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BY

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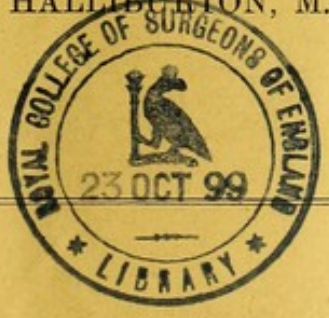

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VII. *The Physiological Action of Choline and Neurine.*

By F. W. Mott, *M.D., F.R.S., and* W. D. Halliburton, *M.D., F.R.S.*

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During the past few years, one of us (F. W. M.) has had exceptional opportunities of seeing patients suffering from various mental complaints, and of comparing the results of post-mortem examinations with the symptoms exhibited during life. One of the commonest complaints seen in the London County Asylums is General Paralysis of the Insane, and it was with a view of elucidating some of the pathological problems connected with this disease, that the present enquiry was originally undertaken. How far this object has been attained will be discussed at the conclusion of the paper.

The disease is one which is characterised by an extensive degenerative and wasting process occurring in the cerebral cortex, especially in the frontal and central convolutions; during the course of the disease there are frequent seizures of a congestive, epileptiform or apoplectiform kind, and after the recovery of the patient from each of these fits, he is, as a rule, worse mentally. Each fit probably indicates the breakdown of a new focus of cerebral matter. The disease is a premature, primary, progressive decay of the neuron affecting especially those structures which have been developed latest. It is a para-syphilitic affection like tabes, with which it is, pathologically speaking, identical.

After death, the brain is seen to be atrophied, especially in the regions already mentioned, and the place of the brain substance within the cranium is taken by an excess of cerebro-spinal fluid. It is often possible to obtain as much as one or two hundred cub. centims. of this fluid.

Microscopical examination of the diseased brains reveals changes which have already been described by other investigators, and which will not be detailed here. It may, however, be mentioned that the perivascular lymphatics are seen (by Marchi's method of staining) in acute cases to contain phagocytes filled with black stained fatty matter.

The main object of the research has been to examine the cerebro-spinal fluid, and to attempt to discover in it some substance or substances derived from the disintegration of the brain matter, which, passing into the general circulation, would produce auto-intoxication, and thus account for some of the symptoms of the disease.

In carrying this out, we have found that the cerebro-spinal fluid from these cases is much richer in proteid matter than the normal fluid is, and that it contains a basic substance, choline, which is easily accounted for from the decomposition of lecithin, and which is absent in normal cerebro-spinal fluid, or present in such small quantities that it cannot be detected.

In our first experiment we took an anæsthetised rabbit, and injected some 30 cub. centims. of the fluid removed, after death, from the cranial cavity of one of these patients. The arterial blood pressure, which was being registered by a mercurial kymograph, fell immediately and continuously, and never rose again. The animal died in the course of a few minutes. This was not due to the nucleo-proteid, which we subsequently found to be present in such fluids, causing intra-vascular coagulation, for the blood was found to be quite fluid; it was due to the choline which is present.

In our subsequent work we used principally dogs and cats, which are hardier animals; hutch rabbits are especially prone to die under circumstances that lead to vascular engorgement of the splanchnic area,* and it is chiefly by dilatation of the vessels in this area that the base choline produces the lowering of arterial blood-pressure, as we shall more fully see in the later sections of this paper.

It will be evident from the foregoing introductory remarks, that as the research proceeded it became necessary to make a complete investigation of the action of the alkaloid in question, to which we added a similar investigation of the closely related alkaloid neurine.

Our proofs that the toxic substance in the cerebro-spinal fluid is choline, rest partly on physiological and partly on chemical grounds. The physiological proof consists in the identity of the action of the alkaloid with that which is present in abnormal cerebro-spinal fluid; the chemical proof consists in the application of the recognised tests for the base, and the separation and analysis of some of its compounds.

Neurine is a far more powerful poison, and differs considerably from choline both in its chemical and its physiological re-actions. It is not present in the cerebro-spinal fluid of these patients.

In order to avoid fallacy from post-mortem changes, or microbic growths, the autopsies were performed as soon as possible after death, in some cases within a few hours. When this was not possible, the bodies were placed in a cold chamber (0° C. or lower) within half an hour after death, until the autopsy was performed. In all cases, too, cultures were made from the cerebro-spinal fluid, and blood of the frontal sinus, and in nearly all instances without result; this precaution we considered to be necessary, as many of these people die with bladder affection, or ulcerative colitis, and microbic toxins might arise. With a little practice also it soon became perfectly easy to obtain the cerebro-spinal fluid without any admixture with blood.

We have never had the opportunity of examining the fluid removed during life;

* Leonard Hill, 'Proc. Physiol. Soc.,' March 12, 1898. ('Journal of Physiol.,' vol. 22, p. liii.)

removal of the fluid by tapping has been recommended for the alleviation of the symptoms; it has, however, been realised that the alleviation that occurs is only temporary, and this method of treatment has in consequence been abandoned in the London asylums.*

We have, however, been sufficiently fortunate to secure on several occasions the blood removed for remedial purposes from such patients by venesection, and we regard one of the most important outcomes of our work the discovery that the blood also contains the same toxic material during a seizure. The choline evidently reaches the blood *viâ* the cerebro-spinal fluid, which functions as the lymph of the brain.

In some cases we have examined the urine also.

A good deal of the work has been carried out at the Claybury Asylum, or in the laboratory attached to it. We have to acknowledge here the assistance frequently given us by Dr. Wakelin Barratt, Technical Research Scholar of the London County Council. The greater amount of the chemical examination of the fluid, and all work which necessitated the use of living animals, has been carried out at King's College, where we have to thank Mr. Arthur Edmunds, B.Sc., for the help he has always been ready to give us during the course of the experiments, and to whom we owe many suggestions and pieces of apparatus that he devised and made. We also owe assistance in special branches of the work, which will be described in later portions of this paper, to Dr. Waller, F.R.S., and Miss Sowton, to Dr. T. Gregor Brodie, Mr. Leonard Hill, and to Prof. W. H. Thompson.

We may add that all the animals employed have been fully anæsthetised during the whole of the experiments. We have sometimes employed ether or chloroform, but generally A.C.E. mixture (alcohol : chloroform : ether : : 1 : 2 : 3). In some few cases, which will be fully described, we have given an injection of morphine, or of a mixture of morphine and atropine as well. If the animals did not die as the result of the injections, they were killed either by asphyxia or chloroform.

We may now pass to the actual description of the details of our work,† and this may be most conveniently done under the following headings :—

1. Previous work on the subject.
2. Chemical examination of the cerebro-spinal fluid.
3. Action of choline, neurine, and cerebro-spinal fluid on blood-pressure and respiration.
4. Experiments for the purpose of determining the substance in the cerebro-spinal fluid which produces the fall of blood-pressure.
5. Experiments with oncometers and plethysmographs.

* See supplementary note A at end of this paper.

† Preliminary communications on our work have been made to the Physiological Society. (See 'Journal of Physiology,' vol. 21, p. xviii.; vol. 22, p. xxxiv.; vol. 24, p. ix.)

6. Experiments to ascertain whether the action of choline and neurine on the blood vessels is direct or indirect.
7. Experiments on nerves.
8. Experiments on the cerebral vessels.
9. Experiments with the blood of General Paralytics.
10. Experiments on the urine.
11. Experiments with other substances.
12. Effect of anæsthetics.
13. Summary of the physiological action of choline and neurine.
14. Concluding remarks on the bearing of the work on the pathology of General Paralysis.

1. Previous Work on the Subject.

Chemical Characters of Choline and Neurine,

Choline was first prepared from bile by Strecker;* it was subsequently obtained as one of the decomposition products of protagon and lecithin, (Liebreich,† Diaconow,‡ Strecker,§ Gilson,‖) and has been prepared synthetically by Wurtz.¶

It forms compounds with various acids, and its hydrochloride forms crystalline compounds with gold chloride and platinum chloride. The platinum compound by which the base is usually identified has the formula:

$$(C_5H_{14}NOCl)_2 + PtCl_4,$$

and contains 31·64 per cent of platinum.

Chemically, choline is trimethyl-oxyethylene ammonium hydroxide, and its constitutional formula is

$$N.OH.(CH_3)_3.CH_2.CH_2.OH = C_5H_{15}NO_2.$$

When heated it splits up into glycol and trimethylamine,

$$C_2H_4\begin{cases}OH\\OH.N(CH_3)_3\end{cases} = C_2H_4\begin{cases}OH\\OH\end{cases} + N(CH_3)_3.$$

In solution it readily undergoes decomposition by bacterial growths, yielding the related alkaloid neurine.**

* 'Annal. Chem. Pharm.,' 123, p. 353 (1862).

† *Ibid.*, 134, p. 29.

‡ 'Centralbl. Med. Wissen.,' 1868.

§ 'Annal. Chem. Pharm.,' 1868, 148, p. 76.

‖ 'Zeitsch. f. Physiol. Chem.,' 12, p. 585.

¶ 'Annal. Chem. Pharm.,' Sup. 6, pp. 116 and 197.

** W. Gulewitsch, 'Zeitsch. f. Physiol. Chem.,' 1898, 24, p. 513. This paper gives a very full account of the chemical properties of choline. See also E. Schmidt, *ibid.*, 20, p. 364.

Neurine has been frequently confused with choline, and although it only differs from choline by two atoms of hydrogen and one of oxygen, its constitution is different. Its structural formula is :

$$HO.N(CH_3)_3.C_2H_3 = C_5H_{13}NO,$$

and the name which expresses this constitution is trimethyl-vinyl ammonium hydroxide. It was first obtained by LIEBREICH* from protagon, and later by BRIEGER† from the putrefactive decomposition of flesh. It has been prepared synthetically by VON HOFMANN‡ and VON BÄYER.§ It forms compounds similar to those which can be derived from choline. A very complete account of the chemistry of neurine and its compounds has recently been published by GULEWITSCH.‖

The two bases choline and neurine are closely related to two others, namely, betaine or oxyneurine, which is a non-toxic alkaloid obtained from the common beet, which has the composition :

$$OH.N(CH_3)_3.CH_2.COOH. = C_5H_{11}NO_2.H_2O,$$

and muscarine, the highly poisonous alkaloid obtained from the *Agaricus Muscarius*, or poisonous mushroom.

Muscarine has the composition :

$$N\begin{cases}(CH_3)_3\\CH_2-COH\\OH\end{cases} = C_5H_{13}NO_2.$$

Previous Work on the Physiological Action of Choline and Neurine.

GAEHTGENS¶ states that choline has no effect on the appearance or coagulation of the blood ; it causes first a rise and then a fall of arterial pressure, and large doses kill by arresting the respiration.

BOEHM** investigated the action of choline obtained from fungi ; in frogs it causes contraction of the pupils, arrest of the heart's action, and in doses of 0·05 to 0·1 gram it produces a curare-like action on the voluntary muscles. In Mammals (cats and rabbits) it produces salivation, paralysis, and finally death ; the toxic dose given by the mouth is 0·7 gram in the rabbit, 0·3 gram in the cat. Intravenous injection of from 0·01 to 0·02 gram causes profuse salivation and rise of blood-pressure ; a dose

* 'Ber. d. Deutsch. Chem. Gesellsch.,' 2, p. 12.

† *Ibid.*, 16, pp. 1190 and 1406 ; 17, pp. 515 and 1137.

‡ 'Jahresb. d. Chemie,' 1858, p. 339.

§ 'Annal. Chem. Pharm.,' 140, p. 311.

‖ 'Zeitsch. f. Physiol. Chem.,' 26, p. 175 (1898).

¶ 'Dorpater Med. Zeit.,' 1870, vol. 1, p. 185.

** 'Archiv f. Exp. Pathol. u. Pharm.,' 1883, vol. 19, p. 87.

of 0·06 gram causes arrest of the respiration, which, however, is resumed after a time.

There can be very little doubt that the choline described by these two investigators consisted very largely of neurine.

V. Cervello* made a more complete investigation of the two alkaloids; he recognised that the neurine of commerce contains both substances; he investigated the action of each; and states that both act in precisely the same way, but that neurine is the more powerful of the two.

Using commercial neurine, he found that the fatal dose in frogs was 3 or 4 milligrams; the most marked symptoms are dilatation of the pupil and paralysis. In rabbits the fatal dose was 5 centigrams injected subcutaneously; it produces salivation and gradual paralysis. In dogs weighing 6 kilograms, 30 centigrams was the fatal dose. In Mammals death is due to cessation of the respiration, which is caused by the curare-like action of the drug on the voluntary muscles.†

In dogs the intravascular injection of 0·02 gram causes the heart to become slower and irregular, a rise of blood-pressure, and an increase in the depth and rate of respiration. After several injections artificial respiration is necessary to keep the animal alive. Previous cutting of both pneumogastric nerves does not modify this action. Cervello's general conclusion is that the chief effect of the poison is on the respiration, and that the vascular changes are mainly secondary to this. He, however, found on the frog's heart (both *in situ* and isolated) that the poison acts like muscarine, and the stoppage of the heart so produced is antagonised by atropine. Moreover, he states that the antagonism, as in the case of muscarine and atropine, is a unilateral one. From experiments on salivary secretion he also concludes that neurine and atropine are antagonistic in their action on the glandular system. He states that neurine is eliminated from the body by the urine.

In the course of an investigation on the effects of suprarenal extract, Schäfer and Oliver‡ investigated the action of neurine on arterial blood-pressure, since some observers were inclined to believe that the active principle in suprarenal extract is neurine. They describe a preliminary fall of pressure, and a subsequent rise. This is what we have found, though in most cases the rise has been greater than in the tracing reproduced by Schäfer and Oliver in their paper.

Schäfer and Moore,§ in a paper on the contractibility of the spleen, found that an extract of dried brain substance made with boiling water or saline solution, produces a fall of blood-pressure and great increase in the extent of the rhythmic splenic movements. From the similar result which we have found to follow the

* 'Arch. Ital. de Biol.,' 1886, vol. 7, pp. 172 and 232.

† Moriggia ('Atti della R. Acad. dei Lincei,' ser. 3; Trans., 8, livr. 10, p. 232) on insufficient grounds denies this curare-like action.

‡ 'Journ. of Physiology,' 1895, vol. 18, p. 266.

§ *Ibid.*, 1896, vol. 20, p. 26.

injection of choline, we believe that the active principle in these extracts of brain was choline.

LEON ASHER* found that the intravenous injection of choline in dogs (3 to 5 cub. centims. of a 5 per cent. solution) causes an increased flow of lymph, but no increase in its concentration; there was no increase in the flow of bile. ASHER also states that he has undertaken, in conjunction with WOOD, an investigation of the action of choline on the circulatory system, but that this is reserved for a later paper, not yet published.†

Previous Work on the Injection of Cerebro-spinal Fluid.

We can find no previous record of work on this subject. One of us (F. W. M.), however, previous to the commencement of our joint work, had injected normal cerebro-spinal fluid obtained from one animal into the circulation of another animal, with entirely negative results; and Professor SCHÄFER tells us that he also has made injections of human cerebro-spinal fluid obtained from cases of meningocele, and he also has only obtained negative results.

In the course of our investigations we have confirmed this; we obtained specimens of human cerebro-spinal fluid from a man who died of cerebral hæmorrhage, from cases of meningocele and of hydrocephalus, and a case (under Dr. STCLAIR THOMSON'S charge) in which cerebro-spinal fluid dripped from the nostril.‡ We also used other serous effusions (hydrocele fluid, pleural effusion, ascitic effusion); but in all these cases injection of the fluid itself, or of a solution in physiological saline solution of an alcoholic extract of the fluid, produced entirely negative results on blood-pressure, heart and respiration.

We may at this point also mention that a saline solution of the alcoholic extract of normal blood (from man, dog and sheep) produces a similar negative result. This is important in view of our results presently to be described with the blood of General Paralytics.

2. CHEMICAL EXAMINATION OF THE CEREBRO-SPINAL FLUID.

Normal cerebro-spinal fluid is alkaline to litmus and contains a very small percentage of solids. In Dr. STCLAIR THOMSON'S case just mentioned, the solids comprised less than 1 per cent. of the total; in cases of meningocele, and still more in the case of hydrocephalus, the percentage is somewhat higher; but if tapping is frequently performed the fluid which accumulates becomes richer in solid, particularly in proteid constituents, and the fluid may ultimately differ but little from that of ordinary serous effusions.§

* 'Zeitsch. f. Biol.,' 1898, vol. 37, pp. 261–306.

† See supplementary note B at the end of this paper.

‡ STCLAIR THOMSON, L. HILL and W. D. HALLIBURTON, 'Proc. Roy. Soc.,' Feb., 1899 (vol. 64, p. 343).

§ W. D. HALLIBURTON, 'Journal of Physiology,' 1889, vol. 10, p. 232. References to the writings of previous workers on the subject are given in this paper.

In normal cerebro-spinal fluid the amount of proteid is especially low; serum globulin, and occasionally proteoses may be present, but fibrinogen and albumin are absent.

The fluid is also characterised by containing a substance which reduces Fehling's solution. This substance, according to the almost unanimous verdict of physiologists, is not sugar,* and one of us (W. D. H.) has advanced the opinion that it is a substance related to pyrocatechin.

Choline is not present in the specimens of normal cerebro-spinal fluid which we have examined. The method adopted is the same as that used in the separation of choline from the pathological fluids. Phospho-tungstic acid causes no precipitate in the fluid after the proteids have been thrown down by alcohol, and we have never succeeded in obtaining from the normal fluid the characteristic platinum double salt of choline.

We may now pass on to our examination of the fluid in cases of General Paralysis. The fluid is very abundant; it is colourless and faintly opalescent; its reaction is alkaline to litmus.

The reducing substance is generally absent; in only two cases out of fourteen specimens examined was it found.

The total solids are more abundant than in the normal fluid; this is principally due to excess of proteid material. On boiling there is quite a large coagulum formed.

In eight cases the amount of proteid was estimated in the following way. The fluid was rendered acid with acetic acid, thrice the volume of absolute alcohol was then added, and the mixture boiled. The resulting coagulum was collected on a weighed filter, dried at 110° C. to constant weight, weighed, then incinerated and the ash deducted.

The results are given in the following table:—

No.	Quantity of fluid.	Weight of proteid less ash.	Percentage of proteid.
	cub. centims.		
1	65	0·212	0·327
2	80	0·211	0·263
3	120	0·281	0·234
4	183	0·388	0·211
5	90	0·153	0·170
6	165	0·361	0·219
7	130	0·326	0·251
8	100	0·242	0·242
Average	...	...	0·239

In all the foregoing cases the fluid was obtained free from blood.

The average percentage of proteid in cerebro-spinal fluid obtained from three cases

* NAWRATZKI has stated in a recent paper ('Zeitsch. f. Physiol. Chem.,' 1897, vol. 23, p. 532), principally from observations on the cerebro-spinal fluid of the calf, that it is sugar.

of spina bifida was 0·088. The amount in the fluid from General Paralytics is thus increased about threefold.

The nature of the proteids was then investigated. Fibrinogen is absent; admixture of the fluid with blood or serum never produces clotting.

Proteoses and peptones are absent; if the fluid is mixed with an equal volume of 10 per cent. trichloracetic acid, boiled and filtered hot, the filtrate never gives the biuret or other reactions for proteoses and peptones.

The greater amount of the proteid present is coagulable by heat between 73°–80° C. The greater amount of the proteid is precipitable by saturation with magnesium sulphate, and therefore consists of globulin or nucleo-proteid or both; there is, however, a small amount of true albumin which remains in the filtrate after removal of the precipitate produced by saturation with magnesium sulphate.

That nucleo-proteid is present was determined in the following way: the cerebro-spinal fluid as each autopsy was performed was mixed with excess of alcohol, until at last over a litre of the fluid had been collected. The precipitated proteid was collected, well washed with alcohol and with water, and then subjected to artificial gastric digestion; at the end of a week the undissolved residue was collected; it was found to have the solubilities of nuclein, and this was confirmed by the fact that it contained a high percentage of phosphorus; this was done in the following way: the residue was well washed with 0·2 per cent. hydrochloric acid to remove any still adherent inorganic phosphates; then heated for some hours on the water bath at 100° C. with fuming nitric acid, to which a small quantity of sulphuric acid was added, and an occasional pinch of potassium chlorate.* The residue was dissolved in nitric acid, and ammonium molybdate added; on warming, an abundant yellow crystalline precipitate settled out. The experiment was not performed quantitatively, but we judge the amount of phosphorus was large, from the abundance of the precipitate relatively to the small amount of material investigated.

There can be little doubt that the nucleo-proteid originates from the disintegration of the brain cells; one of us has already shown the presence of nucleo-proteid in the cerebral tissue,† and this nucleo-proteid when injected intravenously in rabbits causes intravascular coagulation.‡

The question will therefore be asked whether injection of the cerebro-spinal fluid from General Paralytics causes intravascular coagulation. We have injected samples of cerebro-spinal fluid from about fifty cases of General Paralysis, but in the majority of these instances, after we had found the fall of blood-pressure to be due to choline, we precipitated the proteids by alcohol first. In those cases where we injected the fluid itself, we, as a rule, obtained no noticeable effect on the coagulation of the

* The method is described in full by one of us, W. D. H., 'Journal of Physiology,' 1892, vol. 13, pp. 814, 815.

† W. D. H., 'Journal of Physiology,' 1893, vol. 15, p. 90.

‡ *Ibid.*, p. 106.

blood. The quantity injected, as a rule, was 5 or 10 cub. centims. To this we had, however, one marked exception; the fluid was particularly rich in proteid, as judged by the heavy coagulum which occurred on boiling; 10 cub. centims. were injected into a cat anæsthetised with A.C.E. mixture; the fall of blood-pressure was at first that which is usually produced, but instead of recovering its usual level again, the fall became greater and greater until the animal died. *Post mortem* the right heart and veins (particularly the portal vein) were found full of clot.

Although we did not discover that choline is the toxic agent in the fluid which is responsible for the fall of pressure usually observed until a comparatively late stage in our research, it will be convenient to complete the account of the chemical side of our work by stating the method by which we separated and identified this base.

The proteids were first precipitated by alcohol in the usual way, and the alcoholic filtrate was evaporated to dryness at 40° C. The residue was taken up with absolute alcohol, filtered and again evaporated to dryness, and the treatment with alcohol repeated. The final residue after evaporation of the alcohol would be by this treatment free from potassium chloride. The residue was crystalline at first, but soon deliquesced on exposure to the air. It has a slightly brownish colour, and is soluble in water, physiological saline solution, absolute alcohol, and ether. If the residue is exposed freely to the air it putrefies in a few days, giving out the odour of trimethylamine and ammonia.

Dissolved in water or physiological saline solution it gives a white precipitate with phospho-tungstic acid, with phospho-molybdic acid, and with mercuric chloride, a brownish precipitate with iodine dissolved in a solution of potassium iodide, a yellow precipitate with gold chloride and platinum chloride. It gives no precipitate with tannic acid; this distinguishes it from neurine.

The alcoholic solution gives with gold chloride a precipitate which consists of tiny yellow crystals, which are soluble in hot water and hot alcohol; these separate out again on cooling; they are insoluble in ether.

In all these points the substance from the cerebro-spinal fluid exactly resembles choline, and in all cases the experiments were controlled by similar experiments with a solution of choline hydrochloride (0·1 per cent.).

They were repeated in six specimens of the cerebro-spinal fluid, and always with the same results. In normal cerebro-spinal fluid they were negative.

The platinum compound* was more fully investigated; it is easily soluble in water, so distinguishing it from the double platinum compounds of potassium and ammonium; it is insoluble in ether, and readily soluble in 15 per cent. alcohol.

On evaporating its aqueous solution it crystallises in six-sided plates and needle-

* On the chemistry of the platinum compound, see HUNDESHAGEN, 'Journ. f. Prakt. Chem.,' N.F. 28, p. 246; E. SCHULZE, 'Ber. d. Deutsch Chem. Gesellsch.,' vol. 22, p. 1829; 'Zeitsch. f. Physiol. Chem.,' vol. 15, p. 149; E. SCHULZE and S. FRANKFURT, 'Ber. d. Deutsch. Chem. Gesellsch.,' vol. 26, p. 2153; E. JAHNS, *ibid.*, vol. 23, p. 2973; W. GULEWITSCH, 'Zeitsch. f. Physiol. Chem.,' vol. 24, p. 513.

like prisms of a yellow colour. On evaporating the solution in 15 per cent. alcohol it crystallises in yellow octahedra. These are very typical crystals, and the accompanying figures show their appearance as drawn under a low power ($\frac{1}{2}$ inch) of the microscope. Fig. 2 are those from cerebro-spinal fluid; fig. 1 those from a 0·1 per cent. solution of choline hydrochloride.

Fig. 1. Fig. 2.

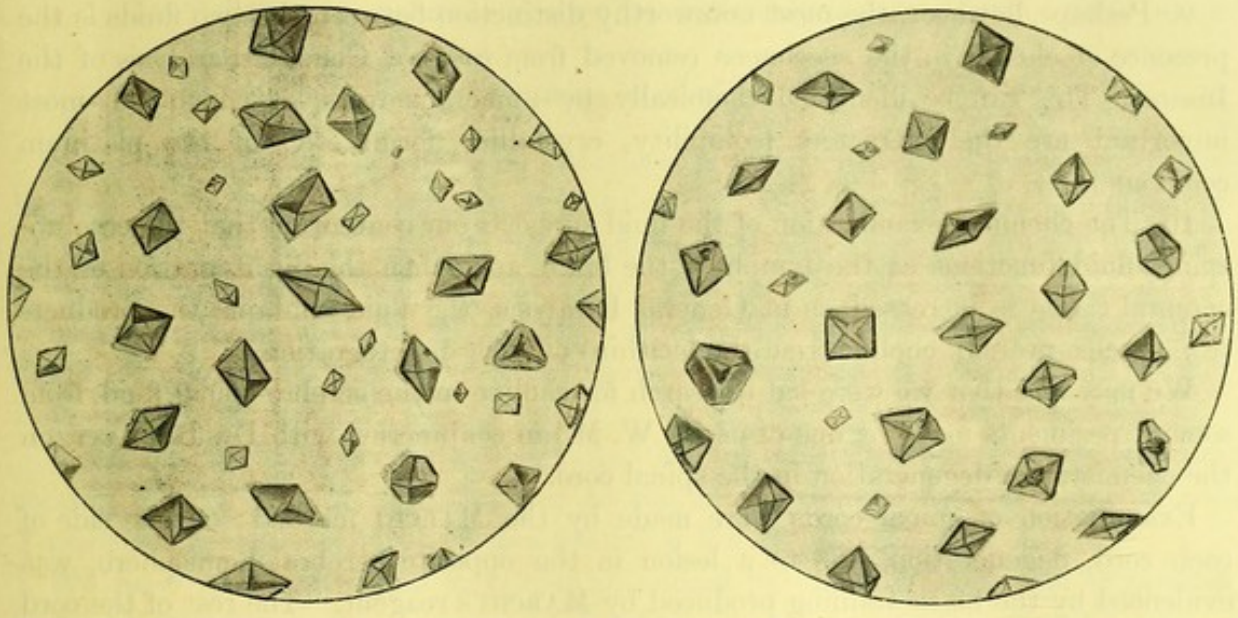


Fig. 1.—Crystals of the platinum double salt of choline, crystallised from 15 per cent. alcohol.
Fig. 2.—Crystals of the platinum double salt of the base separated from cerebro-spinal fluid in cases of General Paralysis of the Insane. Crystallised from 15 per cent. alcohol.

In one case we obtained crystals visible to the naked eye about the size of small pins' heads.

In another case we obtained a sufficient quantity of the compound to enable us to estimate the platinum. From 290 cub. centims. of cerebro-spinal fluid we obtained 0·1153 gram of the platinum compound; this yielded 0·0401 gram of platinum; the percentage of platinum was therefore 34·8; this is a little higher than the correct number (31·64 per cent.), but considering the small amount of material at our disposal, the difference is probably within the limits of experimental error.

The following is a summary of the preceding section on the chemistry of the cerebro-spinal fluid :—

1. The fluid from cases of General Paralysis is richer than the normal fluid in total solids.

2. This is particularly true for the proteid constituents (see table p. 218).

3. The proteids present are in great measure precipitable by salts, as globulins are.

4. There is, however, a small quantity of albumin in addition; in the normal fluid albumin is absent.

5. Fibrinogen is absent, as in the normal fluid.

6. Proteoses and peptones are also absent.

7. Nucleo-proteid is present, in one case in sufficient quantity to produce intra-vascular coagulation when 10 cub. centims. of the fluid were injected into the jugular vein of a cat.

8. A marked distinction between the normal and pathological fluid is the presence in the former of a reducing substance, and the absence of this, as a rule, in the latter.

9. Perhaps, however, the most noteworthy distinction between the two fluids is the presence of choline in the specimens removed from cases of General Paralysis of the Insane. This can be identified chemically by numerous tests, of which the most important are the characters (solubility, crystalline form, etc.) of the platinum compound.

10. The chemical examination of the fluid supports our contention that the cerebro-spinal fluid functions as the lymph of the brain, and when the disintegration of the cerebral tissue is increased, as in General Paralysis, the fluid contains the products (*e.g.*, nucleo-proteid, choline from the lecithin) of such disintegration.

We may add that we were led to search for choline in the cerebro-spinal fluid, from some experiments made by one of us (F. W. M.) in conjunction with Dr. BARRATT, on the chemistry of degeneration in the spinal cord.*

Examination of spinal cords were made by the MARCHI method; on one side of each cord, degeneration, due to a lesion in the opposite cerebral hemisphere, was evidenced by the black staining produced by MARCHI's reagent. The rest of the cord was divided into two halves longitudinally, and each half extracted with ether in a SOXHLET's apparatus. The residue of the ethereal extract on the degenerated side was more abundant, but contained less phosphorus than on the healthy side; its consistency was like that of butter. On the healthy side the residue consisted chiefly of protagon crystals. The degenerated half of the cord was also more watery. Here there was distinct evidence that in the degenerative process the lecithin had, in great measure, disappeared and been replaced by neutral fats. It was this which first suggested to us that we should seek for the products of lecithin in the cerebro-spinal fluid.

3. ACTION OF CHOLINE, NEURINE, AND CEREBRO-SPINAL FLUID ON BLOOD-PRESSURE AND RESPIRATION.

In order to investigate the effects of these substances on the circulatory and respiratory systems, the carotid artery in an anæsthetised dog or cat was exposed on one side, and the external jugular vein on the other. A cannula was inserted into each vessel; that in the carotid artery was attached to a mercurial kymograph; that in the jugular vein was used for injecting the material.

* 'Proc. Physiol. Soc.,' February, 1899; 'Journal of Physiol.,' vol. 24, p. iii.

The dogs used were mostly fox terriers, weighing 7 to 10 kilogs. The cats weighed about 5 to 6 kilogs.

The volume of fluid injected varied from 1 to 10 cub. centims. At first we warmed the fluid to be injected to the temperature of the animal's body, but as this did not modify the action of the drugs we subsequently omitted this procedure.

The fluids first used were :—

1. Cerebro-spinal fluid.
2. A 0·2 per cent. solution of choline hydrochloride in physiological saline solution.
3. A 0·1 per cent. solution of neurine in distilled water.

We selected choline hydrochloride in preference to the base itself, because it keeps better both in the solid state and in solution. We however made a number of control experiments with choline itself, and found that it produced exactly the same results as the hydrochloride.

Neurine is not soluble in physiological saline solution, so we were obliged to use distilled water. Injection of the same quantity of distilled water without any neurine in it produces no result.

The respiratory movements were recorded by the tambour method : two tambours connected by a T-piece were strapped to the animal's chest, the third limb of the T-piece was connected by tubing to a recording MAREY'S tambour.

We have never succeeded in killing an animal by injection of choline or choline hydrochloride.

The fatal dose of neurine in dogs is less than a decigramme ; respiration ceases before the heart.

Choline produces practically no effect on the respiration ; the arterial pressure falls considerably ; the heart at the same time becomes somewhat slower, but this slowing is not usually a marked symptom. In a short time the pressure recovers its normal level, doubtless because the injected material is so diluted as it gets mixed with the whole volume of blood that it no longer can produce any noticeable effect ; possibly, also, it is broken up into simpler materials and excreted. (See further under Urine.)

The cerebro-spinal fluid from cases of General Paralysis of the Insane acts in a precisely similar way. Normal cerebro-spinal fluid has no effect. We have chiefly confined our attention in pathological cases to the disease just mentioned, but we have also occasionally tested the fluid from cases of other diseases. In a case where a man died from cerebral hæmorrhage the fluid produced no result. In a case of melancholia (very probably General Paralysis), and in one of senile dementia, the fluid produced the same results as that from cases of General Paralysis. In both these cases there was, however, great and evidently acute wasting of the brain tissue, such as is met with in General Paralysis.

Neurine produces results which are quite distinct from these. The injection pro-

duces a great exacerbation of the respiratory activity ; this shortly afterwards returns to normal ; after several injections, however, the respirations become feebler, and finally cease, and if one wishes to continue the experiment, the animal has to be kept alive by artificial respiration. This confirms the observations previously made by CERVELLO;* we have not paid particular attention to the curare-like action he describes on the voluntary muscles, and to which he ascribes the cessation of respiratory movements. As confirmatory of his statements in this direction, we may mention that in the case of those animals which we killed by asphyxia on the termination of an experiment, convulsions were usually absent if neurine has been injected previously.

The effect on arterial blood-pressure varies somewhat. The usual effect is a preliminary fall of blood-pressure (doubtless, as we shall see later, cardiac in origin) ; this is followed by a rise which is often considerable, and then a fall again to the normal, or somewhat below it.

In some cases, especially if the dose is a small one, the preliminary fall is absent, and the sole effect is a rise.

In other cases again, especially when the dose is large and the animal a feeble one, the secondary rise is absent, and the blood-pressure falls, the slowing of the heart being a marked symptom.

In all cases, whether choline, neurine, or cerebro-spinal fluid is employed, the effect of successive injections becomes progressively lessened ; the animal, in other words, acquires a certain degree of immunity, but if a considerable time (10 or 15 minutes) is allowed to elapse between the injections, this immunity is not so evident.

In no case, even when much stronger solutions were employed than those mentioned, did we observe anything in the shape of fits or convulsions. In order to test this thoroughly, we injected the material into the carotid artery towards the brain, but here again there were no convulsions, though the usual effects were produced on respiration and blood-pressure. In the case of a monkey we painted both weak and strong solutions on various parts of the surface of the brain, but again with negative results. It is therefore evident that the convulsive seizures of General Paralysis cannot be explained by the presence of choline in the cerebro-spinal fluid or the blood.

The accompanying tracings illustrate the general effects on blood-pressure and respiration just described.

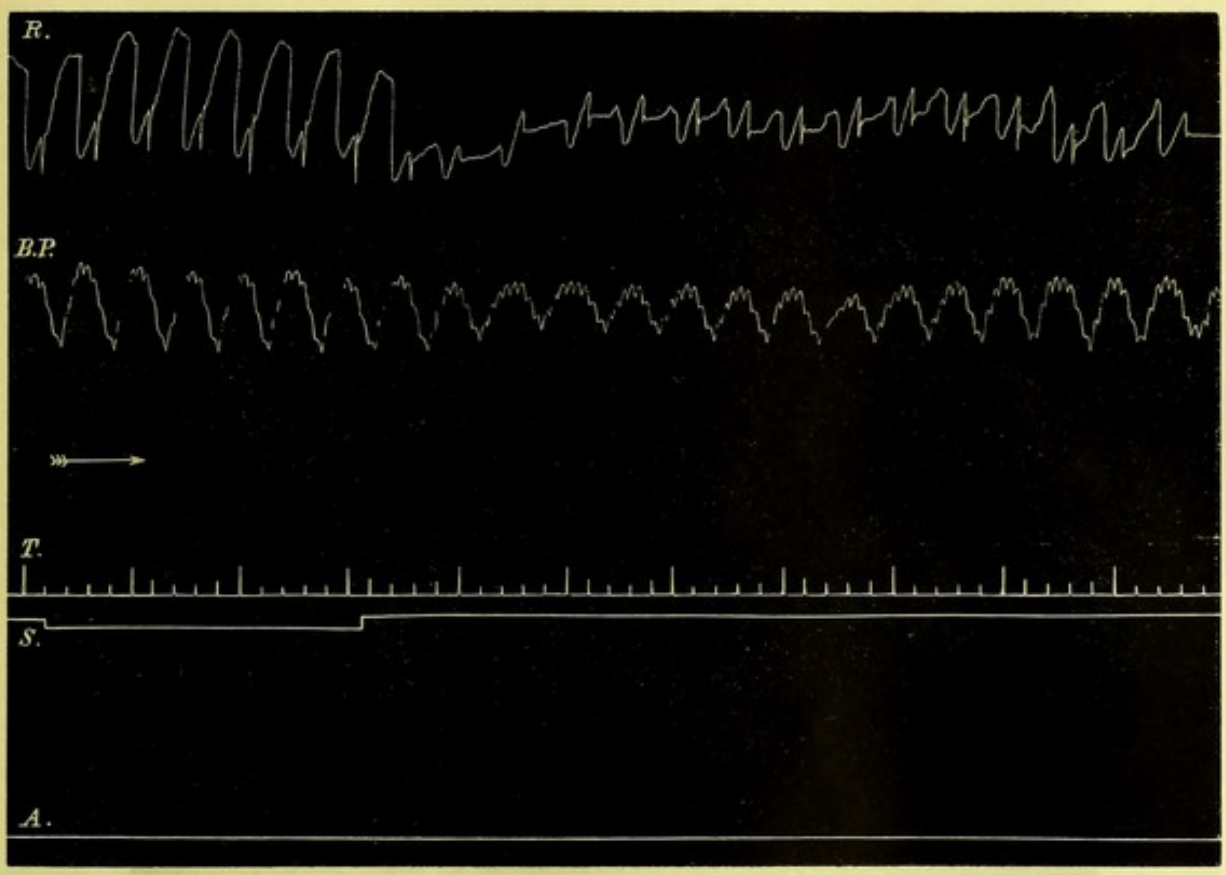
In each case the top tracing (R.) shows the respiration ; the next (B.P.) is the arterial blood-pressure from the carotid ; the bottom line (A.) is the abscissa ; the next line (T.) is a time-tracing in seconds ; and the last (S.) is written by a signal, the period of injection being indicated by its writing at a different level.

Fig. 3 shows the effect of injecting normal cerebro-spinal fluid in a dog. 7 cub. centims. were injected. There is a slight alteration in the respiratory movements, but no fall or rise of blood-pressure.†

* *Loc. cit.*

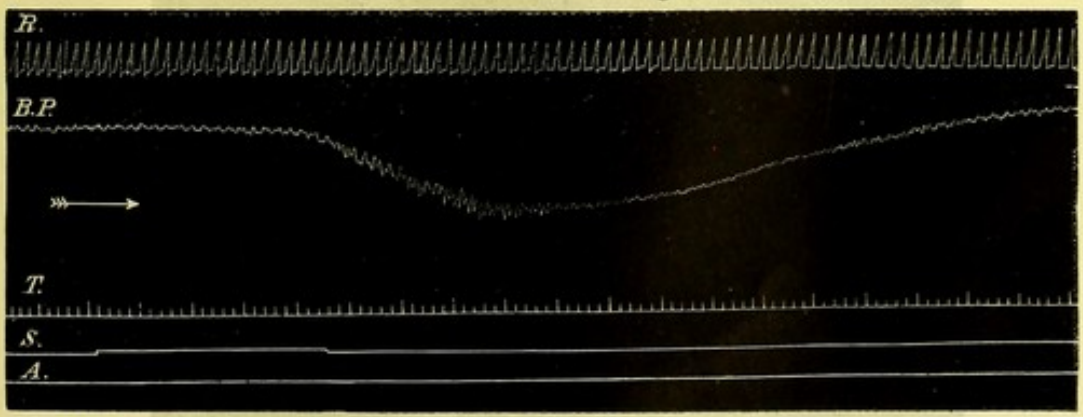
† Tracings which in other cases show merely negative results have been omitted at the suggestion of the Referees.

Fig. 3. Original size.



Effect of injecting 7 cub. centims. of normal cerebro-spinal fluid in a dog. R., tracing of respiration; B.P., tracing of blood-pressure from carotid artery; A., abscissa of blood-pressure; T., time in seconds; S., signal, the lowering of the level of which indicates the period of injection. The same letters have the same significance in subsequent tracings. The arrow in each tracing indicates the direction in which the tracing is to be read; in nearly all cases it is from left to right.

Fig. 4. Reduced to half original size.



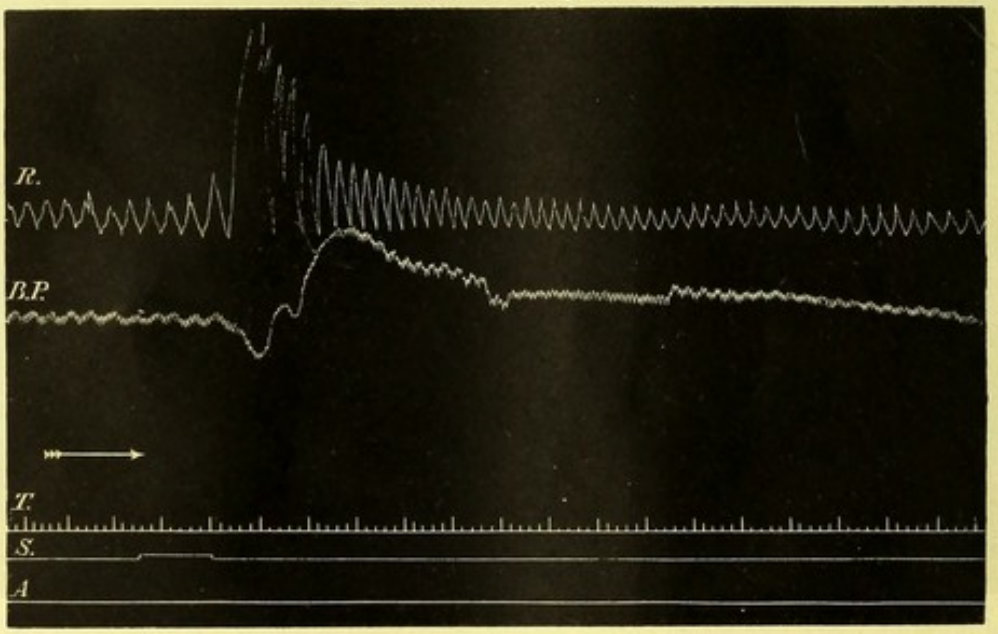
Fall of blood-pressure in a dog produced by injecting 5 cub. centims. of a 0·2 solution of choline hydrochloride. No effect on respiration.

Fig. 4 shows the effect of 5 cub. centims. of a 0·2 per cent. solution of choline

hydrochloride in physiological saline solution. The effect (fall of blood-pressure, no effect on respiration) is just the same in the case of pathological cerebro-spinal fluid (see figs. 8 and 9).

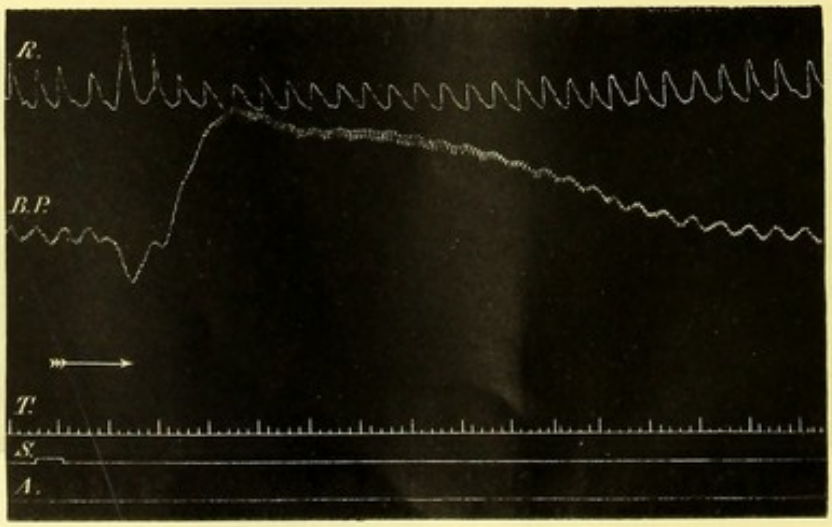
Fig. 5 shows the usual effect of 2·5 cub. centims. of a 0·1 per cent. solution of neurine in distilled water; there is first a fall, then a rise, and finally a fall again in blood-pressure. The exacerbation of the respiratory movements is well seen.

Fig. 5. Half the original size.



Effect of injecting in a cat 2·5 cub. centims. of a 0·1 per cent. solution of neurine. In the tracing, R., each upstroke is caused by inspiration. Note the increase in respiratory efforts, followed by a decrease. In the blood-pressure tracing there is a fall, followed by a pronounced rise.

Fig. 6. Half the original size.

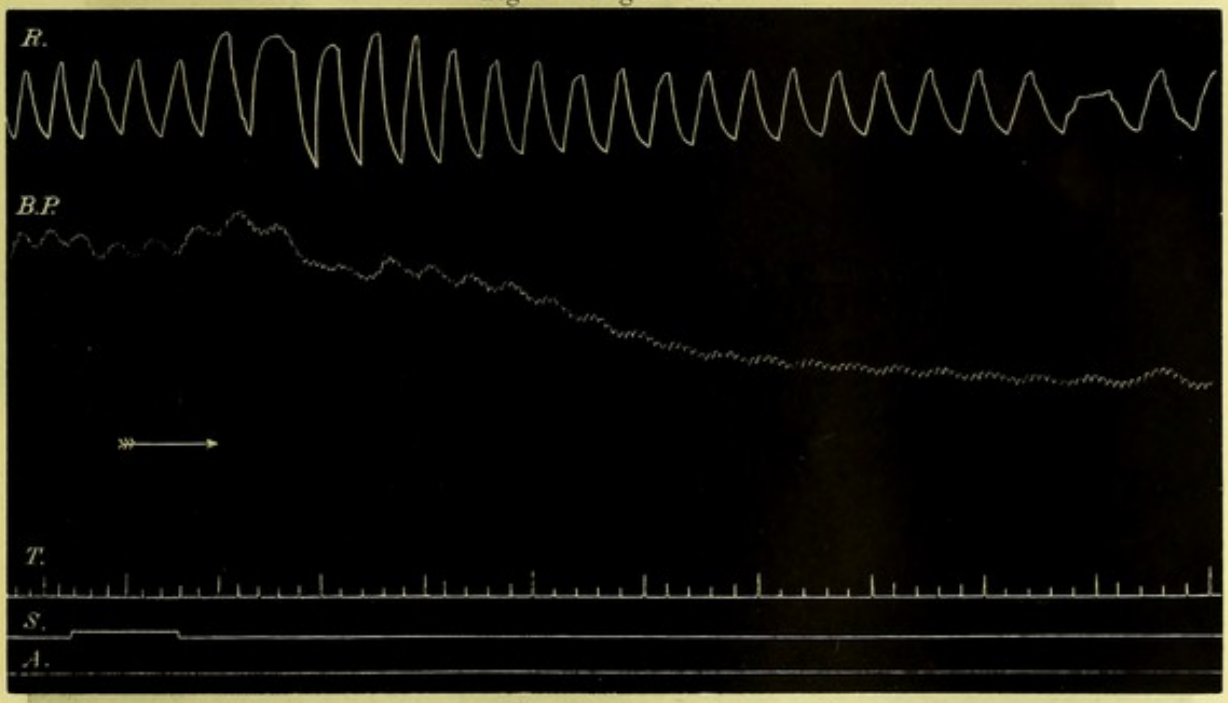


Same animal as in fig. 5. 1 cub. centim. only of the solution was injected; the effect on respiration is not so marked; the rise of blood-pressure is the most important effect of small doses.

Fig. 6 shows the effect of injecting 1 cub. centim. of the same solution, in which the main effect is a rise of blood-pressure. The respiratory increase was not so marked as usual, the animal having received several injections previously.

Fig. 7 shows the effect of injecting 5 cub. centims. of the same solution in another animal. The secondary rise is absent; there is only a fall of blood-pressure; the respirations are at first increased, and then return to normal.

Fig. 7. Original size.



Effect of injecting 5 cub. centims. of a 0·1 per cent. solution of neurine in a dog. The effect on respiration is well seen. The effect on blood-pressure is only a fall. This does occasionally occur in some animals, especially with large doses.

These experiments with neurine indicate that the rise of blood-pressure in the arteries is not merely secondary to the respiratory change as CERVELLO considered. No doubt the increased respiratory activity will assist in causing the rise of pressure, but we shall later see that it is constriction of peripheral arterioles which is the main factor in producing that rise.

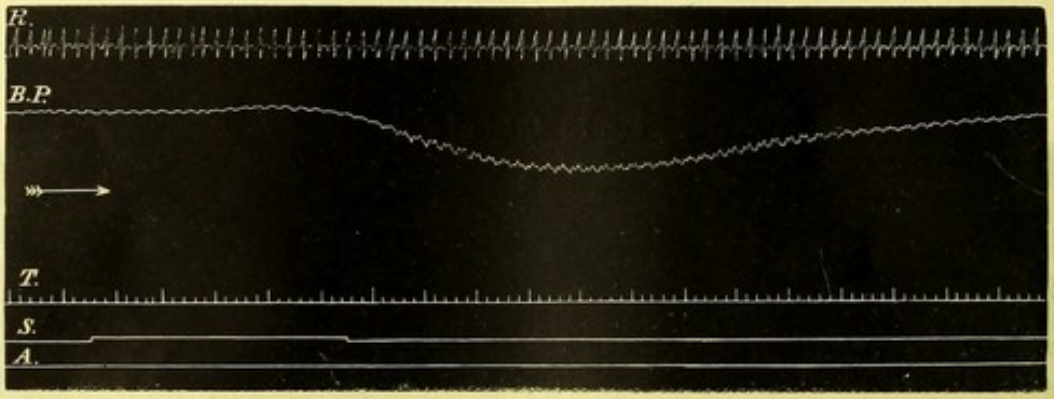
4. EXPERIMENTS FOR THE PURPOSE OF DETERMINING THE SUBSTANCE IN CEREBRO-SPINAL FLUID WHICH PRODUCES THE FALL OF BLOOD-PRESSURE.

We at first thought it was the proteid material in the fluid which caused the fall of arterial pressure. But we found that removal of the proteid by heat, or by alcohol, makes little or no difference in the result observed.

This is illustrated by the two next tracings. Fig. 8 shows the effect of injecting

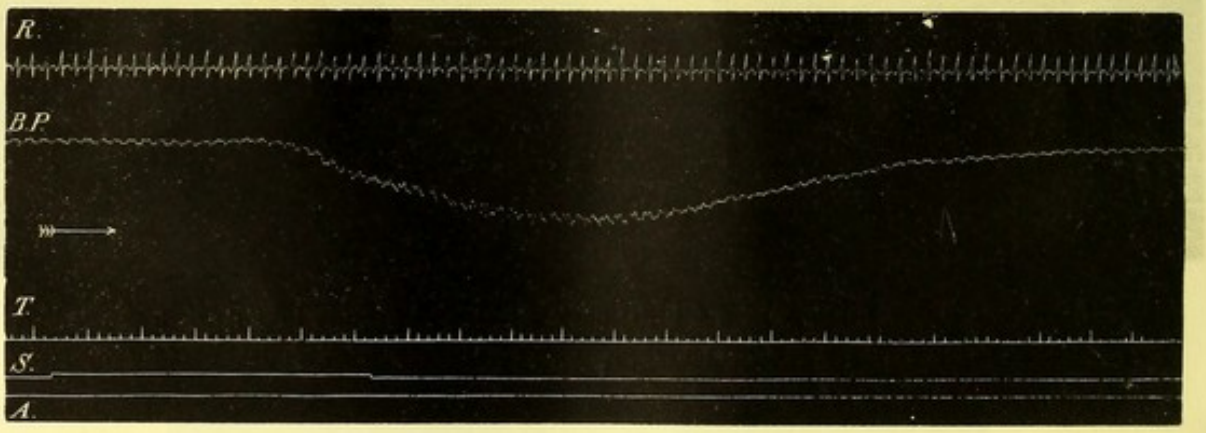
10 cub. centims. of cerebro-spinal fluid; fig. 9 shows the effect of the same amount of the fluid after removal of the proteids by boiling and filtering

Fig. 8. Half the original size.



Effect of injecting 10 cub. centims. of cerebro-spinal fluid in a dog. The fluid was removed from a patient who died from General Paralysis of the Insane.

Fig. 9. Half the original size.



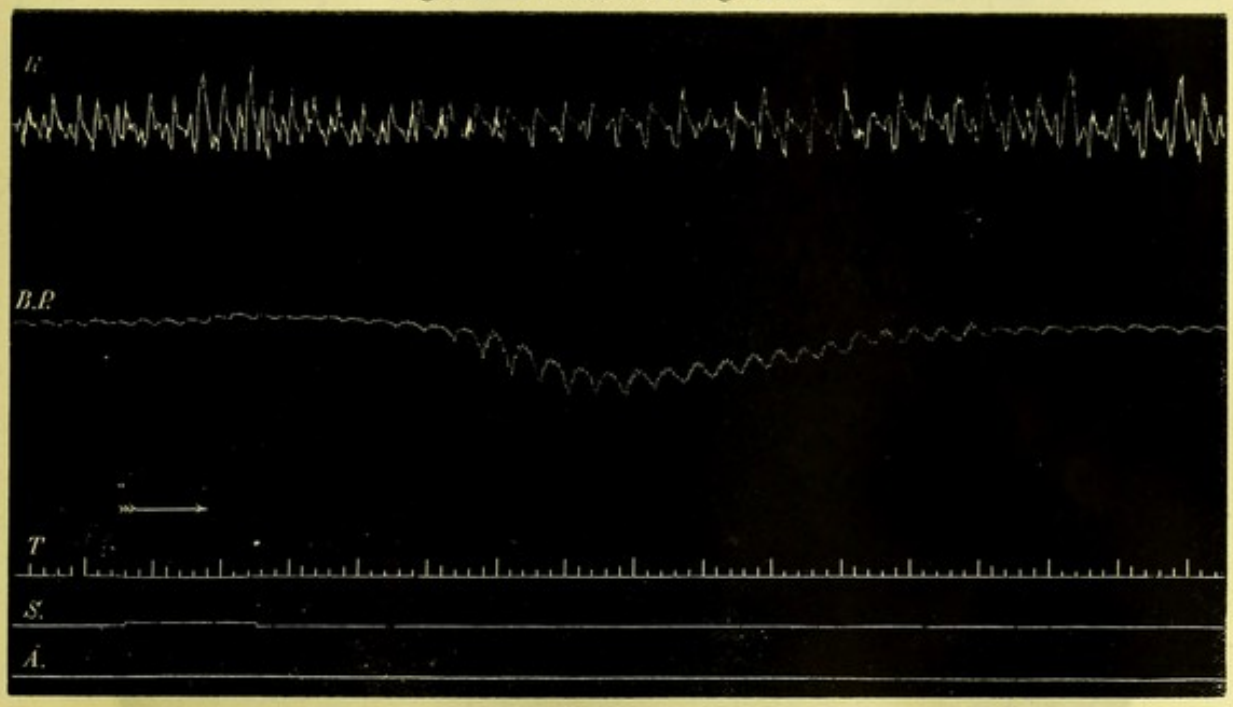
Effect of injecting 10 cub. centims. of the same fluid, after the proteids had been removed by boiling and filtering. Same animal.

Our next supposition was that it might be the inorganic salts which are responsible for the effect observed. We therefore took a large quantity of the fluid, evaporated it to dryness, and incinerated the residue. The ash was dissolved in physiological saline solution and injected without result.

The active physiological substance is therefore of organic nature, but is not proteid.

We then took the alcoholic extract of the fluid, evaporated off the alcohol at 40° C., took up the solid residue with absolute alcohol, filtered it, and again evaporated off the alcohol at 40° C. The residue was taken up with physiological saline solution and injected; the next figure (fig. 10) shows the result. A fall of blood-pressure is produced like that caused by the original fluid.

Fig. 10. Two-thirds the original size.



The cerebro-spinal fluid was freed from proteids by the use of alcohol; the alcoholic filtrate was evaporated to dryness, and the residue dissolved in physiological saline solution. A quantity of this, equivalent to 10 cub. centims. of the original fluid, produces the effect shown in the figure, fall of blood-pressure. Dog.

The active substance is therefore of organic nature, and one which is soluble both in absolute alcohol and in physiological saline solution.

It then occurred to us that the substance might be alkaloidal in nature. We accordingly took a solution of it in physiological saline solution prepared as just described, and added to it phospho-tungstic acid in the presence of sulphuric acid until no more precipitate occurred; the precipitate was an abundant one, and we filtered it off, preserving both precipitate (a) and filtrate (b).

The precipitate (a) was treated in the following way in order to separate out any base that might be present. It was suspended in hot water, and excess of boiling saturated barium hydroxide added. A stream of carbonic anhydride was passed through it until most of the excess of barium was precipitated as carbonate; this was filtered off. To the filtrate dilute sulphuric acid was added carefully to precipitate the rest of the barium; this was filtered off, and if the reaction of the filtrate was acid, the small excess of sulphuric acid was carefully neutralised with dilute sodium hydroxide; this fluid would contain the alkaloid together with a minute amount of sodium sulphate. Such a small amount of sodium sulphate we determined produces no effect on blood-pressure.

The filtrate (b) was treated in a corresponding way; excess of the acid was precipitated by baryta water and filtered off. Excess of barium was carefully precipitated

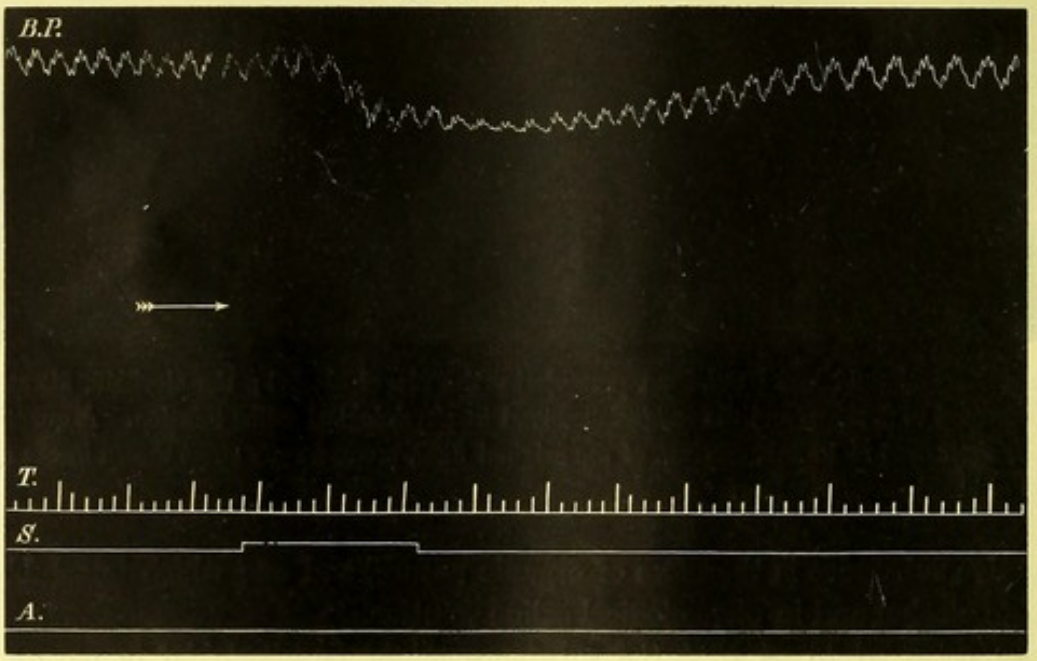
with dilute sulphuric acid and filtered off. Excess of sulphuric acid was then carefully neutralised by soda.

The final liquid in each case was then injected.

The liquid from the filtrate (*b*) produced no fall of arterial-pressure.

The solution of the alkaloid separated from the precipate (*a*) produced a distinct fall of blood-pressure (fig. 11). The effect is not so great as that shown in previous tracings, for doubtless in the chemical operations just described there is some loss and destruction of the alkaloid.

Fig. 11. Original size.



A solution of the base separated from the phospho-tungstic precipitate, as described in the text, was injected. It produces a fall of blood-pressure. Dog.

Having in this way confirmed our suspicion that the active material in these pathological cerebro-spinal fluids is basic, we naturally next thought of the bases which would most likely be present, and accordingly we tested the result by the injection of weak solutions of choline and neurine. The result of these experiments was to convince us that choline is the material of which we were in search. Neurine is much more toxic, and the most marked action it has on blood-pressure is the rise that it produces. Whereas choline produces a fall exactly like that caused by the cerebro-spinal fluid, or the basic substance separated from the fluid.

How exact is the similarity became more evident as our work proceeded.

Having found that the active principle in the cerebro-spinal fluid is soluble in alcohol, our work was rendered considerably easier; for now it was not necessary to inject the fluid as soon as possible after removal from the corpses, but the fluid, when collected, was immediately mixed with alcohol, and a solution in physiological saline

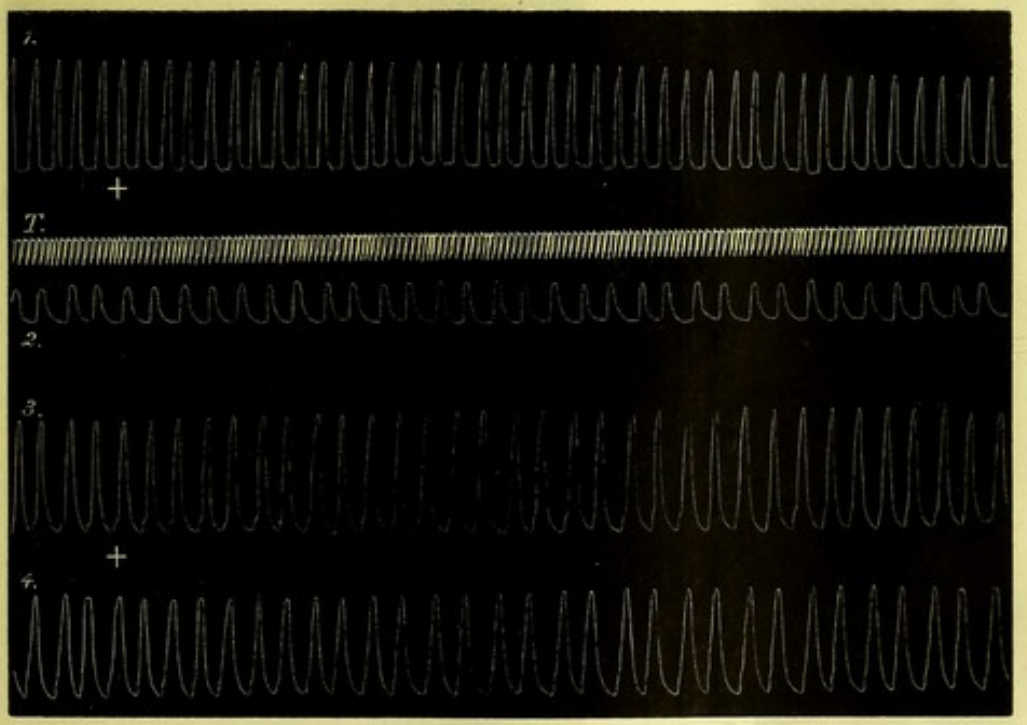
of the residue of the alcoholic extract could be prepared at our leisure, and injected whenever an experiment was convenient. In the majority of the experiments still to be described it was fluids prepared in this way which were injected.

In order to determine the cause of the fall of arterial pressure, it was necessary to make experiments both on the heart, and by the use of plethysmographs or oncometers on the peripheral organs. It will be convenient to take our work on the heart first.

5. Action of Choline, Neurine, and Cerebro-spinal Fluid on the Heart.

We first did experiments on the frog's heart. The animal was pithed, the body cavity freely opened, and the heart's apex was tied by a thread to the short arm of a

Fig. 12. Original size.

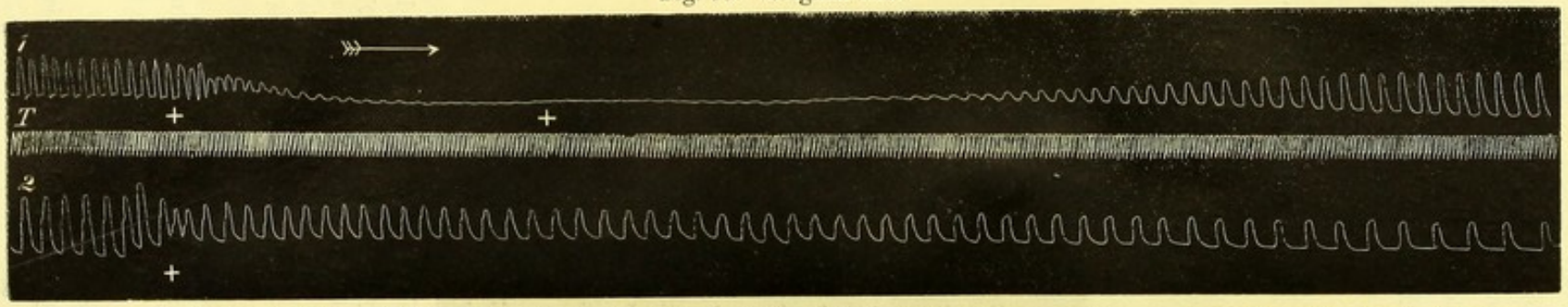


Tracing of frog's heart taken as described in text. Choline hydrochloride (2 per. cent.) was dropped on the heart at the point + in line 1; the contractions gradually became smaller, until they reached the size shown in line 2. Atropine was applied, and the heart recovered as seen at the commencement of line 3; at + line 3, choline hydrochloride was again applied, but the heart is but little affected; see remainder of line 3 and line 4. Time (T.) in half seconds. Read from left to right.

lever placed above it; the long arm of the lever was provided with a writing point, and the tracings were taken on a slowly revolving drum.* The three substances in 2 per cent. solutions were then dropped upon the heart.

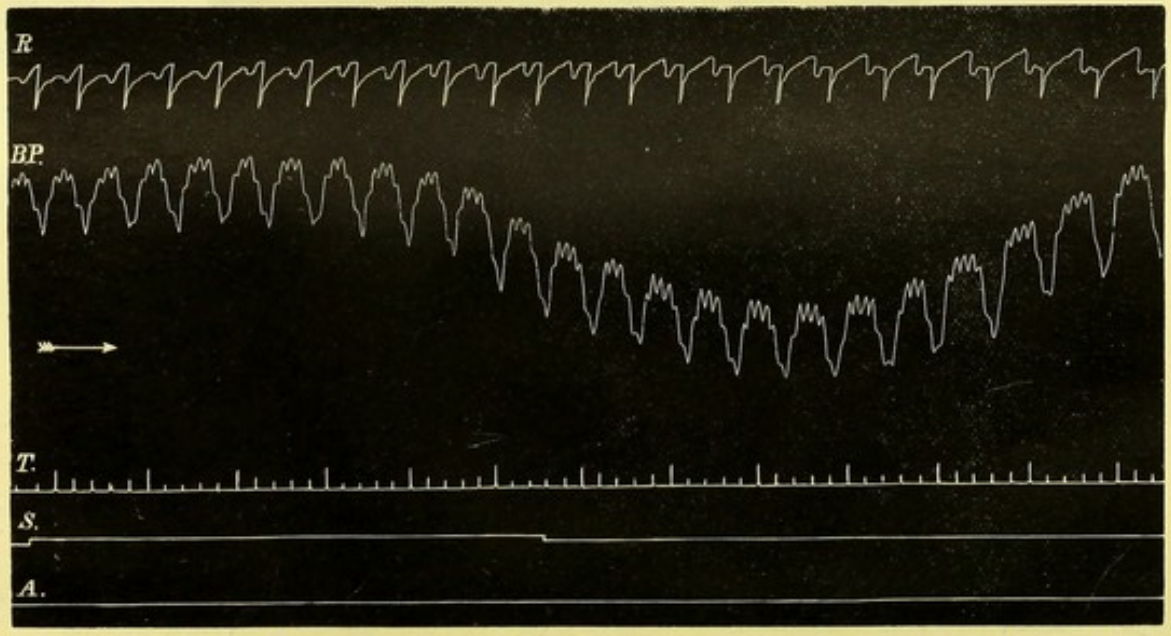
* This simple frog-cardiograph is figured by one of us (W. D. H.) in 'Kirkes' Physiology,' 15th Edition, fig. 229, p. 231.

Fig. 13. Original size.



Tracing of frog's heart. Time (T.) in half seconds. In line 1, at the first + neurine (2 per cent.) was dropped on the heart; this soon brought it to a standstill; at the second + atropine was applied, and the heart soon recovered. At + in line 2, a second dose of neurine was applied, with comparatively little result.

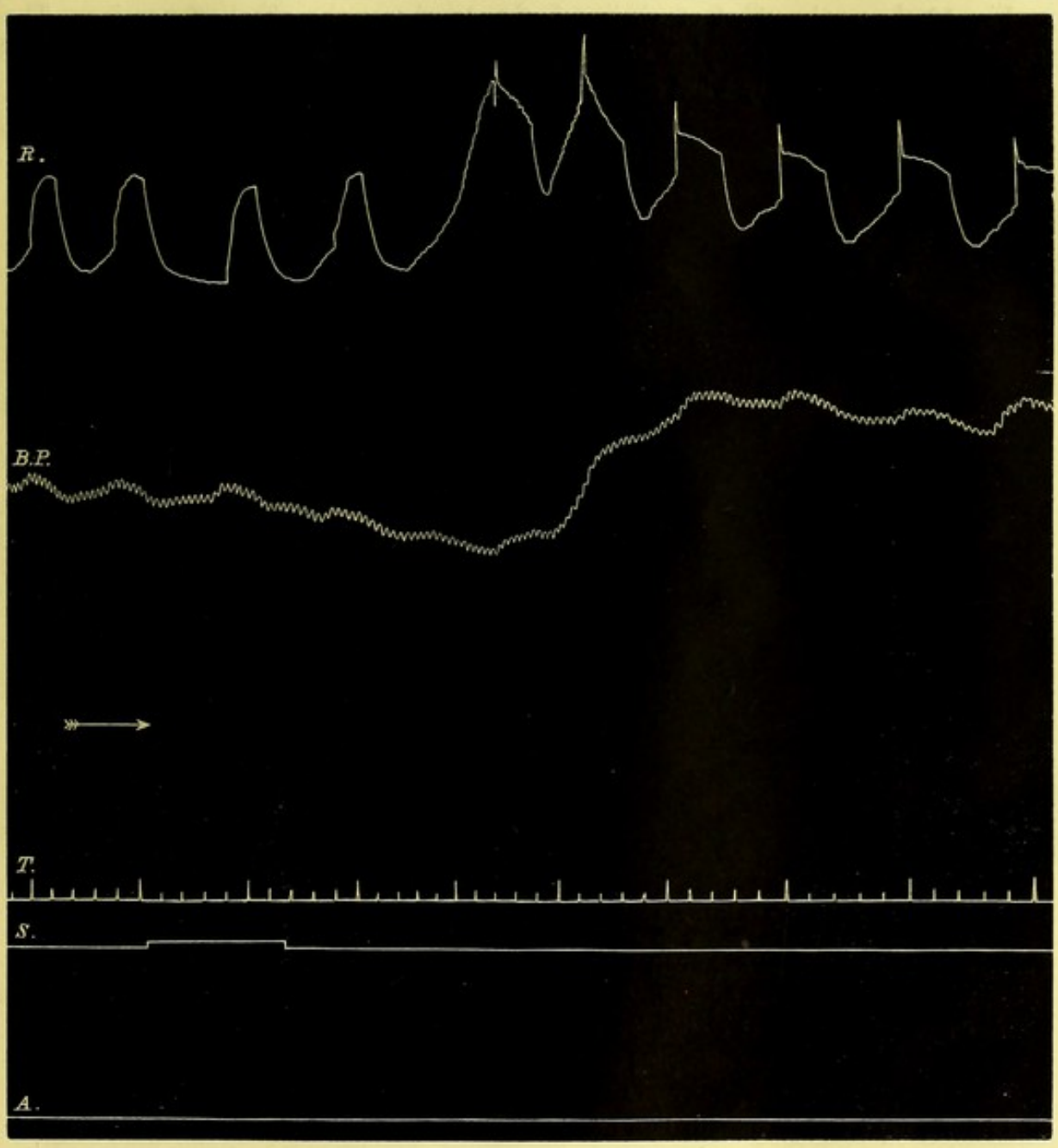
Fig. 14. Three-quarters the original size.



Dog, vagi cut. Effect of injecting the pathological cerebro-spinal fluid (10 cub. centims.) gives the usual fall of blood-pressure; no effect on respiration.

Choline hydrochloride and the cerebro-spinal substance both produce a slight diminution of the heart's force. This is antagonised by atropine.

Fig. 15. Original size.



Cat, vagi cut. 2·5 cub. centims. of a 0·1 per cent. solution of neurine injected; the usual result occurs, first a fall, then a rise of blood-pressure. There is also increase of the respiratory movements.

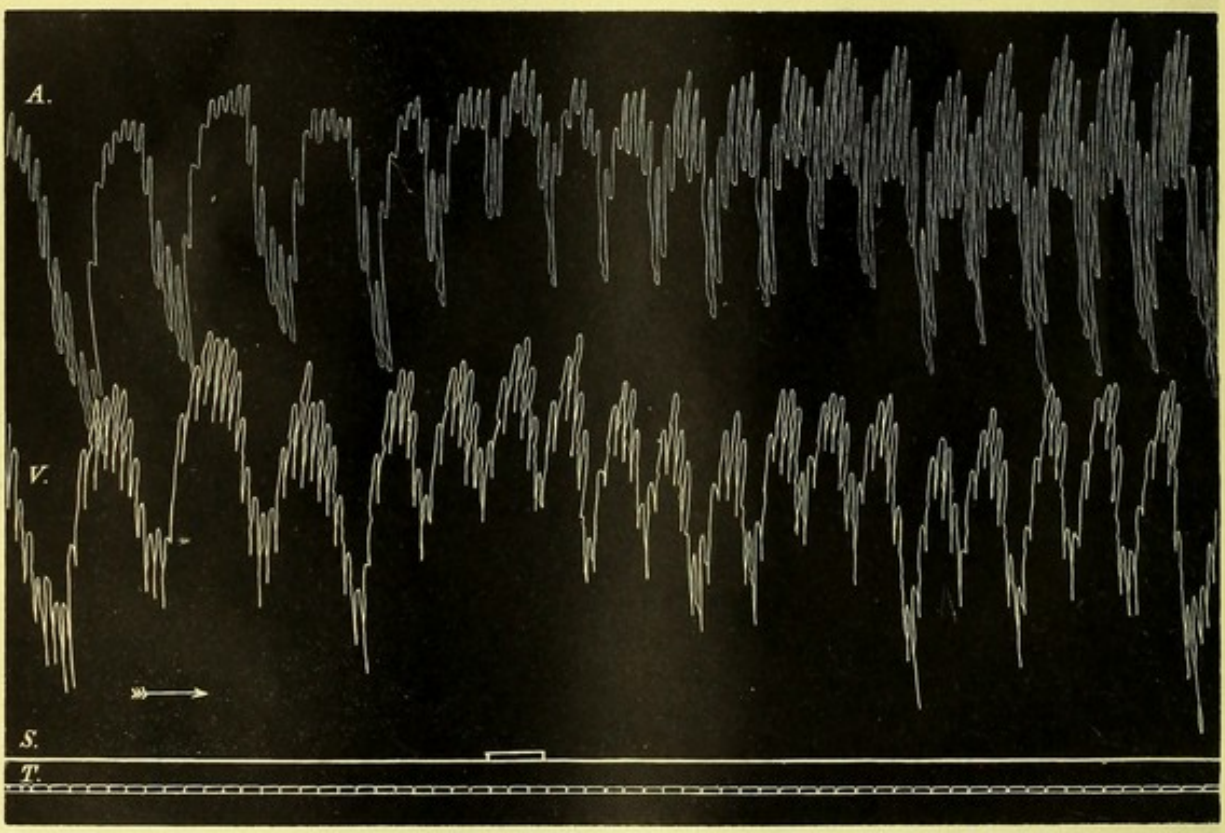
The action of neurine is more powerful, and the effect may be complete stoppage of the heart. This effect is also antagonised by atropine.

The antagonism between these drugs and atropine is not absolutely unilateral, as previously stated by CERVELLO; after the heart is atropinised the other drugs still produce a little effect.

The accompanying tracings illustrate the action just described.

Fig. 12 shows the effect of choline;* fig. 13 shows the effect of neurine. The antagonistic effect of atropine is also shown. The tracing showing the effect of neurine is almost exactly what one obtains on the frog's heart with muscarine.

Fig. 16. Original size.



Tracing of a dog's heart, taken by BRODIE'S levers. A., tracing from right auricle; V., from right ventricle; S., signal showing time of injection; T., time in seconds. At the rise of the signal, a quantity of the cerebro-spinal substance corresponding to 10 cub. centims. of the original cerebro-spinal fluid was injected. The effect is similar to that produced by 5 cub. centims. of a 0·2 per cent. solution of choline, but not quite so marked; the force and rate of the heart is lessened. This and the succeeding tracing were taken by Dr. BRODIE.

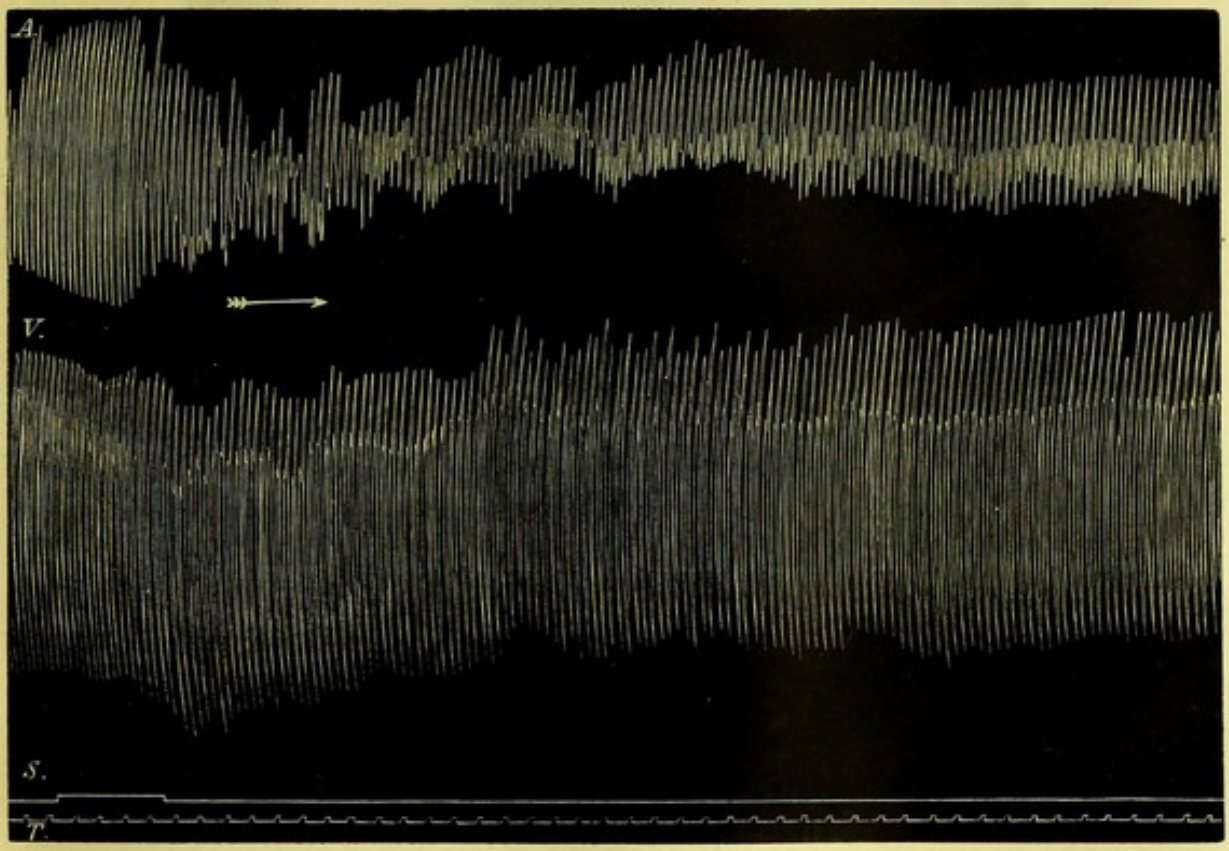
Coming next to the mammalian heart, we found on examining our tracings of blood-pressure, that the effect of choline and cerebro-spinal substance on the rate of the beats is to produce slowing; this is usually slight, the effect of neurine is more marked; the slowing in this case is often followed by acceleration.

* The effect of the cerebro-spinal substance is precisely similar; in order to reduce the number of figures, it has been deemed unnecessary here and later to give more than one of each pair of tracings.

Section of both vagi makes no difference in these experimental results. The accompanying tracings give the results after section of both vagi. Fig. 14 shows the effect of the cerebro-spinal fluid, and fig. 15 of neurine. The effect of choline is exactly like that of cerebro-spinal fluid.

In order to obtain tracings of the heart itself, the thoracic cavity was opened, and the animal kept alive by artificial respiration. The right auricle and ventricle were

Fig. 17. Original size.



Tracing of dog's heart taken in a similar way to the foregoing. 2 cub. centims. of a 0·1 per cent. solution of neurine were injected. The force of the heart, especially of the auricle, is lessened. There is also considerable slowing.

attached, by hooks, to springs like those used by SCHÄFER and OLIVER.* In other experiments we used levers, as described and figured by BRODIE.† In these experiments we have to acknowledge the assistance given us by Dr. BRODIE. In other experiments again we used the simple air cardiometer of BARNARD,‡ connected either to a MAREY's recording tambour, or to a HÜRTHLE's piston recorder.

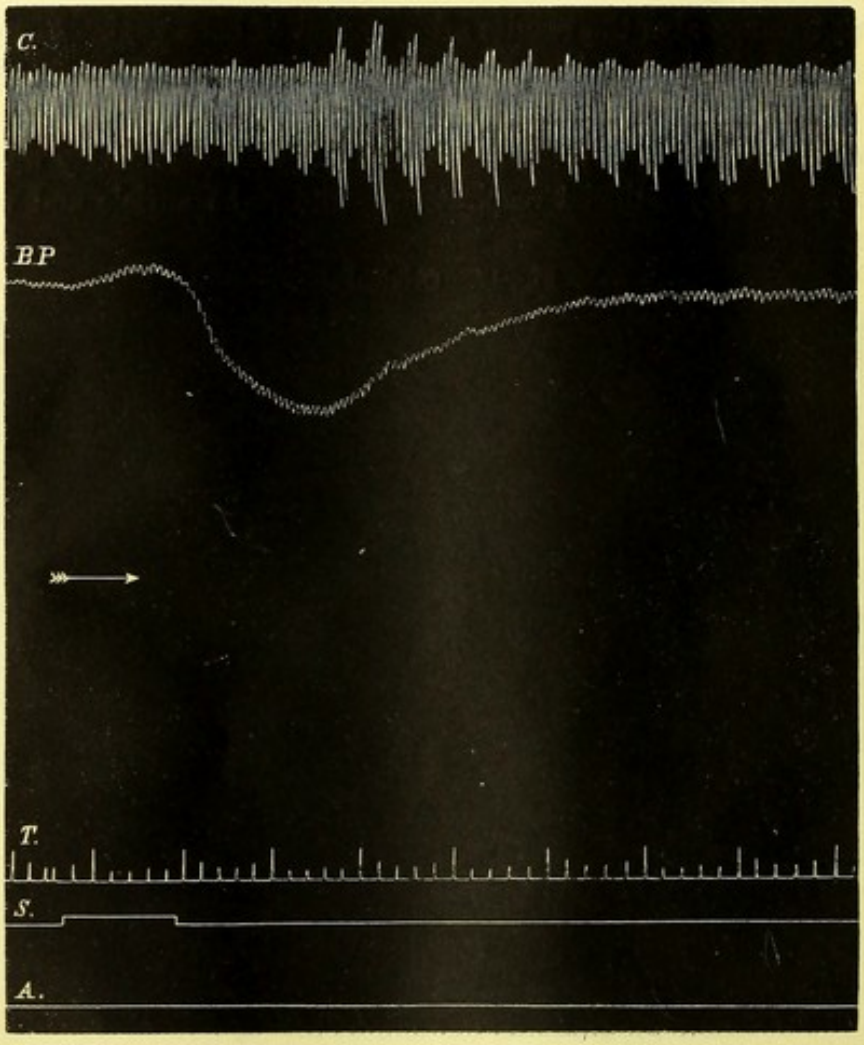
We give some illustrative tracings of the effects obtained.

* 'Journal of Physiology,' vol. 18, p. 256, fig. 13 (1895).

† 'Essentials of Experimental Physiology,' p. 140, fig. 110. (1898.)

‡ 'Proc. Physiol. Soc.,' Dec. 11, 1897, p. xv. ('Journal of Physiology,' vol. 22.)

Fig. 18. Original size.



Injection of choline hydrochloride (5 cub. centims. of a 0·2 per cent. solution) in a cat. The uppermost line, C, is the tracing of the cardiometer of BARNARD; each down stroke represents systole. The fall of arterial pressure is accompanied by an increased output of the heart; the heart's rate is also rather slower. The cerebro-spinal substance produces exactly the same result.

These tracings show :

1. With choline and cerebro-spinal substance a slowing (fig. 16) and diminution of force in the heart's action.

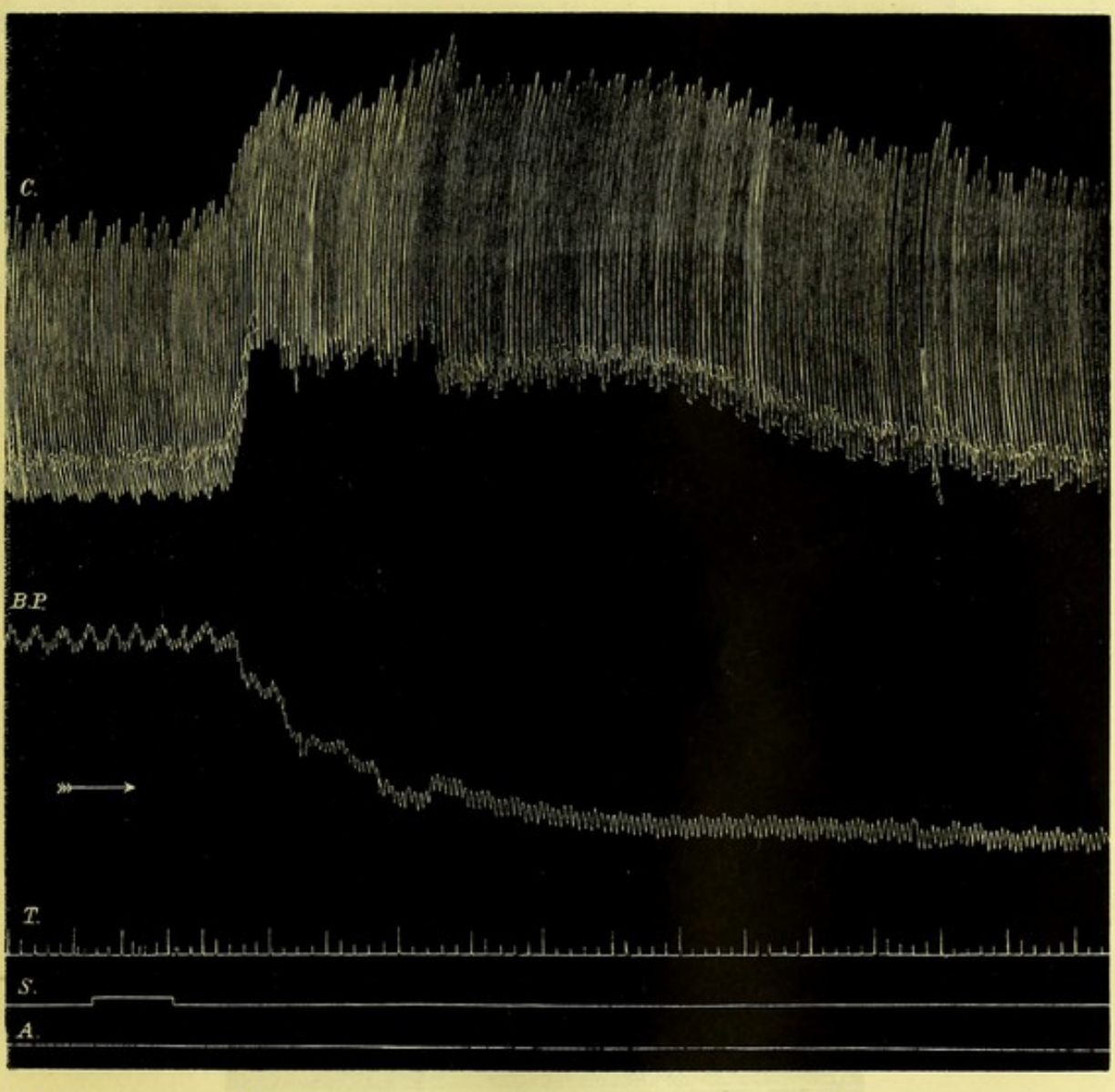
2. With neurine the effect is more marked, especially in the auricle (fig. 17).

3. In spite of this, in case of choline and cerebro-spinal substance the output of the heart is increased with each beat (fig. 18).

4. In case of neurine the output of the heart is lessened, and its tone diminished (fig. 19).

We thus learnt, that in the case of choline, the fall of blood-pressure is partly of cardiac origin, especially at the commencement of the fall; the increased output of the heart points also to a lessening of the peripheral resistance. We then proceeded by the plethysmographic method to investigate this side of the subject.

Fig. 19. Original size.



Effect of injecting 2·5 cub. centims. of a 1 per cent. solution of neurine in a cat. In this case the usual rise of blood-pressure, which occurs after the initial fall, did not take place. The tracing from the cardiometer shows a decrease in the heart's force, and subsequently in the heart's rate; the rise of the level of the tracing indicates that each systole is incomplete, and that the heart is dilated.

6. Experiments with Oncometers and Plethysmographs.

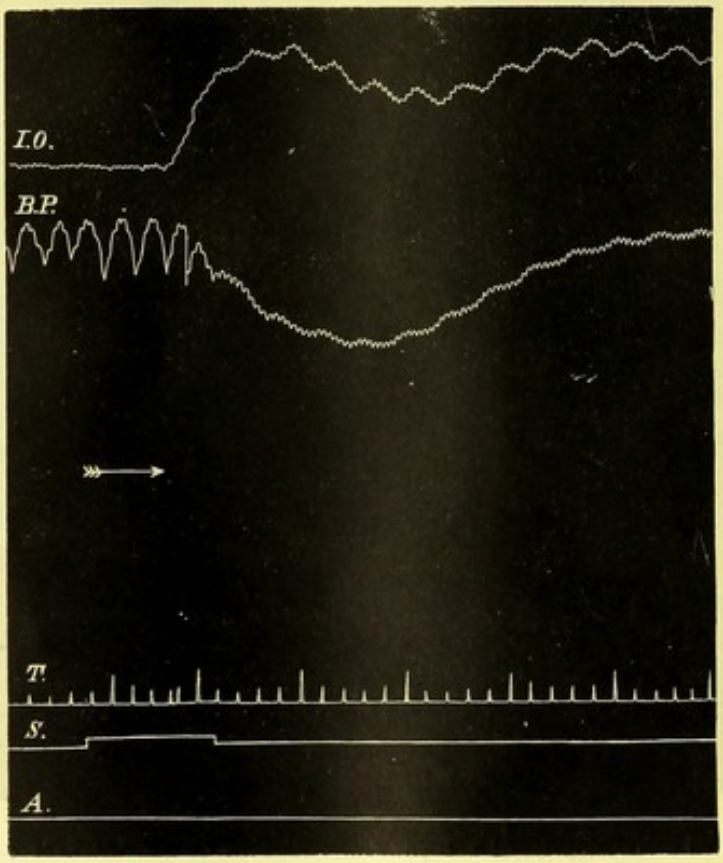
Throughout these experiments we used air oncometers; the box, in which the organ or limb investigated was enclosed, was made air-tight with vaseline and vaseline wool, and connected to the recording apparatus (a Marey's tambour) by thick india-rubber tubing. Schäfer was the first to introduce the use of an air oncometer in

connection with the spleen;* we have extended the method to the kidney and intestines;† we can thoroughly confirm SCHÄFER's contention that the air instrument gives better results, and can be more easily used than the oncometers of ROY, in which oil was used as the transmitting medium.

The accompanying tracings will show the results we have obtained.

(a) *On the Intestines.*—The fall of blood-pressure which accompanies the injection of choline (fig. 20) and of cerebro-spinal substance, is accompanied with a rise of the oncometer lever (I.O.), which indicates an expansion of the blood vessels in this region.

Fig. 20. Original size.



Effect of injecting 5 cub. centims. of a solution of choline hydrochloride (0·2 per cent.). The fall of blood-pressure is accompanied by a dilatation of the intestinal vessels; this is shown by the rise of the lever of the Marey's tambour connected with the intestinal plethysmograph (I.O.). Dog.

In some animals the oncometer lever does not rise synchronously with the fall of blood-pressure; but at the very commencement of the arterial fall the oncometer lever falls also; it, however, soon begins to rise. The preliminary fall of the oncometer lever

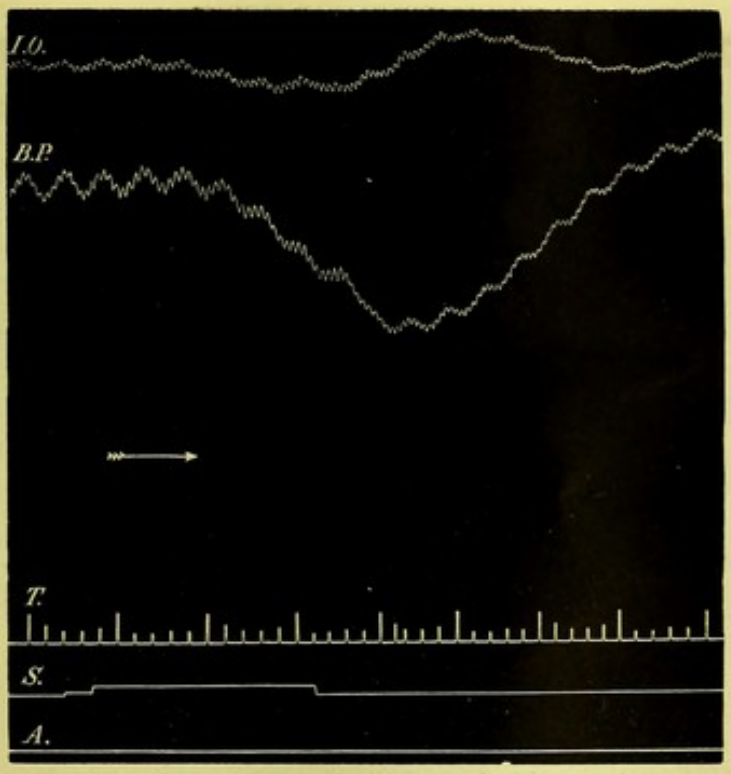
* SCHÄFER and MOORE, 'Journal of Physiology,' vol. 20, p. 1, 1896.

† The intestinal oncometer we used was devised by Mr. A. EDMUNDS, who has described the instrument and the method of using it in the 'Journal of Physiology,' vol. 22, p. 380, 1897–8.

indicates that the fall of blood-pressure is partly of cardiac origin, and the peripheral vessels follow the course of general blood-pressure passively.

In those animals in which choline produces this effect, the cerebro-spinal substance acts in exactly the same way (fig. 21).

Fig. 21. Original size.



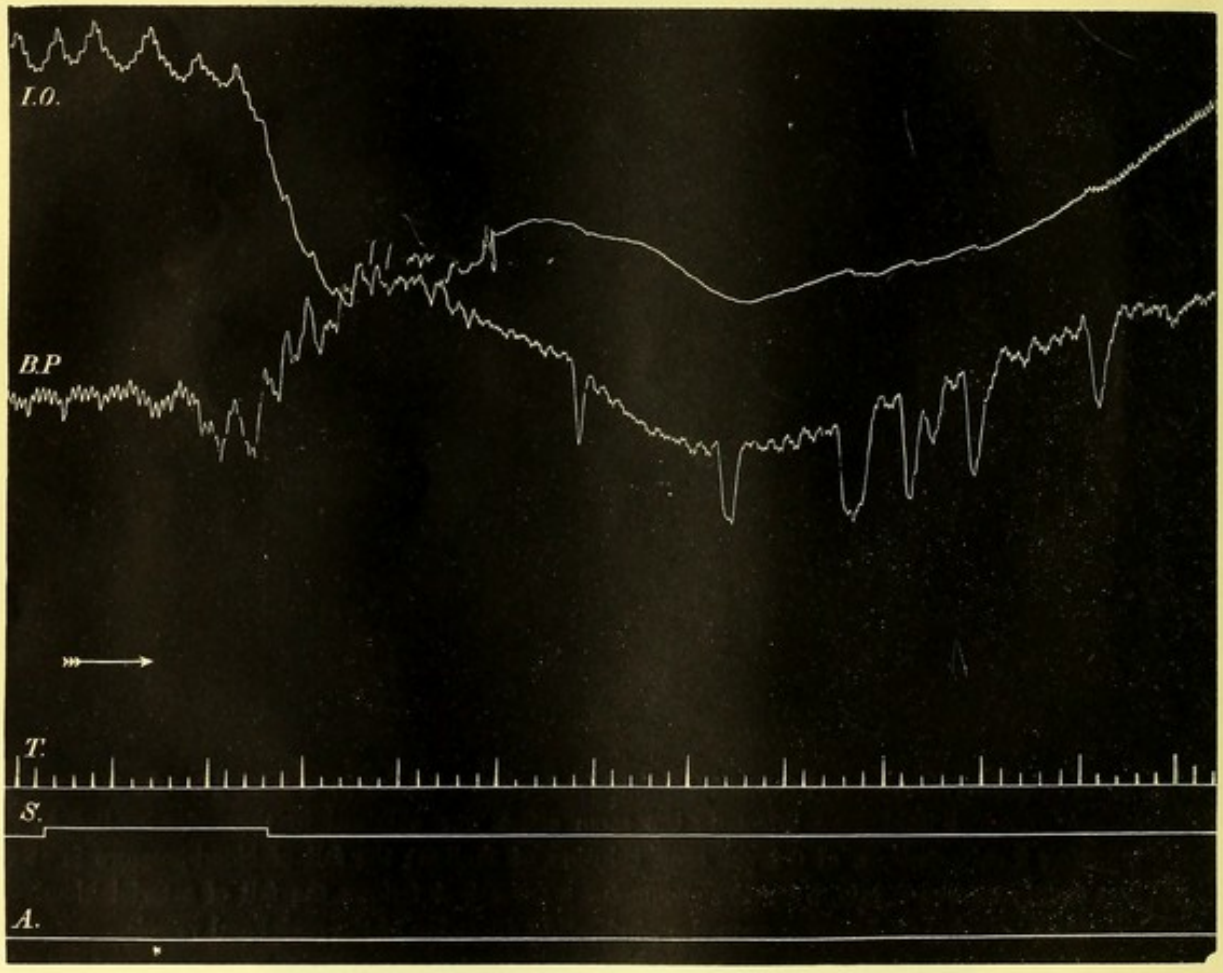
Tracing of intestinal oncometer (I.O.) and arterial blood-pressure (B.P.) in a cat. 10 cub. centims. of cerebro-spinal fluid were injected; the same effect was obtained in the same animal by injecting 2 cub. centims. of 0·2 per cent. solution of choline; the fall of blood-pressure is at first mainly cardiac in origin, for the oncometer tracing first follows the fall of arterial blood-pressure passively; it, however, soon rises, indicating dilatation of the peripheral vessels.

The effect of neurine is very different; the blood-pressure falls, and then, as a rule, rises. The oncometer lever falls with the fall of blood-pressure, but continues to fall and remains down as the blood-pressure rises. This shows the cause of the rise of blood-pressure to be constriction of the peripheral blood vessels (fig. 22). In some cases the preliminary fall of blood-pressure is absent, and the rise of blood-pressure is accompanied by a well-marked fall of the oncometer lever, due to constriction of the blood vessels (fig. 23).

In our early experiments with choline and cerebro-spinal fluid, we noted *post-mortem* the engorgement of the splanchnic blood vessels. This is confirmed by the more exact graphic method. An advantage which EDMUNDS' air-oncometer for the

intestines possesses is that the intestines and their blood vessels can be watched readily through the glass plate which forms the lid of the instrument, and the rise of the oncometer lever is seen to be accompanied by flushing, and the fall (in the case of neurine) by paling, due to constriction of the arterioles.

Fig. 22. Original size.



Effect of injecting 2·5 cub. centims. of a 0·1 per cent. solution of neurine in a cat. There is first a small preliminary drop of arterial pressure, and the heart is distinctly slowed; in spite of this, however, the pressure rises; later, the heart shows an occasional missed beat. The tracing of the intestinal oncometer shows the great but somewhat irregular constriction of the splanchnic blood vessels.

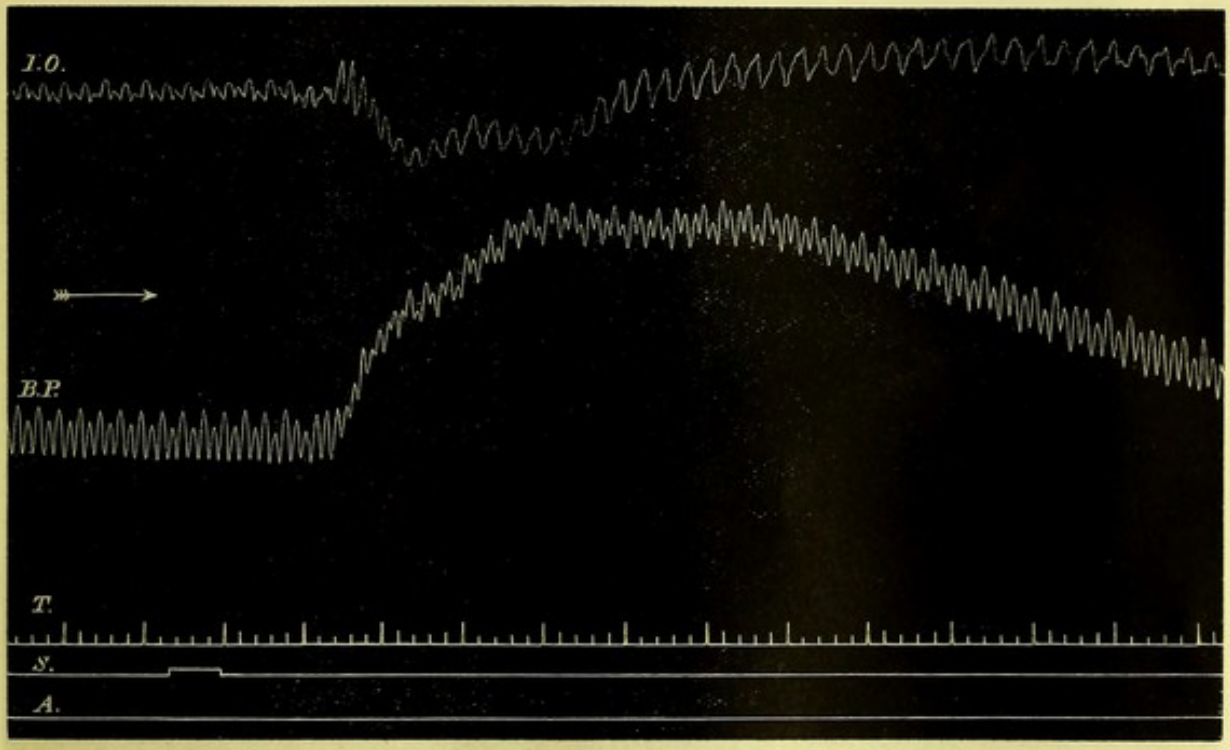
(b) *On the Kidney.*—There is but little evidence of any active dilatation on the part of the kidney vessels under the influence of choline. The lever of the kidney oncometer (K.O.) falls with the blood-pressure, and though there may be a slight rise (fig. 24) before the blood-pressure has resumed its usual level, it is never very marked.

Injection of the cerebro-spinal substance produces the same result. In both cases

the fall of blood-pressure occurs, and the kidney volume follows passively this fall, which is produced, as previously stated, partly by cardiac weakness and partly by dilatation of the vessels in the intestinal area.

In the case of neurine, however, the effect is similar to that found in the intestines;

Fig. 23. Three-quarters of the original size.



Effect of injecting 1 cub. centim. of the same solution in a dog. The preliminary fall of arterial pressure is absent; the rise is accompanied by the constriction of the peripheral blood vessels, as shown by the fall of the oncometer lever.

the rise of blood-pressure is accompanied with great constriction of the renal vessels (fig. 25).

(c) *On the Limbs.*—Here also with choline and cerebro-spinal substance there is no evidence of active dilatation in the peripheral vessels. The plethysmograph lever (L.P.) simply follows passively the general fall of blood-pressure.

Fig. 26 shows the effect of cerebro-spinal substance; the effect of choline is exactly the same.

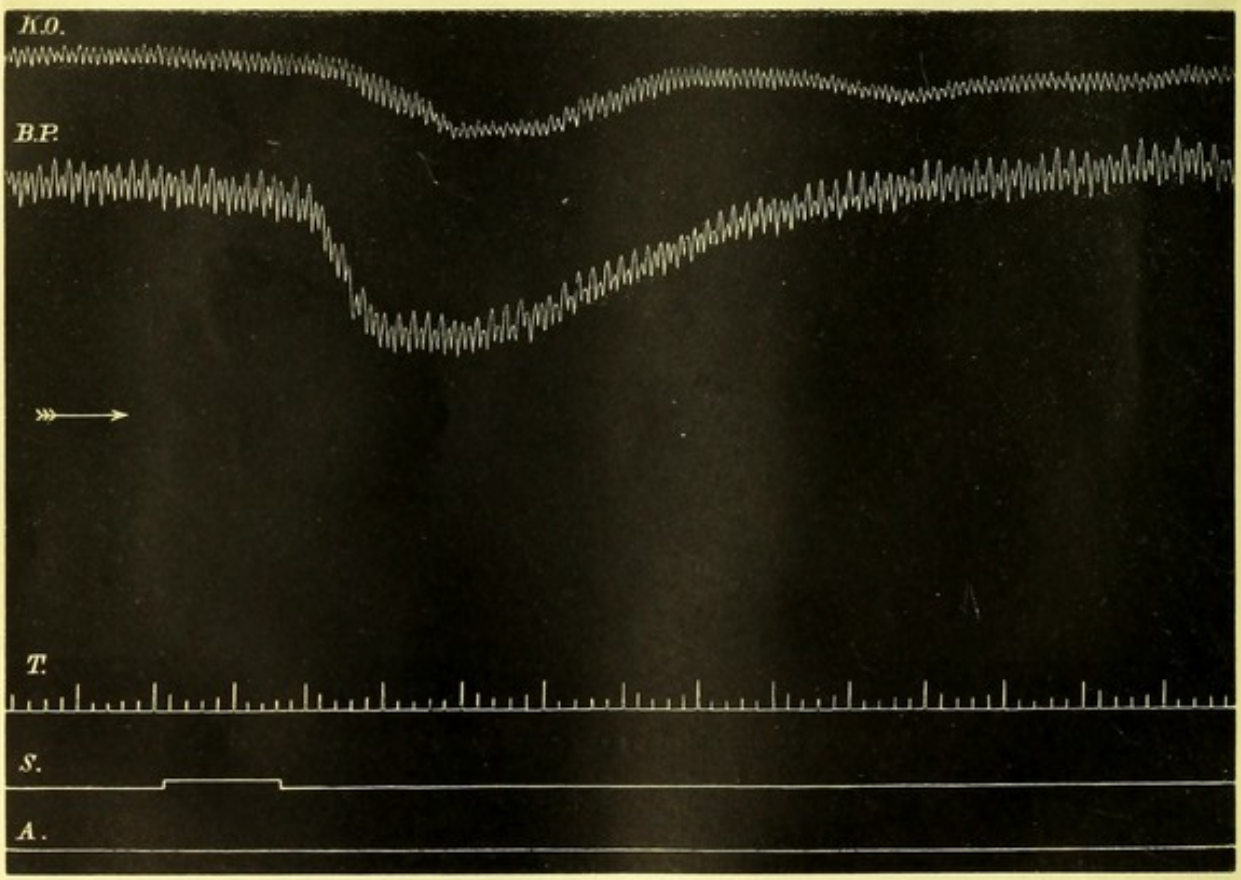
(d) *On the Spleen.*—We have now seen that the fall of blood-pressure which follows the injection of choline and cerebro-spinal substance is partly due to dilatation of the blood vessels in the intestinal section of the splanchnic area. We naturally expected the spleen to show a corresponding dilatation. But this is not the case.

When the blood-pressure falls the lever of the spleen oncometer falls also; the fall

of the oncometer lever is usually so pronounced that it is difficult to suppose it can be merely a passive fall due to such slight cardiac weakening as is produced by the action of choline. We therefore consider that choline produces a specific effect on the spleen; the spleen constricts; and this view is confirmed by what happens a few seconds later.

For then the splenic waves become much more vigorous than they were before.

Fig. 24. Original size.



Fall of blood-pressure produced in a dog by injection of 10 cub. centims. of a 0·2 solution of choline. The tracing at the top (K.O.) is that of the kidney oncometer. The lever of the oncometer first follows the blood-pressure passively; later, there is a slight indication of a dilatation of the kidney vessels, for the lever rises before the blood-pressure resumes its normal level.

It is a striking confirmation of our position that the cerebro-spinal substance is choline, in that the cerebro-spinal substance produces the same specific effect on the spleen.

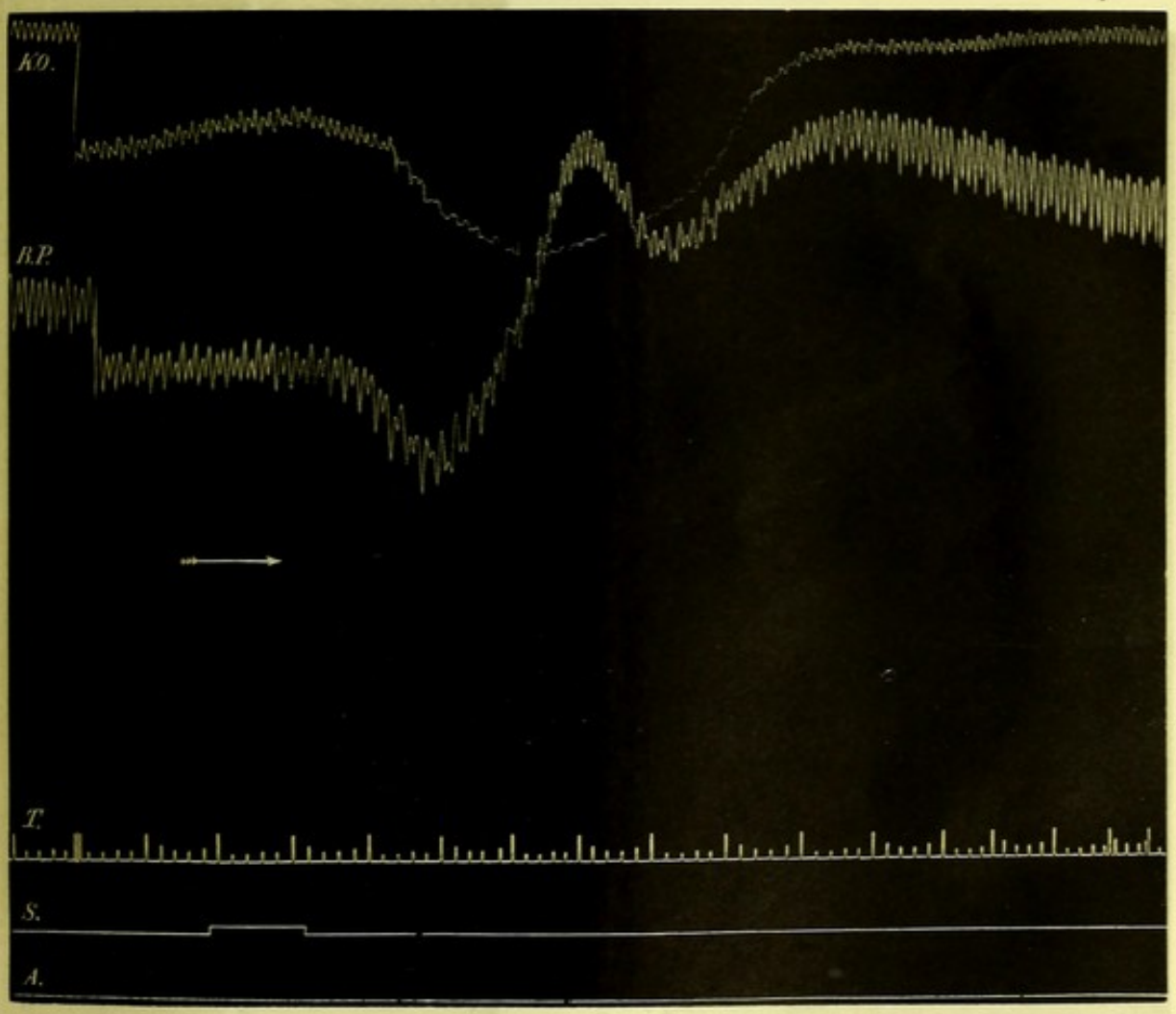
The two next figures illustrate this: fig. 27 shows the result of the injection of choline; fig. 28 of the injection of cerebro-spinal substance.

As previously stated, SCHÄFER and MOORE obtained the same results with extract

of brain; there can be little doubt that the active substance in their extracts was choline.*

Neurine produces a much more intense and lasting constriction of the spleen, as it

Fig. 25. Original size.

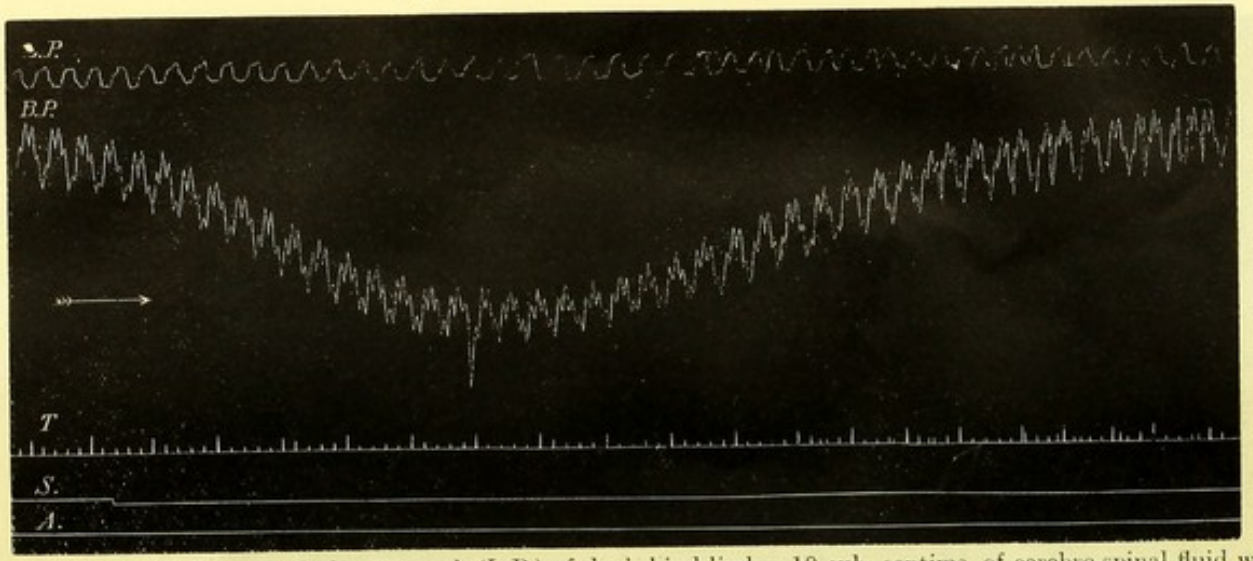


The same animal was used as in the preceding experiment. 2·5 cub. centims. of a 0·1 per cent. solution of neurine were injected. The effect on arterial pressure is that which is usually seen, viz., a fall succeeded by a rise. The oncometer tracing (K.O.) shows great constriction of the kidney vessels.

does with the intestines and kidney. A subsequent increase of the splenic waves does not occur (fig. 29).

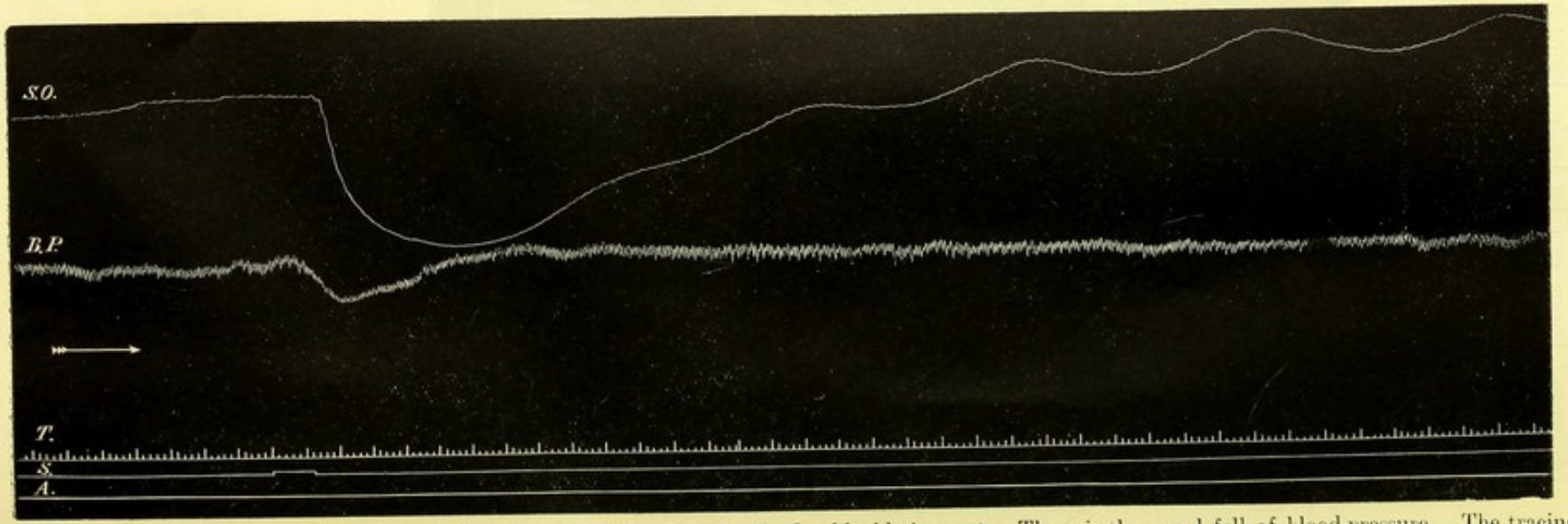
* This hypothesis is fully confirmed by the recent work of GULEWITSCH ('Zeitsch. f. Physiol. Chem.,' 1899, vol. 27, pp. 50–82); he shows that extracts of brain contain choline, but neurine is absent.

Fig. 26. Two-thirds of the original size.



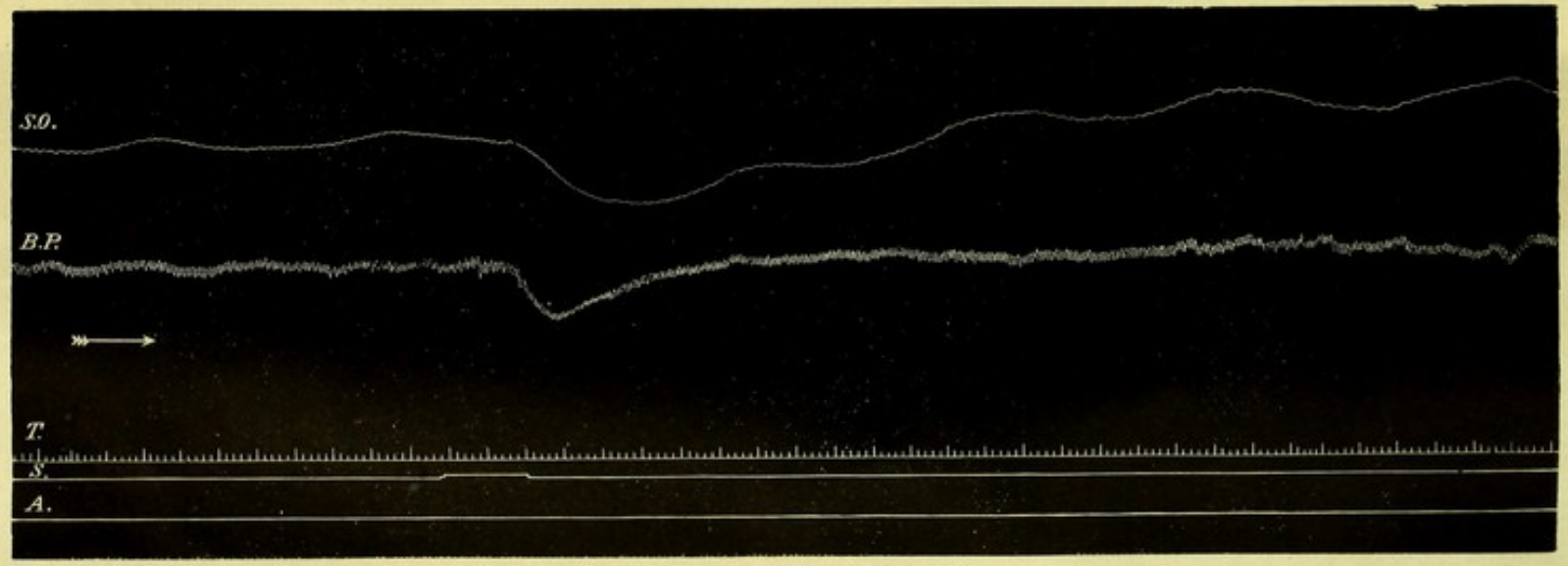
Tracing of carotid blood-pressure (B.P.) and plethysmograph (L.P.) of dog's hind limb. 10 cub. centims. of cerebro-spinal fluid were injected; choline produces the same effect. There is a very slight (passive) reduction of the volume of the leg accompanying the fall of blood-pressure.

Fig. 27. Two-fifths of the original size.



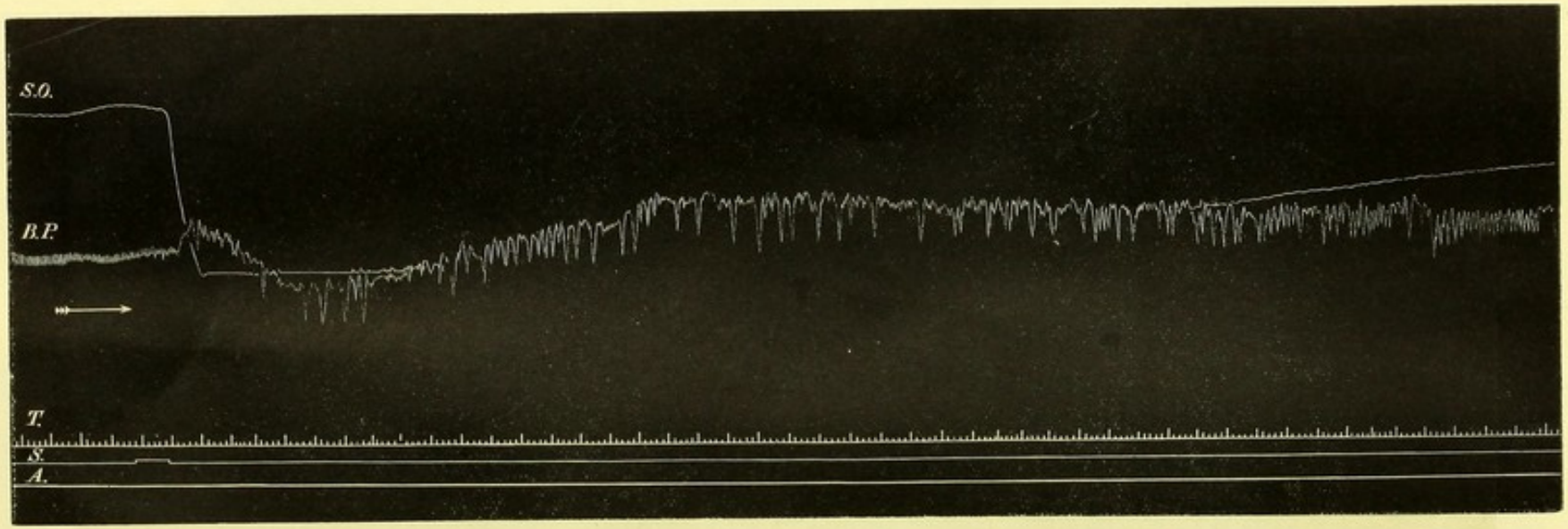
Effect of injecting 2·5 cub. centims. of a 0·2 per cent. solution of choline hydrochloride in a cat. There is the usual fall of blood-pressure. The tracing of the spleen oncometer (S.O.) shows a marked constriction of the spleen, followed by an exaggeration of its normal rhythmic contractions.

Fig. 28. Two-fifths of the original size.



Same cat. Effect of injecting a quantity of cerebro-spinal substance obtained from 10 cub. centims. of cerebro-spinal fluid. The effect is not quite so marked as with choline (see previous tracing); otherwise it is the same.

Fig 29. Two-fifths the original size.



Same cat. Effect of injecting 2·5 cub. centims. of a 0·1 per cent. solution of neurine. The blood-pressure shows the usual fall and rise, with a rather more pronounced effect on the heart than usual. The spleen (S.O.) contracts suddenly and forcibly, so that the lever of the recording tambour rested on the rim of the instrument; the few upward marks seen on the horizontal line so written are due to the writing style of the kymograph jerking against it. The greater part of the horizontal line was not traced. As the spleen recovers, no increase in the normal rhythmic waves are seen. The piece of tracing reproduced in the figure does not show the complete return of the spleen to its previous volume; complete recovery did take place about a minute later, and the tracing was continued for another five minutes; but in this time there was no exaggeration of the splenic waves.

7. Experiments to Ascertain whether the Action of Choline and Neurine on the Blood Vessels is Direct or Indirect.

Our plethysmographic experiments having shown us the effect that the substances we were investigating have on the peripheral vessels, it next became necessary to ascertain whether the drugs act directly on the blood vessels, or indirectly through the nerve centres.

Choline.

We will take choline first; and here, as before, the cerebro-spinal substance has exactly the same action. Our results may be briefly stated as follows: choline produces the fall of blood-pressure by acting directly on the neuro-muscular apparatus of the peripheral vessels; for after the action of the vaso-motor centre and of the central nervous system generally have been eliminated, the injection of the alkaloid still continues to produce the usual fall of blood-pressure.

The methods by which we came to this conclusion are four in number.

1. *The spinal cord was divided* between the first and second thoracic segments in the cat. This is above the situation of the vaso-motor outflow from the cord, and below that of the nerves supplying the diaphragm, so it was not necessary to have recourse to artificial respiration. Stimulation of the central end of the cut sciatic nerve was found to cause reflexly only a very slight rise of blood-pressure, doubtless due to the action of subsidiary vaso-motor centres in the cord.

Injections of choline produce a fall of blood-pressure (fig. 30), so do injections of cerebro-spinal substance.

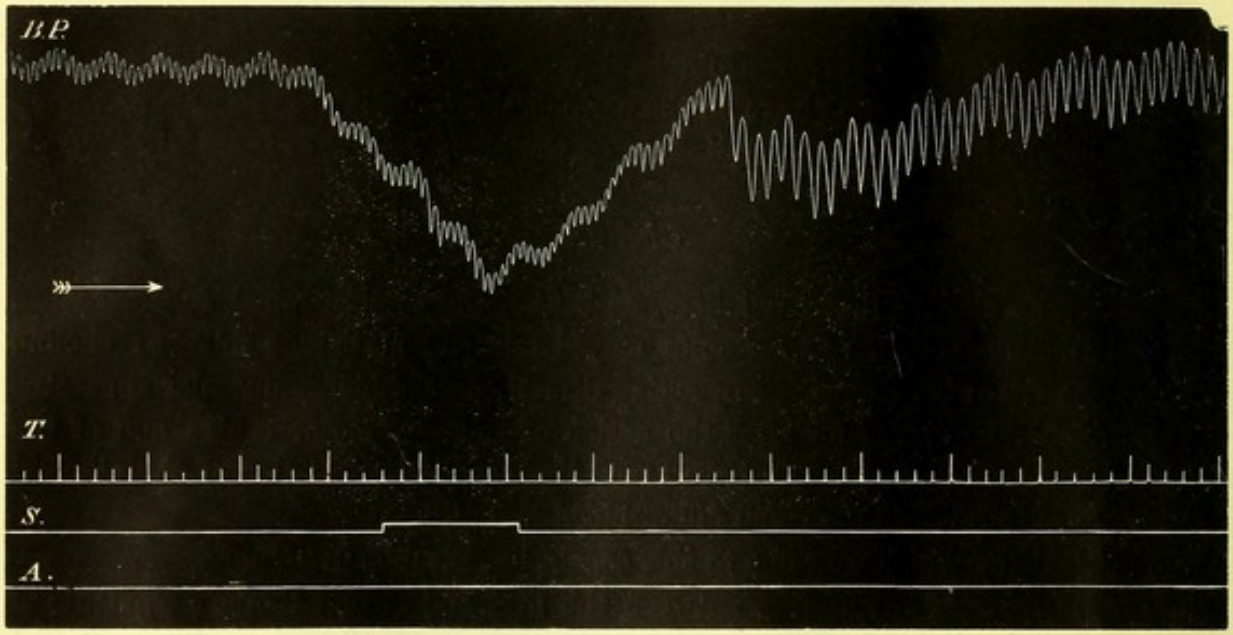
2. *The splanchnic nerves were divided.*—This operation was performed in dogs, and we have to thank Professor W. H. Thompson for assistance in doing some of the operations. In order to be quite certain that we had cut the right nerves we tested the nerves by stimulating them, and noting the rise of arterial pressure which occurs; we also made afterwards a careful *post-mortem* examination so as to see exactly what nerves had been cut.

We are not altogether sure that the operation is completely satisfactory, for even if one cuts through all the strands into which the nerve divides below the diaphragm, it is impossible to be quite sure all vaso-motor nerves to the abdominal area are severed, for branches often originate above the diaphragm which are liable to be missed.

In all cases, however, even after the most complete section of the splanchnic nerves on both sides, injection of choline or of cerebro-spinal substance still produced the usual fall of blood-pressure (fig. 31).

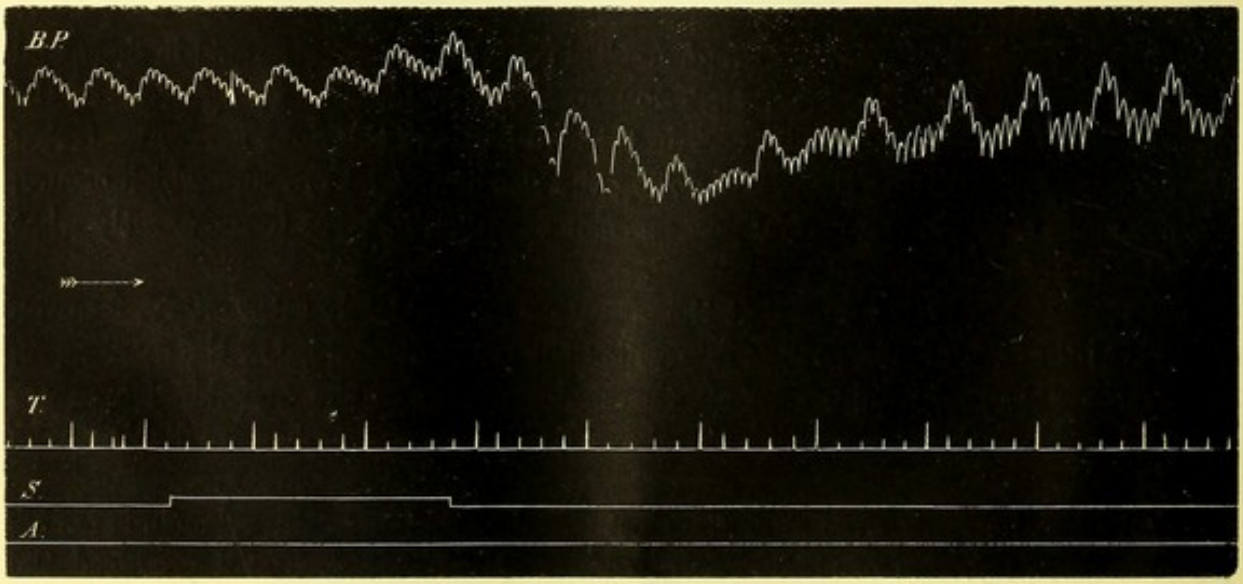
3. *The animal was poisoned with nicotine;* this will cut off the influence of all nerve fibres originating from the central nervous system, as well as the influence of the sympathetic ganglia.

Fig. 30. Original size.



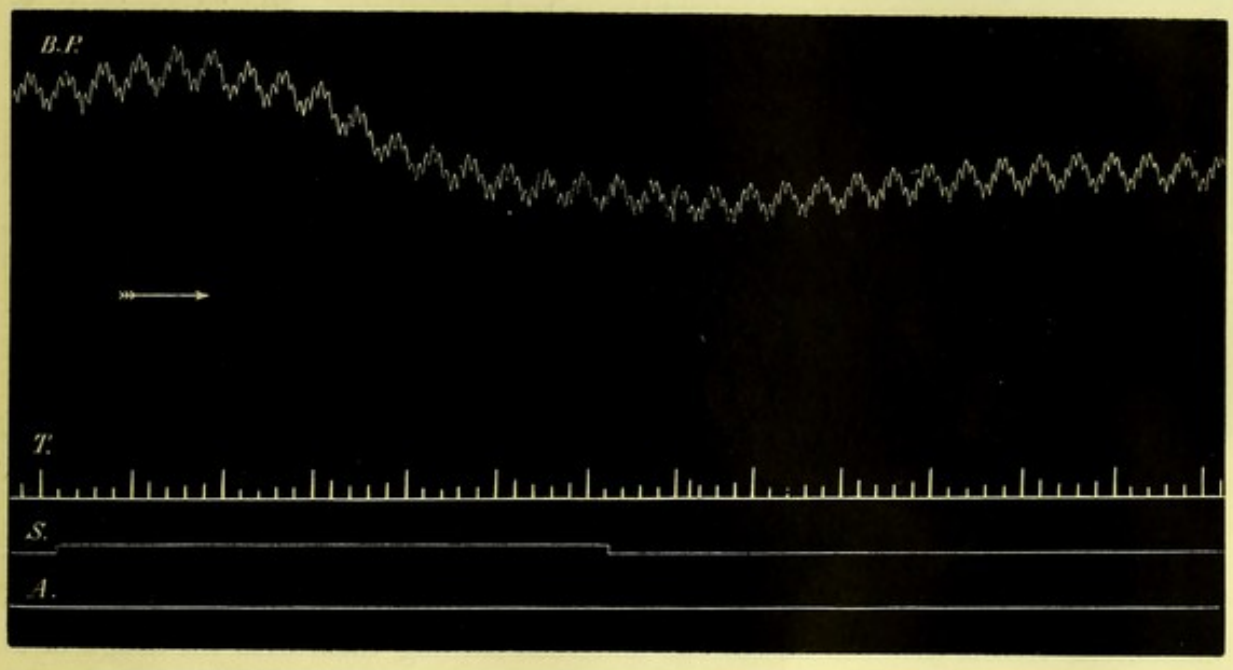
Cat. Spinal cord divided, as described in text. Injection of 2·5 cub. centims. of 0·2 per cent. choline produces the usual fall of blood-pressure. There is subsequently marked slowing of the heart.

Fig. 31. Original size.



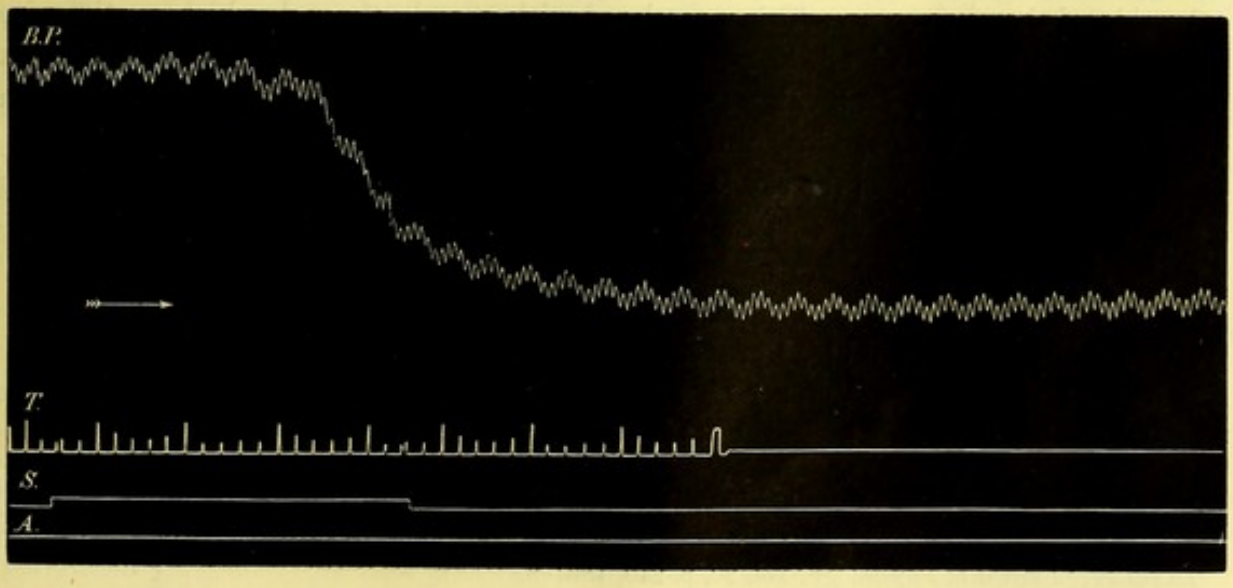
Dog. Both splanchnic nerves divided. The injection of choline hydrochloride (5 cub. centims. of a 0·2 per cent. solution) produces the usual fall of arterial blood-pressure.

Fig. 32. Original size.



Cat, under the influence of nicotine. The stimulation of the central end of the divided sciatic nerve was followed by no effect on blood-pressure. This showed the animal was fully under the influence of the drug. A quantity of cerebro-spinal substance (corresponding to 10 cub. centims. of the original fluid) was injected later, and the usual fall of blood-pressure, but with a slow recovery to normal, was produced.

Fig. 33. Original size.



Same cat. Choline (2·5 cub. centims. of a 0·2 per cent. solution) produces the same result as shown in the preceding figure.

The injection of nicotine is followed by increased respiratory actions, and rise of blood-pressure, but if the dose is repeated again and again, this effect becomes less and less, and finally *nil*, and the animal has to be kept alive by artificial respiration.*

But when this stage has been reached, the animal must not be considered to be fully nicotinised, for stimulation of the central end of the divided sciatic still continues to produce a great rise of blood-pressure. At last, however, the animal is fully under the influence of the drug. In the case of one cat we had to inject 115 milligrammes of nicotine, in another 275 milligrammes of nicotine before this stage was reached.†

Even after this stage is reached, the blood-pressure still continues to be comparatively high; the subsequent injection of choline (fig. 33), or of cerebro-spinal substance (fig. 32) produces the usual fall of arterial pressure.

It will be noticed in this, as in some of the previous tracings obtained after the elimination of the central nervous system, that the recovery of the blood-pressure to its usual level is exceedingly slow.

4. The last method employed in this branch of the research has been to *apply the drug locally* to the intestinal vessels. This can be done in the frog by watching the mesenteric vessels with the microscope and then bathing them with the drug; or in the mammal the drug can be applied locally to the loop of intestine within the oncometer, and the effect can be watched with the eye through the glass lid of the instrument, or the effect can be registered graphically. These effects confirm the statements already made that choline and cerebro-spinal substance act locally on the neuro-muscular mechanism of the blood vessels.

In order to obtain the effect with the oncometer, it was first necessary to warm the drug to the temperature of the animal's body.‡ A hole was previously made through the side of the oncometer. This was closed with a cork, through which a stiff wire was passed; at the end of the wire was a little open porcelain vessel filled with a solution of the drug; on turning the wire through 180°, the contents of the porcelain spoon were upset over the intestinal loop.

The next figure shows the result. There is a rise of the oncometer lever, indicating dilatation of the vessels; this continues to increase, until the glass lid of the oncometer is raised, and the drug washed away with saline solution.

The local effect on a loop of intestine, however, is not sufficient to cause any appreciable change in general blood-pressure.

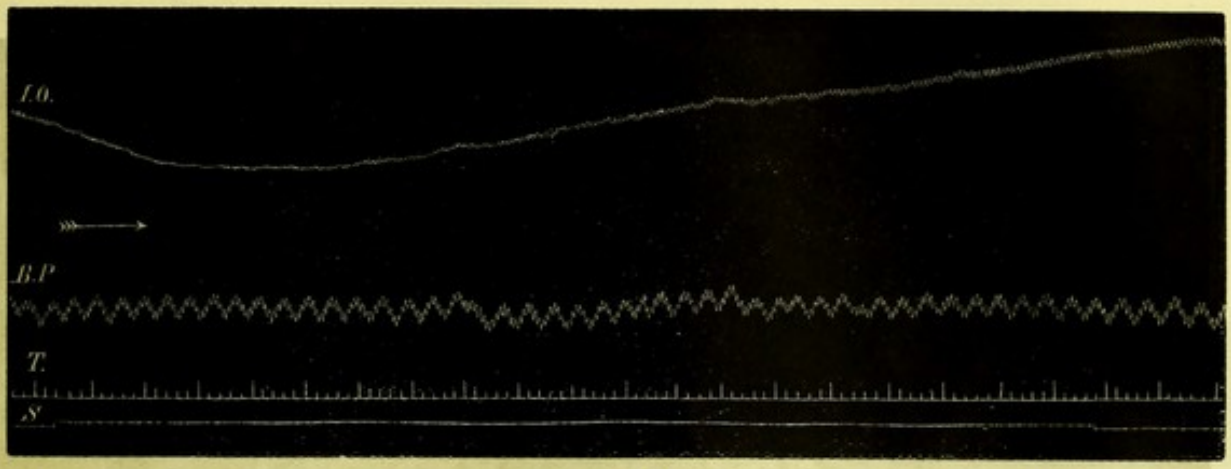
Fig. 34 shows the effect of 10 cub. centims. of cerebro-spinal fluid; a 0·2 per cent. solution of choline hydrochloride acts in exactly the same way.

* See MOORE and ROW, 'Journal of Physiology,' vol. 22, p. 275, 1897–8.

† The resistance of the cat, and still more so, the dog, to nicotine has already been pointed out by LANGLEY, 'Journal of Physiology,' vol. 20, p. 240, 1897–8.

‡ In order to minimise the effects of shock when using the intestinal oncometer, we always kept the animal on a hot water tin, and surrounded it with cotton wool.

Fig. 34. Two-thirds the original size.



10 cub. centims. of cerebro-spinal fluid were upset over loop of intestine of cat within the intestinal oncometer as described in text. The solution was gradually poured over the loop, as indicated by the signal line. There was a steady dilatation of the intestinal vessels, as shown by the tracing I.O. The dilatation continued until the glass lid of the oncometer was removed, and the alkaloid washed away with physiological saline solution. The general blood-pressure was not affected. The abscissa of the blood-pressure in this tracing was accidentally omitted.

Neurine.

The effect of neurine is also a local one, though not quite in the same sense as that of choline is. We used the same four methods.

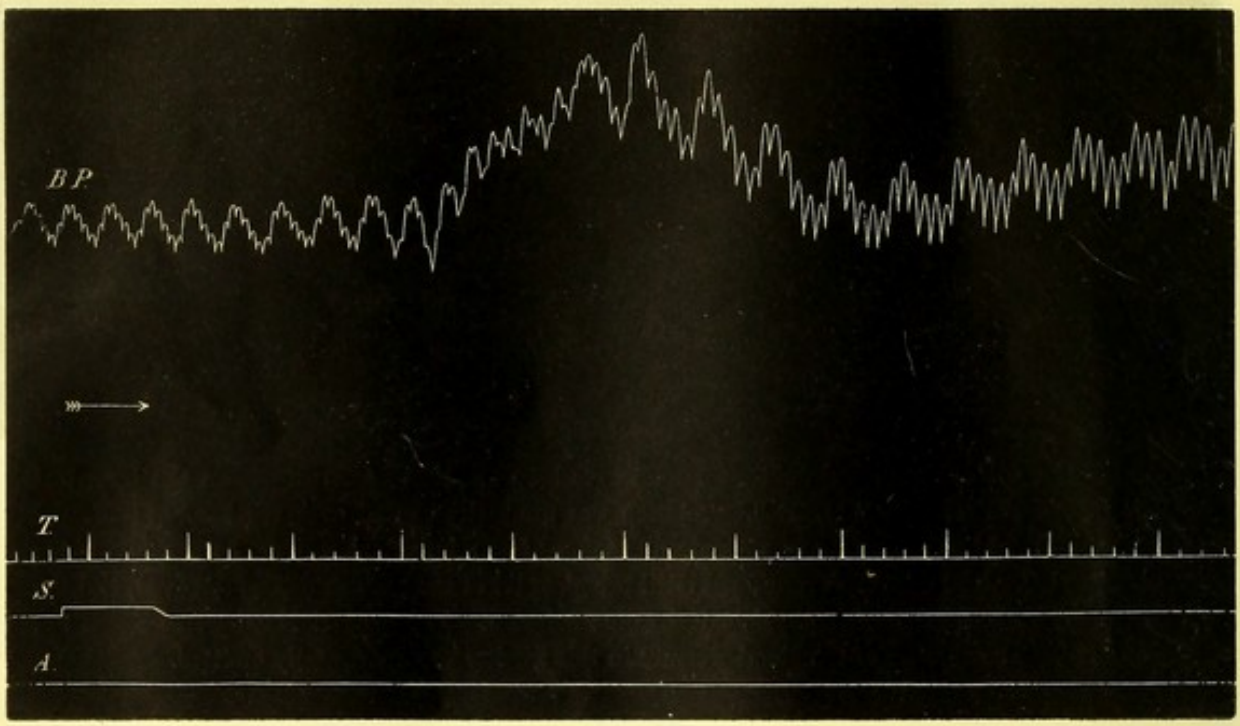
1. *Section of the Cord.*—Fig. 35 shows the effect of injecting 2·5 cub. centims. of a 0·1 per cent. solution of neurine after the spinal cord has been divided. The preliminary fall of arterial pressure was slight and was followed by the usual rise in spite of the slowing of the heart; the rise was, therefore, produced by a constriction of the peripheral vessels, and this occurred independently of the bulbar vaso-motor centre.

2. *Section of both Splanchnics.*—The usual dose of neurine produced the usual result after both splanchnic nerves had been divided. Fig. 36 shows the effect. The preliminary fall was followed by a rise of arterial pressure. There was the usual effect on respiration, but this was not traced.

3. *Action of Nicotine.*—The experiments just given under headings 1 and 2 show that the effect of neurine does not depend on the action of the drug on the central nervous system. Two possibilities yet remain; either the drug acts on the local neuro-muscular mechanism of the vessels themselves, or on the sympathetic ganglia. We have arrived at the conclusion that the main effect, or, at any rate, the rise of pressure, is due to the action of the drug not on the vessels themselves but on the peripheral ganglia.* In this neurine differs from choline. Our principal ground for

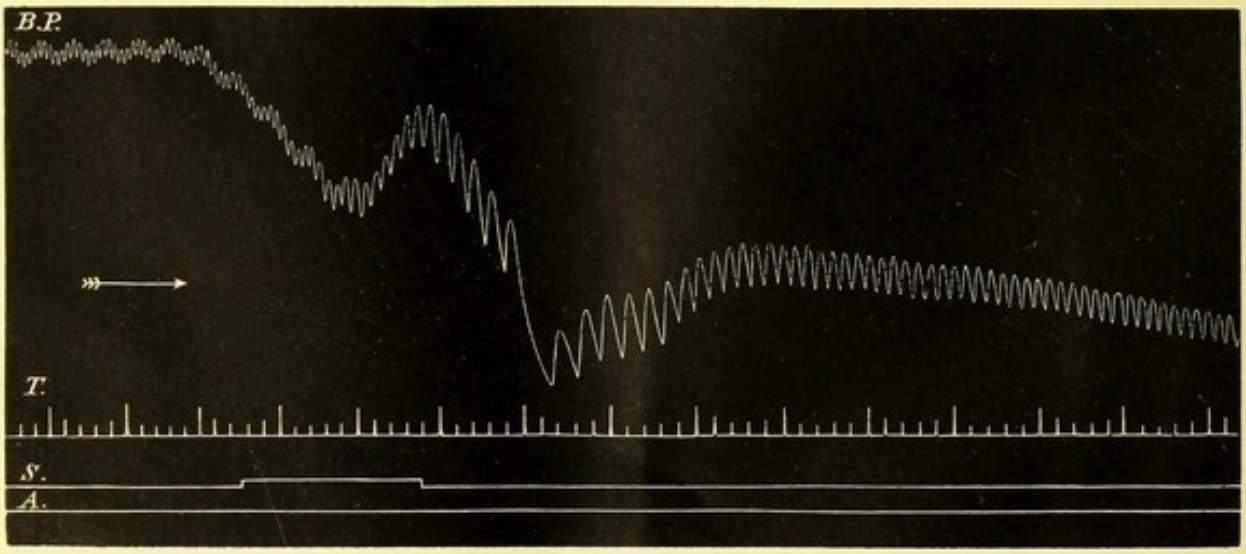
* A similar conclusion respecting the action of nicotine, piperidine, and coniine has been arrived at by Moore and Row. 'Journal of Physiol.,' vol. 22, p. 273, 1897–8.

Fig. 35. Original size.



Cat; cord divided. Injection of 2·5 cub. centims. of a 0·1 per cent. solution of neurine. The preliminary fall of blood-pressure is slight; this is followed by the usual rise of blood-pressure. Heart markedly slowed later.

Fig. 36. Original size.

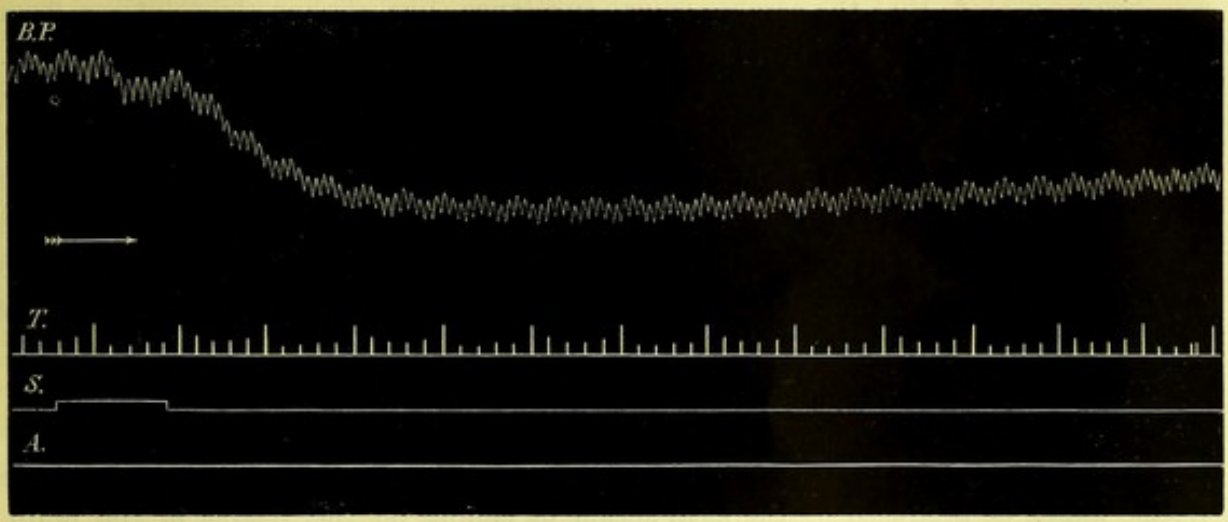


Dog; both splanchnics divided. 2·5 cub. centims. of a 0·1 per cent. solution of neurine injected. The effect is the usual one, except that the rise which follows the initial fall is rather less marked than it often is. The heart here, however, shows marked slowing.

this conclusion is that after the animal has been nicotinised, in the manner already described, injection of neurine produces only a fall of blood-pressure (fig. 37).

It may be that the preliminary fall of blood-pressure, which we have generally noted after injection of neurine, is not wholly of cardiac origin, but is in part produced by the action of the alkaloid on the neuro-muscular apparatus of the vessels; this is soon counterbalanced by the opposite effect produced by the action of the drug on the peripheral ganglia.

Fig. 37. Original size.



Cat, under the influence of nicotine. 2·5 cub. centims. of a 0·1 per cent. solution of neurine were injected. The result is a fall of blood-pressure without any secondary rise.

4. *Local Flushing with a Solution of Neurine.*—These conclusions are confirmed by the effect of the local application of neurine solution; there is some preliminary constriction of the vessels, but the most marked effect is flushing of the intestines, and a corresponding rise of the oncometer lever. It was a little difficult to be quite sure in these cases that one had to deal merely with a local effect, for such small doses of the alkaloid are efficacious that the absorption of even a small amount of it would produce general effects. The tracings we obtained show, in fact, that the drug was absorbed in sufficient amount to produce a general effect on the arterial pressure. We have, therefore, considered it hardly worth while to reproduce any of these tracings.

8. Experiments on Nerves.

We have stated that the effect of choline is upon the neuro-muscular mechanism of the blood vessels. The question still remains whether the alkaloid acts on the muscular fibres, or the nerves that supply them.

We know of no method which will enable us to distinguish between activity of the muscular fibres and that of the nerve-endings. We can, however, say that

choline has no effect on nerve-trunks. We are able to make this statement from the researches of Dr. Waller. Dr. Waller and Miss Sowton have made a very complete study of the action of the neurine group of alkaloids on frog's nerves; the nerve is bathed in a solution of the alkaloid, and its electrical response to a stimulus is registered. Dr. Waller and Miss Sowton have been good enough to include our cerebro-spinal substance among the materials they have investigated, and have already made a brief communication to the Physiological Congress at Cambridge* on the subject. They intend to publish their results fully in a separate paper. We can, however, state that the cerebro-spinal substance, like choline (in solutions from 0·2 to 4 per cent.), produces little or no alteration in the electrical response of nerve. The non-toxic action of choline on nerve contrasts with the action of neurine, which is more poisonous to this tissue.

It also happened that in some of their experiments they employed a solution of the cerebro-spinal substance which had been kept some days and had undergone decomposition; it is well known that choline is readily changed into neurine by this means, and the solution was now more toxic, giving the same results which solutions of neurine exhibit.

9. Experiments on the Cerebral Vessels.

It appeared possible to us that some of the symptoms of General Paralysis of the Insane might be produced by the local action of choline on the vessels of the brain, similar to that which it produces on the mesenteric vessels. It appeared to us highly important to investigate this question, for it is in the neighbourhood of the cerebral vessels that the poison is being continually produced. At the same time, we were aware of the fact that, although nerve-fibres have been shown by histological methods to exist in these vessels, it has not yet been possible for physiologists to discover by experimental methods any evidence of vaso-motor action in this region of the body.

In carrying out these experiments, we were fortunate enough to secure the assistance of Mr. Leonard Hill, whose name is so closely associated with this branch of research.

The animals used were dogs, and the anæsthetics employed were morphine and chloroform. A tracing of arterial blood-pressure was obtained from the carotid by a mercurial kymograph. Manometers filled with salt solution coloured with methylene blue were connected with the torcula and with the jugular vein; these manometers were placed against a scale, and the extent of the rise and fall of the fluids was written down by one of the observers. The injection of the solutions was made into the femoral vein. The injection of fluid of any kind caused always a slight preliminary rise in both venous manometers, due to the increase in the volume of the vascular contents. This was not sufficient to affect the arterial pressure.

* 'Journal of Physiology,' vol. 23, 1899 (Supplement), p. 35.

5 cub. centims. of choline (0·2 per cent. solution) caused the usual fall of arterial pressure; the fluid in the torcula manometer fell with the arterial pressure; the fluid in the jugular manometer rose with the arterial fall.

5 cub. centims. of choline hydrochloride (0·2 per cent. solution) caused the same effects.

5 cub. centims. of cerebro-spinal substance (of approximately the same strength) caused the same effects. Among the specimens of cerebro-spinal fluid used in these experiments was one from a case in which the fluid was removed by lumbar puncture only half an hour after death.

2·5 cub. centims. of neurine (0·1 per cent. solution) was also injected. The preliminary fall of arterial blood-pressure was accompanied, as usual, with intensely deep respirations, which caused a great fall in the venous pressure: there was a corresponding fall, but not so marked, of the fluid in the manometer connected with the torcula. In both venous manometers the fluid rose again with the rise of arterial pressure; in the torcula, the pressure follows passively the rise of general blood-pressure; the rise of pressure in the jugular vein is accounted for by dilatation of the heart, the rate of which was very great.

The experiments just recorded indicate that the drugs injected have no direct effect on the cerebral vessels, but that the results obtained are due to the pressure in these vessels following that in the general circulatory system in a passive manner.

10. Experiments with the Blood of General Paralytics.

As stated in the introductory section of this paper, we have had the opportunity of examining the blood from several cases of General Paralysis. The blood removed by venesection as a remedial measure during a seizure, was mixed with excess of alcohol, and the alcoholic extract was examined for choline by the method already given in connection with cerebro-spinal fluid; we succeeded in obtaining the characteristic crystals of the platinum double salt.

The alcoholic extract was freed from alcohol by evaporation; the residue was taken up with physiological saline solution and injected into animals. The material at our disposal did not allow us to make so complete an investigation in the experimental direction, as we have done with cerebro-spinal fluid; but so far as we have gone, the results obtained are exactly the same.

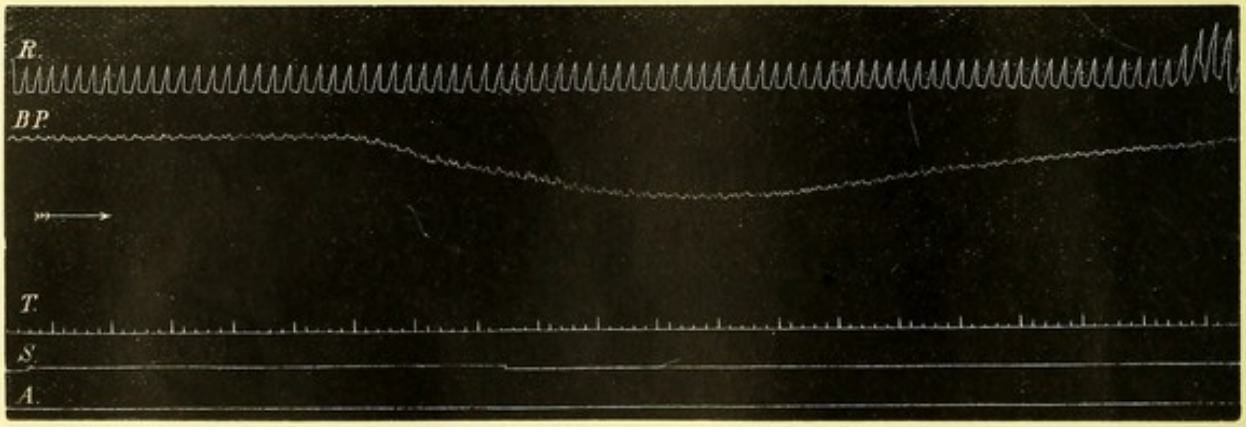
We prepared in a similar way blood from normal individuals, and from animals. With one exception, the injection of such fluids was entirely negative. The one exception can hardly be called quite normal blood, as it was removed from a patient suffering from adeno-sarcoma of the breast. The result of the injection of this fluid was a very insignificant fall of blood-pressure.* Fig. 38 shows the marked result

* This tracing shows such a slight fall of pressure, that the Referees of this paper have judged it to be a negative result, and therefore not worth reproducing.

which follows in the injection of a preparation from the blood of a general paralytic.

We have also, thanks to the kindness of Dr. PATRICK MANSON, had the opportunity of investigating the action of the blood removed by venesection from a case of Beri-beri. This is a nervous disease, accompanied by great cardiac failure, vascular

Fig. 38. Half the original size.



Effect in a dog of saline solution of the alcoholic extract of 70 cub. centims. of blood removed during a seizure from a patient suffering from General Paralysis of the Insane. There is no effect on respiration (R.), but there is a well-marked fall of blood-pressure (B.P.). In the same blood choline was identified chemically.

depression, and œdema. A saline solution of the residue of the alcoholic extract of the blood produced a marked fall of blood-pressure with cardiac depression, and dilatation of the peripheral vessels of the splanchnic area. We were, however, not successful in this case in obtaining any chemical evidence of the presence of choline.

11. EXPERIMENTS ON THE URINE.

In the few cases in which we have examined the urine of the animals in which injections of choline and neurine had been performed, we did not succeed in obtaining any evidence of the presence of these alkaloids in that secretion. It was, perhaps, hardly to be expected that we should, for the doses we administered were always minute ones.

These alkaloids are stated by the observers, whose work we have already referred to, to stimulate secretion, and CERVELLO lays considerable stress on the salivation produced. We have not studied this aspect of the question minutely, but we have noticed, especially in dogs, that considerable secretion of saliva does occur.

We have also sought for choline in the urine of patients suffering from General Paralysis, but again with negative results. It may be that the choline does pass as such into the urine, but that our tests are not sufficiently delicate to detect minute

quantities; it, however, appears to us more probable, as choline is a substance which is so easily decomposed into simpler products, that it is similarly broken up and oxidised by metabolic processes in the body, or during excretion by the kidneys and other excretory organs.

The urine of General Paralytics does produce a fall of arterial pressure when injected into a vein; so also does a saline solution of the residue from the alcoholic extract; so also does the basic substance (or substances) which can be separated from the abundant precipitate produced by phospho-tungstic acid.

But exactly the same statements are true for perfectly normal urine; the amount of arterial fall is sometimes greater in the urine from General Paralytics, and sometimes greater in normal urine.

We are therefore unable to draw any conclusions regarding these experiments, beyond the fact that all urines contain certain bases which cause a fall of arterial blood-pressure. The fall of pressure which occurs when the urine itself is injected must also be in part attributed to its inorganic salts. A solution of the ash of normal urine produces a great fall of blood-pressure.

12. Experiments with Other Substances.

In the course of our work we have from time to time injected several other substances, and the results obtained may be briefly summarised as follows:—

(a) *Products of Decomposition of Choline.*

Choline is readily decomposed into trimethylamine and glycol.

Trimethylamine was injected in 0·25 and 1 per cent. solutions made with water. 5 cub. centims. of such solutions produced either no result, or an almost imperceptible fall of blood-pressure.

Glycol was injected in three animals. The strength of the solutions was 0·2 and 1 per cent. In one animal the result was entirely negative; in the other two there was a very slight fall of arterial pressure, accompanied with a correspondingly slight dilatation of the vessels of the intestinal area.

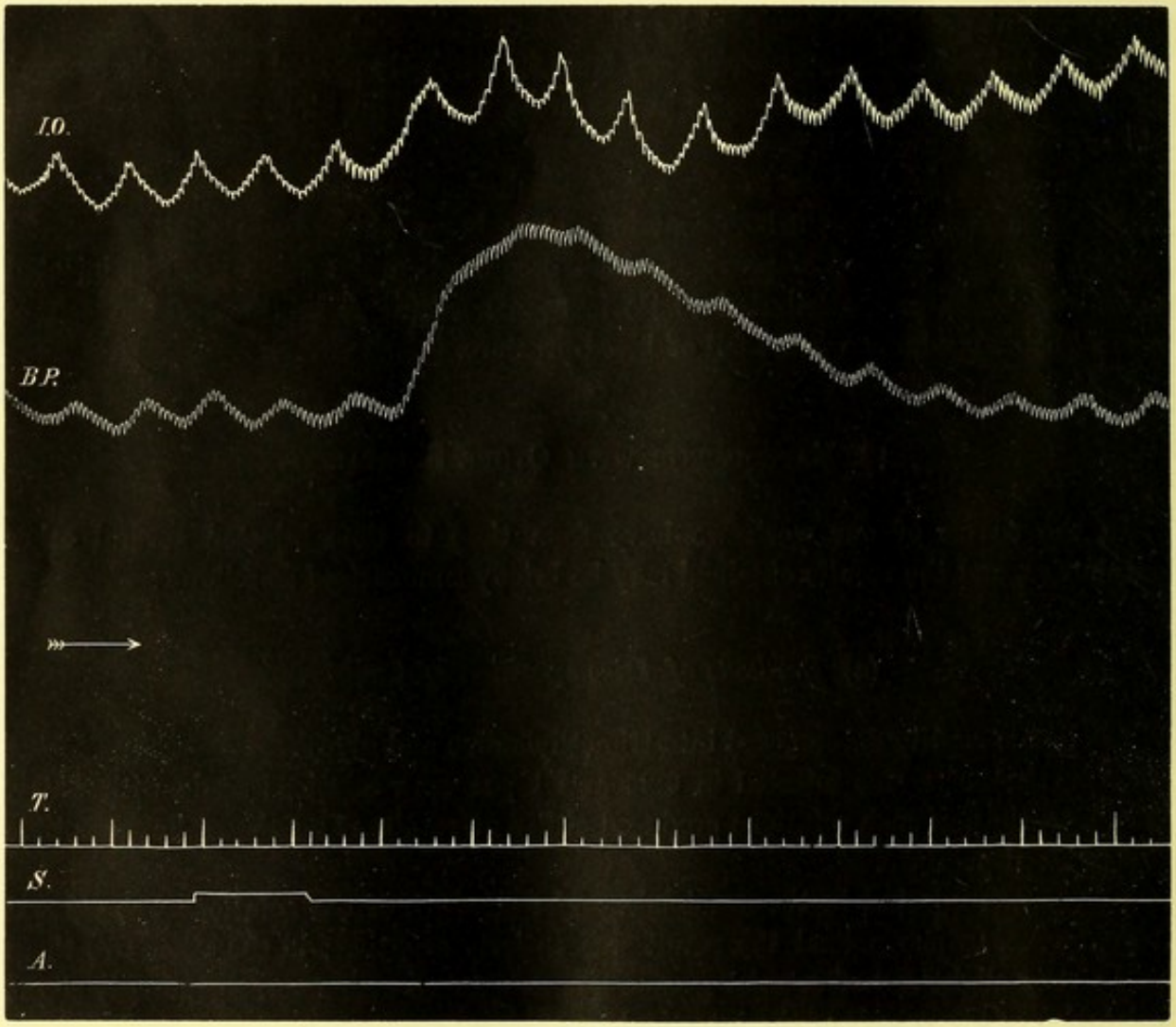
(b) *Acids.*

Sarcolactic acid was used in solutions varying in strength from 0·2 to 2 per cent. It had been suggested to us that the presence of this acid in cerebro-spinal fluid might explain the fall of blood-pressure which followed injections of that fluid. Cerebro-spinal fluid is, however, always alkaline to litmus, so there cannot be any free lactic acid in it. The result of injecting the weaker solutions was wholly

negative; even the strongest solutions produced only a very insignificant fall of blood-pressure.

Glycero-phosphoric acid was investigated because this is a product of the decomposition of lecithin. Injection of solutions of strengths varying from 0·2 to 2 per cent. produced wholly negative results.

Fig. 39. Original size.



Cat, anæsthetised with A.C.E. mixture, but had also previously received a subcutaneous injection of morphine and atropine. Injection of choline hydrochloride (5 cub. centims. of a 0·2 per cent. solution) now produces a rise instead of the usual fall of blood-pressure; the lever of the intestinal oncometer (I.O.) also rises.

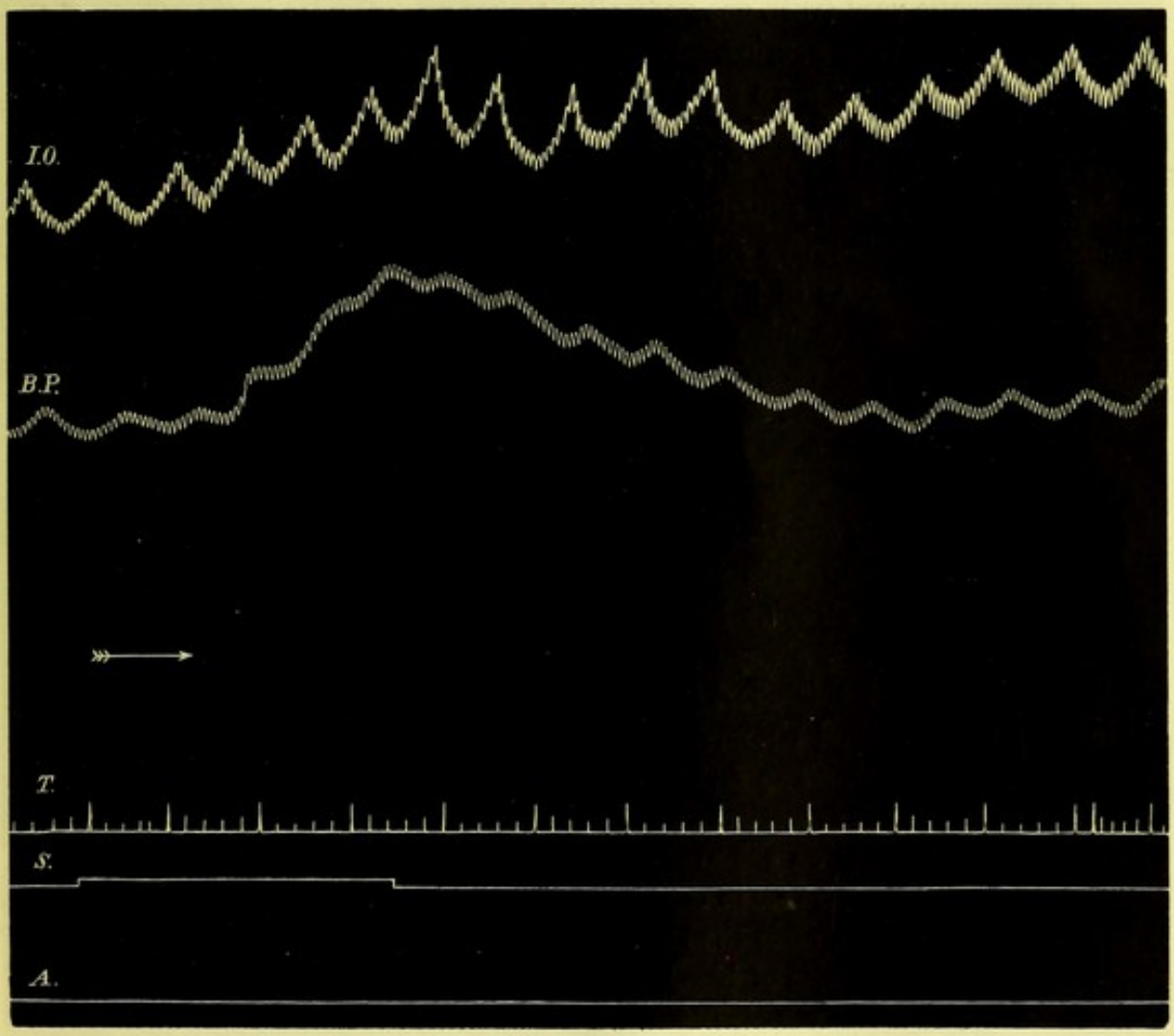
(c) *Pyro-catechin.*

We investigated this substance because one of us has already stated he considers it probable that the reducing substance of normal cerebro-spinal fluid is related to that

body. The reducing substance, however, is usually absent in cases of General Paralysis.

The strengths of the solutions used varied from 0·2 to 2 per cent. The weaker solutions produce no effect; the stronger ones cause an insignificant rise, or sometimes an equally insignificant fall of blood-pressure.

Fig. 40. Original size.



Same effect in the same animal produced by the injection of a corresponding amount of cerebro-spinal substance.

(d) *Suprarenal Extract.*

The action of choline and of suprarenal extract is so directly opposite, that it appeared to us interesting to see what would be the result of injecting a mixture of the two. The suprarenal extract was kindly supplied to us by Professor SCHÄFER.

The result of injecting the mixture (mixtures were made in various proportions) is that the suprarenal effect (rise of blood-pressure and constriction of peripheral vessels) is alone seen. The active principle in the suprarenal extract is therefore much more powerful than choline.

13. Effect of Anæsthetics.

As previously stated, all the animals used have been completely anæsthetised; the kind of anæsthetic used (with one exception) makes no difference in the effects of the injections of choline and neurine. We have sometimes used chloroform alone, sometimes ether alone, sometimes a mixture of the two, but usually A.C.E. mixture. The additional subcutaneous injection of morphine, or of curare, makes no difference.

The exception to the rule is atropine. When Professor W. H. Thompson was helping us in one of our experiments on the cutting of the splanchnic nerves, he suggested that a preliminary subcutaneous injection of a mixture of morphine and atropine would assist us in the subsequent anæsthetisation with A.C.E. mixture. We accordingly, in a dog, injected about $\frac{3}{4}$ of a grain of morphine acetate, to which about $\frac{1}{10}$th of a grain of atropine was added. To our surprise, after the nerves were severed, injection of choline and cerebro-spinal substance produced, like neurine, a rise of blood-pressure.

Remembering, however, the antagonism which is well known to exist between atropine and choline, it occurred to us that the unusual results might be due to the employment of atropine. This we later fully confirmed. In animals to which even such a small dose of atropine had been previously given, injection of choline, or cerebro-spinal substance, always produced a rise of arterial pressure. We give illustrative tracings of this effect (figs. 39 and 40). The uppermost line shows that the lever of the intestinal oncometer also rises. The effect appears to be mainly due to action on the heart. The fact is not without importance as showing that the action of one poison may materially modify the action of another, and this is not entirely without bearing on the study of the disease we wished specially to investigate. We shall return to this in our concluding remarks.

In the case of neurine, after the animal has been anæsthetised with morphine and atropine, there is only a rise of blood-pressure, and this is accompanied by the usual constriction of peripheral vessels (fig. 41).

14. Summary of the Physiological Action of Choline and Neurine.

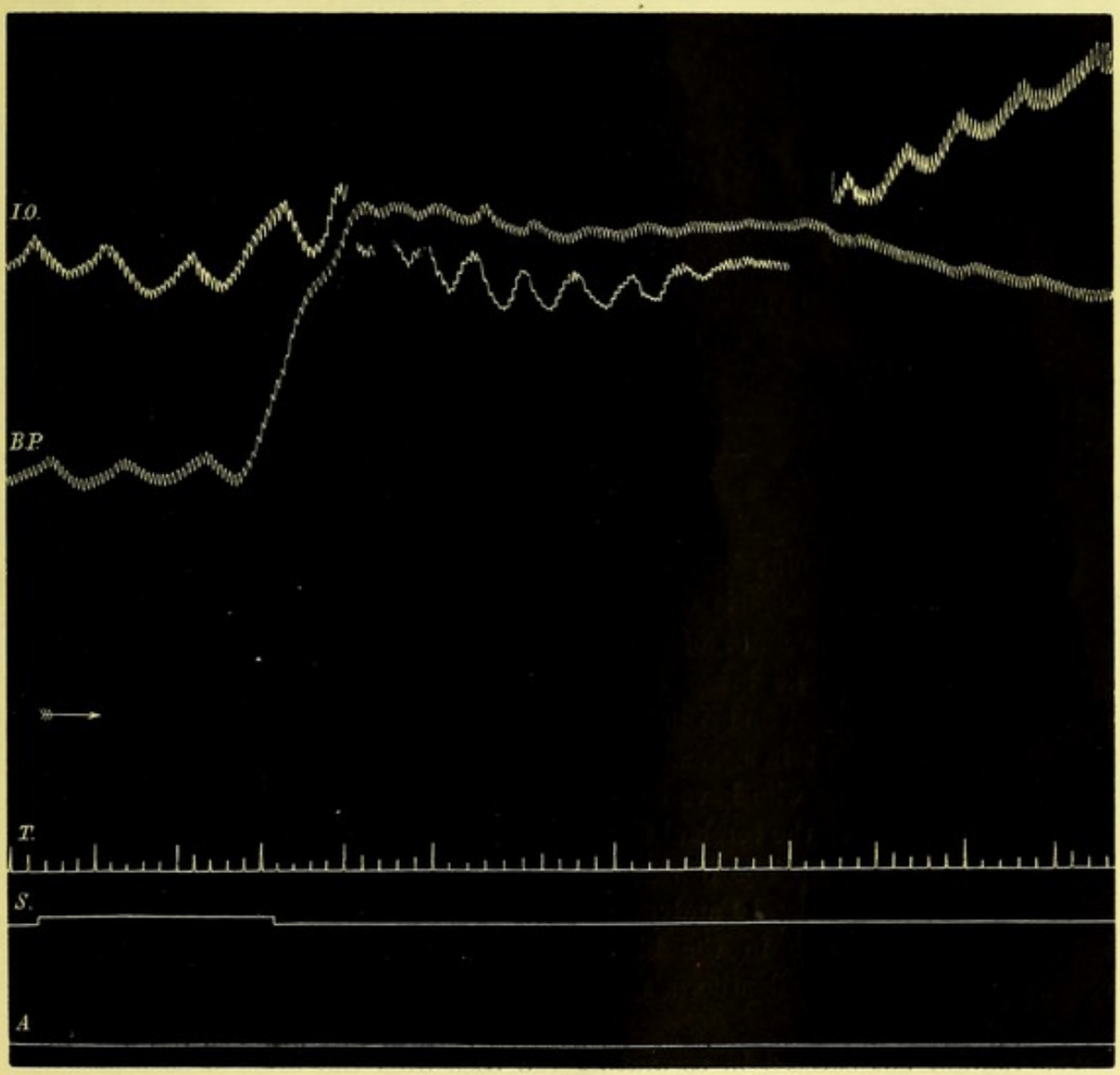
A summary of the chemical side of the investigation has already been given on pp. 221, 222.

Our experimental researches have completely confirmed our chemical experiments. The cerebro-spinal substance acts precisely like choline in every way. The action of neurine is different.

The principal physiological actions of choline (or choline hydrochloride), using the small doses we have adhered to throughout (1 to 10 cub. centims. of a 0·2 per cent. solution), are as follows :—

1. Choline produces a fall of arterial blood-pressure.

Fig. 41. Original size.



Same animal ; 2·5 cub. centims. of a 0·1 per cent. solution of neurine injected. Rise of blood-pressure, with a constriction of the vessels in the intestinal area.

2. This is in some measure due to its action on the heart.

3. It is also, and probably mainly, due to dilatation of the peripheral vessels, especially in the intestinal area.

4. The volume of the kidney and limbs is somewhat lessened with the fall in

arterial pressure; this appears to be merely a passive lessening of volume secondary to the fall in general arterial pressure.

5. With the spleen there is a marked lessening of volume, which we consider is due to the action of the drug on the muscular tissue of that organ. This is followed by an exaggeration of the curves due to the alternate systole and diastole of the spleen.

6. We have obtained no evidence of any direct action of choline on the cerebral blood vessels.

7. The action on the splanchnic vessels is due to the direct action of the drug on the neuro-muscular apparatus of those vessels, for after the influence of the central nervous system has been removed, by section of the cord, or of the splanchnic nerves, choline still causes the typical fall of arterial pressure.

8. The action of peripheral ganglia may be excluded by poisoning the animal with nicotine. After this has been done, choline still causes a fall of arterial pressure.

9. Section of the vagi produces no effect on the results of injecting choline.

10. Choline has little or no action on nerve trunks, as tested by their electrical response to stimulation.

11. If the animal has been anæsthetised with a mixture of morphine and atropine, in addition to chloroform or ether, the effect produced by choline is a rise of arterial pressure, accompanied by a rise of the lever of the intestinal oncometer. Other anæsthetics cause no change in the usual results.

12. The effect of choline soon passes off, and the blood-pressure returns to its normal level rapidly. This is due partly to the great dilution of the substance injected by the whole volume of the blood, and may be partly due to the excretion of the alkaloid, or to its being broken up into simpler substances by metabolic processes. We could not find it in the urine.

13. Choline is absent in normal cerebro-spinal fluid, but is present in the cerebro-spinal fluid of patients who have died from diseases of the brain in which there is great disintegration of the cerebral tissues. It doubtless originates from the lecithin present in those tissues. The disease to which we have paid special attention from this point of view is General Paralysis of the Insane.

14. It is present also in the blood of such patients removed during life by venesection during the epileptiform seizures which form a prominent symptom in this disease.

15. We did not succeed in separating the base from the urine of such patients. The urine, however, does produce a fall of blood-pressure; but the same is true for normal urine; and this effect is to be attributed partly to the inorganic salts and partly to certain basic substances which are precipitable by phospho-tungstic acid.

16. Choline produces little or no effect on the respiration.

The principal physiological actions of neurine are much more intense, and are different from those of choline. Neurine is absent from the cerebro-spinal fluid in the cases we have examined. The main physiological actions, using the small doses

we have adhered to throughout (1 to 5 cub. centims. of a 0·1 per cent. solution), are as follows:—

1. Neurine produces a fall of arterial blood-pressure, followed by a marked rise and subsequent fall to the normal level.

2. With small doses the preliminary fall may be absent.

3. With larger doses, by which presumably the heart is more profoundly affected, the rise may be absent.

4. The effect of neurine on the heart of both frog and mammal is much more marked than is the case with choline; in the case of both alkaloids it is antagonised by atropine.

5. The slowing and weakening of the heart appear to account for the preliminary fall of blood-pressure; though in some cases this is apparently combined with a direct dilating influence on the peripheral vessels.

6. The rise which occurs after the fall is due to the constriction of peripheral vessels, evidence of which we have obtained by the use of oncometers for intestines, spleen and kidney.

7. After the influence of the central nervous system has been removed by section of the spinal cord, or of the splanchnic nerves, neurine still produces its typical effects.

8. After, however, the action of peripheral ganglia has been cut off by the use of nicotine, neurine produces only a fall of blood-pressure. It therefore appears that the constriction of the vessels is due to the action of the drug on the ganglia; in this neurine would agree with nicotine, coniine and piperidine.

9. Section of the vagi produces no influence on the results of injecting neurine.

10. In animals anæsthetised with a mixture of morphine and atropine in addition to chloroform or ether, injection of neurine causes only a rise of blood-pressure.

11. Neurine produces no direct results, so far as we could ascertain, on the cerebral blood vessels.

12. Neurine is more toxic to nerve trunks than choline.

13. Neurine produces a marked effect on the respiration. This is first greatly increased; but with each successive dose the effect is less, and ultimately the respiration becomes weaker and ceases altogether. The animal can still be kept alive by artificial respiration.

14. The exacerbation of respiratory movements will not account for the rise of arterial pressure; the two events are not synchronous, and an intense rise of arterial pressure (due, as previously stated, to constriction of peripheral blood vessels) may occur when there is little or no increase of respiratory activity, or during artificial respiration.

15. As confirmatory of Cervello's statement that neurine acts like curare on the nerve endings of voluntary muscle, and to which he attributes the cessation of

respiration, we may mention that after an animal has been poisoned with neurine, asphyxiation causes little or no convulsive efforts.

15. Concluding Remarks on the Bearing of the Work on the Pathology of General Paralysis.

Our chief interest in this work has centred round the discovery that the cerebro-spinal fluid of patients dying from General Paralysis of the Insane contains toxic material. We are inclined to believe that this toxic material, which undoubtedly originates from the disintegration of nervous tissue, is probably not a single substance. We have obtained chemical evidence of the existence of nucleo-proteids in the fluid, and though the amount of nucleo-proteid is not, as a rule, sufficient to cause massive intravascular coagulation when the fluid is injected into animals, we consider that the presence of even small quantities continuously being poured out into the cerebro-spinal fluid, collecting in the peri-vascular lymphatics and passing thence to the blood, will produce harmful effects. The idea has suggested itself to our minds that an increase in the coagulability of the blood in the small vessels of the cerebral region, which nucleo-proteid would produce, might form a determining factor in promoting venous stasis, and thus the acute manifestations or seizures of apoplectiform or epileptoid nature which the patients exhibit. We have, however, no direct evidence of this, nor have we any evidence that the seizures may be due to the direct action of poisonous products or the contraction of cerebral vessels.

We are almost driven to the conclusion that the toxic substance is not a single body, for the only other poisonous material which we have succeeded in isolating, namely choline, will not account for even the majority of the symptoms of the disease. In the completeness with which we have been enabled to identify (both on chemical and physiological grounds) choline in the cerebro-spinal fluid and blood of these patients, we consider our work has been satisfactory only up to a certain point. As affording a complete explanation of the pathology of the disease it has been a disappointment; we regard the fact that choline does exist in these fluids, as an indication merely of the disintegration of the brain-tissue, and if the majority of the symptoms are to be explained on the basis of auto-intoxication due to such disintegration, we must confess that the other poisonous substances have eluded our search: certainly glycero-phosphoric acid and lactic acid are not highly poisonous; their action is even less so than that of choline.

An apparently feeble circulation and fatty degeneration of the heart are very frequent concomitants of the terminal stages of the disease, especially after a series of epileptiform seizures, and the idea certainly seemed feasible at one point in our research, that choline might explain these. A single dose of choline in a dog or cat produces but little effect on the heart; still, there is some effect, and it did not appear

a far-fetched idea to suppose that the continual pouring of small doses of choline into the cardiac tissue might in time produce cardiac weakness and even degeneration. This possibility certainly still remains, but our study of the disease has now shown us that choline will not explain the fits, but that the fits will explain the degeneration in heart and other muscles. Such fatty degeneration probably never takes place unless fits have occurred before death; and if similar fits occur in other diseases, as in the *status epilepticus* of epilepsy, there is the same fatty degeneration of the muscular tissues, even though no massive disintegration of nervous tissue has been present.

Take again the point of enfeebled circulation; the pulse is often small and might be supposed to be associated with low tension. If this were the case, choline again would serve as the explanation. But on testing with the Hill-Barnard sphygmometer the arterial tension in these patients, low tension was not the rule but the exception.* Evidently choline is not the cause of this; choline alone would produce low tension from dilatation of peripheral vessels. Our experiments with atropine are most instructive from this standpoint; we have seen (p. 260) that the previous subcutaneous injection of a minute dose of atropine, mixed with morphine, will modify the result of the injection of choline very considerably, for, under these circumstances, choline now produces a rise instead of a fall of arterial blood-pressure. We do not, of course, mean to suggest that atropine, or even a similar alkaloid, is present in these patients, but these experiments do suggest that with a plurality of causes we undoubtedly obtain an intermixture or even a reversal of effects.

We have been able to work out fairly thoroughly the physiological action of choline and neurine, but, so far as we can see at present, the result is rather of academic than of direct practical value in the elucidation of pathological problems. No doubt, with fresh light which future work may throw on our own, it may be possible to see the *modus operandi* of these pathological processes more clearly, and our three years' work will not have been thrown away if it forms some sort of guidance to others, or to ourselves, in unravelling the plurality of causes to which we have just alluded, and which we must assume is present in General Paralysis as in so many other obscure diseases.

The expenses involved in this research have been defrayed from grants made by the Government Grant Committee of the Royal Society, and by the British Association.

* See Supplementary Note C at the end of this Paper.

Supplementary Notes.

Added June 8, 1899.

A. The cerebro-spinal fluid with which our work has been performed was removed from the cranial cavity after death. Since the foregoing paper was written, we have, however, had the opportunity of examining four specimens removed during life by lumbar puncture from cases of General Paralysis, and the results of our experiments with these corroborate the conclusions previously arrived at. Two of the specimens we used for injection experiments, the other two for chemical analysis. We are indebted to Dr. John Turner, of the Essex County Asylum, for these specimens. Dr. Turner has also published some observations on the reaction of cerebro-spinal fluid in such cases in 'Brain,' vol. 22, 1899, p. 100.

B. Since the foregoing paper was written, Asher and Wood have published the results of their observations on the influence of choline on the circulation.* The doses they injected were very large compared to those we have used. We injected from 1 to 5 cub. centims. of a 0·2 or 0·1 per cent. solution of choline, or choline hydrochloride; they injected in dogs from 3 to 10 cub. centims. of a 5 per cent. solution of choline. We used weak solutions, for we sought as far as possible to note the effects produced by solutions of such strength as would be comparable to the amount of the base presumably present in pathological cerebro-spinal fluid; and Professor Schäfer stated at the discussion which followed the reading of our paper that even smaller doses than those we had employed would cause a fall of blood-pressure. This we have confirmed. The effects described by Asher and Wood closely resemble those which we have found to follow the injection of neurine, and they attribute their results on respiration and blood-pressure to the action of the alkaloid on the medullary centres which control these actions. It is quite possible that the enormous doses of choline, such as were employed by Asher and Wood, will produce effects different from those produced by our small doses, and similar to those caused by neurine. It is, on the other hand, equally possible that the so-called choline which they used was contaminated with neurine, and a very small dose of neurine is all that is sufficient to cause the typical effect of that base; thus 1 cub. centim. of a 0·1 per cent. solution of neurine will cause an enormous rise of blood-pressure, which was the result they usually obtained with their samples of choline.

Their conclusion that the action of the drug is chiefly on the vaso-motor centres in the central nervous system appears to us to be unwarranted by their experiments, especially as they did not employ the nicotine method.

* 'Zeitsch. f. Biologie,' vol. 37, 1899, p. 307.

C. Since the completion of the experiments described in the body of our paper, one of us (F. W. M.) has made a more extended use of the Hill-Barnard sphygmometer in cases of General Paralysis, and from the careful examination of the results in twenty cases we feel inclined to modify the statement we have expressed that our physiological work has but little bearing on the pathology of the disease. It is quite true that in the first and second stages of the disease (*i.e.* before the patient becomes bedridden) the arterial pressure is as a rule higher than normal; at the beginning of epileptiform seizures the pressure is still high, but after prolonged convulsions the pressure falls considerably, to rise again a few days after the convulsions have ceased. There can be but little doubt that the convulsions are associated with the breaking down of nervous tissue, and we are now inclined to think it possible that choline so liberated is responsible for the fall of blood-pressure which occurs then.