine as 1:200,000. All the others contained it nearly in a strength of 1:1,000,000; some a little more, some a little less. They were kept at 38.5° or 39.5°. The value underwent a somewhat rapid reduction. At the end of a half hour 70 to 90% of the initial was found, but a half hour later 30 to 60 per cent remained. At the end of two hours only 20 per cent of the initial was detected as the mean. The velocity with which the evanescence of epinephrine in the Locke progressed was decidedly greater than in the normal saline solution on the one hand, but also plainly smaller than the adrenalin chloride of Sankyo Co. in the Locke. When the solution of epinephrine of the strength of 0.0012 mgrm. in 1 c.c. was kept at 39.5° and tested at the end of 2 hours 20 minutes, no inhibitory action was elicited. The solution of the strength 1:200,000 was found one hour and 39 minutes after dilution as holding only 8 per cent of the initial strength. Cold retarded the evanescence to a great extent, so that in one experiment with epinephrine of 1:200,000 on ice 87 per cent of the initial remained at the end of 6 hours.

That the evanescence of adrenaline advances more rapidly in the Tyrode than in the Locke was also here realized in the experiments with the Folin's extract. Five experiments were of the epinephrine concentration of about 1:1,000,000, three of 1:400,000. The dilute solutions, 5 in number, were estimated at the end of a half hour as containing only a little or practically no epinephrine. Three samples of the greater strength were found at the end of a half hour holding one tenth or one fifth of the

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Chart 7. Bovine medullary extract by Folin, Cannon and Denis in Locke's solution.

- ○... 31. VII. '25; 1:200,000; 38.5° C.
- □... 12. III. '26; 1:1,176,000 (0.00086 mgrm. in 1 c.c.); 39.0° C.
- △... 15. III. '26; 1:1,000,000; 39.5° C.
- ×... 17. III. '26; 1:1,111,000 (0.0009 mgrm. in 1 c.c.); 39.5° C.
- ○... 19. III. '26; 1:833,000 (0.0012); 39.5° C.
initial. Ten to twenty minutes later they became nearly or totally inert. When kept cold the solution of 1:400,000 was found retaining the full strength at the end of about three hours. The evanescence of the active principle of the extract of Folin in the Tyrode was unquestionably slower in comparison with the adrenaline chloride of the same concentration in the same vehicle.

The medullary extract was diluted with the dog defibrinated blood to 1:1,400,000 to 1:400,000, and placed in the incubator at 39 to 39.5°C. About forty to sixty per cent of the initial activity was found destroyed at the end of one hour; the velocity of the evanescence of epinephrine in the defibrinated blood in the course of one hour is midway between that in the Tyrode and that of the Locke. The velocity decreased however a half hour later, so that the course of the evanescence of epinephrine in the defibrinated blood describes a curve at the vicinity of one and half hours of preservation at 39–39.5°C, while in the Locke or the Tyrode it goes nearly in a straight line. And the present curve of evanescence is quite similar to that of adrenalin hydrochloride in the dog defibrinated blood a peculiar feature from those with the other vehicles, as the normal saline, Locke or Tyrode.

Further it may be also readily seen from the charts and Chart 11, which is constructed to show at a glance the average velocity of evanescence of adrenaline or epinephrine in the strength of about 1:1,000,000 in every kind of vehicle, that the destruction of the active principle in the medullary extract by Folin is unmistakably slower than adrenalin chloride of Sankyo.
Co. when preserved in various kinds of vehicle, as normal saline, Locke or Tyrode. Adrenalin chloride of Sankyo Co. contains NaCl in 0.7\% and chloretone. The quantity of the salt is so small as to account for the latter fact, that is for the different behaviour of both the materials in the vehicles except the defibrinated blood, provided the great degree of diluting with re-distilled water and the vehicles prior to the preservation in the incubator be taken into consideration.

**III.**

**EPINEPHRINE LIBERATED FROM SUPRARENAL GLANDS OF DOGS AND CATS IN DEFIBRINATED DOG BLOOD.**

O'Connor\(^{23}\) applied the suprarenal vein blood serum of a rabbit, obtained after standing for a half hour in the incubator and kept for six hours at the body temperature under oxygen supply, to the frog leg vessel pre-

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paration; the effect was by no means different from the carotid blood serum, similarly treated. A similar experiment with the suprarenal vein plasma and carotid plasma yielded a similar result.

By preserving the serum of the cava pocket sample from an etherized dog collected during stimulation of both the splanchnici, in the ice chest for 48 hours Stewart\textsuperscript{24} came to see the disappearance of a great part of epinephrine. The same sample kept in the ice chest for three days was also found reduced to not more than $1:5,000,000$ at most from nearly $1:1,000,000$. The rabbit intestine segment was employed in the former experiment and the uterus preparation in the latter.

\begin{chart}
\centering
\includegraphics[width=\textwidth]{chart10.png}
\caption{Epinephrine liberated from suprarenal glands of dogs and cats in de-fibrinated blood.}
\end{chart}

\begin{itemize}
\item \textbullet{} 27. VI. '25; de-afferented dog; lumbar route preparation without anaesthesia, fastening experiment; 0.004 mgm. in 1 c.c.; 37.5-38.5°C. (Specimen collected on 26. VI. '25).
\item \textcircled{$\triangle$} 25. IX. '25; cat, ether, cava pocket, under light anaesthesia; 0.00225 mgm. in 1 c.c.; 38.0°C.
\item \textcircled{$\triangle$} 25. IX. '25; cat, ether, cava pocket, splanchnic stimulation; 0.003 mgm. in 1 c.c.; 38.0°C.
\item \textcircled{\textbullet} 26. IX. '25; cat, ether, cava pocket, without ether under suprarenal massage; 0.0015 mgm. in 1 c.c.; 37.5-38.5°C.
\item \textcircled{\textbullet} 26. IX. '25; cat, ether, cava pocket, without ether under suprarenal massage; 0.00225 mgm. in 1 c.c.; 37.5-38.5°C.
\item \textcircled{\textbullet} 2. X. '25; cat, ether, cava pocket, without ether under suprarenal massage; 0.0035 mgm. in 1 c.c.; 37.5-38.5°C.
\item \textcircled{\textbullet} 6. X. '25; dog, ether, cava pocket, under light anaesthesia with suprarenal massage; 0.004 mgm. in 1 c.c.; 38.0°C.
\item \textcircled{$\textcircled{\circ}$} 12. X. '25; dog, ether, cava pocket, without anaesthesia; 0.0009 mgm. in 1 c.c.; 38.0°C.
\item \textcircled{$\textcircled{\diamond}$} 19. X. '25; dog, ether, cava pocket, under light anaesthesia; 0.0009 mgm. in 1 c.c.; 38.5°C.
\item \textcircled{$\textcircled{*}$} 21. X. '25; cat, ether, cava pocket, without any manipulation; 0.002 mgm. in 1 c.c.; 38.0-38.5°C.
\end{itemize}

\textsuperscript{24} G. N. Stewart, J. Exp. Med., 1912, 15, 557 f.
Kodama\textsuperscript{25} parenthetically noted that no diminution occurred in the epinephrine concentration of the cava pocket blood by preserving it on ice for ten hours.

Epinephrine in the suprarenal vein blood was now tested for its stability in the defibrinated blood at 37.5 to 39.5°. The blood was obtained from the cava pocket prepared from cats and dogs under ether; only in one experiment the suprarenal vein blood from a non-anaesthetized dog was tested. The dog, which was previously de-aferented for the operation field, was fastened prone, and preparation for collecting the suprarenal vein blood was carried out by the lumbar route. The dog cried and struggled very furiously and uninterruptedly throughout fastening. It was really Dog 7, cited and especially illustrated in a previous paper of ours,\textsuperscript{26} and the sample collected during this stage was found containing much epinephrine. It was just the one utilized for the present experiment.

The cava pocket samples were obtained either without any other special manipulation, under stimulation of the splanchnic nerve on one side, or under massage of one side gland. The specimen was kept on ice and first assayed as usual at a given time. Then it was diluted with the dog defibrinated blood to the desired concentration of epinephrine; in fact the latter was taken as from 1:250,000 to 1:1,100,000.

As may be readily seen from the Chart 10 on p. 114 the destruction progressed quite similarly to that of adrenalin chloride and the active principle in the bovine medullary extract by Folin in the dog defibrinated blood. At the end of a half hour of keeping at 37.5–39.5° twenty to forty per cent of the initial activity disappeared and a half hour later forty to sixty per cent still remained. At the end of one and a half hours as well as two hours thirty to forty five per cent was recovered, and no further destruction was observed till at the end of four hours, when the experiment was discontinued.

This peculiar curve of the destruction of adrenaline or epinephrine in the defibrinated blood expresses unquestionably the true course of the evanescence of the active principle in the defibrinated blood itself, since the indifferent blood used for preparing the control fluid for matching was also warmed in the water bath for exactly the same interval of time as the test blood fluid containing adrenaline or epinephrine.

In this connection we may mention some experiments which were carried out in order to see whether or not the defibrinated blood when preserved for some time will develop some

\textsuperscript{25} Kodama, Tohoku J. Exp. Med., 1923, 4, 186.
\textsuperscript{26} Satake, Watanabé and Sugawara, Tohoku J. Exp. Med., 1927, 9, 6 ff.
intestino-inhibitory property as the production of the vaso-constricting power in the blood serum or plasma, although it is by no means difficult to answer this question from our daily experiences without any special experiments.

The blood specimens were taken from the carotid of a non-anaesthetized dog, defibrinated, and a portion preserved at 39° and the rest on ice. Two hours later the same manipulation was repeated and all the four specimens were applied with addition of a certain amount of adrenalin chloride (1:6,600, 000 to 1:5,000,000) to the intestine segment. No material difference was found in the blood samples preserved in the different manners.

The duration of the preservation was 1 hour 20 minutes to 5 hours. In the second experiment the blood was also taken from the carotid of a non-anaesthetized dog and defibrinated forthwith; one half was laid on ice and the other in the water bath of 39°. 20 minutes later the application of the samples to the intestine was started and it lasted about 2 hours. The specimens were preserved partly with adding adrenalin chloride (1:20,000,000 to 1:6,000,000) and partly without it. There was detectable no variation in both specimens, kept cold or warm, in relation to the effect upon the tone as well as movement of the intestine segment. The third experiment was run nearly as the second, and yielded quite similar results.

Thus no appreciable alteration was observed in the effect of the defibrinated dog blood upon the rabbit intestine segment movement by keeping the blood at 39° or on ice for 2 to 5 hours.

When kept cold, the active principle in the suprarenal vein blood, diluted with the dog defibrinated blood, remained unaltered for a considerable time. The solution with the strength of about 1:40,000, kept on ice, was found at the end of 32 hours 50 minutes containing the entero-inhibitory power, not one whit less than the normal. The solutions of 1:1,000,000, kept cold, were estimated at the end of 7, 8, 10 or 29 hours, the same concentration prior to the warming being again found.

In conclusion a figure is inserted to demonstrate with ease the comparative influence of the various kinds of vehicles upon the adrenalin chloride and epinephrine extracted by Folin or the liberated from the gland. It is compiled of the average curves constructed from every series of the experiments, in which the adrenaline or epinephrine was taken in the strength of about 1:1,000,000. Only the results with Folin extract in the defibrinated blood and with suprarenal vein blood are not expressed in this chart because of a too small number or of lacking experiments with strength of 1:1,000,000. Further it may be added here for caution's sake that Folin extract preserved in warm re-distilled water indicated no trace of decomposition even at the end or 10 hours or later.

IV.
Summary.

Adrenalin chloride solution (of Sankyo Co.), epinephrine in the bovine medullary extract prepared by Folin, Cannon and Denis and that liberated from the suprarenal bodies of dogs and cats were diluted with re-distilled water, 0.85% NaCl solution, Ringer-Locke's fluid or Tyrode's fluid or the dog defibrinated blood to the strength of 1:1,000,000 or 1:200,000, etc., and kept at the body temperature, that is 37 to 39.5°. The entero-inhibitory power was tested from time to time.
The stability of the substances was considerably great in re-distilled water. In the normal saline solution they were also highly stable, whereas they were incomparably rapidly destroyed in the other vehicles; among them the deterioration took place most rapidly in the Tyrode's fluid. In the Locke's fluid and the dog defibrinated blood the velocity with which the evanescence progressed was nearly similar. The average velocities of the deterioration of adrenaline and epinephrine in the various vehicles are diagrammatically demonstrated in the last chart on p. 116.

While in the other vehicles the deterioration went on nearly in the straight, the defibrinated blood modified the progress of destruction in the form of a curve, further destruction being arrested at the end of nearly one and a half hours of keeping warm. This is the result of the protection of the deterioration by the warmed defibrinated blood itself.

The deterioration curves of adrenalin chloride, epinephrine extracted and epinephrine liberated in the dog defibrinated blood at body temperature were similar to each other.

The epinephrine in the Folin's extract was somewhat more stable in the re-distilled water, normal saline, Locke and Tyrode in comparison with the adrenalin chloride of Sankyo Co.