

FURTHER INVESTIGATION UPON THE INFLUENCE  
OF ORGAN EXTRACTS OF COLD-BLOODED  
ANIMALS ON THE BLOOD PRESSURE.<sup>1</sup>

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In the spring of 1906, Brown and Joseph,<sup>2</sup> working together, showed that there were either pressor or depressor substances or both present in several organs of various cold-blooded animals. The material collected for that paper was insufficient to make anything more than a very superficial test of the organs used, but during the summer of 1906, Doctor E. P. Lyon, while at Beaufort, North Carolina, and Woods Hole, Mass., kindly collected more material from the same species, and also material not before tested.

The material was preserved in two ways: (1) The fresh organs, such as shark testis, ovary, pancreas and spleen, were cut into thin strips, placed upon filter paper and exposed to the rays of the sun till the water had evaporated and the fat if present in quantity had exuded. The dried material was then wrapped up and shipped immediately to St. Louis, where it was used in a short time. The small amount of oil which remained in the tissue seemed to prevent any deterioration. Before use, this dried tissue was cut into fine pieces and ground up with clean sand in a mortar. (2) Other organs when removed were immediately placed in jars of ninety-five per cent. alcohol, and the jars tightly closed. Before use these organs were put through a meat grinder and ground very fine. They were then spread over a clean glass plate and dried. The alcohol in which the organs had been preserved was evaporated to dryness. The dried residue after this evaporation was added to the dried organ and the whole run through a pulverizer and reduced to a fine powder. No difference in action was observed

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<sup>2</sup>Brown and Joseph, *Jour. of Physiol.*, 1906, xxxiv.

between the material dried at the seashore and that preserved in alcohol and dried afterwards.

*Methods of Preparing Extracts.*—The extracts were prepared in various ways. Definite weighed quantities of the dried glands were used in making up the various solutions. Sometimes they were made up to a given percentage in normal saline solution and warmed to  $41^{\circ}$  C. for fifteen to twenty minutes and then filtered. These were termed “warm extracts.” The residue was sometimes made up to its original percentage, either in more normal saline or in distilled water, boiled for from ten minutes to two hours, and then filtered. Such extracts were termed “sediment extracts.” Again, a solution was made by boiling a fresh mixture of organ and normal saline for from ten minutes to two hours, then filtering. These were termed “boiled extracts.” Extracts were made in ninety-five per cent. alcohol, and also absolute alcohol, filtered, the filtrate evaporated to dryness, and the residue made up to the original percentage in normal saline, so as to make the relative solubilities in physiological salt solution, distilled water, and alcohol apparent by comparing the results obtained upon injecting them into an animal. By the expression “made up to a certain (or original) percentage,” I mean this: a three per cent. extract of an organ is one in which three grams of the dried gland were added per hundred cubic centimeters of water. Therefore, a three per cent. extract is the “extract” of three grams of gland in one hundred cubic centimeters of water. In the case of the alcoholic extract, if three grams of gland were added to one hundred cubic centimeters of alcohol, then to the dried filtrate after extraction, would be added one hundred cubic centimeters of physiological salt solution.

*Method of Recording Results.*—These experiments were all performed upon dogs. The A. C. E. mixture was the anesthetic used in all experiments except when a subcutaneous injection of extracts was made to determine any changes of temperature, in which cases no anesthetic was necessary.

In those cases where tracings of the respiration were taken a “T” tube connected with a tambour was inserted between the tracheal cannula and anesthetic bottle. The blood pressure was recorded by

means of a three-way cannula in the carotid artery, connected with a pressure bottle, and mercury manometer. Sodium citrate (two per cent.) was used to fill the tubing of the blood pressure apparatus. The extracts were injected by means of a burette and a cannula inserted in the central end of the external jugular vein. All injections were made at body temperature. The burette was surrounded by a large glass tube which was kept full of warm water to insure a constant temperature of the injected extracts. In all cases where different extracts were tested upon the same dog, the tubing and burette were disconnected from the venous cannula, and both cannula and burette carefully cleaned out with warm distilled water or physiological salt solution. The moment of injection was recorded by a magnetic signal connected in an electric circuit with the automatic make and break of an induction machine. The time was recorded in seconds by another magnetic signal, and the writing point of this lever was adjusted beforehand so as to show also the line of zero blood pressure. All levers were arranged to write in the same vertical line. Control injections were frequently made by injecting an equal quantity of the physiological salt solution used in making the extracts, but these made it plain that the results obtained when injecting an extract were not due to the salt solution used in making up the extract.

Extracts of the following organs were tested: liver, spleen, pancreas, ovary and testis of shark; liver, kidney, spleen, and testis of swordfish; and spiral valve of dog fish. Care was taken to see that solid particles were absent from the injected substance, except in those cases where the effect of such particles, if present, was under consideration. More than one experiment was performed to determine the different points, but only a single representative effect to show each point will be described. Since the effects of shark and sword fish testes were among the most potent of those examined, an effort was made to determine the following facts regarding them:<sup>3</sup>

I. How small an amount of extract will produce a noticeable physiological effect?

<sup>3</sup>Because of a limited amount of material, however, I have not determined in every case the same point for both shark and sword-fish testis. I might say that in those cases where the same point was determined for each, the results were very similar.

- II. Will either the pressor or depressor substance dialyze?
- III. Can the pressor and depressor substances be separated:
  - A. By dialysis?
  - B. By difference in solubility in absolute alcohol?
  - C. By difference in solubility in cold normal saline solution?
- IV. What is the effect of long-continued boiling?
- V. To what physiological change is the marked fall of blood pressure due?
- VI. What is the effect of extracts after cutting the vagi?
- VII. What is the effect upon respiration?
- VIII. What is the effect of subcutaneous injections?
- IX. What is the effect of intrastomachic injections?
- X. Is the effect of a single extract exactly the same on different animals, or does a personal idiosyncrasy of the animal cause a variation?

Experimental results on these points are as follows:

- I. *How small an amount of extract will cause a noticeable physiological effect?*

An extract of shark testis was used in which one cubic centimeter of extract equaled one milligram of the dried gland. An injection of twenty-five cubic centimeters of this extract into a dog weighing eight kilos caused an initial rise of blood-pressure of thirteen millimeters of mercury beginning two seconds after the commencement of injection. In fifteen seconds the pressure had fallen to normal again, and fifteen seconds later was eight millimeters of mercury below normal. The total duration of this depression was thirty seconds. This was the smallest amount of extract used, but it gives a very noticeable though transient effect.

- II. *Will either pressor or depressor substance dialyze?*

*Method.*—An extract of swordfish testis was made as follows: A three per cent. solution of swordfish testis in normal saline was boiled for one hour and forty-five minutes and then filtered through cotton. The filtrate was placed in a dialyzer and dialyzed against a physiological salt solution for five and one half hours.

*Results.*—Fifty cubic centimeters of the dialysate were injected

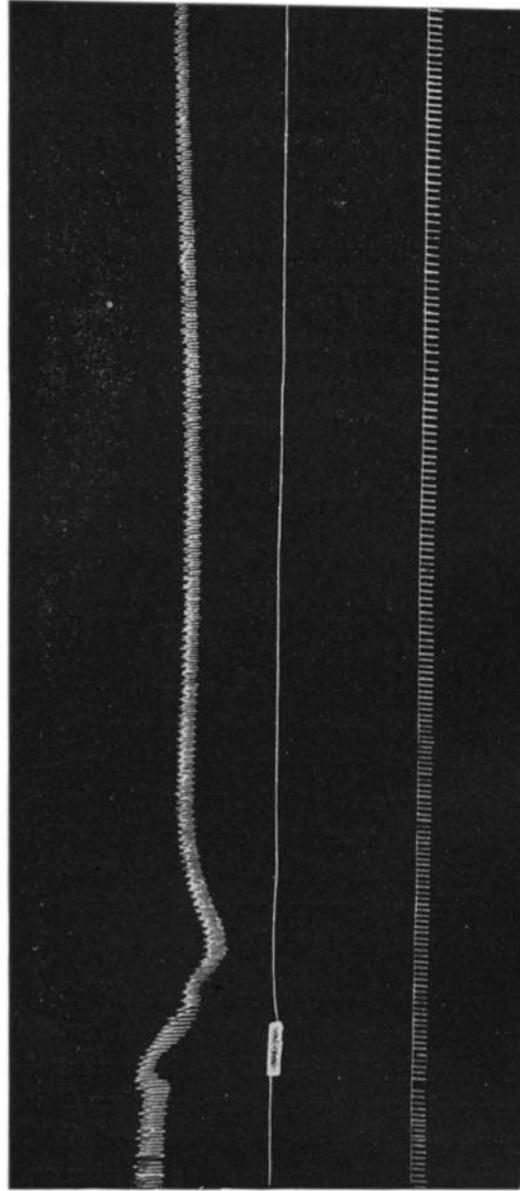


FIG. 1. Injection of 50 c.c. of the dialysate from a three per cent. solution from testis of swordfish, dialyzed against 0.9 per cent. sodium chloride for five and a half hours.

(see Fig. 1) into a dog weighing seven kilos. At the beginning of the injection the blood-pressure was 102 mm. of mercury. In twenty-one seconds the pressure had fallen 23 mm. The return of pressure to normal was, for a few seconds, rather rapid, but became slower as it rose, and the normal level was not regained for two and two thirds minutes. There was practically no pressor effect shown. A control injection of an equal quantity of the physiological salt solution used in making the extracts gave neither pressor nor depressor effects. It is, therefore, concluded that the depressor substance will dialyze, but that the pressor very probably will not.

### III. *Can the pressor and depressor substances be separated?*

#### A. *By Dialysis.*

*Method.*—A three per cent. solution of swordfish testis in 0.9 per cent. sodium chloride solution was warmed at 41° C. for thirty-five minutes, and after standing for some time until solid particles had settled, it was filtered through cotton. The filtrate, which at that time showed the presence of both pressor and depressor substances, was divided into two portions (*a*) and (*b*); (*a*) was put upon ice as it was; (*b*) was put in a parchment tube for dialysis, surrounded by normal saline, and placed upon ice also. Once every twenty-four hours both (*a*) and (*b*) were heated to 76° C. in a water bath, and (*b*) placed in a fresh parchment tube with fresh normal saline surrounding it. This process was kept up for five days, when the extracts were tested.

*Results.*—(*a*) An injection of twenty-five cubic centimeters of this solution (see Fig. 2) lowered the blood-pressure from 114 mm. to 20 mm. Hg in forty-eight seconds, and it was impossible to get a recovery of the animal. An injection of five cubic centimeters of the same extract into another dog caused the following results: The blood-pressure fell 23 mm. Hg in seventeen seconds, and seven minutes afterward it was not quite back to the level existing at the time of injection. The rate of heart beat just before injection was 115 per minute. One minute afterwards 96 per minute, and four minutes after injection the normal rate of beat had been reestablished. There was no evidence of the presence of a pressor substance.

(*b*) An injection of fifteen cubic centimeters of this solution into the same dog used for the latter part of (*a*) above caused a fall of 22 mm. Hg, which lasted about four minutes. There was almost no evidence of the presence of a pressor substance. The heart

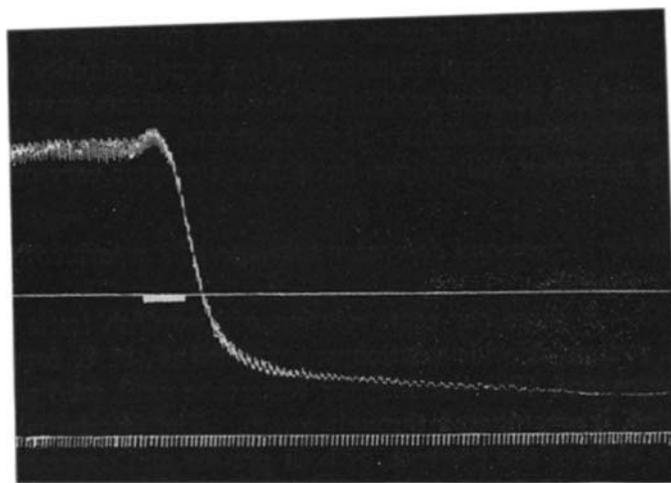


FIG. 2. Injection of 50 c.c. of a three per cent. extract of testis of swordfish in 0.9 per cent. sodium chloride prepared at 41° C. for thirty minutes.

rate was 158 just before injection, 183 at the point of lowest blood-pressure, and 152 three minutes after injection.

Apparently it is not practical to try to separate the two active substances in this way, inasmuch as the pressor substance is lost or destroyed by the method used. It may have been destroyed by the repeated heating to 76° C., the long standing in watery solution, or (possibly though not probably) by bacterial action. I do not think the cause was bacterial action, since no putrefactive processes were detectable. The depressor action of equal doses of (*a*) and (*b*) is considerably less prominent in the case of (*b*). This is probably the result of removal of a considerable amount of depressor substance by the long-continued dialysis of solution (*b*).

*B. By difference in solubility in absolute alcohol.*

*Method.*—It has been found in organ extracts of warm-blooded animals,<sup>5</sup> that the depressor substance is soluble in absolute alcohol and the pressor substance insoluble. To see if this would hold

good for these cold-blooded animal organ extracts, solutions were made as follows: A three per cent. solution of sword fish testis in absolute alcohol was made and left standing at room temperature ( $21^{\circ}$  C.) for fifty minutes. It was then filtered through cotton, the filtrate evaporated to dryness and the residue, after evaporation, made up to three per cent. in normal saline. This will be called solution (*a*), and if the results are the same as in the case of warm-blooded animals it should contain depressor and no pressor substance (or at least a less amount of pressor than the normal). The original residue remaining after filtering through cotton was dried, made up to three per cent. in normal saline, boiled for one hour, and then filtered through cotton. This will be called solution (*b*), and it should give pressor and no (or little) depressor effect.

*Results.*—Without giving exact figures for the effects of these two solutions, I will say that it does not seem possible to separate the two active substances in this way. The pressor effect is about equal in each solution. The depressor action is, however, only half as strong in the absolute alcohol extraction as in the physiological salt solution, seeming to indicate that the depressor at least is less soluble in absolute alcohol than in physiological salt solution.

*C. By difference in solubility in cold normal saline.*

*Method.*—A four per cent. solution of sword fish testis in 0.9 per cent. salt solution was allowed to stand for seven minutes at room temperature ( $21^{\circ}$  C.) and then filtered. The filtrate was called solution No. 1. The residue was again made up to four per cent. in more normal saline and allowed to stand at room temperature for twenty-five minutes, to see if most of the pressor substance had been dissolved out in solution No. 1. The filtrate this time was called No. 2. The residue made up to the original percentage again, was boiled for some time and then filtered. The filtrate was called solution No. 3.

*Results.*—The results from this method also are negative. Both substances are found in all the solutions. The depressor substance, however, seems to overshadow the pressor in every case. I believe that the depressor substance is not very highly soluble in water, for I have noticed repeatedly that injections of solutions made up

as (1), (2) and (3) above show almost the same amount of depressor action, and also, that if the length of time that the normal saline is allowed to stand upon the powdered organ is varied, there is not as a rule, a corresponding difference in the amount of depressor action. This is true particularly if the saline is exposed to the organ for longer than about fifteen minutes.

IV. *What is the effect of long continued boiling upon the pressor and depressor substances?*

*Method.*—A three per cent. solution of swordfish testis was made up in 0.9 per cent. salt solution by warming to 41° C. for thirty-five minutes and filtering. This filtrate was divided into two portions, (1) and (2). No. 1 was left standing unchanged, while No. 2 was boiled for two hours.

*Results.*—These two solutions, when injected, gave approximately the same amount of fall of blood-pressure, but there was a very marked difference in the length of time the effect lasted in the two cases. Three minutes after injecting No. 1 (see Fig. 3) the pressure had regained a normal level. At the time of injecting No. 2 (see Fig. 4) the pressure was 108 mm. Hg. Immediately following the injection there was the usual sudden fall of blood pressure, but the recovery, though gradual, was exceedingly slow, so slow in fact that twelve minutes after the injection the pressure was only 98 mm. Hg. It was still gradually rising and apparently would have reached the normal level in three minutes more. In No. 2 there was no apparent pressor action, while in No. 1 there was an initial rise of pressure equal to 10 mm. Hg. In neither (1) nor (2) was there any marked effect upon the respiration. The heart rate in (1) was 178 just before injection, 170 at point of lowest pressure, and 174 about two minutes after injection. In No. 2 the rate at time of injection was 123, at point of lowest pressure 101, and twelve minutes after injection 121 beats per minute.

The most apparent thing shown by this line of experiments, is that the pressor substance is rendered inactive by long continued high temperature. The more prominent depressor action following continued boiling of an extract may be interpreted either as a result of the disappearance of the pressor substance alone, or to

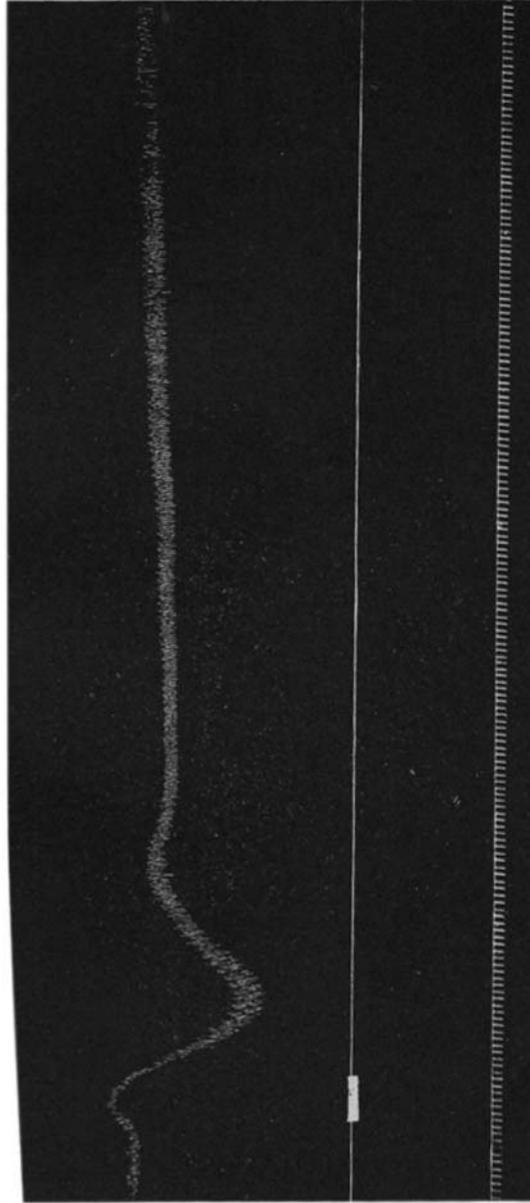


FIG. 3. Injection of 50 c.c. of a three per cent. extract of testis of swordfish, showing effect when extract was boiled for twenty minutes.

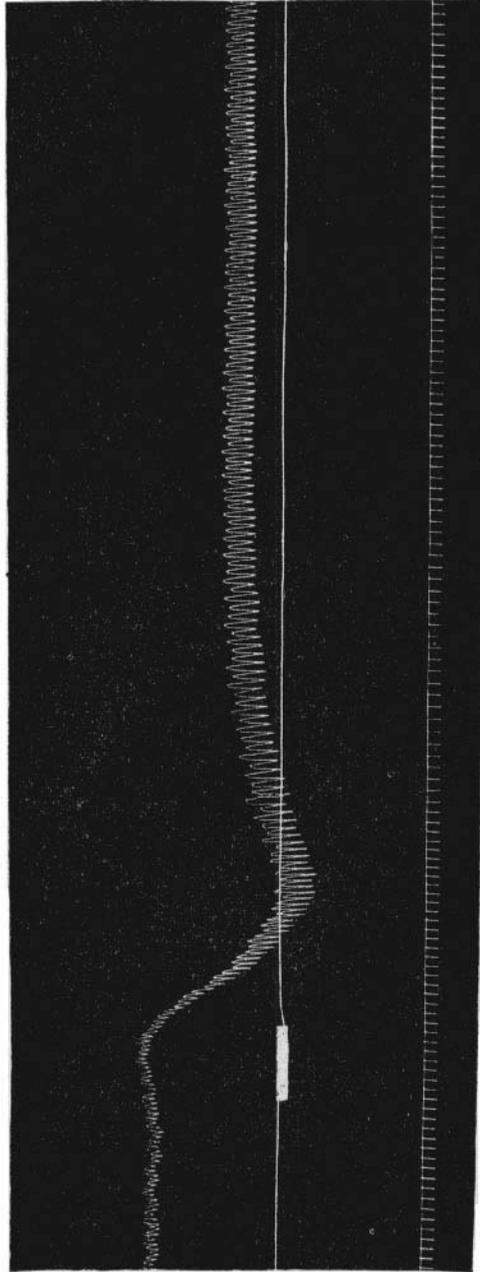


FIG. 4. Injection of a three per cent. extract of testis of swordfish, showing the effect when the extract was boiled for one hour and three quarters. In twelve minutes the recovery was not complete.

that disappearance coupled with the development of another depressor substance not before present.

V. *To what physiological change is the marked fall of blood pressure due?*

*Method.*—A hind foot and part of the leg of a dog were enclosed in a Mosso's plethysmograph during the time that intravenous injections of extracts were being made.

*Results.*—An increase in the volume of the enclosed limb followed an injection of twenty-five cubic centimeters of a boiled extract of shark testis. Since in this extract the depressor action is so much more prominent than the pressor, I think it is safe to say that the result obtained, *i. e.*, the vaso dilation was due to the action of that substance. No experiments were performed to determine whether these effects were due to action on the nerve centers, or on the blood-vessels direct.

VI. *What is the effect of extracts after cutting vagi?*

*Results.*—After cutting both vagi (see Fig. 5) the chief effect noticed is that there is a much more rapid return of pressure to

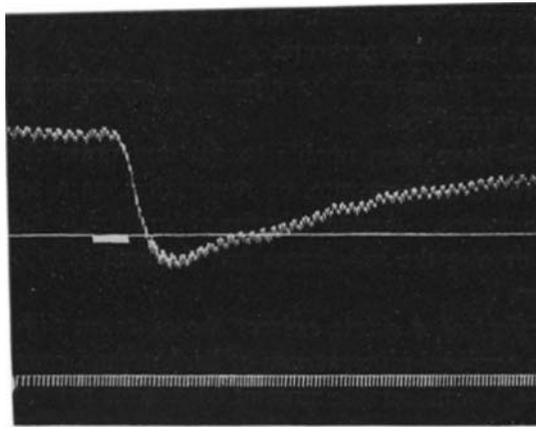


FIG. 5. Vagi cut; injection of 50 c.c. of a three per cent. extract of testis of swordfish (boiled extract). This injection followed the one shown in Fig. 4 and shows the difference in the curve obtained after cutting the vagi.

normal than when the vagi were intact. The fall is about as great as before, but where normally it took three minutes to recover, it

now takes but little over one minute. The rate of heart beat was little affected either before or after cutting the vagi. I am therefore unable to account for the quick recovery after injecting with vagi cut.

VII. *What is the effect upon respiration?*

*Results.*—I may say at the beginning that I have been unable to reproduce with these extracts the remarkable results which Doctor Brown and I obtained last year from “Sex-gland of Shark.” In the case of which I speak an injection of twenty-five cubic centimeters of extract of sex-gland of shark increased the rate of respiration from forty-eight per minute before the injection to eighty-four one minute after injection. There was also a remarkable increase in the heart rate (from 132 per minute to 264 per minute). I cannot explain last year’s results in the light of the effects I have obtained since that time. It was unfortunate that we had so little tissue on hand at the time that work was done. We have been unable to learn either the species or sex of the shark whose sex-gland was examined last year. I have since frequently noticed a slightly increased respiratory rate at the time, after an injection when the pressure was lowest, but I have considered it as a natural result of the low blood-pressure.

VIII and IX. *What are the effects of subcutaneous and intrastomachic injections?*

*Results.*—I have been unable to get any noticeable effect upon the blood-pressure, heart-rate, or respiration, as a result of injecting by these means. Apparently the absorption is too slow to give any result, or else the substances are changed before reaching the blood-stream.

X. *Is the effect of a single extract the same on different animals, or does a personal idiosyncrasy of each animal cause a variation?*

*Method.*—A considerable amount of a three per cent. solution of swordfish testis was made up in 0.9 per cent. salt solution and tested upon three dogs.

*Results.*—In every case the general effect was similar. Individual parts varied slightly. The variation was more marked in the “first” injection in each dog than in the succeeding injections. I also found that where a number of injections were made into

the same dog, the effect of a given dose became less and less pronounced with each injection after the "first" injection. In a few dogs there was a very slight and transient response from the blood-pressure, following the ninth or tenth injection of extract. I had thought that possibly some individual variation due to the animal itself might account for the results which we obtained last year.

The remaining organs were studied only in a preliminary way, and a brief description will be given merely to show their general action.

I. *Shark Organ Extracts:*

(a) *Shark Ovary*.—The extracts were made from ovaries containing well developed eggs, and were used in solutions as strong as five per cent. They were tried repeatedly, but no effect worth recording was obtained. This result would indicate that the "Sex-glands of Shark" which Doctor Brown and I used last year was the male organ and not that of the female.

Our records at that time did not show which we were using.

(b) *Shark Spleen*.—An injection of twenty-five cubic centimeters of a two and one half per cent. solution of shark spleen in 0.9 per cent. salt solution shows no initial pressor effect. The fall of blood pressure is very rapid, and in this particular case amounted to 54 mm. Hg. The recovery is also very rapid—the normal level being regained in fifty-five seconds after injection. The respiration was not noticeably affected either in rate or depth. The heart rate at commencement of injection was 87 per minute, at the point of lowest pressure 110 per minute, and one minute after injection had slowed down to 104 per minute.

(c) *Shark Liver*—There is nothing particularly interesting to say about extracts of this organ, except that there is a depressor substance present, as in the other organs already described. The effect produced by extracts of this organ is even more transient than in the case of shark spleen. Twenty-five cubic centimeters of a five per cent. solution caused a 42 mm. drop of blood pressure, and in twenty-eight seconds the pressure had returned to its normal level. The respiratory rate was practically unchanged throughout. The heart rate was 132 just before injection, 138 at point of lowest pressure and 156 one minute after injection.

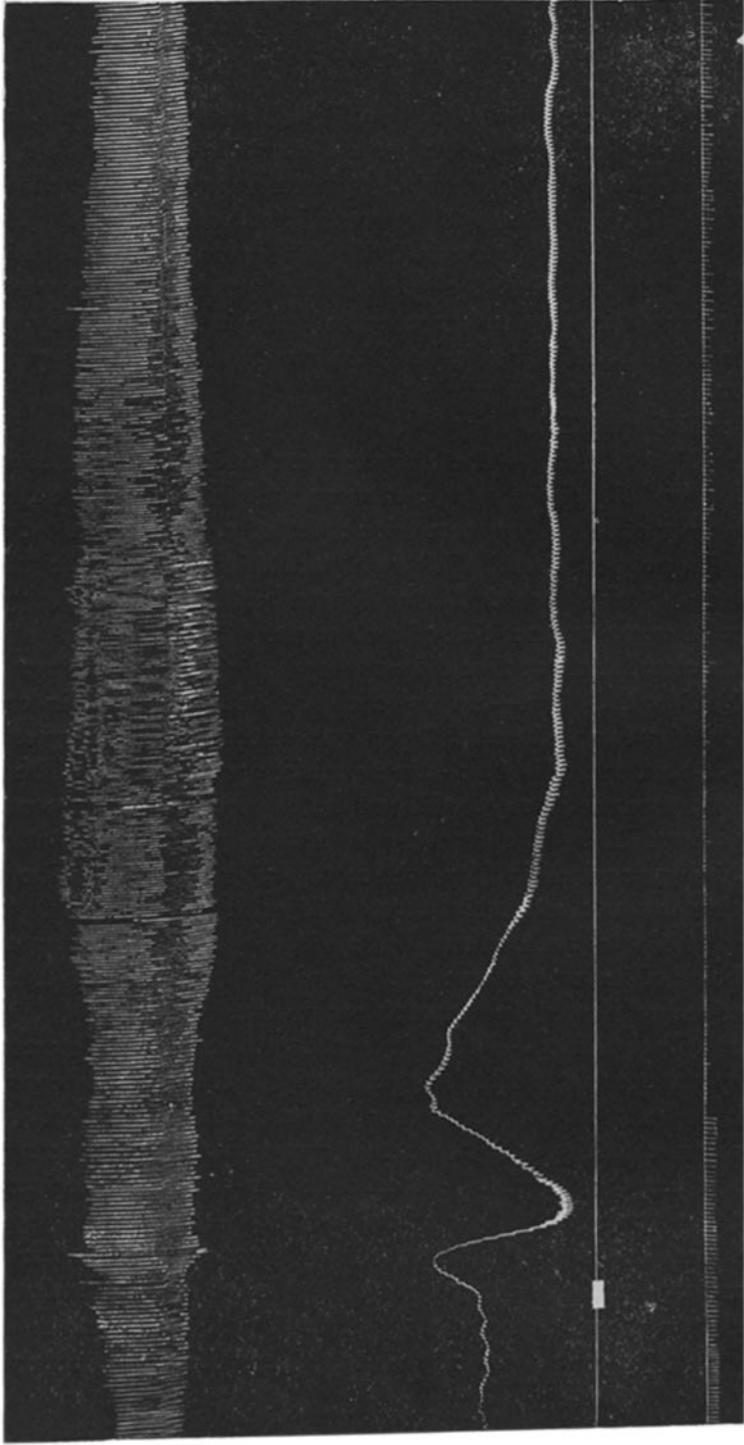


FIG. 6. Injection of 35 c.c. of a two and one half per cent. warm extract of pancreas of shark.

The heart in this case was slower than usual in resuming its original rate.

(d) *Shark Pancreas*.—This is one of the most interesting of the organs tested. An injection of twenty-five cubic centimeters of a 2.5 per cent. solution in normal saline (see Fig. 6) caused first an initial rise of blood pressure of 18 mm. Hg, ten seconds elapsing before normal level was regained; second, a fall of 40 mm. which was not recovered from for twenty seconds. In recovering, the pressure did not stop at the normal level, but continued to rise until it was at a height equal to that caused by the initial pressor effect. From this point the pressure gradually fell until in sixty-five seconds it was at the lowest level attained by the first depression. Here the pressure remained (40 mm. below normal) without beginning to rise for over four minutes. The heart rate before injection was 72, during the first depression 90, and during the second depression, two minutes after injection, 66 beats per minute. The rate of respiration before injection was 54, immediately succeeding the injection 66, and two minutes after injection (when the heart rate was 66) 120 per minute. The depth of respiration was almost doubled at the time when its rate was most rapid.

These results might be explained by supposing that there are present two pressor substances and either one or two depressors. If we suppose two pressors and one depressor the order of action would be as follows: The first pressor causes the initial rise, and the depressor action following close upon that of the pressor overcomes the pressor and causes the initial fall. Next the second pressor action shows up and for a few seconds it overcomes the depressor action and causes the second rise in blood pressure. The effect of this second pressor substance is quite transient, and as it wears off the depressor substance resumes its action and holds the pressure at a low level for some time.

## II. *Swordfish Organ Extracts*:

(A) *Swordfish Liver*.—Not essentially different from shark liver.

(B) *Swordfish Spleen*.—Not different from shark spleen except that the depressor substance appears to be a little less potent.

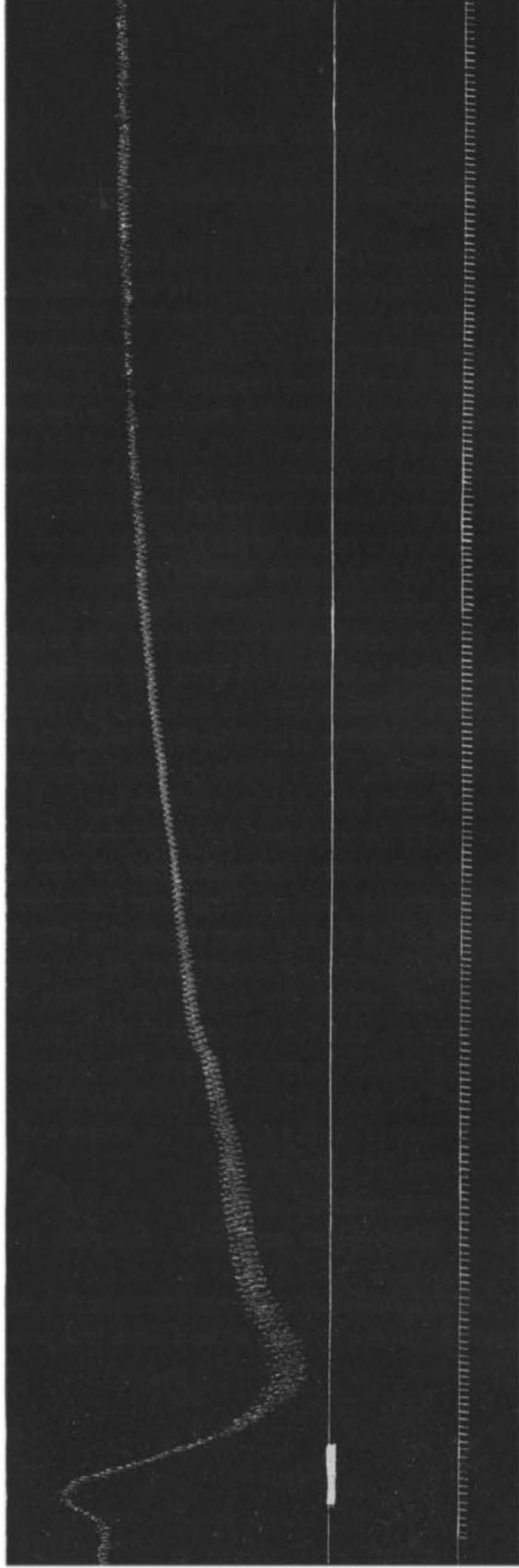


FIG. 7. Injection of 50 c.c. of a three per cent. boiled extract of spiral valve of dogfish.

(C) *Swordfish Kidney*.—This organ also contains a depressor substance. It is not, however, potent enough to warrant a definite description of its effects, except, perhaps, to say that recovery from twenty-five cubic centimeters of a three per cent. solution of boiled extract takes over three and one half minutes. The fall of blood pressure is not very great.

### III. *Spiral Valve of Dogfish*.

This organ furnishes an interesting extract. It contains both pressor and depressor substances. An injection of fifty cubic centimeters (see Fig. 7) of a three per cent. solution of spiral valve in 0.9 per cent. salt solution (boiled one and a half hours) produced an initial rise in blood pressure of 18 mm. Hg, followed by a fall of 80 mm. Hg. The recovery was very gradual and it took over four minutes to accomplish it. The only difference between the effect of this boiled extract and a "cold" extract, made by allowing the powdered organ and physiological salt solution to stand at room temperature for twelve minutes, is that the depressor action is more permanent in the case of the boiled extract. Recovery occurs in the case of the cold extract in one and a half minutes. The heart rate was 180 just before injection, 210 when the pressure was lowest, and had practically returned to 180 by the time the normal blood pressure was regained.

#### SUMMARY.

1. None of the extracts examined are very potent in small doses. A noticeable effect, however, is produced upon the blood pressure by the extract of fifteen milligrams of shark testis.
2. A small dose of a five per cent. shark testis extract seems to show more pressor action than a larger dose of the same solution.
3. The fall of blood-pressure is due to a vaso-dilation.
4. After repeated (seven or eight) injections of almost any extract, an injection of more of the same extract, or of another extract, which would be rather potent as a "first" injection, causes very little if any response from the blood-pressure.
5. Continued boiling of an extract destroys the pressor substance.
6. The depressor action is then very pronounced and especially prolonged.

7. The depressor substance will dialyze.

8. The pressor and depressor substances cannot be separated by dialysis, because in the repeated sterilizations the pressor substance disappears.

9. The pressor and depressor substances cannot be separated by absolute alcohol, as each seems to be soluble. The solubility is not, however, very great, at least in the cold.

10. It seems impossible to separate the pressor and depressor substances by means of a difference in solubility in cold physiological salt solution, since the depressor substance is even more soluble in that solution than the pressor. And yet the depressor substance is not nearly all removed by this means, even though it is allowed to stand for some time.

11. After cutting the vagi, the recovery from the effect of the depressor substance is much more rapid than with the vagi intact. The amount of fall of blood-pressure is about equal in either case.

12. Extracts of the testis and pancreas of shark; of the testis and spleen of swordfish; and the spiral valve of dogfish show the presence of a pressor substance.

13. Extracts of the testis, spleen, pancreas and liver of shark; of the testis, liver, spleen and kidney of swordfish, and the spiral valve of dogfish show the presence of a depressor substance.

14. Extracts of shark ovary gave no result. I believe, however, that if sufficient of the parenchymatous tissue of the ovary had been obtained a result would have been given by this organ also.

## A COMPARISON OF THE PROPERTIES OF ORGAN EXTRACTS OF COLD- AND WARM-BLOODED ANIMALS.

*Cold-blooded Animals.*

1. Most organ extracts show the presence of both pressor and depressor substances.

2. The pressor substance is best obtained by extraction with normal saline at the room temperature, but this is not a very successful means of separating the pressor and depressor substances.

3. The pressor action is more transient than that of the depressor.

4. Boiling, if continued for any length of time, destroys the pressor substance.

5. Long continued boiling causes a more prominent depressor action either by forming new depressor substances or by removing the pressor.

6. The depressor substance is much more prominent in its action than the pressor and is probably present in larger amounts.

7. The fall in blood-pressure seems to be due to a dilatation of peripheral vessels.

8. The effects of "second" and later injections are not the same as those produced by "first" injections.

9. Both pressor and depressor substances are soluble in absolute alcohol, and they cannot be successfully separated in this way.

10. The depressor substance is dialyzable.

11. No effect is obtained by injecting subcutaneously.

12. The only difference in the effect of injections made before and after cutting the vagi, is that the normal blood pressure is regained sooner after cutting the vagi than it was before cutting.

*Warm-blooded Animals.*

Same.

Vincent and Sheen, state that this is the best way to *obtain* and *separate* the two.

Same.<sup>4</sup>

Same.<sup>4</sup>

Same.<sup>4</sup>

Same.<sup>4</sup>

The fall of blood-pressure is due to a direct action upon the blood-vessels and not to an action upon the vaso-motor center.

Same.<sup>5</sup>

According to Schäfer and Vincent,<sup>5</sup> the pressor substance is insoluble in absolute alcohol and the depressor soluble. They used this means to separate the two.

Same.<sup>5</sup>

Same.<sup>5</sup>

No difference in the action of extracts injected before and after cutting vagi.

<sup>4</sup> Vincent and Sheen, *Jour. of Physiol.*, 1903, xxiv, 242.

<sup>5</sup> Schäfer and Vincent, *Jour. of Physiol.*, 1899, xxv, 87.

<sup>6</sup> Osborne and Vincent, *Jour. of Physiol.*, 1899, xxv, 283.