THE ANTAGONISM OF ACETYL CHOLINE BY METHYLENE BLUE.

By R. P. COOK.

(*From the Department of Pharmacology, University College, London.*)

STRAUB(1), after working with muscarine and atropine on the hearts of *Aplysia* and the frog, concluded that their action depended on the concentration of the drug around the cells and that the entrance of the drugs into the cells actually antagonised the action, which they produced on the surface. Recently Clark(2) showed that the amount of action produced by acetyl choline did not appear to be proportional to the amount of drug taken up by the cell and concluded that two independent processes occurred when acetyl choline acted on the frog's heart, viz. (*a*) the action of the drug on the cell, probably a surface action, and (*b*) the entrance of the drug into the cell.

This relation can best be studied with a dye, methylene blue being a very suitable agent because in moderate concentrations it paralyses the vagus (Heymans and Maigre(3), (4)) without producing any injury to the heart cells. In the experiments described below isolated hearts of *Rana temporaria* were used. They were prepared with a Straub cannula containing 1 c.c. of Ringer's solution. The Ringer's solution was of the same composition as that used by Clark(2). The isotonic movements of the ventricle were measured.

Methylene blue was found to antagonise the action of acetyl choline

TABLE I. Antagonism of acetyl choline by methylene blue.

The table shows the percentage reduction in response of the heart produced by varying the concentration of acetyl choline.

Molar concentrations of methylene blue	Molar concentrations of acetyl choline							
	9×10^{-8}	$2{\cdot}6 \times 10^{-7}$	8×10^{-7}	$2{\cdot}4 \times 10^{-6}$	7×10^{-6}	$2{\cdot}2 \times 10^{-5}$	6×10^{-5}	2×10^{-4}
Nil.	30	47	67	70	85	—	—	—
6×10^{-7}	32	43	60	70	—	—	—	—
3×10^{-6}	15	20	30	63	71	—	—	—
6×10^{-6}	—	—	9	21	56	71	94	—
3×10^{-5}	—	—	—	14	23	28	50	67

on the frog's heart in a manner similar to atropine. Table I gives a series of measurements of the effects produced by acetyl choline in the presence

of varying amounts of methylene blue, and Fig. 1 gives a graphical interpretation of these results.

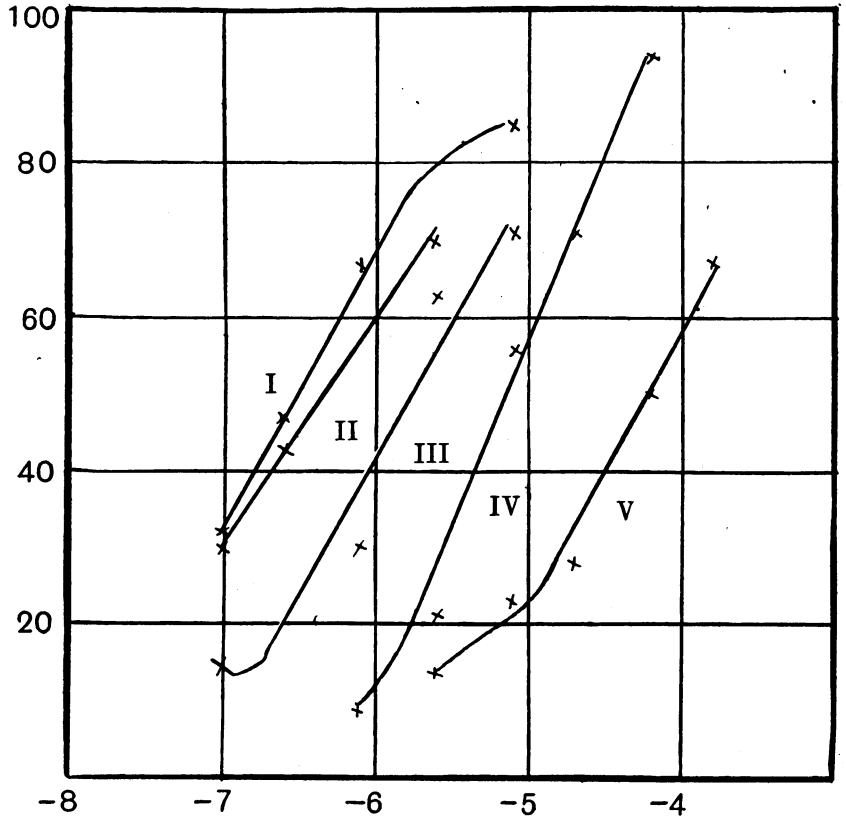

Fig. 1. Antagonism of acetyl choline by methlyene blue.

Action of acetyl choline (I) in absence of methylene blue; (II) in presence of methylene blue 6×10^{-7} molar; (III) in presence of methylene blue 3×10^{-6} molar; (IV) in presence of methylene blue 6×10^{-6} molar; (V) in presence of methylene blue 3×10^{-5} molar.

Ordinate: percentage reduction in response of heart.
Abscissa: log molar concentration of acetyl choline.

The quantities of acetyl choline which produce a 50 p.c. reduction in the response of the heart in the presence of methylene blue are shown in Fig. 2. Clark(5) showed that, when atropine and acetyl choline act on the frog's heart, equal effects are produced as long as the ratio

$$\frac{\text{concentration of acetyl choline}}{\text{concentration of atropine}}$$

remains constant. The curve in Fig. 2 actually follows the relation that constancy of the ratio

$$\frac{\text{(concentration of acetyl choline)}}{\text{(concentration of methylene blue)}^{1\cdot25}}$$

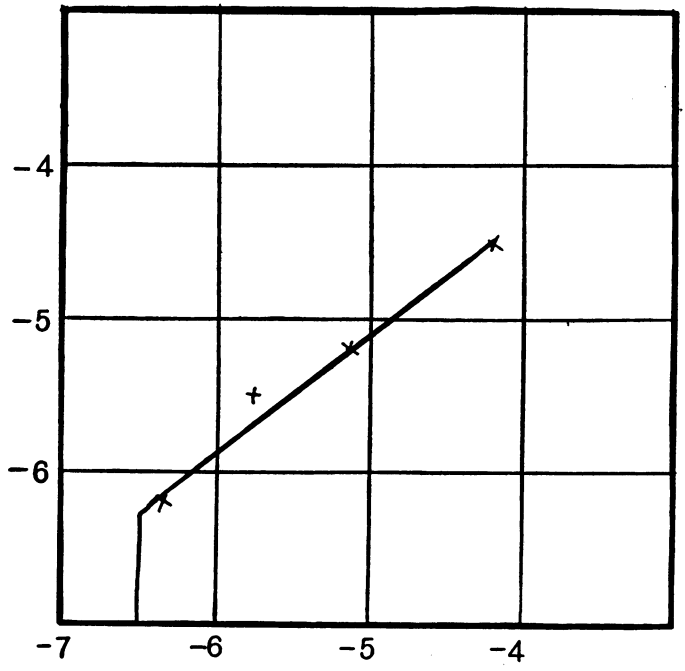

Fig. 2. Quantities of acetyl choline necessary to produce a 50 p.c. reduction in the heart's response.

Ordinate: log. molar concentration of methylene blue.
Abscissa: log. molar concentration of acetyl choline.

leads to constant results. The experiments, however, were not sufficiently extensive to justify any definite conclusion as to whether in the formula

$$\frac{\text{(conc. Ac. Ch.)}}{\text{(conc. M.B.)}^n} = \text{constant},$$

n is unity or more than unity.

The frog's heart is stained a deep blue with the concentrations of dye used in these experiments and it is not possible to wash out a noticeable quantity of the dye with frequent changes of Ringer in several hours. When the dyed hearts were examined microscopically the dye was found to be present in the interior of the muscle cells and also the nerve endings were deeply stained. This condition remained unchanged

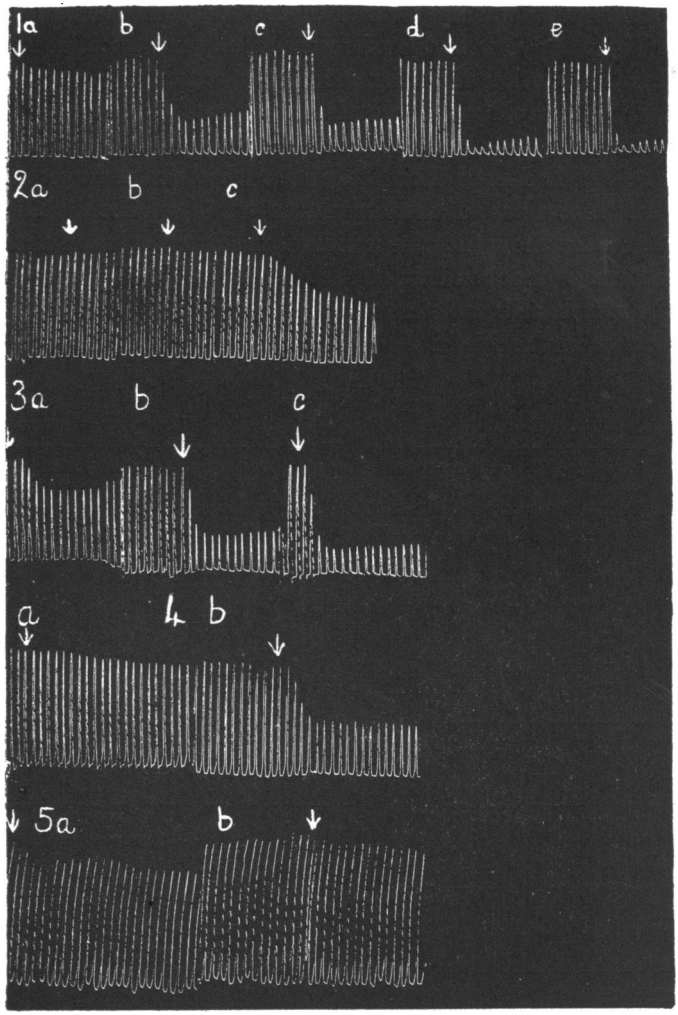

Fig. 3. Heart under artificial rhythm of 20 shocks per min.

(1) Action of acetyl choline in absence of methylene blue. Molar concentrations: (*a*) 5×10^{-8}; (*b*) 5×10^{-7}; (*c*) 5×10^{-6}; (*d*) 5×10^{-5}; (*e*) 5×10^{-4}.

(2) Methylene blue 3×10^{-5} molar and acetyl choline: (*a*) 5×10^{-7}; (*b*) 5×10^{-6}; (*c*) 5×10^{-5} molar.

(3) Methylene blue washed out with Ringer's solution for the following times: (*a*) 1 min.; (*b*) 7 mins.; (*c*) 17 mins.; acetyl choline 5×10^{-5} molar added in each case.

(4) Methylene blue 3×10^{-5} molar (following 3) and acetyl choline: (*a*) 5×10^{-5}; (*b*) 5×10^{-4} molar.

(5) Heart washed out after 4 and then methylene blue 3×10^{-6} added: (*a*) acetyl choline 5×10^{-6} molar added at once after methylene blue, and (*b*) the same 4 mins. later.

even after frequent washings with Ringer's solution. The staining effects produced by the dye were therefore practically irreversible, but in spite of this fact, the action of methylene blue in antagonising acetyl choline was found to be rapidly and completely reversible.

Fig. 3 shows the action of acetyl choline on a frog's heart after methylene blue had been alternately introduced and removed. The heart during the greater part of the experiment was dyed deeply blue.

These results show that the action of methylene blue in antagonising acetyl choline is independent of the quantity of methylene blue taken up by the muscle cells and nerves, and appears to depend entirely on the concentration of the dye in the fluid around the cells. This suggests that

TABLE II.

Rate of action of methylene blue		Rate of wash-out of methylene blue	
Time (in mins.) since introduction of drug	Percentage reduction produced by 5×10^{-5} molar acetyl choline	Time (in mins.) since removal of drug	Percentage reduction produced by 5×10^{-5} molar acetyl choline
0	83	1	36
2	43	3	43
5	19	5	53
10	13	7	64
—	—	9	71
—	—	13	73
—	—	17	80

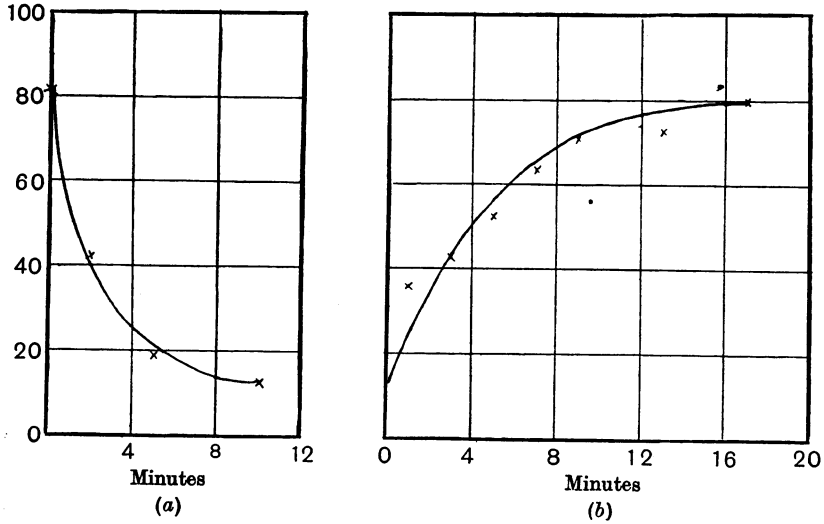

Fig. 4. (*a*) Rate of action of methylene blue; (*b*) rate of wash-out of methylene blue.

Ordinate: percentage reduction in response of heart when 5×10^{-5} molar acetyl choline was added.

antagonism is due to the methylene blue producing some freely reversible surface action on the cells.

Table II and Fig. 4 show the results of experiments made to measure the rate of action and the rate of wash-out of the methylene blue as indicated by the response to acetyl choline. These figures show that methylene blue produces half its final action in about 2 minutes and that the abolition of action on removal of the dye proceeds at a similar rate.

Accurate measurements of the rate of adsorption of methylene blue by the heart were very difficult to obtain. 1 c.c. of fluid containing methylene blue was introduced into the heart, the amount of dye remaining in the fluid was determined colorimetrically, and the amount of dye taken up by the heart was thus estimated. The results showed that the concentration of dye in the heart cells was from 20 to 200 times the concentration in the fluid around the cells. The adsorption of the dye proceeded slowly but was nearly complete at the end of an hour. The wash-out of the dye, if it occurred at all, proceeded too slowly to be measurable.

Conclusions.

(1) Methylene blue antagonises the action of acetyl choline, and equal effects are produced by acetyl choline in the presence of methylene blue when the ratio $\frac{\text{concentration of acetyl choline}}{(\text{concentration of methylene blue})^n}$ remains constant. The value of n is either unity or slightly more than unity.

(2) The heart cells adsorb methylene blue slowly and the action is practically irreversible.

(3) The action of methylene blue in antagonising acetyl choline is produced rapidly and is removed equally rapidly by washing out the methylene blue. A heart can regain its full sensitivity to acetyl choline although deeply stained with methylene blue.

(4) This antagonism therefore is independent of the entrance of the dye into the nerve and muscle cells; the dye appears to produce its antagonism to acetyl choline by a freely reversible action on the surface of the cells.

I should like to take this opportunity of thanking Prof. A. J. Clark for the suggestions and help he has given me in this research.

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