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THE ACETYLCHOLINE CONTENT OF THE CEREBRO-SPINAL FLUID OF DOGS.

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Dikshit [1934] has suggested that the effects of central vagus stimulation might be transmitted in the medullary bulb by the liberation of acetylcholine. This theory was based on the following observations: (1) injections of minute amounts of acetylcholine into the lateral ventricle of a cat had effects on respiration and heart activity similar to those of central vagus stimulation; (2) extracts from the basal ganglia of the brain yielded a higher amount of a substance resembling acetylcholine than extracts from either the cortex or the cerebellum; (3) in a few experiments out of many, cerebro-spinal fluid (c.s.f.) of cats collected during central vagus stimulation contained more acetylcholine than the fluid collected before stimulation.

These last experiments appear to have been carried out without previous administration of eserine. In its absence it would hardly be expected that any acetylcholine which might be liberated during stimulation would escape destruction during its passage into the c.s.f. We have therefore made similar experiments on dogs and prevented the destruction of acetylcholine by intravenous injections of eserine; atropine was injected simultaneously in order to prevent the disturbing effects of eserine on the circulation. In addition to vagus stimulation, the effect of asphyxia was examined. In atropinized dogs both central vagus stimulation and asphyxia are associated with a large rise of arterial blood-pressure in which an output of adrenaline from the suprarenals may be an important factor. Control experiments were therefore carried out in which either the effect of injected adrenaline was studied, or the suprarenals were removed and the rise of pressure was prevented by use of a compensator included in the arterial system.

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Methods.

Dogs of 13–30 kg. were anæsthetized with chloralose. A cannula was tied into the trachea, both vago-sympathetic nerves were cut and the arterial blood-pressure was recorded from the right femoral artery. In most experiments the large and small intestine, spleen and omentum were removed, but the stomach left *in situ* with the portal circulation intact. Both splanchnic nerves were cut, and in some experiments the suprarenal bodies were also removed. If a compensator was used the lower end of the abdominal aorta was dissected for a length of about 2–3 cm., all branches from the isolated portion being tied and cut. The blood was rendered incoagulable by intravenous injection of chlorazol-fast pink and a T-shaped cannula was tied into the freed part of the aorta and connected to the compensator by a rubber tube, which was kept clamped until shortly before the test.

The c.s.f. was collected from the subcerebellar cisterna. The skin and muscles over the occiput were divided in the middle line as far as the third cervical vertebra; the head was strongly flexed and a cannula with a sharp trocar was thrust through the occipito-atlantal membrane and dura mater. The cannula was fixed in position by a thread secured to the muscle layers. When the trocar was removed, clear c.s.f. dropped freely from the cannula. Samples of $1\frac{1}{2}$–$2\frac{1}{2}$ c.c. were withdrawn at every 10–20 min., the rubber tube from the cannula being clipped in the intervals. After the removal of some 10 c.c. slight suction with a syringe had to be applied to obtain further fluid.

The central end of the vago-sympathetic nerve was stimulated with an induction coil and ordinary metal electrodes for periods of 6–10 min. The adrenaline was slowly injected into the femoral vein with a pipette or with the slow infusion apparatus of Burn and Dale. Asphyxia was induced by clamping the trachea. The administration of eserine was always preceded by an injection of a tenth of the amount of atropine.

The c.s.f., in 5–75 p.c. dilution, was tested on the eserinized leech muscle, and, without dilution, on the arterial blood-pressure of a cat under chloralose.

Experimental.

(*a*) *The effect of eserine.* The c.s.f. collected from dogs, which had not received eserine, was without effect, even in a 75 p.c. dilution, on the eserinized leech muscle. As the leech commonly reacted to a solution of acetylcholine 1 in 10^9, the fluid contained certainly less than $0{\cdot}001\gamma$ of acetylcholine per c.c. The fluid was still without effect upon the leech

after an intravenous injection of 0·5–1 mg. of eserine per kg. After the dog had received a somewhat larger dose, such as a total of 1·5–2 mg. of eserine per kg., injected over a period of 20–30 min., the c.s.f. became active, so that a sample taken 10 min. later caused contraction of the leech. With such intermediate dosage of eserine, however, subsequent samples always showed a progressive decline in activity. The administration of a further quantity of eserine to the animal then evoked a renewed increase of activity of the c.s.f.

After a larger dose, such as 4–4·5 mg. of eserine per kg., injected in the course of 20–30 min., the action on the leech of subsequent samples of the c.s.f., collected at intervals of 10 min., was not only greater than after smaller dosage with eserine, but increased progressively in the second and

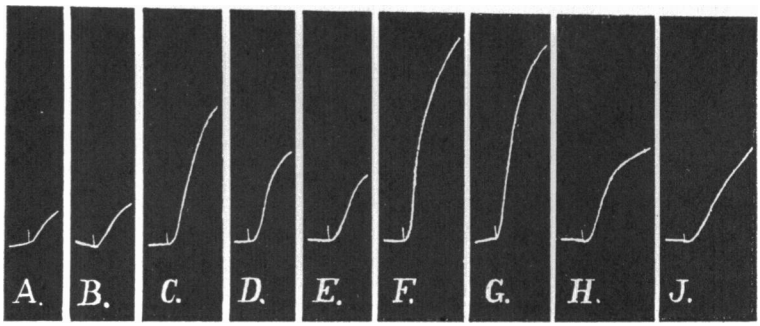

Fig. 1. Leech in eserine 1 in 5×10^5. A–H, c.s.f. of dog in 20 p.c. dilution. J, ACh. 1 in 5×10^8.

third samples. Fig. 1, A–E, shows the effect of five successive samples of c.s.f. in 20 p.c. dilution, taken at 10–20 min. intervals. The dog had been given 3 mg. of eserine per kg. in all, the injection being completed 15 min. before sample A was taken. The activity of the most potent samples of such c.s.f. corresponded in some experiments to an acetylcholine concentration of 0·015γ per c.c., or about 1 in 7×10^7.

The fact that the c.s.f. only became active after the intravenous injection of eserine strongly suggested that the activity was due to acetylcholine. This was further substantiated by the following observations. The fluid was inactive on the non-eserinized leech preparation; it caused a fall of pressure in the cat, which was abolished by atropine; the depressor action and the action on the leech were destroyed by alkali; and, finally, the assay of the same sample of fluid on the leech and the blood-pressure gave closely corresponding values in terms of acetylcholine. We

shall see later that adrenaline causes an increase in, or the appearance of acetylcholine in the c.s.f. This action of eserine, however, is not due to adrenaline output, since it was unchanged by removal of the suprarenals.

(*b*) *Adrenaline.* Slow intravenous infusion of 5–15 c.c. of adrenaline 1 : 10,000 or 1 : 20,000 into dogs treated with eserine, caused the appearance of acetylcholine in the c.s.f. The samples were taken at the end of the adrenaline infusion or shortly afterwards. If the c.s.f. had already some activity, due to the eserine, adrenaline caused a significant increase. Subsequent samples taken at 10–15 min. intervals showed a gradual disappearance of the acetylcholine in the c.s.f. Adrenaline was ineffective if injected prior to the administration of eserine; the effect could sometimes

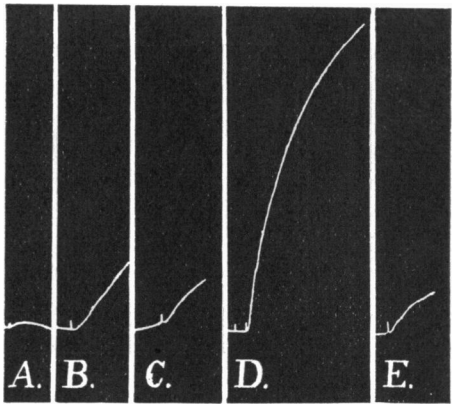

Fig. 2. Leech in eserine 1 in 5×10^5. B and C, 30 p.c. arterial blood; A, D and E, 30 p.c. c.s.f. A and B collected before, C and D during, E, 50 min. after adrenaline infusion.

be observed, however, after 0·5–0·8 mg. of eserine per kg. The greater the amount of eserine injected before the adrenaline infusion, the stronger the effect of adrenaline and the slower the disappearance of acetylcholine from subsequent samples. Concentrations of acetylcholine as high as 1 in 4×10^7 were observed. The effect of adrenaline could be repeatedly obtained in the same dog.

The acetylcholine appearing in the c.s.f. after adrenaline does not simply diffuse from the blood into the c.s.f., as no simultaneous rise in the acetylcholine content of the arterial or venous blood could be observed after adrenaline. Fig. 2 shows the effect on the eserinized leech of blood collected from the femoral artery and of c.s.f., both in 30 p.c. dilution. A dog weighing 18·6 kg. had received three injections of 10 mg. of eserine and 1 mg. of atropine, the last injection being given 15 min. before the first

samples were taken. The blood (B) and the c.s.f. (A) produced only slight effects. Then 15 c.c. of adrenaline 1: 20,000 were slowly injected into the dog, causing a rise of blood-pressure. During the last minute of the adrenaline infusion new samples of blood and of c.s.f. were taken. The c.s.f. now had (at D) an intense action on the leech, whereas the blood (at C) had an effect a little weaker even than that of the first sample. A sample of c.s.f. taken 50 min. later shows that the effect (at E) had now practically disappeared.

The effect of adrenaline is not due to the rise of arterial blood-pressure, as it could be obtained if the pressor effect was compensated, although in these conditions the appearance and disappearance of acetylcholine in the c.s.f. was definitely delayed. For instance, Fig. 1, F–H, shows the assay on the leech of samples of c.s.f. taken after the slow infusion into a dog of 15 c.c. of adrenaline 1: 20,000, the rise of pressure being compensated. One sample (F) was taken immediately at the end of the infusion, a second (G) 10 min., and a third (H) 30 min. later. Sample G contained as much acetylcholine as sample F. In many experiments the second and third samples actually contained more acetylcholine than the first, whereas in the absence of a compensator the second sample was always weaker. This difference might be due to the fact that the rise of pressure normally caused by adrenaline mechanically expresses the c.s.f. [cf. Dixon and Halliburton, 1913]. It was always observed that the fluid flowed from the cannula more quickly during an adrenaline rise of pressure.

(*c*) *Asphyxia.* Asphyxia causes the appearance of acetylcholine in the c.s.f. of dogs, to which eserine has been administered. In the absence of eserine, asphyxia has no effect of this kind. The amounts of eserine required were the same as those used for adrenaline, and insufficient by themselves to cause a progressive rise in the acetylcholine content of the fluid.

Asphyxia causes a rise of arterial blood-pressure in which an output of adrenaline may participate. The appearance of acetylcholine in the c.s.f. is, however, not due to these factors. It could be obtained in dogs from which the suprarenals had been removed and the effect of a rise of pressure could be excluded by taking a sample of c.s.f. before the blood-pressure rose. The pressor effect of asphyxia did not appear until the trachea was opened. This is shown in Fig. 3 which is taken from a 30 kg. dog. The suprarenals had been removed, and 17 and 10 mg. of eserine had been injected 45 and 15 min. before the collection of the first sample. This contained no acetylcholine (A). Sample II taken just before, and sample III taken just after the clamp had been removed from the trachea

caused a contraction of the leech (B and C) nearly as great as that produced by acetylcholine 1 in 10^8 (D). The effect of sample III is slightly

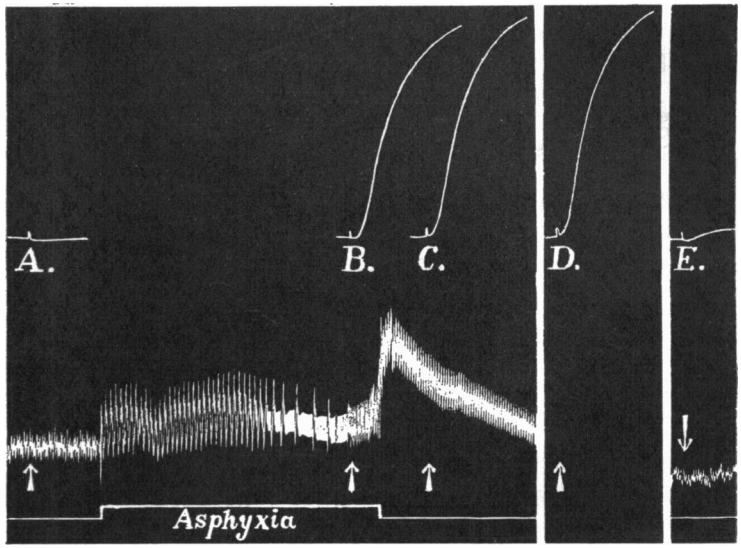

Fig. 3. Leech in eserine 1 in 5×10^5 and dog's blood-pressure. Effects of c.s.f. in 50 p.c. dilution on leech (A) before (B, C) during and (E) 50 min. after asphyxia. D, ACh. 1 in 2×10^8.

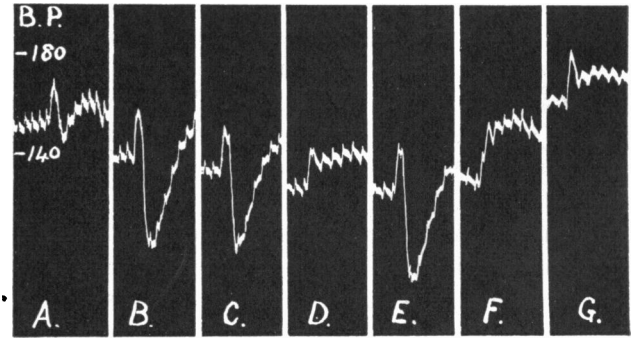

Fig. 4. Cat, chloralose, eviscerated. Blood-pressure: A, B, D, E, G, 2 c.c. c.s.f. A before, B, D, E and G during asphyxia. D made alkali, E and G made acid. F, 2 c.c. arterial blood. C, 0·04γ, ACh. in 2 c.c. Between F and G, 2 mg. of atropine.

greater than that of sample II. A similar delay in the appearance of acetylcholine has been described for the experiments with adrenaline, in which the rise of arterial pressure was compensated. An hour later, the activity of the c.s.f. had again disappeared (E).

In Fig. 4 c.s.f. and blood from the femoral artery were collected simultaneously during asphyxia and tested on the cat's arterial blood-pressure. The experiment shows that the acetylcholine in the c.s.f. does not originate directly from the blood. The 22 kg. dog had received 45 mg. of eserine in all. A shows the effect of the c.s.f. collected before the asphyxia was started, the slight depressor action being due to acetylcholine which had not completely disappeared from a previous asphyxia, carried out 50 min. previously. The slight effect contrasts well with the powerful depressor action of the sample collected during asphyxia and tested at B. Parts of the same sample were made alkaline and acid, kept at room temperature for 20 min., then neutralized and injected at D and E. The alkali had removed the whole depressor action. F shows the effect of 2 c.c. of blood collected during the asphyxia; it had no depressor action. G shows the abolition of the depressor action by atropine. The depressor effect corresponded in this experiment to that of acetylcholine 1 in 5×10^7. Asphyxia always caused the appearance of more acetylcholine than did adrenaline; a concentration as great as 1 in 2×10^7 was sometimes found in the c.s.f.

(*d*) *Central vagus stimulation.* After the intravenous injection of eserine in doses (0·5–2·5 mg. per kg.), sufficient to cause the appearance of acetylcholine in the c.s.f. after adrenaline or asphyxia, central vagus stimulation had no such effect. In certain earlier experiments results were obtained which suggested that, after larger doses of eserine, central vagus stimulation caused the appearance of acetylcholine in the c.s.f., even when the suprarenals had been removed and a rise of blood-pressure prevented. It was later found, however, that we had been misled by the fact, then not known, that such large doses of eserine might by themselves produce a progressive increase of acetylcholine concentration in the successive samples (see, for instances, Fig. 1). Repetition of the central vagus stimulation after the acetylcholine content of the fluid had begun to decline did not cause a renewed rise of acetylcholine in the c.s.f., so that the earlier observed effects were due to the eserine and not to the vagus stimulation.

Discussion.

So far as concerns the suggested effect of sensory impulses in the vagus nerve, in causing the appearance of acetylcholine in the c.s.f., the results of our experiments are negative. We have put them on record, partly as a warning to others who may work in this field, of the possibility of being misled by effects of excessive doses of eserine in themselves. The appearance of acetylcholine in the c.s.f. in response to adrenaline or asphyxia was

a genuine effect, being observed after doses of eserine which were not themselves effective in causing its appearance.

We have as yet no data which would warrant speculation as to a relation between this output of acetylcholine after adrenaline and asphyxia and any functional changes in the nerve centres.

SUMMARY.

1. After intravenous injections of eserine, acetylcholine appears in the c.s.f. of dogs, disappearing again after some time; its concentration is dependent on the amount of eserine injected previously.

2. *Adrenaline* and *asphyxia* cause the appearance of acetylcholine or an increase of its concentration in the c.s.f., but only after the administration of eserine. *Central vagus stimulation* had no effect of this kind after eserine in doses which were not sufficient by themselves to produce a progressive increase of acetylcholine in the fluid.

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