MECHANISM OF SPREADING CORTICAL DEPRESSION

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INTRODUCTION

SPREADING DEPRESSION, which results from strong stimulation of the cerebral cortex, was first described by Leão in 1944 (15). In the intact brain of rabbits, cats, or monkeys, this phenomenon manifests itself in the disappearance of the spontaneous electrical activity of the cortex, starting at the stimulated point, and spreading in all directions over the cortex at the rate of about 3 mm./min. (26, 23, 27). Simultaneously, all cortical responses to electrical and physiological stimulation disappear, and a large potential gradient develops across the cortical grey matter, as a result of which the surface of the cortex becomes first negative with respect to an indifferent cortical point and then positive (17). The negative phase attains an amplitude of 3–15 mV., and the positive phase is usually considerably smaller. Sometimes the negative wave is also preceded by a small positive deflection. These slow potential changes last for several minutes at any one point, and the spontaneous electrical activity of the cortex does not return to normal for several minutes after these changes have disappeared.

It has been found that the spread of depression depends on a mechanism which operates within the cerebral cortex (20), but the nature of this mechanism is still unknown. It appears unlikely that synaptic transmission is responsible, since the phenomenon is highly resistant to anoxia (20) and to anaesthesia (26, 29). The slow time course of the depression suggests that a chemical transmitter may be involved, but there has been no evidence of the liberation of any substance that might serve as a transmitter. Many substances, including ACh, eserine, adrenaline, histamine, cocaine, strychnine, KCl and CaCl$_2$ have been applied to the surface of the cortex, but only KCl, CaCl$_2$ and strychnine have been found to start depression (20, 26).

It has been suggested that an "electrotonic" mechanism of some kind may be involved (26, 25), but there is no evidence which unequivocally supports this point of view. It has also been suggested that spreading depression may be an asphyxial phenomenon, produced by a travelling wave of vasoconstriction (28), but the evidence that a period of vasoconstriction
occurs at the onset of depression has not been confirmed by measurements of the heat production of the cortex (24).

In the experiments described below, an attempt was made to determine the mechanism of propagation of depression in the isolated cerebral cortex of the cat. This preparation, which has been described by Burns (2, 4), was particularly advantageous for the investigation, since it eliminated the complications which might have been produced by the presence of sulci, and by activity originating in extracortical centres. The results obtained in these experiments indicate that the propagation of depression depends on the liberation of K⁺ from the cortical neurones.

METHODS

Preparation. Cats weighing 1.5-2.0 kg. were anaesthetized with ether, decerebrated according to the method described by Burns and Grafstein (4), then taken off anaesthetic. An area of cortex 0.5 cm. × 1.5 cm. was isolated in the suprasylvian gyrus, so that the isolated region was free from sulci. After the slab had been cut, the skin flaps were tied to a metal ring to form the walls for a bath of mineral oil which covered the cortex to a depth of about 5 mm.

Recording system. Non-polarizable wick electrodes were used for recording from the surface of the cortex, and saline-filled glass microelectrodes with an external tip diameter of 30-40 μ were used for recording single unit potentials and slow potential changes below the surface of the cortex. The microelectrode was fixed in an adjustable rack, and could be inserted vertically through the pia, or parallel to the surface of the cortex. Monopolar recording was used throughout, with the reference electrode on a small patch of killed cortex at one end of the slab. The electrodes were connected through cathode followers to push-pull amplifiers which could be used either DC- or RC-coupled. Two channels of amplification were available. The slow potential changes during spreading depression were recorded with the amplifier DC-coupled. In order to eliminate amplifier drift, an air-driven chopper of the type described by Burns (3) was inserted between the cathode follower and amplifier, and the chopped signal from the output end of the amplifier was fed simultaneously into one beam of a Cossor double-beam oscilloscope, and into a full-wave germanium rectifier. The rectified signal was recorded on a Sanborn Twin-Viso recorder, with a paper speed of 0.5 mm./sec., while the trace displayed on the oscilloscope face could be recorded on film with a camera attachment. For some of the figures, photographs of the actual records have been used. Most of the figures, however, have been traced or redrawn from the film or Sanborn records. An inset diagram in each figure shows the arrangement used for stimulating and recording. Negativity of the focal electrode is recorded as an upward deflection.

Stimulation. Electronically controlled rectangular potential changes, 1 msec. in duration, were applied through a pair of fine platinum wire electrodes, with their tips about 1 mm. apart, resting lightly on the surface of the cortex. Single stimuli were used for testing the surface-negative and surface-positive responses. For starting depression, a 5-10 sec. burst of stimuli with a frequency of 10-20/sec. and strength 8-15 V. was used.

Polarization. Polarizing currents were applied through non-polarizable (Ag-AgCl) wick electrodes with cotton wool wicks 2 mm. in diameter. For polarization in a radial plane, one of these electrodes was applied to the surface of the cortex, and the circuit was completed through a diffuse lead in the cat's mouth. In the case of tangential polarization, the source of potential was connected to two wick electrodes, one placed at each end of the slab. Constant polarizing currents were delivered through a resistance of several megohms with the electrodes and were measured with a microammeter.

Arterial occlusion. In order to avoid the gross changes in brain volume which occur when the whole blood supply to the brain is occluded, the middle cerebral artery only was clamped with a pair of mosquito-forceps, as low down as possible on the lateral side of the hemisphere. All branches of the artery below the point of clamping were sealed by diathermy in order to prevent blood from reaching the slab through anastamoses with these branches.

N₂ administration. The animals were immobilized with d-tubocurarine (Burroughs
Wellcome) and placed under artificial respiration, in order to prevent asphyxial convulsions when \( \text{N}_2 \) was admitted from a reservoir into the artificial respiration apparatus.

**Application of chemicals.** Small drops of 1 per cent solutions of KCl or of CaCl\(_2\) were applied to the surface of the cortex from the tip of a fine-bore hypodermic needle. These solutions were usually made up in 0.9 per cent NaCl to increase their specific gravity, so that the drops fell readily onto the cortex and did not remain suspended in the paraffin oil bath. NaCl solutions having concentrations of up to 3 per cent were applied in the same way. Intravascular injection of 1 per cent KCl solution was performed through the common carotid artery in cats immobilized with d-tubocurarine.

**RESULTS**

I. **Characteristics of spreading depression in isolated cortex**

In the intact brain, the most obvious indication of the onset of spreading depression is the disappearance of the spontaneous electrical activity of the cortex (15). Since a completely isolated cortical slab is not usually spontaneously active (2), this criterion of depression was obviously unsuitable for the present experiments. However, certain other changes which have been found to be equally characteristic of the depression response could be observed. These included the appearance of slow potential changes and the disappearance of the responses to electrical stimulation.

a) **Slow potential changes.** Figure 1 (upper record) is a typical record of the slow potential changes which appeared a few seconds after a 5 sec. burst of stimulation had been applied to the slab, recorded with an electrode several mm. away from the stimulated point. These changes appear to be identi-
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...cal with those observed in intact brain (17). After a brief preliminary positive deflection (which was not present in all preparations), the potential of the cortical surface became negative with respect to its resting level. The negativity developed over a period of 30 sec. to 1 min., and in some instances attained a peak amplitude of 19-20 mV., recorded with the focal electrode on the surface of the cortex, and the reference electrode on a small patch of killed cortex a few mm. away. The negative potential was usually followed by a smaller positive phase. In most of the experiments described below, the slow negative shift of the cortical potential served as criterion for the presence of spreading depression. The wave of potential change moved across the cortex at a rate of 2-5 mm./min. The rate of propagation and the amplitude of the potential at any one point were independent of the stimulus used to start depression.

b) Effect on responses to electrical stimulation. A single electrical stimulus applied to an isolated cortical slab produces two types of response (4). The first is a short-lasting, surface-negative response, which is transmitted with decrement for a distance of about 10 mm. from the stimulating electrodes. A stronger stimulus produces, in addition, a synaptically propagated surface-positive response which spreads over the slab in all directions from the stimulated point. The changes in amplitude of these responses to a single stimulus during the slow potential changes of spreading depression are shown in Fig. 1 (lower record). The surface-negative response usually began to decrease in size as the slow negative shift of the potential of the cortical surface began, and disappeared when the negativity had attained a height of 3-5 mV. The time at which the surface-positive response began to decrease was more variable. Sometimes it declined together with the surface-negative response. In other cases, as in Fig. 1, its amplitude began to fall off even before the cortical surface started to become negative. This may be due to a decrease in the number of functioning neurones in the self-re-exciting chains which transmit the surface-positive response (2) resulting from the depression of adjacent regions of the slab.

The variability of the time course of disappearance of the two responses might be partly explained by the fact that an increase in cortical impedance occurs during depression (9, 19), so that the effective strength of the single testing stimuli would not really have been constant. However, even when testing stimuli of considerably more than threshold strength were used, both responses did eventually disappear completely, so that the decreased cortical excitability cannot be entirely attributed to changes in cortical impedance.

The surface-negative response often reappeared as early as 15-20 sec. after the peak of the slow negative potential, and steadily increased in size over the next 3-4 min., reaching a peak amplitude of almost twice the control value recorded before depression, before it began to decrease again. The surface-positive response began to return at about the same time as the surface-negative response, but was at first restricted to the region close to the...
electrodes through which the single stimuli were delivered. Propagation of this response did not occur until 3–4 min. later, and the amplitude of the propagated response did not return to its pre-depression value for at least another 4–5 min., so that this response did not appear normal for at least 10 min. after the onset of depression. Frequently the response remained depressed in amplitude, or could not be elicited at all, during the whole subsequent course of the experiment, which lasted for several hours. The slow recovery of this synaptically propagated response, as compared with the recovery of the non-synaptic surface-negative response, suggests that either the structures which form synaptic connections among the cortical neurones or the synaptic mechanisms themselves recover last from depression.

c) Initiation of depression. Depression could be started in the isolated slab by most of the methods which have been used in preparations with an intact brain (15, 20). Strong repetitive stimulation, negative polarization of the cortical surface, mechanical stimulation, and the application of KCl were all effective. It was found that positive polarization of the surface could also start the response, although the threshold current strength was much higher than for negative polarization. Moreover, if subthreshold positive polarization was applied to the cortex in the path of a depression response which was already in progress, the spread of the depression was arrested.

Depression responses could be easily and regularly elicited by all the above means at 10–20 min. intervals for a period of 4–9 hours, although the threshold tended to rise somewhat during the progress of the experiment. As the experiment went on, there was also a greater tendency toward epileptiform after-discharge (31) following stimulation, so that the same stimulus strength, which produced only depression at the beginning of the day, might several hours later produce an epileptiform discharge followed by depression. It should be noted that there was never any epileptiform activity other than that associated with the initiating stimulus (cf. 15).

II. Single unit activity during spreading depression

When a microelectrode was inserted into the cortex, it was found that a short period of intense neuronal activity occurred at the onset of depression. In the experiment shown in Fig. 2, it was possible to record single unit “spikes” and slow potential changes with the same saline-filled microelectrode which was inserted to a depth of about 1 mm. below the cortical surface. Before the appearance of the slow negative wave (Fig. 2a), no single unit discharges were seen, but just as the slow negative potential began to rise, there occurred a burst of intense activity, lasting about 2–3 sec. (Fig. 2b). After this, there was no further detectable activity during any phase of the slow potential changes (Fig. 2c). The burst of activity did not appear to be restricted to the cells at any single level in the cortex, since it could be recorded with the microelectrode at any depth in the grey matter below 0.2 mm., i.e., below layer I. It is probable that the unit spike poten-
tials in layer I were too small or too sparse to be detected (cf. 21). As a result of this activity, a series of surface-positive responses sometimes occurred, originating at the front of the depression wave and spreading into the undepressed regions of the slab.

In the present study, a great deal of importance has been attached to the observation that a brief period of intense activity precedes the neuronal depression. As will be shown below, if this activity is interfered with by stimulation of the cortex, the spread of depression may be arrested. It is therefore believed that the phase of neuronal excitation is an essential link in the spreading depression mechanism.

III. Effect of repetitive stimulation

In order to determine whether artificial activation of the cortex would affect the spread of depression, repetitive stimulation was applied to the isolated slab in the path of a depression response. Although this stimulation was in itself not strong enough to initiate spreading depression, it did produce in the stimulated region a decrease in the amplitude of the slow negative potential of the oncoming depression wave. Moreover, if somewhat stronger stimulation was used, although it was still subthreshold for starting depression, the spread of the oncoming depression wave could be completely

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**Fig. 2.** Unit neuronal activity during spreading depression. Simultaneous recording with microelectrode of 1: unit activity recorded at high gain with 1 msec. TC (indifferent lead on cortical surface over tip of microelectrode); 2: slow cortical potential changes recorded at low gain with DC-coupled amplifier (indifferent lead on killed cortex). Samples of unit activity are shown a) preceding negative potential wave, b) as negativity begins, c) after peak of wave.
arrested. In order that these effects might be produced, it was necessary to start the repetitive stimulation at least 1 min. before the onset of depression at the stimulated point.

These results may be interpreted in accord with the idea that the propagation of depression depends on a substance which is present in resting cortical neurones, and which is released from the neurones during activity. Prolonged repetitive stimulation of the cortex would exhaust this substance, or at any rate, would cause a decrease in the ratio of its intracellular and extracellular concentrations. Thus the release of the substance during the period of intense activity at the onset of depression would be interfered with, and the spread of depression would be impeded. One substance which is known to be released during nervous activity in the manner postulated above is K+ (14). The experiments described in the following sections provide some evidence for the view that K+ actually plays a specific role in the depression mechanism.

<table>
<thead>
<tr>
<th>Table 1. Effects of 1% KCl solution applied to surface of cortex</th>
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<td><strong>Amplitude of negative potential</strong></td>
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<td>mV.</td>
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<td>a)</td>
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<td>1. Before KCl (Fig. 3A)</td>
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<td>2. After KCl (Fig. 3B)</td>
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<td>1. Before KCl (Fig. 3C)</td>
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<td>2. After KCl (Fig. 3D)</td>
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* In all cases, the potential level before stimulation was taken as the baseline.

IV. Application of KCl

When a series of depressions was produced in a single preparation, it often occurred that the amplitude of the negative potential recorded at any one point progressively decreased in size. The decrease was gradual and irregular, and was to some extent irreversible. However, it was possible to restore the amplitude of the potential at least partially by the application of KCl to the surface of the cortex. Photographs of the actual records obtained in an experiment of this kind are shown in Fig. 3. The changes in amplitude and the corresponding changes in conduction velocity are summarized in Table 1. It is seen that the amplitude of the negative potential and the conduction velocity were much less affected by the application of KCl at the beginning of the experiment than when the KCl was applied after the slow negative potential had declined. These effects cannot be attributed to the mode of application of the KCl solution, since it had the same effects when it was injected intravascularly. Other ions, even in hypertonic solution, did not have the same effect as K+. NaCl applied superficially in concentrations
up to 3 per cent did not affect the amplitude of the negative potential, while 1 per cent CaCl₂ was found to decrease it.

These results suggest that the observed decrease in the amplitude of the slow negative potential during a series of depressions was actually due to the depletion of K⁺, which could be compensated for by the application of KCl to the cortex. This points to the importance of the role of K⁺ in the mechanism of spreading depression.

V. Effects of polarization

The effects of applied potential gradients on the rate of spread of depression were found to be consistent with the idea that the propagation of depression depends on the movement of a positively charged particle such as K⁺. In these experiments, tangentially oriented potential gradients in the cortex were created by connecting a source of constant current to two non-polarizable electrodes applied one at each end of the isolated slab. After the polarizing current had been turned on, depression was initiated by repetitive stimulation at one end of the slab, and the conduction velocity of the response was determined by measuring the time interval between the arrival of the peak of the slow negative potential at two recording electrodes. The conduction velocity showed considerable variation even when no polar-
izing current was flowing, so that no single control value could be used. However, it was possible to demonstrate that the polarization was actually effective by plotting the conduction velocity of each of a series of responses, produced during or in the absence of polarization, against the time at which the particular response was initiated, as has been done in Fig. 4. It is apparent from this figure that the conduction velocity increased considerably between the beginning and the end of the experiment. In spite of this, it can be seen that in each instance the conduction velocity was decreased when the front (rostral) end of the slab was polarized negative relative to the back (caudal) end, and increased when the front end was polarized positive relative to the back. In addition, it was found that under the conditions in which the conduction velocity was increased, the amplitude of the slow negative potential was also increased. This increase in amplitude was observed to occur in regions close to both the polarizing anode and cathode. Similarly, polarizing currents which produced a decrease in conduction velocity also produced a decrease in the amplitude of the slow negative potential in regions close to both polarizing electrodes. In the argument which follows, it will be shown that these results make it appear unlikely that the propagation of depression depends on an electrical mechanism involving either radially or tangentially oriented dipoles.

First, let us consider the cortex as consisting of a set of radially oriented neuronal elements, subjected to polarization as shown in Fig. 5A. Under these conditions it would be expected that the effects produced at one polarizing electrode would be opposite in direction to those produced at the other electrode. In the region of the anode, for example, the membrane potential of the superficial ends of the radial elements would increase and that of their
deep ends would decrease, while in the region of the cathode the superficial ends would become depolarized and the deep ends hyperpolarized. Thus, if the propagation of depression depended on current flow between the deep and superficial ends of these elements, a mechanism analogous to that proposed by Gerard and Libet for the propagation of caffeine waves in the cortex (10), it would follow that the rate of propagation of depression would be increased in the neighbourhood of one of the polarizing electrodes, and decreased in the neighbourhood of the other. It would also be expected that the amplitude of the potential generated by the radial current flow would change in opposite directions at the two electrodes as a result of the polarization.

However, it was actually found that the effects produced by polariza-

Fig. 5. Effects of tangential polarization on radially oriented (A) and tangentially oriented (B) sets of hypothetical neuronal structures. Neuronal structures in question are represented as appropriately oriented rectangles. The + and − signs represent regions in which neuronal membrane potential is increased and decreased respectively as result of polarization in the sense indicated. Arrow shows direction of propagation of spreading depression when polarization causes an increase in its rate of spread.

tion were qualitatively the same throughout the whole length of cortex between the two polarizing electrodes (although the effects were most intense close to the electrodes because of the greater density of current flow). It might seem, therefore, that the elements involved in spreading depression, which determine the rate of spread of the response and the amplitude of the slow negative potential, are arranged parallel to the surface of the cortex rather than at right angles to it.

Let us now consider such a set of tangentially oriented elements, subjected to polarization in the direction which was found to increase the rate of spread of depression, i.e., positive-to-negative in the direction of spread (Fig. 5B). Because of the polarizing current, that end of each element which is closer to the front of the oncoming wave of depression has its membrane relatively hyperpolarized with respect to the resting state, while the distal end is relatively depolarized. If the propagation of depression depended
solely on the flow of electrical currents at the wave front (cf. 10), it appears likely that the rate of propagation would be *slowed down* under these conditions of polarization, since in the transmission of depression from one element to the next, the relatively hyperpolarized portions of the elements would have to be depolarized first, and a heavier current flow would thus be required. However, it was actually found that polarization in this direction increased the rate of spread of depression. Thus the hypothesis that a current flow mechanism is involved does not appear to be tenable, regardless of which arrangement of electrical dipoles is postulated. An alternative explanation, which appears to fit the facts better, is that the propagation of depression depends on the movement of a positively charged particle. This particle would tend to move along the applied potential gradient from the positive toward the negative pole. On this basis, it would be expected that the rate of propagation of depression would be increased when the applied gradient was positive-to-negative in the direction of propagation, and this was actually found to be the case.

While there is no direct evidence for assuming that the positively charged particle is \( K^+ \), this view is in accord with the evidence which has been presented above. Moreover, it has been found that a potential gradient applied to peripheral nerve (13) or to muscle (12) will cause the migration of \( K^+ \) in the manner which has been postulated. It is not possible from our present data to estimate whether the electrophoretic effects of the gradients which were used in the present experiments would have been of sufficient intensity to account for the observed changes in the rate of spread of the depression response. Qualitatively, however, the results obtained bear out the hypothesis that the spread of the response is determined by the movement of \( K^+ \).

VI. Effects of anoxia

It has not been possible to find any direct evidence that \( K^+ \) or any other chemical agent is actually liberated during depression. A number of attempts were made to collect a "transmitter substance," i.e., a substance liberated during depression which would produce depolarization when reapplied to the cortex, on pieces of filter paper inserted into the cortex, and in saline pools on the cortical surface. The results of the filter paper experiments were all negative and the results of the saline pool experiments were equivocal, since it was found that a depolarizing substance was liberated in about equal amounts from resting cortex and from cortex undergoing depression.

However, the results of experiments with arterial occlusion seemed to provide some indirect evidence that a chemical mechanism was involved in depression. When the middle cerebral artery was clamped for 35 sec. with the cortex in a resting condition, the surface-positive responses to single stimuli disappeared within 15 sec., indicating that at least some of the corti-
cal synaptic connections had ceased to function, but the DC potential of the cortex did not markedly change (Fig. 6A). If the period of occlusion occurred during the falling phase of the slow negative potential of spreading depression, the cortical negativity was greatly prolonged (Fig. 6B, C). If the period of occlusion occurred during the rising phase of the negative potential, the cortical negativity developed more rapidly, and attained a greater amplitude (Fig. 7).

One possible interpretation of these results, which confirm those obtained by Leão (18) and by van Harreveld and Stamm (30), is that occlusion of the vascular supply prevents the removal by the bloodstream of some chemical agent which is responsible for the appearance of the slow negative potential. This view had to be rejected, however, when it was found that asphyxiation with $N_2$ produced the same effects as arterial occlusion, although the blood flow was not arrested.

While these experiments ruled out one possible argument in favour of
the participation of a chemical transmitter, they did not provide any positive evidence about the actual nature of the propagation mechanism. They did, however, demonstrate an important feature of spreading depression which has not previously been emphasized—namely, that recovery from depression involves an oxidative process. In conditions of O₂ lack, this recovery process is interfered with, and the slow negative potential is consequently prolonged.

**Discussion**

It is necessary that any hypothesis concerning the mechanism of spreading depression should be able to account for all the cortical changes which occur during this response. The most important of these in the isolated cortical slab include the slow shift of the cortical DC potential, the disappearance of all cortical responses, and, as the present experiments have shown, the occurrence of a brief period of intense activity before the cortical neurones become depressed. In addition, the postulated mechanism must be in accord with the facts that spreading depression is a cortical phenomenon, that it spreads at a very slow rate, and that it is resistant to anaesthetics and anoxia.

From the experiments described above there is some evidence that the depression mechanism depends on a substance which is present in resting neurones and which is liberated during activity, and there is reason for believing that the substance involved may be K⁺. These facts are all in accord with the hypothesis that the release of K⁺ from the cortical neurones is the essential mechanism involved in the propagation of spreading depression. It appears possible that the intense neuronal activity which accompanies the onset of depression results in the liberation of K⁺ into the interstitial spaces in sufficient quantity to depolarize adjacent cells. These cells are in turn forced into intense activity, and thus further K⁺ leakage occurs, so that the cycle is repeated. In the discussion which follows, it will be shown how the characteristics of spreading depression might be accounted for on the basis of this hypothesis.

a) **Initiation of depression.** If the liberation of K⁺ during intense neuronal activity is responsible for the spread of depression, it may be expected that depression would be initiated by any condition which produces intense activity in a localized region of the cortex. Repetitive stimulation, surface-positive polarization (3), injury, the application of strychnine, and the depolarization produced by KCl application or by surface-negative polarization would all fall into this category.

The initiation of depression in the isolated slab appears to be considerably easier than in the intact brain. Marshall (23), for example, has reported that in the intact brain of the anaesthetized cat the reaction was "capricious" and could be elicited only with difficulty when the cortex was covered with oil. No such difficulty was encountered in the isolated cortex. This difference does not appear to be due solely to the fact that the isolated preparations were unanaesthetized, since even in these preparations it was
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found to be considerably more difficult to produce depression outside the isolated region. It is possible, therefore, that the greater ease with which depression is produced in the isolated cortex is due to the fact that the spontaneous neuronal activity is reduced by the isolation. It has been shown above that in the isolated slab the spread of depression may be arrested in a region which is subject to activation by repetitive stimulation. A similar effect might be produced by cortical activity resulting from impulses arriving from extracortical centres. Thus the continual "spontaneous" activity which is usually present in the cortex of the intact brain (21) may make depression more difficult to elicit than in the isolated slab, in which neuronal activity occurs in the unstimulated preparation only during surface-positive bursts (3).

The mechanism by which surface-positive polarization stops the spread of depression may also be the same as that involved in the arrest of depression by repetitive stimulation, since surface-positive polarization has been shown to produce intense neuronal activity (3).

b) Propagation of depression. The propagation of depression, according to the scheme which has been proposed, depends on the transmission of excitation from one group of neurones to the next. However, the K+ liberated during activity could produce further excitation only if the neurones involved were sufficiently close together. This limitation may explain why spreading depression is confined to the cerebral cortex. In this tissue, the complex arrangement and interlacing of the neuronal structures, their profuse branching and proximity to one another, would provide the appropriate conditions for the spread of the response. In the white matter, on the other hand, the spread of depression may be prevented by the separation of the individual elements from one another by their myelin sheaths. Moreover, it is possible that a reduction of the neuronal separation may have been the essential factor underlying Marshall's observation that depression could be more readily elicited in cats which had been subject to internal dehydration (23).

c) Slow potential change and disappearance of cortical activity. There has been a tendency to consider the slow potential change as being of different origin from the depression of cortical activity, emphasis having been placed on the fact that the return of activity frequently did not occur for some time after the slow potential changes had disappeared (25). In the present experiments it has been shown (Fig. 1) that some of the neuronal structures are actually inexcitable only during the period in which the slow negative potential is present, and recover rapidly thereafter. On the other hand, the structures which are responsible for the transmission of synaptic responses become depressed at about the same time, but recover much more slowly, possibly because of their smaller size. It appears likely, therefore, that the active phase of the depression process coincides with the period during which the slow changes in cortical potential occur, but since complete recovery does not occur for some time afterward, the record of cortical activity does not
appear normal for a considerable period of time beyond the duration of these potential changes. Thus it appears to be adequate to postulate a single mechanism to explain both the slow potential shift and the disappearance of cortical activity.

In keeping with the hypothesis which has been here advanced, both these phenomena may be attributed to an increase in extracellular K+ by active neurones. This lowers the membrane potential of adjacent neurones, causing them to fire at first, and then, as their membrane potential falls still further, to become inexcitable (6). The question arises, however, of whether this process can adequately account for the large amplitude of the slow negative potential, which in the present series of experiments was sometimes as great as 19–20 mV. at the surface of the cortex. Davies (7) has found that the complete depolarization of a small region of cortex, produced by the injection of KCl into the cortical arteries, generated a maximum negative potential of 21 mV. at the cortical surface. It seems, therefore, that the large negative potential observed during spreading depression could reasonably be attributed to neuronal depolarization by K+.

However, the precise manner in which this negative potential is generated is still not clear. Two possibilities suggest themselves. One is that the superficial ends of the neurones become depolarized while the lower ends are still polarized. It seems quite likely that in the case of the pyramidal cells, for example, the terminal branches of the apical dendrites lying near the surface might become depolarized more readily than the larger dendritic trunks lying deeper in the cortex. An alternative explanation which may be considered is that the superficial ends of resting cortical neurones are normally positive to their deep ends, and that this potential difference is wiped out when the neurones become depolarized. There is some indication that a potential gradient of this kind does actually exist, since Goldring and O'Leary have found that in resting cortex the pial surface is usually positive to the ventricle (11); but at the present time there does not appear to be sufficient evidence to reject one of these mechanisms in favour of the other, and it is possible that both of them may be responsible for the development of the slow negative potential associated with spreading depression.

d) Recovery from depression. It has been shown above that recovery from spreading depression involves an oxidative process, which presumably results in the restoration of the intraneuronal K+. This ties in with the fact that a period of increased oxygen utilization begins at about the time that the slow negative potential is at its peak (24). It appears likely that the increased production of CO₂ and of acid metabolites during this period accounts for the vasodilatation which has been observed during depression (16).

This increased oxygen consumption associated with spreading depression may be related to the increased oxygen consumption which occurs in brain tissue slices on the addition of excess K+ to the Ringer medium surrounding them (1, 8). It has been suggested (22) that this effect of K+
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might be due to the fact that this ion enters somehow into the tricarboxylic acid cycle. However, it has been found that K+ stimulates only the respiration of brain and skeletal muscle, and depresses the respiration of other tissues (5). Thus it does not seem likely that the stimulating effect of K+ could be the result of its direct action in such a mechanism as the tricarboxylic acid cycle, which probably plays a part in the carbohydrate metabolism of all tissues. In the case of skeletal muscle, the stimulation of respiration has been attributed to the presence of contracture (5), and it is now suggested that the stimulation of brain respiration in a high K+ medium is due to the fact that the cortical tissue becomes depolarized, bringing into play the oxygen-consuming processes which are also involved in recovery from depolarization during spreading depression.

SUMMARY

1. Spreading depression has been investigated in the isolated cerebral cortex of the cat. In this preparation, the presence of spreading depression manifests itself in a decrease in cortical excitability and slow changes in the cortical DC potential similar to those which have been reported for intact brain by other workers.

2. By recording with microelectrodes it was shown that a brief phase of intense neuronal excitation precedes the depression.

3. The amplitude of the slow negative depression potential was found to be decreased in a region which was subjected to strong repetitive stimulation. This suggests that the propagation of depression depends on a substance liberated from the neurones during intense activity.

4. The amplitude of the slow negative potential often decreased during a series of depressions, but could be restored at least partially by the application of KCl to the cortex. Since other ions did not have the same effect it appears likely that K+ plays some specific role in the depression mechanism.

5. Polarizing currents applied along the surface of the cortex caused an increase in conduction velocity and in the amplitude of the slow negative potential when the applied potential gradient was positive-to-negative in the direction of spread of depression. When the direction of the polarizing current was reversed, the conduction velocity and the amplitude of the slow negative potential were decreased. These results may be taken to indicate that the propagation of depression depends on the movement of a positively charged particle such as K+

6. The effects of anoxia on the slow negative potential were found to be identical when the anoxia was produced by arterial occlusion or by N2 administration. It was concluded that recovery from depression involves an oxidative process.

7. It was shown that the above findings are consistent with the hypothesis that the propagation of spreading depression proceeds according to the following mechanism. The intense neuronal activity preceding depression results in the liberation of K+ into the interstitial spaces in sufficient quan-
tity to depolarize adjacent cells. These are in turn thrown into intense activity, and liberate more $K^+$. The manner in which the chief characteristics of spreading depression might be accounted for on the basis of this hypothesis has been discussed.

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REFERENCES