CCLXIII. THE INHIBITORY ACTION OF ESERINE UPON CHOLINE-ESTERASE *IN VIVO*.

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LOEWI [1921] showed that on stimulation of the vagus supply to the frog's heart a substance, indistinguishable from acetylcholine, could be demonstrated in minute amounts in the fluid filling the heart. More recently Dale [1934] and Dale and Feldberg [1934] have been able to demonstrate that parasympathetic impulses in the peripheral nervous system are transmitted by the liberation of a substance which they have definitely identified as acetylcholine. Further, it has been shown [Feldberg and Gaddum, 1934; Feldberg and Vartiainen, 1935] that stimulation of the pre-ganglionic fibres of the superior cervical ganglion of the cat causes the liberation of acetylcholine in the immediate neighbourhood of the ganglion cell, and this appears to be responsible for the transmission of the impulse to the post-ganglionic fibres.

Feldberg *et al.* [1934] have shown that a stimulus to the splanchnic nerves is chemically transmitted to the effector cells in the suprarenal medulla by the liberation of something indistinguishable from acetylcholine and is, in all probability, the direct stimulant of the medullary cells to secrete adrenaline. Thus the importance of acetylcholine as a humoral transmitter of parasympathetic effects and, in some instances, also of sympathetic impulses has been fully demonstrated. It has been shown by Loewi and Engelhart [1930] that acetylcholine is destroyed in the body by an agent of an enzymic nature. This point has been investigated by Stedman and Stedman [1931] who have called this enzyme choline-esterase and suggested that its action is specific. It has been shown further [Stedman and Stedman, 1932; Loewi and Navratil, 1926; Matthes, 1930] that eserine inhibits *in vitro* the action of choline-esterase upon acetylcholine whereas pilocarpine [Stedman and Stedman, 1931] does not.

In the present paper the choline-esterase is measured by a modification of the method described by Stedman and Stedman at the Glasgow meeting of the Biochemical Society over a year ago, and published by them in another paper in this number of the *Biochemical Journal*. The method is based on that of Krebs and Henseleit [1932], but using the Barcroft differential manometer. It depends on the evolution of CO_2 from physiological salt solution by the acetic acid split off from the acetylcholine used as substrate. The experimental details are as follows:

One ml. of a 1:5 dilution of blood serum is placed in the reaction flask together with 1 ml. of the physiological salt solution and in the control flask 2 ml. of the salt solution. In the side-bulb of each flask is placed 1 ml. of a 2·5 % solution of acetylcholine chloride in distilled water. The apparatus is filled with a gas mixture of 95 % O_2 and 5 % CO_2 composition and is then shaken very gently

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in a thermostat at 37° until equilibrium is attained (about 10 min.). The taps are then closed and the acetylcholine solution is added to the main flask. The apparatus is shaken at about 120 oscillations per minute and readings of the manometer are taken at $2\frac{1}{2}$ minute intervals. The activity of the serum is then calculated from the formula:

$$\text{C.E.} = \frac{h \times k \times P/760 \times c}{T}.$$

In this formula, h is the difference of levels in mm. for the time interval T in minutes, on the straight line portion of the curve when h is plotted against T; P is the barometric pressure; c the concentration of the serum and k the constant for the apparatus found by the Münzer and Neumann [1917] method, quoted by Dixon [1934]. The unit thus represents μl. CO_2 per minute per ml. of serum, the volumes being corrected for pressure but not for temperature as they are all estimated at 37°.

With a view to demonstrating this inhibitory action of eserine *in vivo* a group of 12 cases was taken, the conditions standardised as to diet, all drug treatment was discontinued, and all cases were investigated while in bed. 5 ml. of venous blood were removed and a subcutaneous injection of eserine sulphate grs. 1/50 then administered. A further 5 ml. of blood was removed when a definite physiological response to the drug had been obtained. There was in some cases an initial lowering of the blood pressure and slowing of the pulse but this was in most cases followed by a rise of pulse rates and blood pressure, and in 4 cases vomiting occurred. Table III shows the variation in blood pressure and pulse rate from the initial values at the time when the second sample of blood was drawn. The times elapsing between the removal of the 2 specimens of blood varied from 10 to 35 min. The results obtained by using eserine are given in Table I.

As pilocarpine stimulates the parasympathetic, but not by the inhibition of choline-esterase, it appeared to furnish an ideal method of control. The same procedure as described above was adopted on the day following the eserine test, this time using pilocarpine nitrate grs. 1/10. The blood pressure did not vary to any marked extent from the initial reading but there were, in most cases, a definite rise in the pulse rate and salivation, while all cases showed sweating. The control figures are shown in Table II. Further, as the rise of blood pressure found in several of the cases after injecting eserine may have been partly the result of an adrenaline response (Sollmann [1932] states that eserine increases the

Table I. *Response to eserine.*

Case	Age	Diagnosis	Choline-esterase		Variation
			Before eserine	After eserine	
1	62	Paranoia	99	81	−18
2	38	Schizophrenia	78	66	−12
3	48	Agit. depression	81	68	−13
4	56	Invol. melancholia	26	19	− 7
5	52	Agit. depression	75	56	−19
6	47	Invol. melancholia	66	56	−10
7	21	Melancholia	70	54	−16
8	18	Melanch. stupor	53	55	+ 2
9	41	Anxiety state	80	63	−17
10	37	Anxiety state	81	68	−13
11	44	Paranoia	54	44	−10
12	55	Diss. sclerosis	93	78	−15

The limit of experimental error is of the order of 2 units.

Table II. *Response to pilocarpine.*

Case	Choline-esterase: Before drug	Choline-esterase: After drug	Variation
1	110	111	+1
2	88	87	−1
3	76	78	+2
4	26	25	−1
5	82	82	0
6	71	71	0
7	61	61	0
8	52	51	−1
9	82	80	−2
10	78	78	0
11	49	49	0
12	92	99	+7

Table III. *Response to eserine.*

Case	Alteration in pulse		Alteration in B.P.		Time in min.
1	120	104	228/125	218/120	20
2	54	52	120/70	110/70	20
3	78	82	116/70	110/70	15*
4	80	90	128/78	140/92	35*
5	90	94	180/110	175/115	20
6	80	68	132/82	120/75	15
7	90	126	125/75	140/90	20*
8	56	62	110/65	116/75	10
9	50	62	105/70	112/65	20
10	60	60	120/70	115/65	20
11	70	70	132/85	128/82	20*
12	64	78	125/105	130/98	15

* Vomiting.

Table IV. *Response to adrenaline after fifteen minutes.*

Case	Alteration in pulse		Alteration in B.P.		Choline-esterase: Before adrenaline	Choline-esterase: After adrenaline	Variation
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	78	84	115/76	120/80	67	77	+10
4	84	96	132/78	140/78	13	14	+1
5	92	94	160/105	184/110	66	66	0
6	86	87	132/72	112/62	68	68	0
7	88	94	132/80	138/80	75	75	0
8	58	74	108/60	130/45	48	56	+8
9	56	60	108/58	114/56	76	83	+7
10	62	66	120/68	130/60	80	80	0
11	76	90	140/85	162/90	54	61	+7
12	68	72	138/90	145/78	84	84	0

adrenaline output in contrast to pilocarpine) it seemed desirable to control the same group of cases with adrenaline. This was done, using m. 7 of a 1 : 1000 solution of adrenaline hydrochloride, and the results obtained are given in Table IV. Two cases were not investigated as, in one case, the patient objected to further interference, while in the other (Case 1) the blood pressure was considered to be dangerously high.

SUMMARY.

1. It has previously been shown *in vitro* that eserine inhibits the action of choline-esterase on acetylcholine and that pilocarpine, though acting in a somewhat similar manner by producing parasympathetic response, nevertheless does not do so by inhibiting choline-esterase.

2. That these facts obtain *in vivo* has been shown by us.

3. Adrenaline, in an amount sufficient to produce definite alteration in the blood pressure, caused no lowering of the choline-esterase activity.

4. The above facts corroborate the theory that eserine acts on the parasympathetic by virtue of its inhibition of choline-esterase, leading to a prolongation of the action of acetylcholine present.

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REFERENCES.

Dale (1934). *Brit. Med. J.* **1**, 835.
—— and Feldberg (1934). *J. Physiol.* **81**, 320.
Dixon (1934). Manometric methods. (Cambridge.)
Feldberg and Gaddum (1934). *J. Physiol.* **81**, 305.
—— Minz and Tsudzimura (1934). *J. Physiol.* **81**, 286.
—— and Vartiainen (1935). *J. Physiol.* **83**, 103.
Krebs and Henseleit (1932). *Z. physiol. Chem.* **210**, 33.
Loewi (1921). *Pflüger's Arch.* **189**, 230.
—— and Engelhart (1930). *Arch. exp. Path. Pharm.* **150**, 1.
—— and Navratil (1926). *Pflüger's Arch.* **214**, 689.
Matthes (1930). *J. Physiol.* **70**, 338.
Münzer and Neumann (1917). *Biochem. Z.* **81**, 319.
Sollmann (1932). Manual of pharmacology. (W. B. Saunders Co., Philadelphia and London.)
Stedman and Stedman (1931). *Biochem. J.* **25**, 1147.
—— —— (1932). *Biochem. J.* **26**, 1214.
—— —— (1935). *Biochem. J.* **29**, 2107.