THE EFFECTS OF INTRAVASCULAR INJECTIONS OF EXTRACTS OF ANIMAL TISSUES[1]. BY SWALE VINCENT AND WILLIAM SHEEN. (Twenty-four Figures in Text.)

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CONTENTS.

1. *Introductory.*

THE effects produced by extracts of different tissues when injected into the blood vessels of a living mammal are not merely of pharmacological interest: the chemical interaction of certain tissues with others more or less remote is only at the present time understood in a few isolated cases[2]. It must not of course be rashly assumed that whenever an extract of a tissue introduced artificially into the circulation produces a distinct physiological effect that the production of a substance or

[1] The expenses involved in this research have been partly defrayed by a grant from the Government Grant Committee of the Royal Society.

[2] Thus the medulla of the suprarenal capsule manufactures a substance which is poured into the blood-stream and probably helps to maintain the tone of muscular structures throughout the body (Oliver and Schäfer, This *Journal*, XVIII. p. 230, 1895) and may control in some way or other glandular secretion (Langley, This *Journal*, XXVII. p. 237, 1902). A more recent example of this chemical interaction is the discovery by Bayliss and Starling of what we may call the chemical mechanism of pancreatic secretion (This *Journal*, XXVIII. p. 325, 1902).

substances with a similar effect is one of the normal functions of the living tissue. At the same time we think that a general survey of the results produced upon a living animal by the injection into its vascular system of various tissue extracts is likely to be of some value, both in regard to observations of the general physiological effects induced and the specific physiological effects of tissue upon tissue.

We have already made a preliminary communication on this subject[1], and although our researches have now extended over the greater part of a year they cannot be regarded as completed. Certain new facts have however come to light, and since the series of experiments has had to terminate we have thought it desirable to publish an account of the work.

2. *Mode of Preparation of Extracts, and Methods employed.*

The extracts employed were made from the tissues of various animals[2]. The names now given to the various extracts will be used throughout this paper.

1. *Saline Decoction.* (This was the form of extract most often employed.) A weighed quantity of the fresh moist tissue was ground in a mortar with sand, three times as many c.c. of ·9 % normal saline solution being added as there were grammes of moist tissue; this "1 in 3" extract was employed throughout. After thorough trituration the mixture was boiled with the addition of a few drops of dilute acetic acid. It was then filtered, and the filtrate after cooling, and filtering again if necessary, used for injection.

When, as sometimes happened, the final filtrate was not used at once, it was sealed with a plug of cotton-wool while boiling in a glass flask, to ensure sterility, and filtered again if necessary immediately before use[3].

2. *Proteid Extract.* Made like the saline decoction except that the mixture was filtered without boiling or the addition of acid. The filtrate was always used at once.

3. *Alcoholic Extract.* The fresh moist tissue was pounded in

[1] *Proc. Physiol. Soc.* July 5th, 1902.

[2] Dogs', cats' and rabbits' tissues were usually employed: occasionally those of the pig, ox, sheep and pigeon. We have seen no reason to suspect any specific effects with these extracts, *e.g.* the action of dog's brain extract upon dog differed in no respect from the action of other brain extracts upon the same animal.

[3] The freedom of these decoctions from proteid or nucleo-proteid was occasionally determined by testing.

a mortar with absolute alcohol and sand, and left in contact with alcohol for some hours. The mixture was then filtered, the filtrate evaporated to dryness and the residue taken up with ·9 % normal saline solution, three times as many c.c. of this fluid being added as there were originally grammes of the moist tissue. The fluid was filtered again and used.

4. *Ether Extract.* Prepared in a similar way to the alcoholic extract except that methylated ether was used instead of alcohol as the extracting agent.

Methods. Dogs, cats, and rabbits were employed for the purposes of the experiments. Anæsthesia was induced by the A.C.E. mixture, and maintained by the same substance and by morphia. In a few cases curare was used in addition.

The blood-pressure was taken from the carotid artery and recorded in the usual way by a mercurial manometer. The saphenous vein or one of the forelimb veins was employed in the dog, and the jugular or femoral or a forelimb vein in the cat or rabbit. A glass plethysmograph containing water at body temperature was used for recording changes in the volume of the limb, and for similar changes in the intestinal wall an air oncometer was used. Each of these was connected with a Marey's tambour for recording purposes[1]. The method described by Oliver and Schäfer was employed for recording the effects upon the heart[2]. A hook is caught in the epicardium of the auricle and another in that of the ventricle. From these pass firm threads over pulleys moving on a horizontal axis: the threads then pass vertically downwards to be attached to long elastic levers of steel. To the ends of the levers writing points are attached.

As a usual dose of our extracts we injected the contents of a syringe having a capacity of 5 c.c. Any variations from this dose are specially noted in the text. The dose of extract was washed in by 5 c.c. of ·9 % normal saline.

3. *Physiological Effects.*

(*a*) *Nervous Tissue Extracts. Historical.* After the discovery of certain peculiar cells which stain deeply with bichromate of potash, not only in the medulla of the suprarenal bodies but also in the

[1] A piston recorder was not available, and Brodie's bellows recorder did not give good results in our hands. The Marey's tambour when its inter-pressure was properly regulated was quite sensitive but somewhat deficient in range.

[2] This *Journal*, XVIII. p. 256. 1895.

sympathetic ganglia[1], it occurred to Cleghorn[2] that an extract prepared from sympathetic ganglia intravenously injected might have a similar powerful action on the blood-pressure to the medulla of the suprarenal bodies. But he found on testing the matter that the effect produced is, on the contrary, a fall of blood-pressure and not a rise[3]. He thought that this effect was confined to extracts of the sympathetic ganglia. Schäfer and Moore[4] had previously observed that an extract of brain substance when intravenously injected "is usually followed by a fall in the blood-pressure." Mott and Halliburton suggested that the depressor substance found by Schäfer and Moore was choline[5]. In 1900 Osborne and Vincent[6] found that extracts of all parts of the nervous system produce a marked temporary fall of arterial blood-pressure which can be obtained after section of both vagi and after administration of sufficient atropine to abolish vagus action. The lowering of blood-pressure is due to dilatation of arterioles, those of the splanchnic area being first affected. The depressor substance acts directly on the blood vessels and not through the agency of the vaso-motor nerves. They came to the conclusion that although choline was present in small amounts in their extracts the depressor effect was not due to that substance. The reason for this view was that whereas, after the administration of atropine to an animal, choline always produced a rise of blood-pressure, these extracts on the contrary always produced a fall[7].

Nearly a year later Halliburton[8] published a paper dealing with

[1] Stilling, *Anat. Anz.* xv. p. 229, 1898; Kose, *Sitz.-Ber. des deutschen naturw.-medicin. Ver. f. Böhmen*, "Lotos," 1898, Nr. 6; Kohn, *Prager medicinische Wochenschrift*, xxiii. Nr. 17. 1898.

[2] *Amer. Journ. Physiol.* ii. p. 472, 1899.

[3] It is interesting to note that Biedl and Wiesel (*Pflüger's Archiv*, xci. S. 434, 1902) find that extracts of the "Nebenorgane des Sympathicus" (Zuckerkandl) produce the same effects upon the blood-pressure as extracts of suprarenal medulla. The material was obtained from new-born children and from embryos. These observers have confirmed the observations made by one of us (S. V.) some years ago as to the existence of the same active principle in the paired suprarenals of Elasmobranchs and the medulla of the mammalian suprarenal capsule.

[4] This *Journal*, xx. p. 1. 1896.

[5] *Proc. Physiol. Soc.* Feb. 1899, This *Journal*, xxv. p. ix; *Phil. Trans.* 1899, pp. 216 and 242.

[6] This *Journal*, xxv. p. 283. 1900.

[7] See the tracings reproduced in the paper of Osborne and Vincent, *loc. cit.*

[8] This *Journal*, xxvi. p. 229. 1901. Preliminary communications on the same subject had however appeared both by Halliburton and by Osborne and Vincent in the *Proc. Physiol. Soc.* Feb. 19th, 1899.

the same subject. His conclusions were the same as those of Osborne and Vincent, except that he considered that the effects of nervous tissue extracts could be explained on the hypothesis that choline was the principal active agent in the solutions used. He arrived at this conclusion because in his experiments after the administration of atropine neither choline nor nervous tissue extracts produced a fall of blood-pressure.

Our Experiments. It will thus be seen, as Halliburton himself states[1], that the point of difference between his view and that of

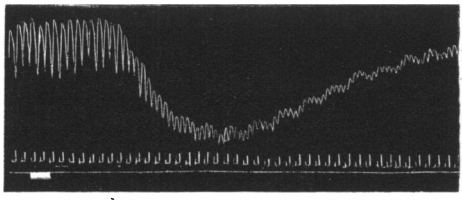


Fig. 1.

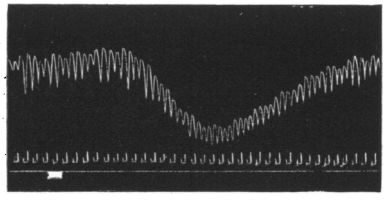


Fig. 2.

Fig. 1. Dog. A.C.E., morphia. Injection of 5 c.c. saline decoction of rabbit's brain—before atropine. Notice the marked increased frequency of heart-beat. Time-marker = seconds.

Fig. 2. Same dog. Injection of 5 c.c. of a 2 % solution of choline hydrochloride—before atropine. Heart frequency unaffected.

Osborne and Vincent is a fundamental one. For this reason it is necessary to lay stress upon a number of new experiments which we have performed, which confirm in every respect the results obtained by

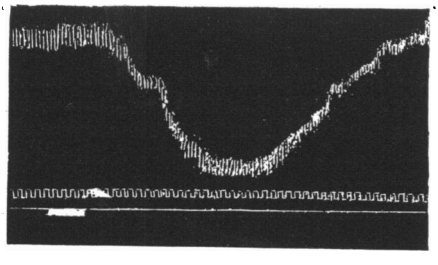


Fig. 3.

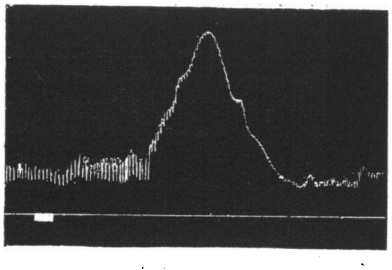


Fig. 4.

Fig. 3. (Same experiment.) Injection of 5 c.c. saline decoction rabbit's brain, after the administration of 3 c.c. of a 1 % solution of sulphate of atropine.

Fig. 4. (Same experiment continued.) Injection of 5 c.c. of choline solution as in fig. 2—*after* atropine.

[1] *loc. cit.*

Osborne and Vincent, viz., that, whereas before the administration of atropine both choline and nervous tissue extracts produce a fall of blood-pressure, after its administration in doses sufficient to abolish vagus action choline produces a rise which is usually marked, while nervous tissue extracts produce a marked fall (see Figs. 1 to 5). This marked fall after atropine was observed in 26 experiments; in 9 of these the contrast between the choline rise and the nervous tissue fall after atropine was noted. The point was tested on dogs, cats, and rabbits. The extracts were saline decoctions usually made from the brains of rabbits, sometimes from those of dogs or cats. Figs. 1—4 are a typical series of tracings from a dog. Figs. 5 and 6 show the results after atropine in a cat and rabbit respectively[1]. How are we to account for this serious discrepancy in experimental results? Halliburton lays no inconsiderable stress upon the chemical evidence of the existence of choline in the solutions employed. This is a point that we did not test, the present matter at issue being, not whether or not choline is present in these solutions, but whether it is the *active substance* present.

Halliburton surmised that the extracts of Osborne and Vincent "contained more nucleo-proteid than they bargained for" and more than his "happened to contain[2]." In our own experiments at any rate we took every precaution to avoid the presence of proteids of any kind

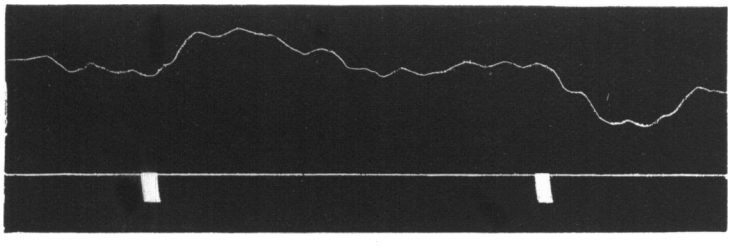


Fig. 5. Cat. A.C.E., morphia, atropine.
1st injection = choline.
2nd ,, = brain decoction.

in our saline decoctions (*vide supra*). Halliburton's experiments were performed exclusively on cats. We particularly noted in cats

[1] In a minority of our experiments we observed that the fall of blood-pressure after atropine was not so great as before, but in these particular experiments the atropine had caused considerable lowering of the general blood-pressure and therefore one would not expect depressor effects to be so marked.

[2] *loc. cit.* p. 241. It is difficult to see how this would explain the difference in results. As a matter of fact the nucleo-proteid fall would be less likely to be persistent than that due to the characteristic depressor substance in brain extracts.

accompanying the general lowering of blood-pressure some diminution of the fall after administration of atropine, but it was never completely abolished. Fig. 5 is from the experiment in which the least amount of contrast between choline and extract was obtained[1].

The saline decoctions used by Halliburton for the purpose of his experiments were weaker than ours (1 in 5 H., 1 in 3 V. & S.)[2]. He sometimes gave 3 c.c. and sometimes 5 c.c. of his solutions[3], *i.e.* his usual dose was a little more than one-third, or a little more than one-half that of ours. This small dose might possibly have no effect in an animal whose blood-pressure was lowered both by full doses of atropine and also possibly by lessened capability to react at the end of a long experiment.

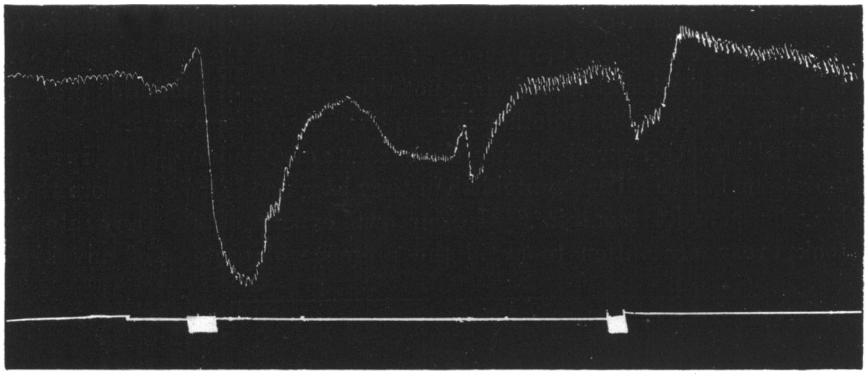


Fig. 6. Rabbit. A.C.E., morphia, atropine.
1st injection = brain decoction.
2nd ,, = choline.
It will be observed that the brain fall is very marked, but that the choline rise is preceded by a sharp fall.

Is it possible that variations in the dose of atropine may explain the discrepancy? Our animals were undoubtedly effectively atropinised. We indeed tried to obtain Halliburton's results by giving large doses of atropine alternately with small doses of the nervous tissue extract, but have never succeeded in doing so; we obtained a marked fall of

[1] In one experiment (on a rabbit) we obtained an anomalous result. The difference between choline and nervous tissue extract was evident even before the administration of atropine, inasmuch as instead of a fall the choline gave a marked rise of blood-pressure while the extract gave the usual fall. After atropine the usual results occurred (Fig. 6).

[2] *loc. cit.* p. 231.

[3] *loc. cit.* pp. 231 and 238.

blood-pressure with the injection of only five-sixth c.c. saline decoction of brain (one-sixth our usual dose) after full doses of atropine.

It is obvious that, If by any means one can bring an animal into such a condition that it reacts differently to choline and to nervous tissue extracts, then the action of the latter cannot be due to the presence in them of the former, and such a condition can be easily and uniformly induced by the administration of moderate doses of atropine[1].

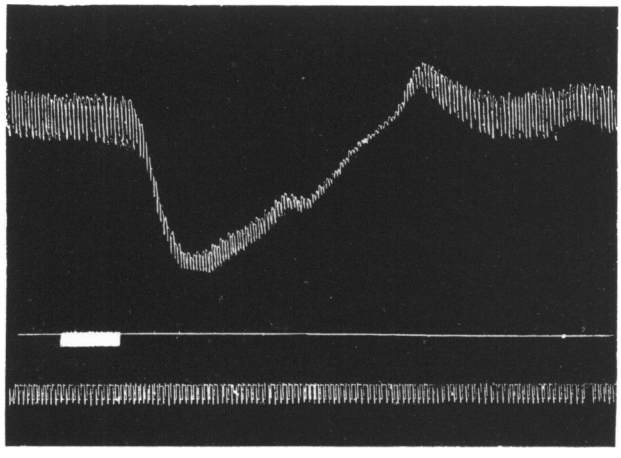


Fig. 7. Dog. A.C.E., morphia. Injection of 5 c.c. alcoholic extract of rabbit's brain.

We find then that saline decoctions of nervous tissues contain a powerful depressor substance[2]. On a few occasions with these decoctions a double effect[3] is produced, a distinct rise preceding a more marked fall of the blood-pressure (*vide infra*, Proteid extracts). Alcoholic extracts contain a substance with a marked depressor effect which is apparent both before and after atropine (Fig. 7). We never noted

[1] Schäfer and Vincent found that the depressor substance contained in extracts from the infundibular portion of the pituitary body produced its usual effect both before and after atropine. (This *Journal*, xxv. p. 87. 1899.)

[2] Very occasionally for the sake of contrast we allowed our extracts to become stale or even to putrefy, or we injected extracts made from stale or putrid tissues. Under all these circumstances we obtained a fall of blood-pressure neither more nor less marked, generally speaking, than that obtained with extracts of fresh tissues.

[3] In all cases where tissue extracts produced this double effect an equal bulk of saline solution was injected as a control.

a double effect with alcoholic extracts. We did not find any active principle in ether extracts of the moist tissue[1].

Ether Extract of Alcoholic Extract. When the nervous tissue was extracted by alcohol, the alcohol evaporated, and the residue left after the evaporation of the alcohol, taken up with ether, the ether again evaporated and the residue taken up with ·9 % normal saline, the filtered extract thus obtained, whether unboiled or boiled and cooled, was found

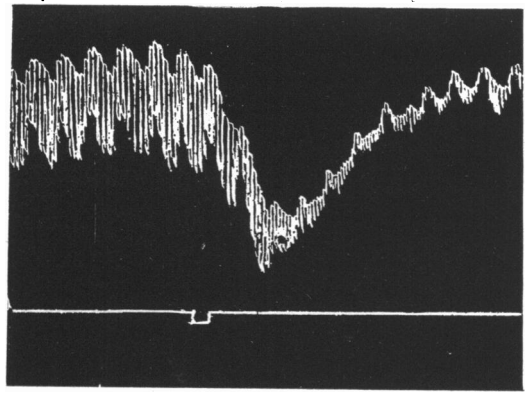


Fig. 8. Dog. A.C.E., morphia. Injection of 5 c.c. of ether extract of alcoholic extract of brain.

to have a powerful depressor effect (Fig. 8). This was constant and was observed in five experiments. We note therefore the curious result that although ether cannot apparently extract the depressor substance direct from the moist tissue, it can dissolve it after it has been previously removed from the tissue by means of alcohol[2].

We further noted a temporary "immunity" effect with nervous tissue extracts, the fall of blood-pressure becoming less and less with repeated injections (Fig. 9). If time be then given for the animal to

[1] Osborne and Vincent state that the residue left after evaporating off the alcohol from an alcoholic extract is completely soluble in ether (*loc. cit.* p. 290). We believe that there is a certain small amount of insoluble material left.

[2] If a saline decoction be evaporated to dryness, and the residue extracted with absolute alcohol, the residue after evaporation of the alcohol and solution in normal saline, has produced marked pressor effects in two experiments (material from sheep's brain) and depressor effects in a third experiment (material from dog's brain). The residue of the saline decoction after thorough extraction with alcohol, will impart to normal saline a substance having very marked depressor effects, and producing very pronounced slowing of the heart beat. The contrast between the alcoholic and saline extracts is more marked after atropine.

recover itself the fall of blood-pressure on injecting will be equal to that originally obtained.

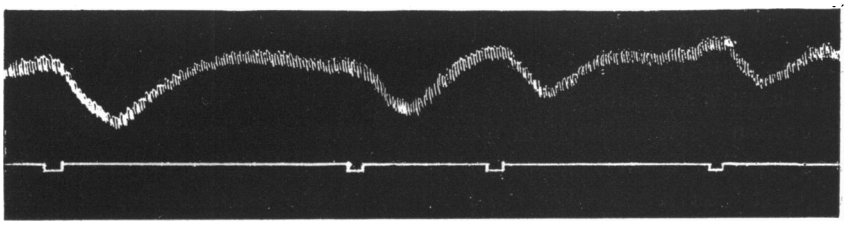


Fig. 9. Dog. A.C.E., morphia. Effect of rapidly repeated injection of 5 c.c. ether extract of alcoholic extract of brain.

"Proteid" Extracts. With regard to what we call "proteid extracts," we found that such extracts produce a rise, slight or marked,

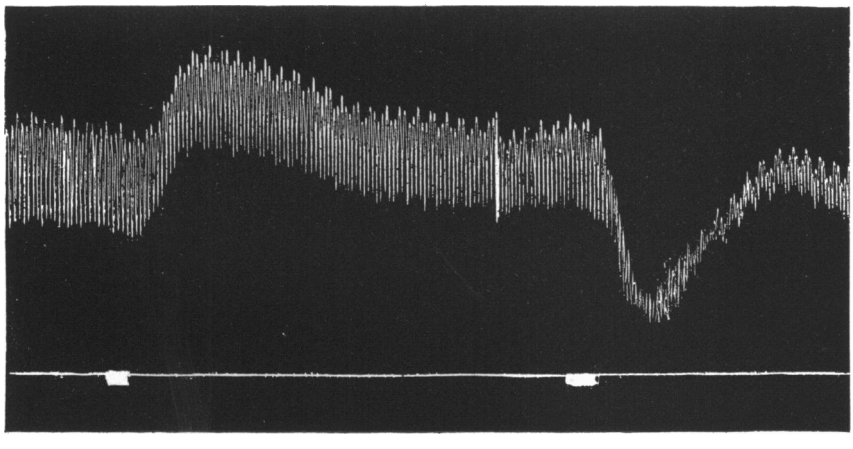


Fig. 10. Dog. A.C.E., morphia. First injection = "proteid" extract of brain of rabbit. Second injection = same extract boiled with a trace of acetic acid and filtered. A similar effect was obtained after administration of atropine and after section of both vagi.

of the blood-pressure (Fig. 10)[1]. This we noted in five experiments[2]. We found that this rise occurs also after the administration of atropine.

In all these experiments a striking contrast was noted between the

[1] Halliburton states that he was able to extract the active principle (depressor) with cold physiological salt solution, but that the effect was not so marked as with the boiled solution (*loc. cit.* p. 238).

[2] In one other experiment a slight fall occurred, in another a very pronounced fall, probably due to the nucleo-proteid, as the effect could not be immediately repeated.

boiled and the unboiled extracts: for if a portion of the same solution which produces a rise of blood-pressure is boiled with the addition of a drop or two of dilute acetic acid, filtered, and the cooled filtrate injected, a distinct fall of blood-pressure is obtained (Fig. 10); while a still more marked fall results when the saline solution with which brain substance is still mixed is boiled with acid, filtered, cooled and injected (*i.e.* when a saline decoction made in the ordinary way is used[1]). On one occasion when the extract was made from spinal cord a similar contrast was noted. We shall return to the consideration of these pressor effects later on.

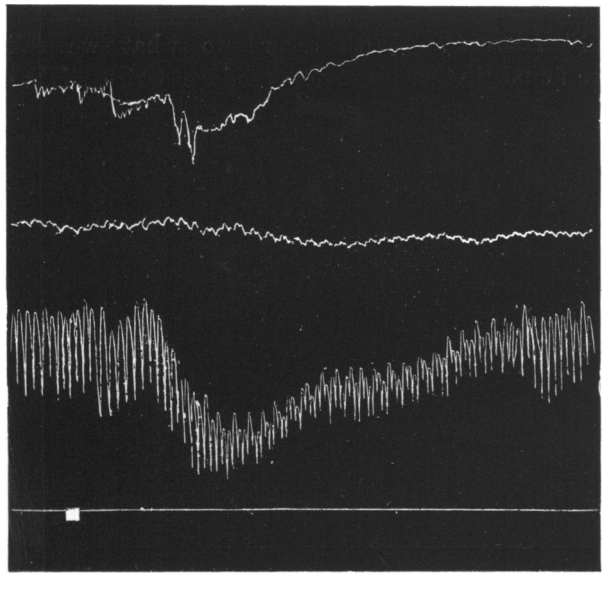


Fig. 11. Dog. A.C.E., morphia, curare, artificial respiration. Hind limb in plethysmograph, intestine in air oncometer. Upper curve = limb volume, middle curve = volume of intestine wall. Lower curve = carotid blood-pressure. Result of injection of 2·5 c.c. saline decoction of brain.

As for the mode of action of the extracts, Osborne and Vincent found[2] that the lowering of blood-pressure by nervous tissue extracts is brought about by dilatation of arterioles throughout the body. The

[1] Frequent controls were made with saline and acid only without any effect on the blood-pressure.

[2] *loc. cit.* p. 286.

vascular dilatation in the splanchnic area is so immediate and so pronounced as to withhold blood from the more distant limb, thus causing in the latter a temporary constriction: a little later the limb also participates in the dilatation. We have, speaking generally, confirmed these results, finding that the effect on both limb and

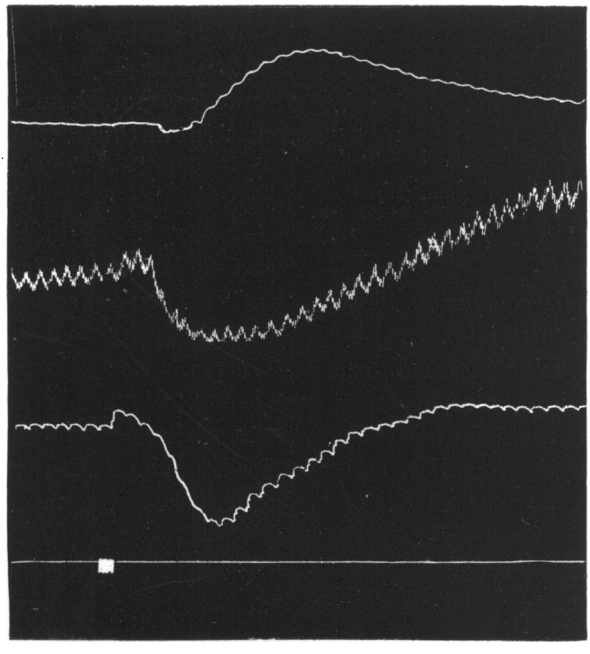


Fig. 12. Dog. A.C.E., morphia, curare, artificial respiration. Upper curve = limb volume, middle curve = intestine, lower curve = carotid blood-pressure. Effect of injection of 5 c.c. alcoholic extract of rabbit's brain.

intestine is one of dilatation, but we have not always found that the splanchnic area is more immediately dilated than the hind limb. Thus in Fig. 11 the limb is distinctly dilated while the intestine is scarcely affected, or if anything shows a very slight constriction. Again, in Fig. 12 the limb is dilated while the intestine volume is diminished, a dilatation of the splanchnic vessels only occurring later. The limb dilatation occurred after alcoholic extracts (Fig. 12), and was observed after doses of atropine sufficient to abolish vagus action. In one experiment the effect on the intestine was constriction without a subsequent dilatation.

Where muscle and nervous tissue extracts were contrasted in the same experiment, they were found to produce their depressor action by vaso-dilator effects on different areas (*cf.* Figs. 11 and 18): for with muscle the limb was constricted and with nervous tissue dilated.

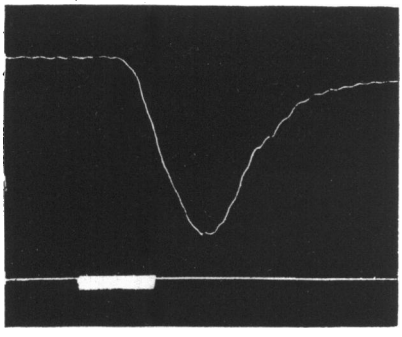


Fig. 13. Cat. A.C.E., morphia. Injection of 5 c.c. saline decoction striped muscle of rabbit.

With regard to the action on the heart we need only say that our results confirm those of Osborne and Vincent[1], viz. that the extent of movement of the auricle lever is markedly diminished, a similar effect being seen to a less extent in the ventricle lever. We have no reason to doubt their conclusion that the cardiac changes are secondary to those in the arterioles.

(*b*) *Muscular Tissue Extracts.* We performed 26 experiments in which the action of muscle extracts was tested. We found that a depressor substance is present in muscular tissues. Saline decoctions of all kinds of muscle, striped, cardiac, and unstriped, produce when injected into the circulation a marked fall of blood-pressure, not however so marked

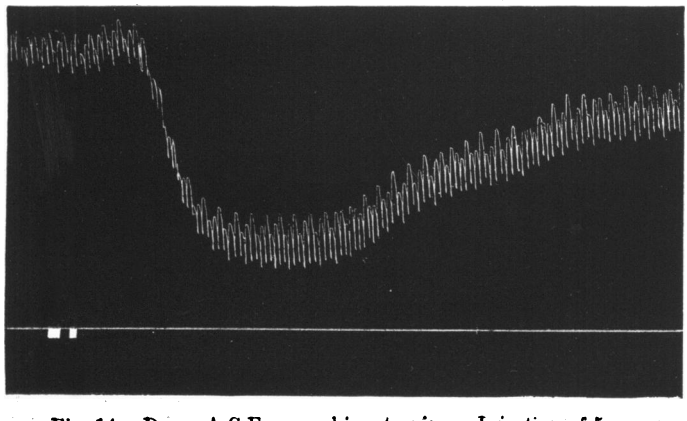


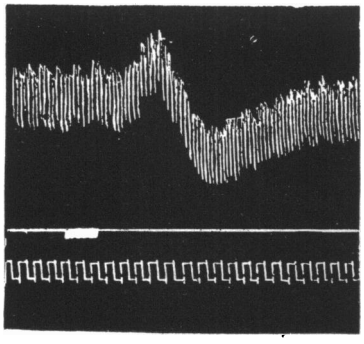
Fig. 14. Dog. A.C.E., morphia, atropine. Injection of 5 c.c. saline decoction smooth muscle.

usually as that which occurs on the injection of nervous tissue extracts (Figs. 13, 14). This fall occurs, as in the case of nervous tissue extracts,

[1] *loc. cit.* p. 289.

after section of both vagi and after the administration of sufficient atropine to abolish vagus action. With saline decoctions of muscle we observed somewhat more frequently than with those of nervous tissue a double effect. When proteid extracts were used this double effect was the usual one (Fig. 15), but we also observed a pure rise, never however so marked as that which occurs with nervous tissue. When the proteid

Fig. 15.



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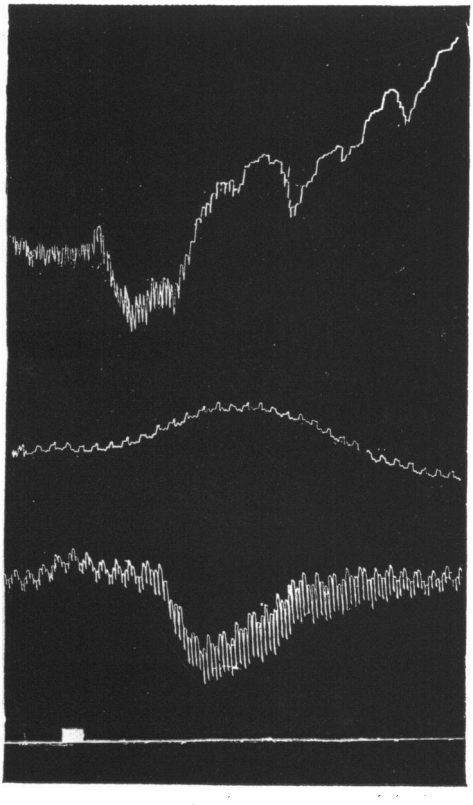

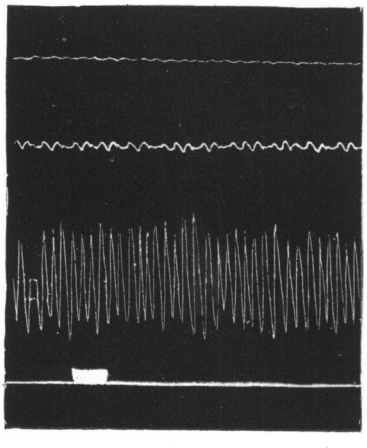

Fig. 17.

Fig. 15. Dog. A.C.E., morphia. Injection of 5 c.c. "proteid" extract of striped muscle.

Fig. 17. Dog. A.C.E., morphia, curare, artificial respiration. Upper curve = limb, middle curve = intestine, lower curve = carotid blood-pressure. Injection of an ether extract of striped muscle.

Fig. 18. Dog. A.C.E., morphia, curare, artificial respiration. Upper curve = intestine, middle curve = limb, lower curve = carotid blood-pressure. Injection of 5 c.c. saline decoction striped muscle of rabbit.

extract was treated like the similar extract of nervous tissue (*q.v.*) the solution only boiled gave results similar to the cold extract: but when boiled with the muscle substance (saline decoction) a fall usually resulted, being occasionally preceded by a slight rise. We note then that the pressor effect is somewhat more persistent with muscle than with nervous tissue extracts, but is less in magnitude.

Alcoholic extracts of muscle always produce a marked fall of blood-pressure (Fig. 16). The double effect was not observed. Ether extracts gave no evidence of containing any active principle[1] (Fig. 17).

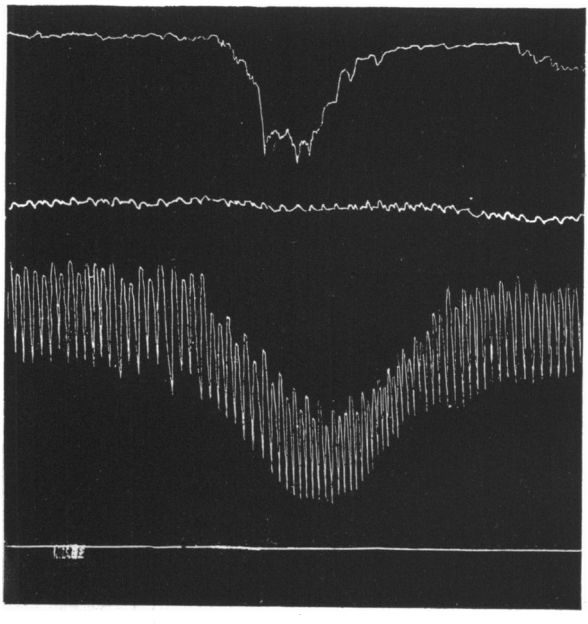

Fig. 16. Dog. A.C.E., morphia, curare, artificial respiration. Upper curve = limb, middle curve = intestine, lower curve = carotid blood-pressure. Injection of 5 c.c. alcoholic extract of ox muscle.

With regard to the mode of action of the extracts the usual effect both on limb and intestine was one of augmentation of volume of these parts. The dilatation of the limb is immediate, but with the intestine there is a slight diminution in calibre of the intestinal blood vessels accompanying the fall of blood-pressure (Fig. 18). In one experiment a marked constriction of the limb accompanied the fall of blood-pressure

[1] A marked depressor effect was obtained (as one would expect) from an alcoholic extract made from the muscle after it had been extracted by ether.

(Fig. 16)[1]. This result should be contrasted with that of nervous tissue extract in the same experiment (Fig. 11)[2]. The dilator effects on limb and intestine were observed with alcoholic extracts and also after the administration of atropine.

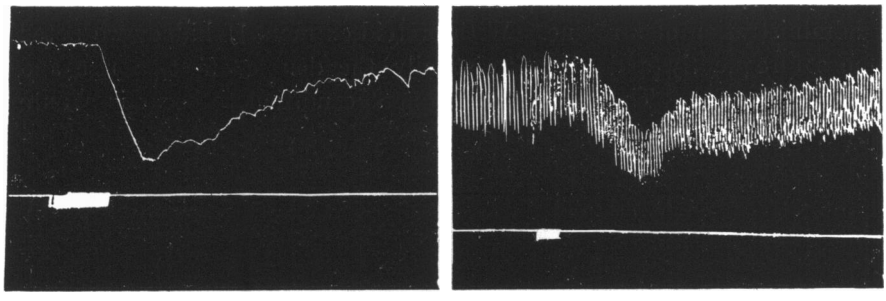

Fig. 19. Cat. A.C.E., morphia. Injection of 5 c.c. saline decoction of kidney of sheep. Dog's kidney extract injected immediately afterwards produced a similar effect.

Fig. 22. Same experiment as Fig. 21. Effect of injection of a saline decoction of same kidney as in Fig. 21, *i.e.* kidney substance boiled with acid saline.

(*c*) *Kidney Extracts.* Saline decoctions of kidney prepared according to our usual method produce as a rule a marked temporary fall of blood-pressure (Fig. 19). This fall is not usually so marked as that

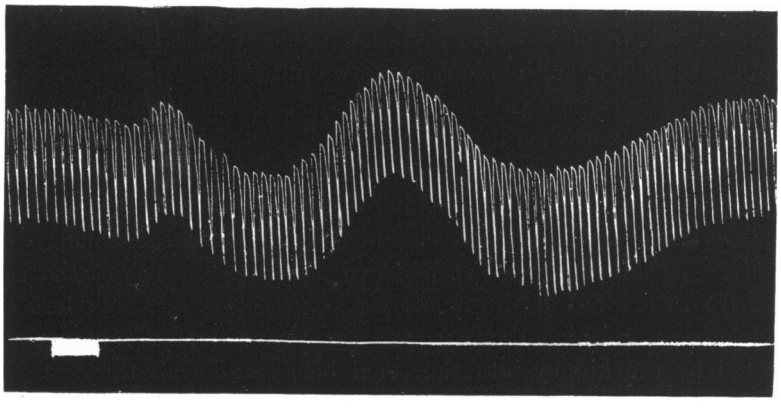

Fig. 20. Dog. A.C.E., morphia. Injection of 5 c.c. saline decoction of kidney of dog. This is a typical kidney effect upon the blood-pressure of a dog.

[1] Possibly the dilating areas were in the other limbs or elsewhere in the body, and the limb investigated underwent a passive diminution of volume.

[2] In this experiment there was little change in the intestinal volume (Figs. 11 and 16).

which occurs with nervous tissue extracts. Frequently a double effect occurs, a rise followed by a fall which may be repeated (Fig. 20), and we have in two out of 14 experiments seen a pure rise follow the injection of saline decoction of kidney. Alcohol extracts the depressor substance, and its effects persist after section of the vagi and the administration of atropine. With proteid extracts of kidney we noted most frequently and characteristically the double effect, a rise and a fall (Fig. 21). The next most frequent condition to occur is a pure

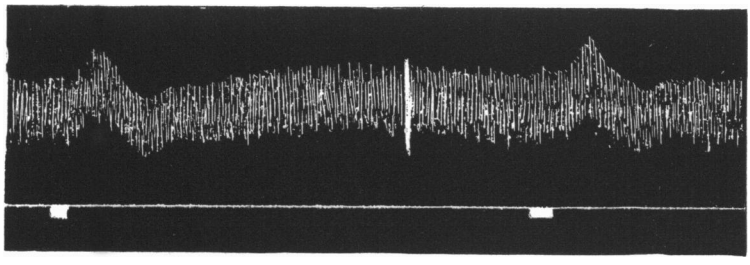

Fig. 21. Dog. A.C.E., morphia. First injection = "proteid" extract of kidney. Second injection = same extract boiled.

rise. On one occasion a fall resulted and on another the line of blood-pressure was unaltered. Where the double effect occurs the rise is the more marked feature, and speaking generally there is with kidney extracts even more than with those of muscle or nervous tissue, distinct evidence of the presence of a pressor substance.

As with muscle and nervous tissue we noted that the pressor effect was more likely to persist when the extract used for injection was boiled after filtering, than when it was boiled first and then filtered. This is well shown by the series of tracings in Figs. 21 and 22.

Tigerstedt and Bergman state[1] that a substance may be extracted from the kidneys of rabbits which when injected into the blood vessels of a living rabbit causes a rise of blood-pressure. They get the same effect from the blood of the renal vein. They conclude therefore that a substance, for which they suggest the name "renin," is normally secreted by the kidney into the renal blood, and that this substance causes a vaso-constriction. It will be seen from what we have written that we have been able to some extent to corroborate this, inasmuch as we have found distinct evidence of the existence of a pressor substance in the kidney. Tigerstedt and Bergman state that this

[1] *Skand. Archiv für Physiologie*, VIII. S. 223. 1898.

pressor substance is destroyed by boiling, and it is certainly true here as with other tissues, with the exception of suprarenal medulla and pituitary infundibulum, that if the extract is thoroughly boiled with normal saline the more is one likely to get a depressor effect from it.

In regard to the mode of action of kidney extracts, accompanying the usual fall of blood-pressure produced by a saline decoction we invariably found that the blood vessels of the splanchnic area are constricted, showing that the fall must be produced by dilatation of vascular areas elsewhere; thus the limb lever rises (Fig. 23).

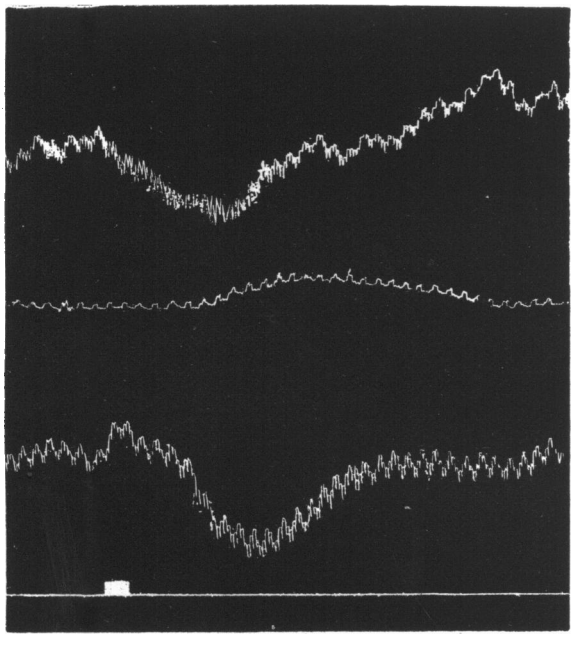

Fig. 23. Dog. A.C.E., morphia, curare, artificial respiration. Injection of 5 c.c. saline decoction kidney of ox. Upper curve = intestine, middle curve = limb, lower curve = carotid blood-pressure.

(*d*) *Liver Extracts.* Saline decoctions of liver produce a marked temporary fall of the blood-pressure. The fall is lessened but not abolished by the administration of atropine. In one experiment in which proteid extract of liver was tried it also produced a distinct fall.

(*e*) *Spleen Extracts.* Oliver and Schäfer[1] found that by spleen extract the blood-pressure is at first lowered and then somewhat raised. This never occurred in our experiments. With saline decoctions we

[1] This *Journal*, XVIII. 3, p. 277. 1895.

obtained a marked fall. With proteid extracts a much smaller but still distinct fall occurred.

(*f*) *Intestine Extracts.* Bayliss and Starling[1] have already described a depressor substance as present in extracts of the mucous membrane of the intestine quite distinct from their "secretin." Our extracts were made both from the separated mucous membrane and from the entire thickness of the gut wall, and the general result obtained is

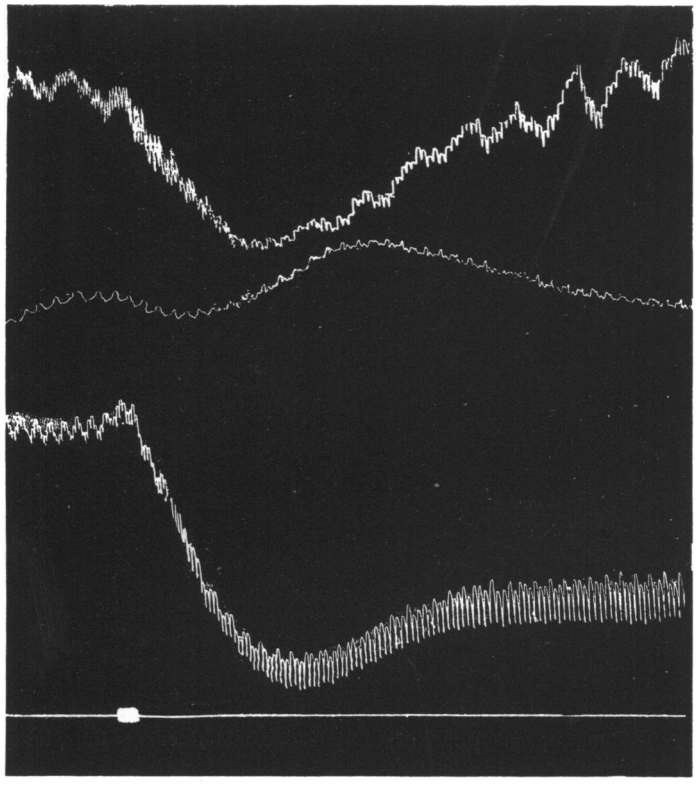

Fig. 24. Dog. A.C.E., morphia, curare, artificial respiration. Injection of 5 c.c. saline decoction made from the whole thickness of intestine of dog. Upper curve = intestine, middle curve = limb.

shown in Fig. 24. With regard to the mode of action of these extracts the limb lever rose in each case with the fall of blood-pressure. The only definite result obtained with the intestinal lever was a fall, but this was

[1] This *Journal*, XXVIII. p. 330. 1902.

not of constant occurrence. The effect of proteid extract was tried on one occasion, when a much slighter fall than that obtained with saline decoction was observed.

(*g*) *Other animal tissue extracts.* We also investigated the effects of extracts of testis[1], pancreas and ovary. We obtained with each a fall of blood-pressure on the injection of a saline decoction. A strong ovarian extract produces a very prolonged lowering of the blood-pressure. The pancreatic effect was also obtained after the administration of atropine. On one occasion a saline decoction of lung was injected and produced a marked fall of blood-pressure.

4. *The effect of injection of animal tissue extracts on the heart.*

An examination of our tracings shows that, speaking generally, such an injection into the circulation produces a quickening of the pulse rate. This is well shown in several of the tracings reproduced in this paper (Figs. 1, 3)[2], though the opposite effect occurred in some cases (Fig. 24). These results however were by no means constant, and not infrequently the frequency remained unaffected. The extent of movement of auricle and ventricle as recorded by the heart-levers was diminished in the case of nervous extracts, the auricle being more affected than the ventricle. The same occurred, though not constantly, with other tissues.

5. *The question as to the existence of a pressor substance in all animal tissues.*

In a footnote to our preliminary communication[3] we stated that "suprarenal medulla and the infundibular portion of the pituitary body appear to be the only animal tissues which yield a pressor substance." We have shown in this paper reasons for altering this opinion. With nearly all the tissues we employed we had evidence, in some cases distinct in others only suggestive, of the presence of a pressor substance. The pressor effect is most marked with extracts of nervous tissue, of muscle and of kidney, and is best obtained from extracts made with cold saline solution[4]. Although a pure rise may

[1] Cf. Dixon. This *Journal*, xxvi. p. 244. 1901.

[2] It is noteworthy in comparing Figs. 1 and 2 that a still further difference between the mode of action of nervous tissue extracts and of choline is shown. The former quickens the heart-beat, while the latter does not affect it.

[3] *loc. cit.*

[4] With spleen extracts Oliver and Schäfer obtained a double effect.

result, the most characteristic result with these "proteid extracts" is a double one, a rise followed by a fall of blood-pressure. Boiling the filtered extract tends to enhance the depressor and mask the pressor effects, while if the tissue itself be boiled with saline, the filtering taking place subsequently, the depressor effect is most usually the only one to be observed.

Our experiments with other tissues have not been sufficiently numerous or varied to enable us to make such definite statements, but with liver, spleen and intestine extracts we noted that the fall of blood-pressure with the unboiled is much less than with the boiled extract. Boiling either tends to destroy the pressor substance or to extract more of the depressor substance, or both of these factors may be concerned in the result.

In the case of brain extract the contrast between the effects of a proteid extract and a saline decoction is well shown in Fig. 10.

We have not investigated the effects of proteid extracts of pancreas or of testis. With regard to testis Dixon has shown[1] that extracts made with saline solution at the body temperature may produce a rise of blood-pressure in the goat. In other animals he found only depressor effects.

The depressor effects of extracts of thyroid[2] and thymus[3] are known, but we have been unable to find any record of a pressor effect.

The presence of both a pressor and a depressor substance in pituitary extracts was shown by Schäfer and Vincent[4]. We know too that even in the medulla of the suprarenal body a depressor substance is present, the effect being manifested on injection of very small doses of the extract[5].

The pressor effect seems to be less in degree, of a more fleeting character, and consequently more difficult to obtain than the depressor effect. The former might easily be missed unless the extracts were made in various ways and a long series of experiments undertaken. The strength of the extract, the dose given, and the stage in the

[1] *loc. cit.*

[2] Schäfer. *Brit. Med. Jour.* 1895, p. 343.

[3] Svehla. *Wiener med. Blätter*, 1896, p. 919.

[4] This *Journal*, xxv. p. 87.

[5] Moore and Purinton. *Pflüger's Archiv*, 81, 1900, S. 483. These observers however do not attribute the depressor effect of minute doses of suprarenal to a separate substance, but consider it the result of the peculiar action of the recognised active principle of the gland when administered in minimal quantities. See also Abel. *Amer. Jour. of Physiol.* II. no. iii. 1899.

experiment at which it is given are all possible factors which may determine whether a pressor or a depressor effect is produced, *e.g.* with suprarenal extract, the depressor effect is only shown with minute doses, while with pituitary the pressor effect is only produced by the initial dose, all subsequent injections giving depressor effects[1]. Further investigations are required on these points.

The best way of obtaining the pressor substance from any tissue and of more or less separating the pressor and depressor substances in that tissue appears to be by extracting the absolutely fresh tissue with physiological saline solution, and after thorough trituration and allowing to stand for a few moments, filtering. From this extract we should expect a pressor or a mixed effect[2]. The tissue substance with the fluid still left on the filter-paper is then thoroughly boiled with the addition of a few drops of dilute acetic acid: from the extract so prepared after filtering and cooling we should expect a marked depressor effect[3].

Our results we think justify us in making the following provisional suggestion. All glandular tissues and probably all animal tissues contain both a pressor and a depressor substance, the former being usually extracted by saline solution at ordinary temperatures, while the latter is extracted by boiling saline solution, which either destroys (wholly or partly) the pressor substance or masks its effect by producing more of the depressor substance[4].

6. *The question as to the existence of specific vaso-dilator and constrictor substances in extracts of animal tissues.*

Bayliss and Starling have put forward the suggestion "that the products of certain tissues would be found to act as vaso-dilators only for certain tissues in functional relation to those in which they arise, or at all events would act to a greater degree on these tissues than on

[1] This at any rate applies to cats (Schäfer and Vincent, *loc. cit.*).

[2] In the case of thymus and brain I have in a series of recent experiments not obtained this pressor effect nearly so often as previously. S. V.

[3] It should be noted in this connection that alcohol in our experiments gave no evidence of being able to extract the pressor substance.

[4] A striking but apparently the only exception to this is of course the medulla of the suprarenal capsule. We have not, except in the case of nervous tissues, discussed the chemical nature of the active substances. The inorganic salts of the various tissues may possibly in some cases help to bring about the results observed. But in a few experiments with tissue ash we have obtained negative results, and we find that potash salts in the amount existing in nervous tissues for example do not cause any appreciable lowering of the blood-pressure.

the rest of the body in general[1].' In two experiments they obtained results which tended to confirm their view, so far as extracts of intestine were concerned. The action of other tissue extracts, *e.g.* muscle, kidney, etc., they do not mention, and presumably have not tried.

Now on looking at our four most typical tracings we observe results which do not tend to confirm this view (Fig. 12, Nervous Tissue; Fig. 20, Striped Muscle; Fig. 24, Kidney; Fig. 27, Intestine). We see in all these tracings an almost identical result, viz. accompanying the fall of blood-pressure an immediate dilatation of the limb, while the intestine at first follows passively the blood-pressure curve, and later undergoes a dilatation, marked and prolonged, except in the case of intestine extract, where it is so slight as to be scarcely noticeable. Thus with intestine extract we have obtained exactly the opposite result to that which Bayliss and Starling figure[2].

We are not however disposed to deny the existence of specific vaso-dilators. Undoubtedly the fall of blood-pressure on the injection of tissue extracts into the circulation is due to vaso-dilatation in very varying areas.

We have already noted that where muscle and nervous tissues were contrasted in the same experiment, the effects on the limb differed (Figs. 11 and 18), for with muscle the more marked effect was dilatation of the limb, while with nervous tissue there was a pure constriction, suggesting in this instance in the case of muscle a specific effect.

We would suggest that the subject may be complicated by the existence not only of specific vaso-dilator but specific vaso-constrictor substances, whose effects should be looked for on those occasions where the injection of a tissue extract produces a rise of the blood-pressure.

It is obvious that a larger series of experiments comprising the investigation of tissue extracts of many different kinds and recording changes in volume of the corresponding organs is necessary before any definite conclusions on these points can be reached.

7. Summary and conclusions.

1. Extracts of nervous tissues produce a marked temporary fall of the blood-pressure, which occurs after section of both vagi and after doses of atropine sufficient to completely abolish vagus action. Since choline always produces a rise of blood-pressure after atropine admini-

[1] *loc. cit.* p. 352.

[2] *loc. cit.* p. 351. But we have not repeated their experiment precisely as they performed it. Thus in our experiments the nerves were intact.

stration, it cannot be the active principle of nervous tissue extracts (confirmatory of Osborne and Vincent).

2. The depressor substance in nervous tissues is found in physiological saline decoctions, in alcoholic extracts dissolved in normal saline, and in ether extracts (dissolved in normal saline) of the alcoholic extracts. The depressor substance is not however extracted direct from the moist tissues by ether.

3. There is distinct evidence of the presence of a second active principle in nervous tissue extracts which produces a pressor effect. This indeed is the usual effect of an extract made with normal saline at ordinary temperatures. (Proteid extract.)

4. All kinds of muscular tissue contain a depressor substance, not usually so marked in its effects as that contained in nervous tissues, but like it equally active after the administration of atropine. A pressor substance appears also to be present, best extracted by normal saline at ordinary temperatures.

5. There is abundant evidence of pressor and depressor substances in kidney tissue. As with other tissue extracts the pressor effect is more readily produced by extracts made at ordinary temperatures, but occasionally by saline decoctions.

6. Many other animal tissues contain a depressor substance. We have shown the presence of such a substance in liver, spleen[1], testis[2], pancreas, ovary and lung. Other observers have found it in thyroid[3], thymus[4], suprarenal[5], and pituitary[6].

With many of these tissues there is evidence more or less marked of the presence of a pressor substance.

7. A depressor substance is present in extracts made from intestinal mucous membrane and from the whole thickness of the gut[7].

8. Possibly all animal tissues contain different substances which when injected into the circulation produce respectively either a rise or a fall of the blood-pressure.

9. The falls and rises of blood-pressure are produced by vaso-dilatation or vaso-constriction of various vascular areas in the body, but further investigations are required before we can state definitely that there is any specific effect of a particular extract upon the tissue from which it arises.

[1] Oliver and Schäfer, *loc. cit.*
[2] Dixon, *loc. cit.*
[3] Schäfer, *loc. cit.*
[4] Svehla, *loc. cit.*
[5] Moore and Purinton, *loc. cit.*
[6] Schäfer and Vincent, *loc. cit.*
[7] Bayliss and Starling, *loc. cit.*