# THE SIGNIFICANCE OF BRAIN ACETYLCHOLINE.*

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Until a few years ago there existed a sharp dichotomy of opinion between the pharmacologists, who, on the basis of their experiments, assigned to acetylcholine the role of universal synaptic transmitter, and the electrophysiologists, who denied that this substance could effect the transfer of excitation from one conducting element to another. It is, however, now generally agreed that, certainly at the neuromuscular junction, and probably also at ganglionic and some—but not all—central synapses, acetylcholine does indeed exert a primary transmitter action. It must be added that recognition of this fundamental action has been hastened as a result of recent experiments by those very electrophysiologists (such as Eccles and his colleagues) who previously supported exclusively electrical hypotheses of nervous transmission. For this reason the assumption which is implicit throughout this paper that the acetylcholine in brain is concerned in maintaining central synaptic transmission is likely to go unchallenged and requires no detailed justification here.

At the same time it is necessary to guard against the acceptance of too simple a theory of acetylcholine action in the brain, and to avoid the unrestricted application to central synapses of results derived from experiments on the anatomically simple neuro-muscular junction. It is, for instance, difficult to believe that the action-currents in the networks of pre-synaptic nerve-endings which form the " synaptic scale " of many central neurones do not influence cerebral excitability and impulse transmission in a way which is impossible at less complex junctions.

Actuated by the desire to find a unifying principle to explain the diversities of nervous activity and by the hope of designing a rational therapeutics of nervous and mental disease, many have unguardedly accepted the hypothesis that anaesthesia occurs when the synthesis of acetylcholine in the brain is inhibited, and that convulsant drugs effect their action by allowing the accumulation of acetylcholine. The experiments to be surveyed in the present paper indicate that this view is untenable, but they also point the way to other investigations which might well lead to the elucidation of one aspect of the transmitter function of acetylcholine in the brain.

A more detailed description of the methods and results of the experiments to be described, as well as a more extensive discussion of their significance, is being published elsewhere.

## Acetylcholine and Anaesthesia.

Several groups of workers had previously obtained evidence which indicated that the amount of acetylcholine in brain increases during anaesthesia (Tobias, Lipton and Lepinat, 1946; Richter and Crossland, 1949; Elliott, Swank and Henderson, 1950), but the present study has carried these results further. They can be briefly summarized as follows:

No change in the concentration of acetylcholine occurred during the induction of anaesthesia, apart from a small and variable fall during the stage of excitement. After anaesthesia developed, the amount of acetylcholine began to rise and reached, within a few minutes, a new stable level. Prolongation of anaesthesia for periods of up to two hours had no further effect on the acetylcholine concentration, which could, however, be increased by deepening the anaesthesia. These effects were observed on rats treated with seven different anaesthetics, on rabbits anaesthetized with ethyl chloride or " Dial," and on mice anaesthetized

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with ether or " Dial." It is evident that the acetylcholine changes occurred secondarily to the development of anaesthesia ; it is also clear that any inhibition of synthesis was insufficient to prevent an appreciable increase in the amount of acetylcholine presumably available for synaptic transmission. The actual percentage increase in the amount of acetylcholine in the brain differed, not only from species to species, but also in different age-groups of the same species. For any age-group of any particular species, however, the fractional increase, at any given depth of anaesthesia, was remarkably constant. Table I, for instance, shows the effect of deep anaesthesia in young rats. A more detailed investigation than that

TABLE I.—*The Effect of Deep Anaesthesia on the Acetylcholine Content of Young Rat Brain.*

(Each anaesthetized animal was paired with an unanaesthetized littermate ; the figures show the mean fractional increase in acetylcholine content, and the standard error of the mean, determined from that number of pairs of animals indicated in brackets.)

| | |
|---|---|
| Chloralose . . . | 1·54 ± 0·09 (10) |
| Chloroform . . . | 1·61 ± 0·10 ( 7) |
| " Dial " . . . | 1·58 ± 0·09 ( 8) |
| Ether . . . . | 1·62 ± 0·08 (13) |
| Ethyl chloride . . | 1·50 ± 0·08 ( 5) |
| Pentobarbitone sodium . | 1·47 ± 0·08 ( 7) |
| Thiopentone . . . | 1·59 ± 0·13 ( 7) |

whose results are recorded in Table I showed a just significant difference ($P = 0.05$) between the effects produced by ether and by pentobarbitone, but this difference probably arose because of the difficulty of preventing some anoxia in the pentobarbitone-anaesthetized animals. It was shown to occur entirely as a result of differences in the concentration produced by the two anaesthetics in the cerebral cortex, and it was not found at all in adult rats, which are known to be less susceptible to anoxia than are young animals. Moreover, the full rise in the acetylcholine concentration of the cortex could be prevented by anoxia.

Not only was there no evidence of a decrease in the acetylcholine content of whole brain during anaesthesia, but other experiments made it clear that all parts of the brain participated, though to different degrees, in the increase noted for whole brain. The pattern of distribution of acetylcholine in different regions of the anaesthetized brain was remarkably similar whether ether or pentobarbitone was used, and this may indicate that these two anaesthetic agents have similar sites of action, though they are usually regarded as acting preferentially on different regions of the brain. Thus, in light anaesthesia there was a marked rise in concentration in the cerebrum and the medulla and a smaller rise in the brain-stem. As anaesthesia deepened, the concentration in the former two regions showed a further but relatively small increase, while that in the brain-stem was more marked than before.

Further experiments excluded the possibility that these changes in acetylcholine content arose from stimulation of its synthesis, or from inhibition of cholinesterase activity, and it seems that they must have been due to the decreased liberation, consequent on the decreased central activity, of acetylcholine from neurones containing it. The fact that the acetylcholine level rose, in light anaesthesia, fairly rapidly to a stable level above that in the normal animal demands the introduction of a second assumption : that the amount of acetylcholine liberated per impulse depends on the amount actually present in the fibre. There is good evidence that this condition obtains in sympathetic ganglia (Rosenblueth, Lissak and Lanari, 1939), and no reason to doubt its validity in the central nervous system ; with deep anaesthesia it seems possible that the brain became completely " saturated " with acetylcholine, and could bind no more in the form of the inactive complex in which it is normally held. This would explain the constant increase found in deep anaesthesia brought about by different agents, as well as the fact that, notwithstanding differences in the normal animal, the acetylcholine content of the brains of anaesthetized adult and young rats were not significantly different from one another.

The changes occurring in anaesthesia are best regarded, then, as adaptations to the decreased central activity, and further consideration of their possible signi-

ficance is best deferred until after the experiments with convulsant drugs have been considered.

### Acetylcholine and Convulsive Activity.

Many attempts have been made in the past to seek evidence that convulsant drugs operate by causing an accumulation of acetylcholine in the central nervous system, but it now seems clear that no such relationship exists (Feldberg, 1945). The specifically anti-cholinesterase drugs form no real exception to this generalization, for they simply preserve endogenously liberated acetylcholine so that it can reach concentrations at which it exerts a convulsant action—and other cholinergic effects—in much the same way as if it were directly injected. It is very doubtful whether any compound with the weak anti-cholinesterase activity of many other types of drugs could, by virtue of this property alone, exert a convulsant action. At the same time it might well be that the potency of an otherwise mild convulsant would be increased if the compound also possessed some anti-cholinesterase activity. It is similarly possible, as will be mentioned later, that convulsive potency might be augmented in convulsants which also stimulate acetylcholine synthesis.

It must be emphasized that, since acetylcholine exerts powerful pharmacological actions, care must be taken in interpreting the results of observations of the effects which follow its parenteral injection. The ease, for example, with which *grand-mal* convulsions can be precipitated in the epileptic subject by the injection of acetylcholine, or of an anticholinesterase drug, bears witness to the abnormal irritability of the brains of these patients to any excitatory substance or stimulus, rather than to the participation of acetylcholine as an aetiological factor in the development of spontaneous convulsions.

Our experiments have shown, however, that, as with anaesthesia, regular changes do occur in the concentration of acetylcholine in the brain during convulsions, but that these changes are secondary to the convulsive activity itself.

We have tried, using rats and rabbits, to correlate changes in the electroencephalogram (E.E.G.) with changes in the concentration of brain acetylcholine and with its liberation into the blood and cerebrospinal fluid. It will probably be necessary later to consider also the mutual effects of the convulsions and other humoral factors.

If the maximum dose of a convulsant drug (we have, for the most part, used leptazol and picrotoxin) is injected into an animal, clonic convulsions appear after a short latent period. As the concentration in the brain rises, these convulsive movements become more and more violent, to culminate in the precipitation of a tonic extensor seizure in which all four limbs show a prolonged and intense extensor spasm. Following this extensor spasm, and after a variable period of relative quiescence, during which the animal lies in a quite flaccid condition, clonic convulsions, much less violent than before, return and continue, with gradually decreasing severity, over a variable period until convulsive activity finally ceases. This last phase is complicated by anoxia, which becomes most severe during the tonic extensor spasm and which is contributed to by several factors: the great metabolic demands made by the early violent convulsions, the apnoea which accompanies the tonic extensor phase and laryngeal spasm.

The work of Goodwin, Kerr and Lawson (1940) showed that the tonic extensor spasm represents the peripheral manifestation of a widespread and intense cerebral excitation, and Toman, Swinyard and Goodman (1946) have adduced evidence that during such a seizure the brain receives the maximum stimulation of which it is capable. The clonic convulsions represent the effect of less intense excitation.

Table II.—*The Acetylcholine Content of Young Rat Brain at Different Times During the Course of Convulsions Induced by Leptazol.*

(Mean, and standard error of mean, of results of experiments on 5 groups of litter-mates.)

| | μg./gm. wet weight of brain. |
|---|---|
| Normal | 2·5 ± 0·22 |
| At onset of convulsions | 2·4 ± 0·20 |
| During first clonic phase | 1·8 ± 0·22 |
| During tonic extensor spasm | 1·4 ± 0·02 |
| During second clonic phase | 1·4 ± 0·14 |

Table II shows the results of a typical experiment on the acetylcholine content of the brains of a series of young rats convulsed with leptazol. Results quantitatively very similar were obtained with picrotoxin and in experiments on rabbits. It can be seen that the concentration of acetylcholine in the brain remained steady until the convulsions set in ; there was then a fall during the clonic convulsions and a further fall during the tonic extensor spasm. It should be added that this latter change was more precipitous than the figures would indicate, for it occurred over the two or three seconds required for the development of the extensor phase : the earlier fall required about fifteen seconds to develop fully. It will also be noted that, following the extensor spasm, the acetylcholine concentration showed no recovery when the second clonic phase began. This continued low concentration of acetylcholine was probably due to the anoxia already mentioned, for it did not occur after the tonic extensor phase of electrically-induced convulsions (Richter and Crossland, 1949) where the experimental conditions were such that deep anoxia was prevented.

These effects were probably due to the increased rate of release from cholinergic neurones, and we have, indeed, been able to detect acetylcholine in the bloodstream during the extensor spasms. Our attention was first drawn to this possibility by the invariable association, in rats, of the extensor tonic spasm and chromodacryorhesis (the secretion of the so-called " bloody tears " from the Harderian glands, which are specifically stimulated by low concentrations of acetylcholine). It has since proved possible to detect acetylcholine in the blood at this time by the usual biological methods. It is assumed that this acetylcholine escaped the normal enzymic barriers in the brain and persisted for a time in the blood because of its low concentration, which would delay its hydrolysis by serum cholinesterase.

The changes in the acetylcholine content of the brain—more especially those occurring during the clonic phase—must probably be regarded as adaptations to the changed physiological conditions and not as manifestations of an exhaustion of the supplies of acetylcholine. This interpretation is best supported by some experiments we carried out on rabbits, anaesthetized with " Dial." The intraperitoneal injection of leptazol into such animals caused the outburst of prolonged spike activity in the E.E.G.,* which exhibited none of the alternating periods of quiescence and activity which occur so often in records taken during convulsions and which, for obvious reasons, lead to equivocal results when used in the type of experiment described here. This continuous spike activity was associated with a fall in the acetylcholine content of the brain, but this occurred early and was thereafter independent of the duration of the experiment. Thus in one series of rabbits (in which the acetylcholine of the cerebral cortex only was studied) the mean content of the cortex of the animals under " Dial " was 2·1 μg./gm. (4 experiments). In animals whose E.E.G. had shown spike-potentials for 10 minutes, the cortical concentration was only 1·3 μg./gm. ; after 35 minutes of this activity it was still 1·4 μg./gm. (4 animals).

Notwithstanding these experiments, it is clearly possible that the brain requires a minimum concentration of acetylcholine in order that normal activity can be maintained, and it is possible, too, that under extreme conditions the amounts available might approach this critical minimum. Such a condition would imply that, other things being equal, those convulsive agents would be most effective which promoted synthesis of acetylcholine, and prevented abnormal falls in its concentration consequent on, for example, circulatory or respiratory embarrassments occurring during the course of the convulsions. There is some evidence of a general tendency for powerful convulsant agents to stimulate acetylcholine synthesis (Torda and Wolff, 1947), but it must be emphasized that this stimulant action is probably only exerted when the supplies of acetylcholine become depleted, for there is no evidence that these convulsant drugs increase the concentration of acetylcholine in brains carrying their normal complement.

Similarly, it seems reasonable to believe that those anti-convulsant compounds might be most effective which tend to inhibit the synthesis of acetylcholine or to accelerate its destruction.

There is as yet no direct evidence for the existence of a critical minimum acetylcholine concentration, but we found that when the convulsive activity of the rabbits

* These records, and others referred to subsequently, were demonstrated at the Cardiff meeting.

under " Dial " and leptazol was abolished by subjecting the animals to artificial respiration with nitrogen, the flattening of the E.E.G. was accompanied by the reduction of the cortical acetylcholine concentration to 0·82 μg/gm. from its previous value of 1·4 μg./gm. It will also be recalled that, following the extensor tonic spasm induced by chemical convulsant agents, the clonic convulsions were but weak, and were associated with a persistently low concentration of acetylcholine. Neither of these observations, of course, gives any real evidence that an abnormally low acetylcholine content and reduced central activity are causally related, and this possibility is, in any event, irrelevant to the main point which has emerged from the studies described here : that, contrary to expectations, the content of acetylcholine in the brain is adjusted in such a way as to increase during depressed central activity and to fall during heightened activity.

An interpretation of the significance of such an adaptive mechanism awaits the results of further experiments, but it does seem reasonable to assume, as a tentative hypothesis, that these changes in acetylcholine concentration during convulsions or other forms of heightened cerebral activity, by reducing the amount liberated at each impluse, reduce the possibility of " over-stimulation " of the nervous system. Alternatively, by preventing the temporary accumulation of too high a concentration of acetylcholine at the synapse, " inhibition " might also be prevented. The converse effects would be found during anaesthesia. One is reminded of the rather similar theories which have been advanced to explain the action of adrenaline on transmission at sympathetic ganglia.

Two lines of approach might help to test these hypotheses : It is hoped to determine first whether the effects of given doses of exogenous acetylcholine on central transmission differ according to the degree of nervous activity already prevailing. The other possible line of approach will attempt to maintain the acetylcholine level in the brain during convulsive and other forms of activity at concentrations other than those normally found. One interesting piece of information of the type to be expected from this latter line of investigation has been obtained from the experiments on rabbits already referred to. If, after the abolition of convulsive potentials by anoxia the animal was given pure oxygen, the convulsive potentials returned after a time with a distinctly higher amplitude than at any time in the entire period preceding anoxia. It appears that during this post-anoxic phase acetylcholine synthesis was stimulated, for the period of enhanced spike potentials was associated with a correspondingly elevated acetylcholine concentration.

Acetylcholine is present in, and is synthesized by human cerebral tissue ; it can also be detected in the cerebrospinal fluid of epileptic subjects following major seizures (Tower and McEachern, 1949). These, and many other lines of evidence, indicate that the conclusions derived from our animal experiments are probably applicable also to man, and may eventually yield results of clinical significance.

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