ACTION OF ACETYLCHOLINE, ATROPINE AND ESERINE ON THE CENTRAL NERVOUS SYSTEM OF THE DECEREBRATE CAT

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The investigations carried out in recent years by different groups of workers [Schweitzer & Wright, 1937 b, c; Schweitzer, Stedman & Wright, 1939; Kremer, Pearson & Wright, 1937; Kremer, 1942; Bonnet & Bremer, 1937; Torda, 1940; Bülbring & Burn, 1941] have proved conclusively that both acetylcholine and eserine have a direct action on the central nervous system in various species (man, dog, cat, frog, toad). There are, however, considerable differences in the results obtained in different species and on the different preparations employed. Thus, Schweitzer & Wright [1937 b, c], working on the knee jerk of cats under chloralose anaesthesia, found that acetylcholine produced generally a transient central inhibition (intensified by eserine), though sometimes an initial stimulation was observed. Bülbring & Burn [1941], working on the isolated perfused spinal cord of the dog, obtained with acetylcholine a motor discharge from the resting spinal cord; the knee jerk showed transient depression followed by stimulation, and the flexor reflex was potentiated. Kremer [1942] injected acetylcholine intrathecally in man in doses from 2 to 500 mg. without result, though, when the drug was administered together with subliminal doses of prostigmine, it depressed muscle tone and reflexes by a central action. Turning to eserine, Schweitzer & Wright [1937 b, c] found that the drug markedly increased the knee jerk and produced convulsions in chloralosed cats. Bülbring & Burn [1941], on the contrary, obtained with the isolated perfused spinal cord of the dog depression of the knee jerk, and potentiation of both the motor and inhibitory effects of acetylcholine. We have examined the actions of acetylcholine, atropine and eserine on the central nervous system in the decerebrate cat.

METHODS

The preliminary procedures were carried out under ether-chloroform anaesthesia. The sciatic nerves to both hindlimbs were cut high up to exclude central effects on the flexor muscles. Drills were passed into the upper and lower ends of both femurs. The left subclavian artery was isolated and its

branches to the muscles of the forelimbs, the costocervical artery and thyro-cervical axis were tied; the only branches left open were the internal mammary and vertebral arteries. Injections of acetylcholine were made into the central end of the subclavian artery so prepared; eserine was injected intravenously. In analysing the site of action of the drugs (whether central or peripheral) two procedures were employed: (a) the contralateral quadriceps muscle was denervated; (b) the hindlimbs were made ischaemic by clamping the abdo-minal aorta, usually after preliminary ligation of its branches below the renal arteries, as described by Schweitzer & Wright [1937c]. After completing these measures, the cat was decerebrated at the level of the anterior colliculi and allowed to rest for $\frac{1}{2}$–1 hr., so that the anaesthetic might be eliminated. Tension changes in the quadriceps muscles were recorded by connecting the ankle by means of threads running over pulleys to Sherrington torsion lever myographs. The knee jerk was elicited by the electrically operated device of Schweitzer & Wright [1937a]. Acetylcholine solutions were made up in 5% NaH_2PO_4 and diluted before use in acid saline (pH 4–4·5). Control injections of the acid saline alone, either intra-arterially or intravenously, had no effect on the nervous reactions studied.

<div align="center">RESULTS</div>

<div align="center">*Action of acetylcholine on quadriceps muscle tone*</div>

Acetylcholine injected intra-arterially into the central end of the subclavian artery increases the tension of the unstimulated innervated quadriceps muscle (Figs. 1, 2). The dose producing the response is 25–100 μg. The effect comes on almost without detectable latent period; the tension rises abruptly to a degree which varies considerably in different preparations. The maximum response recorded showed a tension of 1·7 kg. above the base-line tension due to the decerebrate rigidity itself. The relaxation phase may be completed rapidly in 10–20 sec. or be more prolonged (40–60 sec., or even up to 120–150 sec.). There may be an initial phase of extensive rapid relaxation followed by a drawn-out phase in which the relaxation gradually becomes complete; or relaxation may proceed uniformly in step-like falls with intermediate short plateaux; or intermediate curves may be obtained (Fig. 1 A–D). No tension change whatsoever takes place in the denervated contralateral quadriceps. The rise of tension coincides with the initial rise of arterial blood pressure, which is mechanically produced by the injection of fluid into the circulation; the muscle-tension peak is attained before the fall of blood pressure due to the acetylcholine sets in. There is no relationship between the degree, duration or general pattern of the blood-pressure fall or the rate of recovery and the character of the relaxation curve of the quadriceps. Respiration is often stimulated, becoming deeper and faster [Schweitzer & Wright, 1937b], and over-ventilation may outlast the changes in the quadriceps; the respiratory response does not seem to influence the reaction of the quadriceps.

In addition to the response in the hindlimb, a widespread reaction is often observed, involving, in the forelimbs, increased extension at the elbow and abduction at the shoulders; the trunk may be thrust into opisthotonus, the animal taking up a posture of 'exaggerated' decerebrate rigidity.
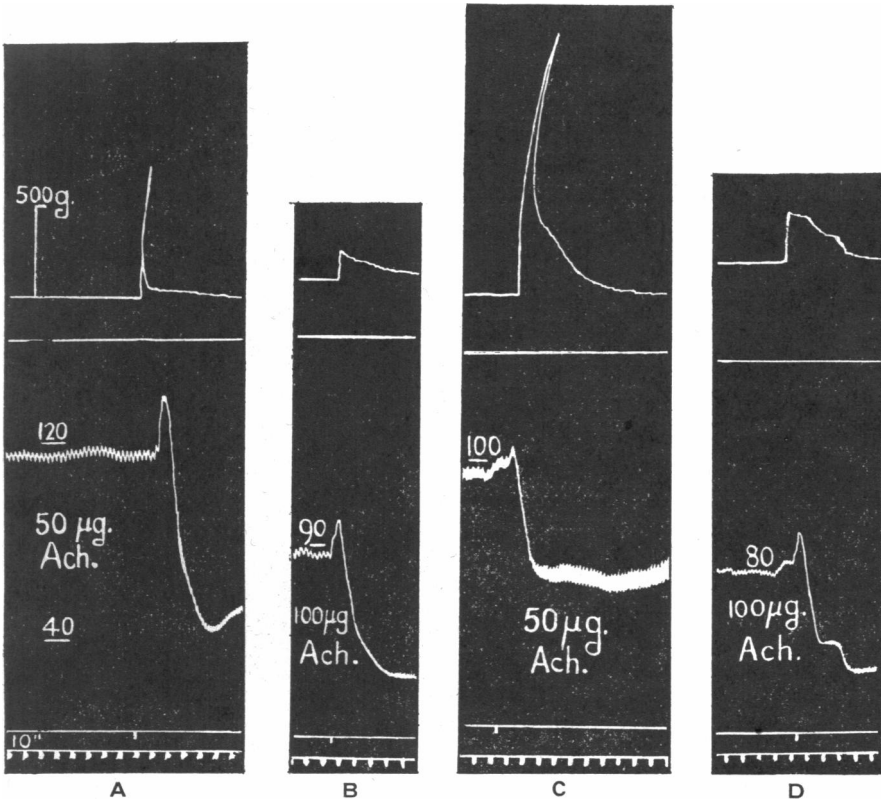

Fig. 1. Cat. Decerebrate. Sciatic nerves cut. Responses obtained in four different animals to injection of acetylcholine (50–100 μg.) into the central end of the subclavian artery. Records from above downwards: tension of innervated quadriceps; tension of denervated quadriceps; blood pressure, mm. Hg; signal; time 10 sec.

Repetition of the initial dose in the same animal may sometimes give comparable responses over a series of injections; in other preparations, however, there may be a progressive decline in the responses. The responses in the two limbs (if both are innervated) are not necessarily identical in magnitude, though similar in general form. The dose commonly producing a response was 50 μg. In about one-third of the experiments no positive reactions were obtained with doses up to 100 μg. We refrained from increasing the dose further, owing to the permanent fall of blood pressure and circulatory failure which results from such a large dose of acetylcholine, in the unatropinized

animal. Equal doses of acetylcholine injected intravenously (into the jugular vein) elicited smaller or no responses in the quadriceps muscle.

The quadriceps changes described can be readily proved to be due to a discharge from the anterior horn cells of the spinal cord:

(1) They are absent in the denervated quadriceps.

(2) They are obtained unchanged when access of the drug to the quadriceps is prevented by cutting off the blood supply to the hindlegs.

(3) As explained, they are not related to the associated circulatory and respiratory changes, but depend on the concentration of acetylcholine reaching the central nervous system.

It may be concluded that the drug acts on elements in the central nervous system, leading to increased motor-cell activity. The response is of the 'd'emblée' type (like the flexor reflex), but the duration of activity in the neurones which are initially excited presumably varies considerably, thus accounting for the gradual relaxation and the different types of relaxation curve.

Action of acetylcholine on the knee jerk

If acetylcholine is injected intra-arterially while the knee jerk is elicited from one limb, the other showing decerebrate rigidity only, both quadriceps muscles initially react in the same way by the rise of tension already described (Fig. 2). The rise of tension produced by tapping the patellar tendon above the new, raised, level of quadriceps tone is diminished initially and may be abolished. As the quadriceps relaxation phase sets in, the knee jerks become progressively bigger, and may, rarely (as in Fig. 2), become greater

Fig. 2. Cat, 2·6 kg. Decerebrate. Sciatic nerves cut. Records from above downwards: knee jerk elicited from right leg once in 9 sec.; tension of innervated unstimulated left quadriceps; blood pressure; signal; time 10 sec. At signal, 50 μg. of acetylcholine injected into the central end of left subclavian artery.

than before the injection. These changes are central in origin, and are also obtained after cutting off the blood supply to the hindlimbs. This type of result presumably depends on central occlusion. The motor-neurone pool (supplying the quadriceps) which is stimulated by the injected acetylcholine, initially includes all (or most of) the anterior horn cells stimulated during the knee jerk. At this stage therefore no (or small) summation of the acetylcholine contraction
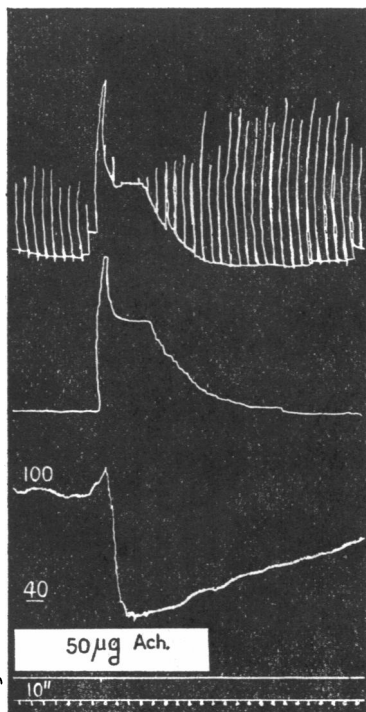
and the knee jerk takes place. As the acetylcholine effect wears off, the occlusion becomes partial; some of the cells of the knee jerk motor-neurone pool remain under sustained acetylcholine stimulation, while others are only activated during the knee jerk itself. The later potentiation may represent, perhaps, a residual acetylcholine 'subliminal' effect.

Effect of atropine on the responses to acetylcholine

Atropine in doses of 1 mg. annuls the central response to acetylcholine; the effect reaches its maximum gradually over a period of 10 min., and then this depression gradually wears off over the next 30–50 min. The results of a typical experiment are illustrated in Fig. 3; (A) shows the type of response obtained prior to atropine. After 5 min., (B), when the depressor action on the circulation was almost completely annulled, repetition of the intra-arterial injection of acetylcholine produced a central effect, which, though little diminished in magnitude, was very transient in character. At 8 min., (C), the response was almost completely abolished, though the general level of blood pressure and the circulatory reactions were similar to those observed at 5 min. At 15 and 25 min., (D) and (E), recovery was progressively taking place. At 50 min., (H), the central motor response initially exceeded the pre-injection level; the duration of the response was, however, still considerably diminished. If the total area of the myogram curve is taken as a measure of the total motor-neurone discharge under the influence of the drug, then even at this late stage some depression by atropine of the central stimulating action of acetylcholine is still evident. Sometimes, 1 hr. after the injection, spontaneous changes in muscle tone set in, consisting of fluctuations rising above the base-line. These effects of atropine are centrally produced, as the doses employed do not modify the response of the denervated muscle to acetylcholine, when appropriate amounts of that drug are given by the method of close intra-arterial injection. Though atropine ultimately annuls the action of acetylcholine, both on the central nervous system and on the circulation, the time course of the effects differs considerably in the two systems.

Action of eserine on quadriceps muscle tone

All the experiments were carried out after the injection of atropine (1 mg.). Positive results on injection of eserine (doses up to 4 mg.) were obtained in only one-third of the cases from the innervated quadriceps. Two types of response were observed:

(a) There may be a gradual progressive step-like increase in tension setting in after a latent period of 1–2 min. and reaching its maximum about 4–6 min. later. Relaxation is likewise very slow and may be incomplete after 10 min. (Fig. 4). In these cases, eserine (unlike injected acetylcholine) is gradually
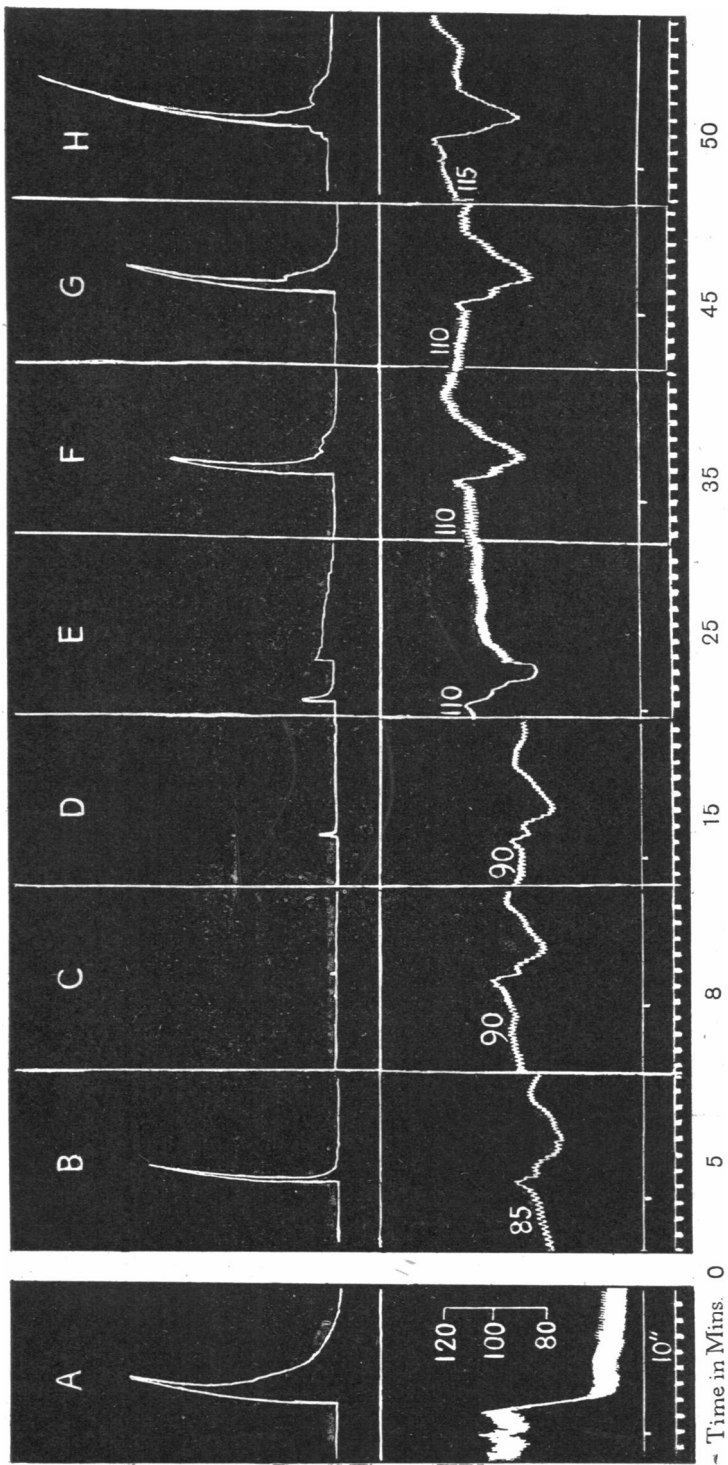
Fig. 3. Cat, 3·2 kg. Decerebrate. Sciatic nerves cut. Records from above downwards: tension of right innervated quadriceps; tension of left denervated quadriceps; blood pressure; signal; time 10 sec. At A, B, C, D, E, F, G, H, 50 μg. of acetylcholine intra-arterially. Between A and B inject 1 mg. of atropine intra-arterially.

'recruiting' motor-neurones into the pool of actively discharging cells; the duration of the discharge likewise varies greatly in different motor-neurones.

(b) On the other hand, after a similar latent period, the tension may rise rapidly to its peak, the rise being accompanied by convulsions (Figs. 5, 6). The relaxation phase may then proceed as in (a), and the decline of tone may be accompanied by the persistence or the appearance of convulsive
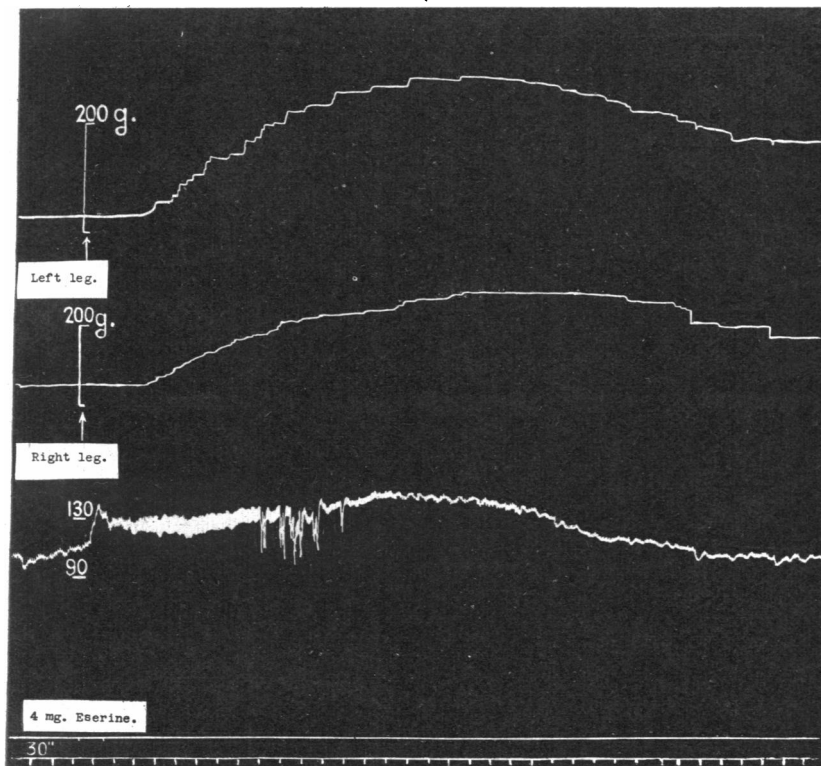

Fig. 4. Cat, 3·5 kg. Decerebrate. Sciatic nerves cut. Atropine 1 mg. Records from above downwards: tension of left innervated quadriceps; tension of right innervated quadriceps; blood pressure; signal; time 30 sec. At signal, inject 4 mg. of eserine intravenously.

movements. The maximal tensions recorded have been about 0·5 kg. above the pre-injection level. The blood pressure usually rises to a moderate extent (e.g. up to 40 mm. Hg) after a very brief latency; sometimes, with the later onset of convulsions, a further rise may take place. Respiration is commonly markedly stimulated. Extension of the forelimbs may occur; micturition or defaecation may be induced.

On repeating the injection of eserine it is unusual to get a further motor response; positive reactions to a third injection are still rarer.

The changes in muscle tone and the convulsions are due to an action of eserine on the central nervous system. They are unrelated to changes in blood pressure or respiration; they are not observed in the denervated limb; they are observed without essential differences if access of the drug to the limb is prevented by suitable occlusion technique (Fig. 6).

These responses to eserine, it must be remembered, took place in the presence of full doses of atropine.
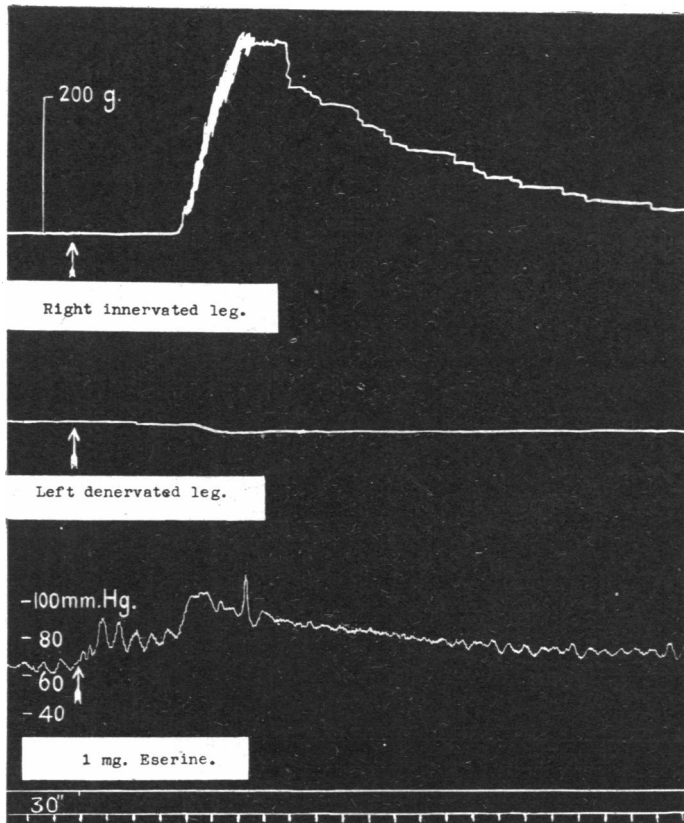

Fig. 5. Cat, 3·0 kg. Decerebrate. Sciatic nerves cut. Atropine 1 mg. Records from above downwards: tension of right innervated quadriceps; tension of left denervated quadriceps; blood pressure; signal; time 30 sec. At signal, inject 1 mg. of eserine intravenously.

DISCUSSION

The results described with acetylcholine on muscle tone are essentially the same as those obtained by Bülbring & Burn [1941] on the isolated perfused spinal cord of the dog. Schweitzer & Wright [1937 *b*, *c*] sometimes noticed an initial stimulation of the knee jerk in the chloralosed cat, but their main

finding was inhibition. In this series of experiments we observed no depression of the reflex arc of the knee jerk by acetylcholine.

Atropine annulled the central excitatory effects of acetylcholine, as it had been observed previously to annul the central inhibitory action of the drug.
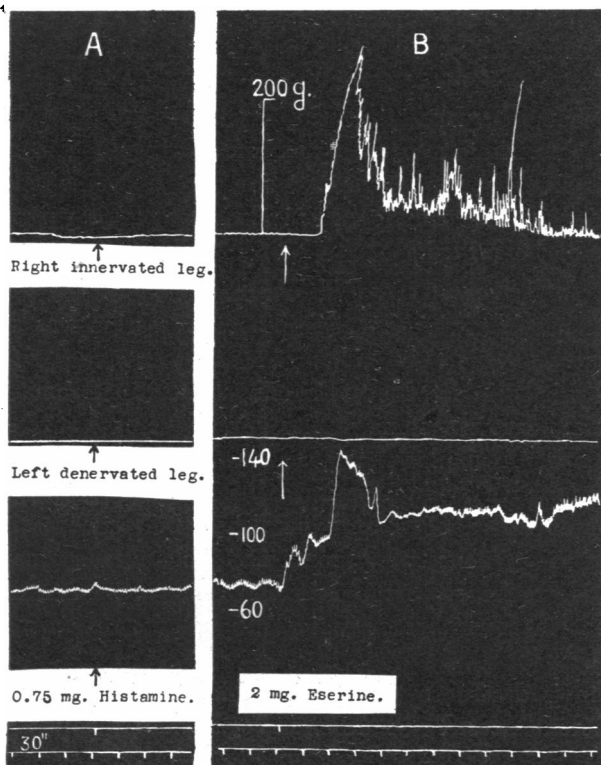

Fig. 6. Cat, 3·0 kg. Decerebrate. Sciatic nerves cut. All branches of the aorta below the renal arteries tied. Clamp on abdominal aorta throughout. Atropine 1 mg. Records from above downwards; tension of right innervated quadriceps; tension of left denervated quadriceps; blood pressure; signal; time 30 sec. At A, 0·75 mg. histamine injected intramuscularly into one leg to test the degree of ischaemia of the limbs. At B, 2 mg. of eserine injected intravenously.

The outstanding feature of the results with eserine was their unpredictability, in the sense that, in many experiments, no changes in muscle tension were produced. In the cat, under chloralose anaesthesia, eserine produces marked and even violent excitatory responses in the great majority of experiments. It seems, therefore, that chloralose in the cat considerably potentiates the central excitant action of eserine. There is evidence that eserine may have a central depressant action; Schweitzer & Wright [1937 b] found an initial central inhibitory action in a few chloralosed cats; this effect was on

occasion marked and prolonged, but was followed by the usual excitation. Bülbring & Burn [1941], working with the isolated perfused spinal cord of the dog and Merlis & Lawson [1939] with chloralosed dogs, found that eserine inhibited the knee jerk. It is conceivable that some balance of central inhibitory and excitatory actions may account for our many negative results with eserine; but we must confess that we never observed a pure inhibitory response in this series. We feel that, at this stage of our knowledge of the pharmacological action of acetylcholine and eserine on the central nervous system, care should be taken to avoid general conclusions based on a single species, or on experiments carried out under one set of conditions. It is probable that the species, the preparation, the anaesthetic employed, the response studied and other undefined factors may play a part in determining the ultimate effect observed.

SUMMARY

1. The action of acetylcholine, atropine and eserine was studied on the decerebrate cat.

2. Intra-arterially injected acetylcholine (25–100 μg.) causes a discharge from the central nervous system, resulting in an increase of tone in the innervated quadriceps. A similar increase in tension was also observed in the quadriceps from which the knee jerk was elicited.

3. Atropine at first abolishes or diminishes the response to acetylcholine for a period of 45–60 min.; later 'spontaneous' changes in tone may occur.

4. Eserine is excitatory to the central nervous system of the atropinized decerebrate cat in a number of cases, although its action is irregular and unpredictable.

5. Attention is drawn to the striking differences in the action of these drugs in different species, in different preparations. and under different anaesthetics.

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