THE EFFECT OF CHOLIN-LIKE SUBSTANCES ON THE CEREBRAL ELECTRICAL DISCHARGES IN EPILEPSY

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IN a recent paper (Williams and Russell, 1941) the effects of anticholinesterases on petit mal epilepsy were described. Clinical and subclinical attacks were recorded on the electro-encephalogram (e.e.g.) in epileptic subjects who were selected either for the frequency of their spontaneous attacks, or for the ease with which attacks could be induced. A dose of eserine insufficiently large to cause symptoms through its effect on the parasympathetic system in man was found to prevent petit mal activity, whereas a larger dose caused an increase in the number and duration of petit mal attacks. Prostigmin on the other hand invariably caused an increase in the epileptic activity seen in the e.e.g. and it reversed the effect of small doses of eserine. The work has been continued in an attempt to establish the mode of action of these drugs and to discover the effect of other substances known to modify transmission at cholinergic endings. The present paper contains observations on the effect of acetylcholine and allied drugs upon the incidence of epileptic cerebral discharges, and the results are discussed in relation to the site of action of the substances.

Methods

As the experimental procedure did not differ greatly from that already described (Williams and Russell, 1941) it will only be referred to briefly. Epileptic patients and normal subjects were used. They sat or lay comfortably at rest with the eyes closed while an e.e.g. record was obtained from two solder electrodes sealed to the scalp over the frontal and parietal lobes with collodium simplex, contact being made with Cambridge electrode jelly. The electrodes led to a condenser coupled amplifying system which was connected to a Grass ink writing oscillograph. This recorded the difference in electrical potential between two widely separated areas of the cortex, and as the cerebral electrical changes associated with clinical fits involve the whole cortex and those seen in subclinical attacks are usually propagated over a wide area, this method of recording proved adequate for the investigation, which only dealt with the number and duration of attacks. Time was recorded in seconds and minutes and in some cases electrocardiographic and respiratory tracings were made simultaneously with the e.e.g. The experimental procedure and clinical signs and symptoms were noted on the moving record as they occurred.

The periodicity of spontaneous epileptic attacks is usually too variable for their incidence to be used as a quantitative index in an experimental method lasting for a few hours only; therefore a standard procedure which would induce attacks at fixed intervals under controlled conditions was used in some cases to provide a measurable criterion of the anticonvulsive effect of the substances under examination.

The most simple and effective method of inducing petit mal is by overbreathing, which eliminates CO_2 and temporarily raises the blood pH (Gibbs, Lennox and Gibbs, 1940). Petit mal outbursts usually occur with the readjustment of the acid base balance after hyperventilation has ceased (Nims *et al*, 1940). Subjects were therefore instructed to overbreathe for a period of 100 seconds every 10 to 20 minutes during the experiment and to maintain the same depth of overbreathing each time. The expired air was collected in a Douglas bag and measured. Repeated records were taken before and after injection of the drugs under similar experimental conditions and the same subject was used to investigate several drugs. A record of a typical series of epileptic discharges after overbreathing has already been published (Williams and Russell, 1941). The duration of these discharges, measured during and after each period of hyperventilation, was taken to represent the total epileptic activity resulting from the preceding hyperventilation, the factor being plotted graphically after each experiment (see Fig. 3).

By selection from a large number of patients, six subjects were found who had very frequent spontaneous epileptic attacks. In these cases the incidence of the spontaneous subclinical attacks was so regular and their number so great that their occurrence in consecutive e.e.g. records could be compared. In these subjects no attempt was made to induce attacks but they merely sat or lay quietly while repeated e.e.g.'s were taken at fixed intervals before and after administration of drugs.

Eserine, prostigmin, pilocarpine, carbaminoylcholine chloride, and atropin sulphate were injected subcutaneously, while acetylcholine alone and with atropin was given intravenously.

In describing the results, the terms epileptic "outbursts" or "attacks" refer to the characteristic discharges seen in the e.e.g. whether or not clinical evidences of the attack were present. This broad conception of epilepsy simplifies the description considerably.

Results

Eserine and Prostigmin.—The observations previously reported (Williams and Russell, 1941) have been made in two further subjects who had frequent spontaneous epileptic attacks, and similar results were obtained.

One subject (G.G.C.), aged 16½, had had attacks since he was 8 months old. Until the age of 8 he had momentary lapses of consciousness when his head fell forwards for a moment and he dropped articles he was holding. He did not fall to the ground. When 12 years of age he began to have nocturnal grand mal attacks every 10 days or so. When seen he was having two to six attacks a day which he described as "losing touch for a moment with what is going on," with a grand mal attack in his sleep about once a fortnight. There was no family history of epilepsy, and the patient was physically and mentally normal.

The e.e.g. showed an abnormal outburst characteristic of petit mal (Fig. 1) at short intervals. The patient was not aware of any subjective change when these outbursts took place, and no clinical attacks were observed during any experiments. A subcutaneous injection of eserine salicylate 1.0 mgm. caused a diminution in the number of outbursts, with complete cessation from 37 to 68

Fig. 1.—The tracing of an electro-encephalographic record taken during a subclinical petit mal attack in the subject G.G.C.

minutes after the injection (Fig. 2). The patient said he felt a little hot but had no other symptoms after the injection. A dose of 0.8 mgm. of prostigmin subcutaneously did not alter the incidence of the outbursts and had no subjective effect, but 1.25 mgm. caused an increase in the number of attacks 45 minutes after injection. The patient did not complain spontaneously of any symptoms but he was a little flushed and there was slight sweating.

Fig. 2.—The effect of a subcutaneous injection of 1.0 mgm. Eserine on the number and duration of petit mal attacks, similar to that illustrated in Fig. 1, in subject G.G.C. Records were run for 10-minute periods, represented by the thick lines along the abscissa. Each vertical line represents one petit mal attack.

Carbaminoylcholine Chloride.—Chloryl (Savory and Moore) was injected subcutaneously in doses of 0.25 mgm. on eight occasions in three epileptic patients who showed petit mal discharges on the e.e.g. and four times in normal controls. In two of the epileptic subjects in whom subclinical attacks were induced by overbreathing in standard conditions there was an increase in the epileptic response to hyperventilation (Figs. 3 and 5). In two patients in whom spontaneous epileptic discharges could be recorded six experiments were performed. In three there was a great increase in the epileptic activity, in two there was a moderate increase, and in one no significant change was seen. An example of the results obtained is shown in Fig. 4. The effect of the drug appeared about 25 minutes after the injection was made. In subjects in whom the attacks were induced by overbreathing the effect lasted for about 30 minutes, but in those in whom spontaneous attacks only were recorded the effect was shorter.

Reference to Figs. 4 and 5 shows that in predisposed subjects epileptic cerebral discharges could be precipitated by the subcutaneous injection of

Fig. 3.—The effect of subcutaneous injection of 0.25 mgm. carbaminoylcholine chloride on petit mal activity induced by overbreathing in an epileptic subject. Each vertical line represents the length of time occupied by petit mal activity, seen in the e.e.g., after 100 seconds of overbreathing. The time of onset of the petit mal attack after beginning overbreathing is constant although the duration of petit mal is increased.

Fig. 5.—Twenty-five minutes after injection of 0.25 mgm. carbaminoylcholine chloride 100 seconds of overbreathing is followed by an outburst of petit mal, although previous overbreathing had not produced any epileptic activity under similar conditions. The graph has the same characteristics as that in Fig. 3. The volume breathed during each period of hyperventilation is fairly constant. Blood sugar is represented in mgm. per 100 c.c.

Fig. 4.—The effect of 0.25 mgm. carbaminoylcholine chloride upon spontaneous petit mal attacks, seen in the e.e.g., in subject K. The columns have the same significance as in Fig. 2. The record was obtained shortly after a meal and blood sugar is plotted in mgm. per 100 c.c. There is no significant change in the blood sugar level as a result of the injection.

carbaminoylcholine chloride although no epileptic activity was present at all during control periods before the injection was given. When records were continued for sufficiently long after the injection had been made—for a period of about an hour—the epileptic activity again disappeared (Fig. 4). Not only was the incidence of the discharges greater, but their duration was increased by the drug and the results showed that the action in spontaneous or artificially induced attacks was identical in this respect. The doses used were not sufficient

Fig. 6.—The effect of subcutaneous injection of 1.5 mgm. of Eserine on the amount of petit mal activity induced by 100 seconds of overbreathing. The columns have the same significance as in Fig. 3 and the abscissa represents time of experiment in minutes. The onset of attacks after beginning overbreathing, the volume breathed during each period of hyperventilation, and blood sugar in mgm. per 100 c.c. are plotted.

to cause any marked clinical effect and none of the subjects complained spontaneously of any discomfort. There was occasionally bradycardia, and the pulse rate fell from 68 to 54 in one subject. Some patients were a little flushed and there was occasional slight sweating.

When subcutaneous injection of atropin sulphate gr. 1/100 was made shortly before the injection of carbaminoylcholine chloride the effect of the second drug was either abolished or greatly diminished. Fig. 7 shows such an effect in a subject who had been given carbaminoylcholine chloride alone with striking effect the previous day. This patient (subject K) was used repeatedly in the examination of many of the substances investigated and a short clinical history is given later.

Petit mal activity is readily modified by alterations in the blood sugar level,

an increase preventing attacks and a decrease inducing them (Gibbs, Gibbs and Lennox, 1939). Changes in the blood sugar level have been reported after injection of cholin-like substances (Hrubetz, 1937), so that blood sugar estimations were made immediately after the e.e.g. records were taken, in case changes had occurred with the doses used in these experiments. Blood was obtained from a superficial vein of the forearm and determinations were made by the Folin Wu method. The results were plotted as in Figs. 5 and 6. There was

Fig. 7.—Inhibition of the effect of carbaminoylcholine chloride upon petit mal by atropin. As in Fig. 6 the thick lines along the abscissa represent periods of recording of the e.e.g. and the vertical lines each represent a petit mal attack.

no relationship between the small fluctuations in blood sugar level and the alteration in the amount of epileptic activity, nor was any constant change in blood sugar level seen after the injections had been made.

Acetylcholine.—Acetylcholine hydrochloride (B.D.H.) was injected intravenously on six occasions in two epileptic patients and six times in normal controls. Intelligent, co-operative subjects were chosen and symptoms of an intravenous injection of acetylcholine (Carmichael and Fraser, 1933) were described to them to allay apprehension. They lay with their eyes closed and a continuous record was made of the e.e.g. and electrocardiogram. After a control run the needle was introduced into a superficial vein of the forearm and a further control run was obtained. Care was taken to prevent entry of any blood into the syringe because of the rapid hydrolytic effect of the blood cholinesterase upon the acetylcholine. Later, at a suitable moment, the injection of acetylcholine in 1 c.c. of water was made rapidly and the needle was left *in situ* until the end of the experiment some minutes later to prevent disturbance of the patient or distortion of the record. The patient's movements and changes in appearance were noted upon the recording paper as they occurred. The observations made upon the two epileptic patients will be described in detail.

Subject K.—An otherwise healthy man, aged 20, began to have attacks 8 years ago. He had had no convulsions in infancy and there was no family history of epilepsy. He had several attacks each day which occurred before breakfast or late in the evening, his last meal being at 5 p.m. The attacks gradually increased in frequency and for the past 2 years he had been having nine to twelve a day although their incidence was variable. The attacks consisted in a momentary loss of consciousness with a liability to drop anything he was holding, followed by a transient sensation of epigastric discomfort, after which he was again quite normal. Three years ago he began to have attacks of similar type but of greater severity in which he fell to the ground without any tongue biting, clonic movements, or incontinence. When

examined he was having three to six minor attacks each morning and a falling attack about once a week. Electro-encephalography showed a normal record interrupted by short outbursts of spike and wave activity (Fig. 8). They occurred every few minutes in fifteen e.e.g. records taken on different days during three weeks. The longest interval seen between these subclinical attacks was 9 minutes and their greatest frequency was three in a minute on one occasion. These petit mal discharges were not accompanied by any subjective or objective disturbance, although an attempt was made to record a clinical attack before breakfast one day.

An intravenous injection of 25 mgm. of acetylcholine given in the way already described caused slight bradycardia 7 seconds after the injection, followed by blushing and a little sweating. The patient (subject K) coughed and had a constricting feeling in the chest, a dry sensation in the throat, and slight substernal discomfort. An e.e.g. was obtained for 5 minutes after the pulse rate had become steady and there was no increase in the number of attacks.

Fig. 8.—Spontaneous subclinical petit mal outburst from the subject used for Figs. 4, 7, and 9.

Fifteen minutes after this first injection 50 mgm. acetylcholine were given under identical conditions. The clinical effect was more marked. In 7 seconds there was an abrupt bradycardia lasting for 4 seconds, with subsequent tachycardia, coughing, flushing of the face, sweating, a constricting sensation in the chest and throat, and slight substernal pain. During the minute following the injection of 50 mgm. acetylcholine there were seven outbursts of epileptic activity of the type shown in Fig. 8. Under no previous circumstances had he had more than three attacks in a minute. The result of this experiment is expressed graphically in Fig. 9.

Ten minutes after this injection of 50 mgm. acetylcholine the same dose was mixed with atropin sulphate gr. 1/150 and given intravenously under identical conditions. There was a very slight clinical reaction with a little flushing and a mild constricting feeling in the chest. There was no increase in the incidence and duration of the epileptic discharges (Fig. 9).

Subject T.—A healthy man, aged 22, began to have attacks 2 years before the experiments. He had had no infantile convulsions and there was no epileptic family history. He became aware of the attacks at work, where he would walk into machinery for no apparent reason. At first he had one attack every few days, but they slowly became more frequent until he was having two to three a day. They always occurred in the day time, were precipitated by going from light into dark, and could be induced sometimes by closing his eyes tightly.

Fig. 9.—The effect of intravenous injection of 50 mgm. of acetylcholine upon spontaneous petit mal attacks seen in the e.e.g. The record is continuous and reads from left to right. Each vertical line represents an attack, seven of which occurred in the first minute after injection of acetylcholine. There is no similar effect when atropin 1/100 gr. is injected after the same amount of acetylcholine under identical conditions.

The attacks were not noticed by others unless the patient was talking to them at the time. He would look vacant and make quite irrelevant remarks such as, "It's over there," or "Why do you do it," in rather an aggressive manner. After a few seconds there would follow a period of confusion lasting for about a minute. He had never bitten his tongue, been incontinent or had clonic movement in an attack. The patient states that he is first aware of a blurred light in the right upper temporal quadrant and that after this has lasted for about 2 seconds he loses touch with his surroundings. He is aware that he is having an attack, and is usually able to force himself to continue what he is doing. He can hear words spoken to him but cannot appreciate their meaning. Similarly he knows that he has spoken, but not what he has said. He has sometimes spilt his tea in an attack and recently fell down in a fit when emerging from a brightly lit cinema into the outside darkness. Electro-encephalography showed scattered abnormal slow waves of low voltage, without any evidence of epileptic activity. No attacks could be induced by closing the eyes, by shining lights and then plunging the room into darkness, or by hyperventilation, nor were any seen in records obtained on three subsequent days.

The patient (subject T) was intelligent and co-operated admirably. The experimental procedure was identical with that adopted throughout. An injection of 60 mgm. acetylcholine was made rapidly into an arm vein, and the characteristic effects of injections of acetylcholine were observed. There was an asystole lasting for nearly two seconds which began abruptly 7 seconds after the injection, with coughing, flushing, sweating, and restlessness caused by discomfort and anxiety. Afterwards the subject described a substernal pain of anginal type, a constricting feeling in the throat, an uncontrollable desire to cough and a sensation of blushing and warmth. He was unable to say whether he had experienced the sensations associated with an attack, for he was too preoccupied with the substernal discomfort to notice this. The e.e.g. was partly obscured by movement artefact but it clearly showed abnormal large slow waves for short intervals. There were no similar outbursts in a 15 minute control period before the injection was made.

The following day an e.e.g. and electrocardiogram were recorded in conditions identical with those in the preceding experiment, and electrodes were also used to record muscular movements to simplify the interpretation of the record. A record was made for 15 minutes without evidence of epileptic activity. The intravenous needle was introduced, a further control period was recorded and without the knowledge of the patient 30 mgm. acetylcholine were injected rapidly. From notes dictated during the experiment and from detailed correlation of the patient's story afterwards the following account of the effects of the injection was obtained. About 7 seconds after the injection the patient was aware of the aura of lights in the right upper temporal quadrant. This increased in intensity for about 2 seconds, and after this the subjective attack commenced. He described this as a sensation of his environment receding rapidly and of his losing touch with reality. The patient's verbatim report reads: "The attack started and I had just time to think 'Is it going to get worse or will it stop?' Then it became worse and at the same time I felt the pain in the chest. The time that I was beginning to lose touch with things coincided with the pain, and I think that the attack would have passed off if the pain had not come. . . . When I was out I heard someone passing remarks and just remember the

Fig. 10.—An e.g. recorded from the left fronto-parietal region of an epileptic subject. Intravenous injection of 30 mgm. acetylcholine was followed by an attack. The exact time of onset of the attack cannot be stated, but the large slow waves seen in the e.g. were associated with unconsciousness and co-ordinated semipurposeful movements such as are seen in psychomotor epilepsy. Time is in seconds, the long signal representing the end of a minute.

words 'mouth moving,' the next I heard was 'coming out.' The sequence of these events had been printed on the e.e.g. record reproduced in Fig. 10. The changes observed in the patient were as follows. After the injection he lay still for 6 to 7 seconds. He then screwed up his eyes and shortly afterwards his face became flushed. Except for small movements of the feet he was still. Automatic smacking movements of the lips and tongue followed, and his expression became tense and anxious. His hands gripped the edge of the bed, but after about 30 seconds he relaxed and remained passive until the observations were completed. There was transient flushing of the face, the patient coughed once and there was slight substernal discomfort. The e.e.g. (Fig. 10) showed a resting record consisting of scattered abnormal waves with a frequency of three to five a second and a low voltage, which were rapidly replaced by large slow waves after a burst of muscle action potentials caused by closing the eyes tightly. These waves persisted throughout the subjective attack and for about 10 seconds afterwards. They then ceased abruptly and the record was indistinguishable from that in the control period. There was negligible bradycardia. The patient's attacks, with automatic actions, speech and a sense of dissociation from his environment, are of the type grouped as "psychomotor" epilepsy and the simultaneous disturbance seen in the e.e.g. is known to occur in this type of fit, whereas it is never found in grand mal or petit mal. It should be observed that repeated records obtained both before and after this experiment did not show any evidence of epileptic activity.

At a later date an intravenous injection of 60 mgm. acetylcholine and 1/150 gr. atropin sulphate in 1 c.c. of sterile water was made under experimental conditions identical with those established previously. There were slight subjective effects of the injection, which the patient thought a little less apparent than after 30 mgm. of acetylcholine injected alone. There was no cough or restlessness, and very slight momentary bradycardia. No aura or symptom of an attack occurred and the e.e.g. did not show any change.

The above experiments may be summarized as follows: Intravenous acetylcholine was given six times to two patients who had petit mal and psychomotor epilepsy. 25 mgm. did not cause an attack, but 30 mgm., 50 mgm., and 60 mgm. were all followed by attacks similar to those usually experienced by the patient. When 50 and 60 mgm. of acetylcholine were injected with atrophin sulphate 1/150 gr. there was no evidence of any increase in epileptic activity.

To control these observations the following experiments were carried out: Six injections of acetylcholine in doses ranging from 30 to 60 mgm. were made in normal subjects in experimental conditions identical with those already described. There were the usual sequelæ of the injections, the intensity of which varied from subject to subject, but no evidence of any epileptic discharge was seen in the e.e.g.

Atropin.—The effective dose of atropin necessary to reduce the number of subclinical and clinical epileptic attacks is variable, and it has been common clinical experience that belladonna has a marked anticonvulsant effect in some epileptics and not in others. In this series of experiments atropin sulphate was used to minimize the parasympatheticomimetic effect of eserine and prostigmin,

but 1/150 gr. and even 1/100 gr. subcutaneously were found insufficient to modify the effect of these substances upon the incidence of epileptic attacks. When 1/100 gr. was injected subcutaneously alone in two instances it reduced the number of attacks and when injected intravenously with acetylcholine or subcutaneously with carbaminoylcholine chloride it completely abolished the epileptogenic effect of these substances (Figs. 7 and 9).

Histamine.—As it was possible that acetylcholine might cause an increase in epileptic activity by modifying the cerebral blood flow (Wolff, 1929) histamine acid phosphate 0.1 mgm. was injected intravenously in two epileptic patients from whom subclinical attacks were being recorded by e.e.g. There were the usual symptoms of headache, flushing, and tachycardia (Pickering, 1933) but there was no change in the incidence of epileptic cerebral discharges. From these observations it appears that cerebral vasodilatation is not alone responsible for the changes induced by acetylcholine, and that the subjective effects of the injections do not necessarily give rise to an increase in epileptic activity. The first conclusion is supported by the observation that CO₂ causes a profound cerebral vasodilatation in man (Carmichael, Doupe, and Williams, 1937), although it suppresses petit mal activity (Lennox, 1928).

Pilocarpine.—To determine whether the increase in epileptic activity resulting from injection of acetylcholine was due to peripheral activity of the parasympathetic system pilocarpine nitrate 1/10 gr. was injected subcutaneously in two epileptic subjects who showed subclinical epileptic discharges upon the e.e.g. There was salivation, flushing, and sweating, but no change in the incidence of the epileptic discharges was noted.

Apomorphine.—In some of the subjects increase in epileptic activity after eserine and prostigmin was associated with nausea, abdominal discomfort, and even vomiting, and it was possible that these symptoms were causally related to the epileptic activity. Similar symptoms were therefore produced by an injection of apomorphine hydrochloride 1/10 gr. in two epileptic patients who showed subclinical attacks upon the e.e.g. There was no increase in the epileptic activity in either case.

Discussion

The outbursts of cerebral electrical activity associated with epileptic attacks are sharply delimited and fall into groups having characteristic rhythmical wave patterns which are easily recognized on the e.e.g. The size and duration of the outbursts are related to the severity of the clinical attack and their form can be correlated with the clinical types of epilepsy (Gibbs, Gibbs and Lennox, 1937). Although the different groups are distinct they may appear in mixed forms in the same subject. This communication is primarily concerned with the disturbance associated with petit mal, which has many well recognized features. In this, as in the other forms of epilepsy, cerebral electrical discharges known as subclinical attacks are frequently seen in the absence of any demonstrable alteration in consciousness. They have a form identical with that seen in a clinical fit, but they have a lower voltage and a shorter duration. Subclinical fits often occur at short intervals, even when the clinical fits are

infrequent, and as in any one subject the ratio of clinical to subclinical fits is fairly constant it follows that they offer a rapid method of observing the effect of drugs upon epilepsy. The incidence and duration of subclinical attacks have been used by Gibbs, Lennox and Gibbs (1940) to investigate the effects upon petit mal epilepsy of changes in various blood constituents and of blood pH. In subjects with petit mal, in whom spontaneous attacks are infrequent, subclinical attacks may be induced by over-breathing, and the application of this fact to the present investigation has already been described (Williams and Russell, 1941).

There has recently been increasing interest in the effect of cholin-like substances upon the activity of the central nervous system. Acetylcholine is present in the grey matter (Stedman and Stedman, 1939) and it is liberated when the isolated brain is perfused with eserinated blood (Chute, Feldberg and Smyth, 1940). Some workers have reported liberation of acetylcholine into the cerebrospinal fluid on stimulation of the central cut end of the vagus (Dikshit, 1934), but others have been unable to confirm this (Adam et al, 1938). Schweitzer, Stedman and Wright (1939) found that prostigmin as well as acetylcholine and allied substances have a direct inhibitory effect upon the reflex activity of the spinal cord, while eserine on the other hand increased the reflex activity and caused convulsive movements (Schweitzer and Wright, 1937). Bremer (1938) introduced acetylcholine into the carotid artery in rabbits and found that minute doses increased the size and frequency of the waves of cerebral electrical activity and that larger doses depressed them. Miller (1937) applied eserine to the exposed cortex in dilute solutions and obtained an increase in the muscular rigidity of the corresponding limb. He thought that the effect was specific and was caused by an enhancement of cortical synaptic transmission. Miller, Stavrakys and Woonton (1940) amplified the experiments and produced a reduction in the size of the waves of electrical potential change by acetylcholine applied locally. They enhanced the effect with eserine and abolished it with atropin, and consequently think that acetylcholine may facilitate cerebral synaptic transmission. More recently McKail, Obrador and Wilson (1941) have produced changes in the responses to electrical stimulation of the cortex by intra carotid injection of similar substances.

It was in view of these observations that the effect of some of these substances upon the cerebral electrical discharges seen in epilepsy were investigated. The results described in the present paper seem to fall into line with the observations already reported (Williams and Russell, 1941), that a small subcutaneous injection of eserine decreases petit mal activity and larger doses increase it, while prostigmin invariably causes an increase in petit mal. The different actions of eserine and prostigmin are analogous to those obtained upon the reflex activity of the spinal cord by Schweitzer and Wright (1937). Schweitzer, Stedman and Wright (1939) suggested that as eserine is a tertiary ammonium base and is fat and water soluble it might enter the nerve cells and cause changes there, while prostigmin, a quaternary base, is only water soluble and acts outside the cell, perhaps in the synapse. This explanation raised the possibility that the effect of eserine and prostigmin upon epilepsy might be central in origin.

That injections of eserine, prostigmin, carbaminoylcholine chloride, and acetylcholine have a similar action upon epilepsy, and that this action is inhibited by atropin, suggests that the changes are related to an alteration in the (acetylcholine transmitting mechanism somewhere in the body. However it is caused, the effect may be an indirect one due to peripheral changes, or it may be directly due to alterations in the central nervous system. The changes to which cholin-like substances give rise in the intact organism are so widespread and complex that it is difficult to single out the factor causing changes such as have been reported in this paper. Coincidental changes which might have been responsible are:

- (1) an alteration in respiratory exchange through bronchiolar constriction or respiratory rate modifying the arterial CO_2 or O_2 .
- (2) a rise in blood pH by glandular secretion, especially that of HCl into the stomach, resulting in an increase in petit mal activity (Nims et al, 1940).
- (3) a change in blood sugar level from adrenal or pancreatic stimulation (Hrubetz, 1937) ; and
- (4) an alteration in cerebral blood flow, either an increase through cerebral vasodilatation (Wolff, 1929) or decrease through fall in systemic blood pressure (Wolff and Cattell, 1935).

Each of these changes, but especially an increase in blood pH or a fall in blood sugar, might increase petit mal activity.

Respiratory exchange was measured and found to be surprisingly constant under the conditions of the experiments, and any changes which occurred bore no relationship to the incidence of epilepsy. Similarly, the small alterations recorded in the blood sugar levels (Figs. 3 and 4) were not related to the changes in epileptic activity. The injections of acetylcholine were followed so quickly by an increase in epileptic activity in subjects K and T that neither blood pH or sugar changes could have had time to be effective. Suggestive evidence that the changes were not caused by alterations in blood pH has already been advanced (Williams and Russell, 1941), but the only conclusive proof is determination of the actual blood pH during the experiments. No observations of the effect of cholin-like substances upon the blood pH have been reported, so that a direct coupled amplifier and a glass electrode system is at present being used for this purpose.

Evidence has been produced that epilepsy might be related to an alteration in local cerebral blood flow (Penfield, 1938). Acetylcholine produces both an increase in cerebral blood flow by cerebral vasodilatation, and a decrease by diminished cardiac output. CO_2 increases cerebral blood flow enormously (Carmichael, Doupe and Williams, 1937), but it prevents petit mal epilepsy, and in the present experiments histamine did not have the same effect on epilepsy as did acetylcholine, although it is an efficient cerebral vasodilator. No cerebral symptoms have been described after injection of acetylcholine in the absence of very prolonged asystole (Carmichael and Fraser, 1933), and in the present experiments there was bradycardia but never asystole for longer than 2 seconds. Furthermore, the same dose given to normal subjects was not associated with

any electro-encephalographic changes characteristic of cerebral anæmia or anoxia. Fig. 10 shows that the cardiac slowing is very slight in the presence of a clinical attack. Carmichael and Fraser (1933) reported the occurrence of a grand mal fit in a normal person after intravenous injection of acetylcholine, but this fit was preceded by a period of asystole lasting for 11·8 seconds.

As the arm-tongue circulation time usually ranges from 13 to 19 seconds, with a low level of 10 seconds (Kremer and Robertson, 1935) the extremely rapid onset of an aura after intravenous acetylcholine shown in Fig. 10 suggests that the attack had begun before the acetylcholine had reached the cerebrum, but the continuation of the effect seen in this figure and in Fig. 9 may have been due to cerebral changes.

The slow waves associated with petit mal may be transmitted across the cortex by neurone chains in such a manner as that described by Adrian (1936) for their rate of propagation is slow, or the rhythmic activity may be spread throughout the hemispheres by a strictly electrical mechanism which is independent of any neuronal co-ordination of cellular activity (Gerard and Libet, 1940). Similar slow waves caused by co-ordinated beats of cell masses in response to introduced substances can spread through cerebral tissue in which the appropriate nerve pathways have been severed (Garard and Libet, 1939), but nothing is known about the physico-chemical mechanism causing the initiation, persistence, spread, or cessation of the rhythmic electrical activity associated with epileptic attacks. The present observations may be interpreted in terms of synaptic transmission, but there is at present little justification for applying this directly to the abnormal central mechanism responsible for epilepsy.

Summary

Acetylcholine and allied substances were administered to patients with petit mal epilepsy. Electro-encephalographic records were made of clinical and subclinical attacks, both spontaneous and induced by overbreathing. Intravenous injection of acetylcholine was followed by an increase in the recorded epileptic activity. This action of acetylcholine was inhibited by atropin, and other substances having somewhat similar pharmacological effects did not alter the incidence or duration of the attacks. No similar results could be obtained in normal subjects.

Experiments were performed in an attempt to elucidate the mode of action of acetylcholine in modifying epilepsy, and several of its peripheral effects were excluded. The results are discussed in relation to recent work upon the effect of cholin-like substances on the central nervous system.

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