Changes in Volume of Cortical Neuronal Elements During Asphyxiation[1]

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ABSTRACT

VAN HARREVELD, A. *Changes in volume of cortical neuronal elements during asphyxiation.* Am. J. Physiol. 191(2): 233–242 1957.—Cortical electrical resistance increases considerably after cortical asphyxiation. After a latent period of about 3 minutes a sudden resistance change occurs during which one-third of the cortical conductivity may be lost. It was postulated that this drop in conductance is due to a transport of ions, accompanied by water, from the extracellular spaces into the cortical cells and fibers. A swelling of cortical elements must thus be expected. The cortex was quickly frozen either before or after the sudden conductivity drop. The frozen cortex was kept in alcohol for 1 week during which the alcohol dissolves the ice in the cortex and fixes the tissue. Histological preparations stained with gallocyanin and with silver for the investigation of cells and fibers respectively were made. In rabbit cortex the mean diameter of nerve cells after the sudden conductivity drop was more than 11% greater than before the resistance change, which represents a volume increase of about 40%. The apical dendrites increased in diameter about 30% implying a volume increase of about 70%. If one assumes that the combined perikaryal and dendritic volume is 30% of the total cortical volume, then the amount of fluid stored by the cortical neurons during the sudden resistance change would amount to about 16% of the cortical volume. Such fluid and ionic movements are ample to explain the resistance changes observed.

IN THE RABBIT arrest of the circulation is followed after a mean latent period of about 3 minutes by a sudden and large increase in the electrical resistance of the cerebral cortex (1, 2). Closely correlated in time with this phenomenon is an asphyxial potential which renders the cortical surface negative with respect to an indifferent electrode (3, 4, 5, 2). These asphyxial phenomena are of interest since they resemble, and are probably closely related to similar phenomena observed during spreading depression. The results of Cole's investigations on the resistance of cell suspensions (6) when applied to a tissue like the cerebral cortex suggest that such large increases in resistance as are observed both after circulatory arrest and during spreading depression can not be due to changes in the membrane resistance of the cortical cells and fibers, but must be due to a removal of intercellular ions which in this way become unavailable for transport of the measuring current (2). Since it has been shown that during the rapid resistance change no marked decrease of the cortical volume takes place, it was concluded that intercellular ions move into the cortical cells and fibers. As a mechanism for this transport a sudden increase in sodium permeability of the cell membranes was proposed, creating a Donnan situation in which the membrane is permeable for sodium, potassium and chloride but remains impermeable for the intracellular anion. This will cause a movement of sodium and chloride ions from the intercellular spaces into the cells and fibers. Such a movement must be accompanied by a transport of water to maintain osmotic equilibrium. The increase in resistance would thus

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be accompanied by a swelling of cells and fibers of the cortex. Increase in sodium permeability is a mechanism generally accepted as the basis for nervous excitation. For this reason the swelling might be expected to occur in the first place in the nervous elements of the cortex, although the possibility is not excluded that similar processes also take place in the glial elements. In the present paper an attempt is made to demonstrate this expected change in the volume of cortical structures.

METHODS

To measure the dimensions of cells and fibers, histological preparations had to be obtained in which the fluid distribution between cellular elements and extracellular spaces in the cortex at the moment of fixation was represented as faithfully as possible. Since the usual histological methods produce considerable shrinking of the tissues, and thus may markedly change the fluid distribution, the following procedure was adopted.

The dorsal aspect of one hemisphere was widely exposed under ether narcosis and the trachea was cannulated. The edges of the skin surrounding the wound which exposed the brain were sewed to a steel ring. By lifting this ring a skin cup was formed. After the surgical procedure was finished, artificial respiration was given, and Squibb's Intocostrin (5 U/kg bodyweight) was administered. When the appropriate moment for fixation of the cortex had arrived, the skin cup was filled with alcohol cooled to its freezing point ($\pm -115°C$) with liquid nitrogen. Nitrogen was then poured on top of the alcohol to keep it cold. The head was severed from the trunk and dumped quickly into a large beaker containing alcohol cooled to the temperature of solid carbon dioxide. The beaker was placed in a refrigerator at a temperature between -17 and $-20°C$, and left there for a week. The alcohol slowly dissolved the ice in the cortex, fixing the tissue where it replaced the ice. Since the alcohol penetrated only a few millimeters of cortex during this time, fixation proceeded slowly and each successive layer of tissue was fixed while the immediately underlying tissue was still frozen in its original shape. One may expect therefore that the original fluid distribution is better preserved by this method of fixation than by the standard methods.

After fixation, one, or in some experiments, two slices of cortex were removed as indicated in figure 1. For the study of nerve cells a slice was embedded in paraffin, sectioned and stained with gallocyanin. For the study of nerve fibers a slice was stained with one of Cajal's silver methods (7, method *B*), then embedded and sectioned.

The cortex was frozen either before or after the resistance change had taken place. The cortical resistance changes were measured with electrodes, consisting of silver chloride plated silver discs 1½ mm across, which were placed on both sides of the region to be investigated histologically, as shown for the rabbit in figure 1 (*A*, *A'*). These electrodes were connected by very thin copper wires with the resistance bridge described in a previous paper (2). The moment of fixation was determined by the observed resistance change. Sometimes a third electrode consisting of a beaded silverchloride plated silver wire mounted on a spring was placed on the cortex in between the resistance electrodes. This electrode led off asphyxial potentials (against an indifferent electrode) which after amplification were recorded with a d'Arsonval galvanometer on photosensitve paper.

RESULTS

First Series of Experiments on Rabbits. In the first series, 10 pairs of rabbits matched in weight were used. In one animal of each pair, serving as control, the cortex was frozen while the circulation of the cortex was intact. When the cortex is normally perfused with blood the resistance remains more or less constant for considerable periods of time. It is markedly increased, however, when a spreading depression passes over the cortex (1, 8, 9). If this were caused by the same mechanism as the asphyxial resistance increase, then the presence of a spreading depression might impair the value of the preparation as a control. To avoid freezing while a spreading depression was in progress, the cortex was not frozen until 15 minutes had elapsed after the preparation was finished, ample time to recover from any spreading depression which might have been elicited in exposing the cortex. Furthermore since an injured area may be the site of spontaneous spreading depressions, only prepara-

tions without visible damage to the cortex were used.

In the other animal of each pair the aorta was severed 10–15 minutes after the preparation was finished, and the cortex was frozen 15 minutes after the circulatory arrest produced in this way. At that time the quick resistance change can be expected to have taken place since the mean latent period of this phenomenon does not exceed 3 minutes; the longest latent period observed was 7 minutes (2). In this series of experiments two slices of cortex were taken from each brain. One was stained with gallocyanin to measure nerve cells, the other with silver for the study of nerve fibers.

A study of the gallocyanin as well as of the silver stained preparations showed that in general the more superficial layers (upper 0.2–0.3 mm) of the cortex are better preserved than the deeper layers. In the deeper layers the tissue in between the nervous elements has a vesicular appearance. This is probably caused by the slower freezing of the deeper layers, resulting in formation of relatively large ice crystals. The slowly penetrating alcohol may have fixed the tissue around these ice crystals, which then remain visible as the clear spaces in the vesicles. In the upper layers the ice crystals are so small that they do not disturb the histological picture. All measurements have been performed in the upper cortical layers.

Changes in Nerve Cell Diameter. Nerve cells were studied with a dry system (Zeiss Apochromat 20) and a Zeiss camera lucida. The magnification of this system was 700 ×. The entity to be measured was marked on the paper acting as a screen of the camera lucida. These marked distances were later measured. The nerve cells of the cortex are not equal in size at all distances from the cortical surface. It is therefore necessary to compare cell dimensions in the same layer or layers. Since the upper layers were least disturbed by ice formation, the following procedure was adopted. The preparation was placed in such a way that the edge of the field (about 250 μ in diameter) coincided with the boundary between molecular and cellular layers. In such a field the width of all cells which showed a clearcut apical dendrite was measured. This insures that the cells measured are nerve cells and also that the largest part of each cell is situated in the

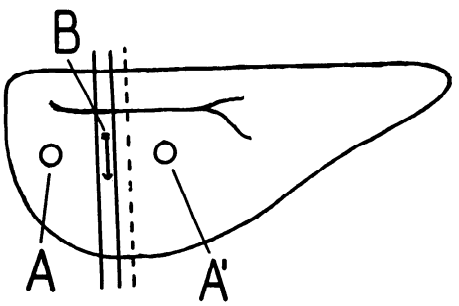

FIG. 1. Dorsal view of a hemisphere of the rabbit. Parasagittal sulcus is indicated. Slice or slices of cortical tissue removed for investigation are indicated by the continuous, respectively interrupted, vertical lines. *A* and *A'* indicate the placement of the electrodes for resistance measurement. *B* indicates the region, 2 mm lateral from the parasagittal sulcus, where measurement of cells and apical dendrites was started.

section viewed. After measuring all the cells satisfying these requirements an adjacent field was measured in the same way. The measurement was started 2 mm lateral from the parasagittal sulcus (fig. 1*B*) and was continued lateral from this point until about 100 cells were measured. The greatest width of the cells was marked on the screen of the camera lucida. This only gives difficulties, when large dendrites arise from the cell in the region to be measured. In order to determine the reproducibility of the measurements two preparations of each cortex were measured in this way with an interval of about 1 month. The preparations were coded to prevent bias by the knowledge of the history of the preparation. The differences between the two determinations of the cell diameter in the same cortex were about what could be expected from the standard error determined of each group of 100 measurements. In table 1 the mean of the combined samples of 200 measurements with their standard errors are given in millimeters, as measured on the camera lucida screen (multiplication with 1.43 gives the cell diameter in μ). The standard error is so small (0.06–0.09 mm), that the variability of the sample is considerably smaller than the differences in mean cell diameters in a group of cortices which have undergone the same treatment. The variability of the sample was therefore neglected in considering the differences in cell diameter before and after the rapid resistance change. The mean cell diameter in all experiments in which the cortex was frozen

TABLE 1. DIAMETER OF NERVE CELLS AND OF APICAL DENDRITES IN RABBITS (FIRST SERIES)

No.	Cell Diameter		Diameter of Apical Dendrites	
	Before resistance change	After resistance change	Before resistance change	After resistance change
1		9.4±0.06		7.1±0.16
2	8.5±0.06		5.0±0.13	
3		9.8±0.06		7.7±0.17
4	8.8±0.07		5.8±0.17	
5	8.7±0.06		5.6±0.14	
6		9.6±0.07		7.8±0.16
7	8.7±0.06		5.7±0.17	
8		9.7±0.07		7.8±0.15
9	8.9±0.08		5.8±0.16	
10		9.8±0.08		7.4±0.14
11	8.9±0.06		5.5±0.14	
12		9.6±0.08		7.3±0.12
13		9.5±0.07		7.3±0.16
14	8.6±0.06		5.3±0.14	
15	8.7±0.07		5.9±0.14	
16		9.9±0.09		7.0±0.16
17		10.4±0.08		7.8±0.16
18	9.2±0.08		5.5±0.13	
19	9.3±0.08		5.8±0.19	
20		10.4±0.08		7.7±0.14
Means	8.8±0.07	9.8±0.11	5.6±0.05	7.5±0.05

while the circulation was intact was 8.8 mm (12.6 μ) with a standard error of 0.07 mm; the diameter after the resistance change can be expected to have taken place was 9.8 mm (14.1 μ) with a standard error of 0.11 mm. The difference between these two groups of cells is 11.2%. This difference is highly significant ($t = 7.5$).

A histogram of the 2000 cell diameters before and after the rapid resistance change (fig. 2*A*) shows that the distribution is almost normal. This figure furthermore shows the variability in cell diameters in the two groups of cortices which were compared. The histogram of the cell diameters from cortices frozen after the resistance change is similar to that frozen before the change, but is moved to the right.

Cortices with intact blood circulation were compared with cortices in which the circulation had been arrested and from which part of the blood had drained away (2). The measurements in the preparations after the resistance change may therefore include cells from a slightly deeper region of the cortex, and since the mean cell diameter changes with distance from the cortical surface, an error may have been introduced. The amount of blood in the cortex was determined by comparing the iron content of cortex, frozen while the circulation was intact, with the iron content of the blood of the same animal. The frozen cortex was collected after the pial vessel had been scraped off so that only the amount of blood in the cortical vessels was determined. Under the conditions of the experiment this proved to be 4–6% of the cortical volume. We may have determined therefore the cell diameters in a layer of cortex about 5% thicker in the experiments after the resistance change than in the experiments in which cortex with intact circulation was frozen. A comparison of mean cell diameter in the superficial half of each field with that of the deeper half showed the latter to be 3.0% ± 0.5% greater. Assuming a regular change of cell diameter, then a larger mean cell diameter of 0.15% due to this factor can be expected if all blood were drained from the cortex. Since the cell diameter after the resistance change is about 11% greater than before the change, this sampling error can be neglected.

Changes in Diameter of Apical Dendrites. Changes in dendritic diameters were investigated in the same cortices in which cell diameters were measured. Dendrites are present in a great variety of sizes and it is difficult to be sure that a group of dendrites in one preparation is directly comparable with a seemingly similar group in another preparation. Moreover the diameters of the smaller dendrites are hard to measure with the light microscope. The measurements were therefore restricted to the diameters of apical dendrites. To insure that the measurements are performed at comparable places, the apical dendrites were measured in a plane just below the boundary between the molecular and cellular layers. The dendrites actually on this boundary are less suitable because at that level many of them are branching. In the region used for measurement the apical dendrites with few exceptions run normal to the cortical surface, whereas in the more superficial regions many of the branches run at an angle. The dendritic surface was seldom smooth, it usually had a more or less wrinkled appearance. The same set up as used for the perikarya was employed, with the exception that the preparations were viewed under oil immersion. The apparatus was ad-

justed to magnify the structures in the field 3000 × on the screen of the camera lucida. The diameter of all the apical dendrites in a field were marked on the paper acting as screen of the camera lucida. The distances between the marks were measured subsequently. About 100 dendrites were measured in each preparation. As in the case of the cells the first field measured was located 2 mm lateral of the parasagittal sulcus (fig. 1*B*). The fields subsequently examined were lateral of this point. As with the preparations of nerve cells the slides were coded to prevent a bias by the knowledge of the origin of the preparation from cortex either before or after the sudden resistance change.

Table 1 shows the results of these measurements. Ten preparations of cortex frozen while the circulation was intact and ten preparations of cortex frozen 15 minutes after circulatory arrest were studied. The mean of each group of about 100 measurements is given, expressed in millimeters on the camera lucida screen (multiplication by ⅓ gives the diameter of the apical dendrites in μ). The standard error of each group of dendritic diameters is considerably larger than that of comparable groups of cell diameters. The variability of each sample, therefore, cannot be neglected in determining the standard error of the mean dendritic diameter of the two groups of cortices which had undergone the same treatment, as was done with the diameters of the nerve cells. All measurements of dendritic diameters in cortices before the resistance change were therefore combined in one group and its standard error computed in the usual way. The same was done for all diameters of the group of cortices frozen 15 minutes after circulatory arrest. The mean diameters of the cortices with intact circulation was 5.6 mm (1.9 μ) with a standard error of 0.05 mm. The diameter of the dendrites in cortices frozen 15 minutes after circulatory arrest was 7.5 mm (2.5 μ) with a standard error of 0.05 mm. The mean diameter of the latter group is about 34% larger than that of the former. This difference is statistically highly significant ($t = 26$).

Second Series of Experiments on Rabbits. The two groups of cortices in the first series of rabbits were frozen while the circulation was intact, and 15 minutes after circulatory arrest. Some time during this interval the sudden resistance change occurs, which may take 2–3 minutes (the beginning but especially the end of this phenomenon are not sharply defined). In a second series of 16 rabbits an attempt was made to decrease the interval between the freezing of the cortices before and after the rapid resistance change. In all these experiments the cortical resistance was recorded. The development of a spreading depression which may impair the value of the cortex as a control can under these circumstances be recognized as an increase of cortical resistance with typical time course. In eight rabbits the cortex was frozen 1 minute after the circulatory arrest by severing the aorta. Since the shortest latent period of the sudden resistance change and asphyxial potential observed was about 1 minute (5) it can be assumed that in these cortices the resistance change had not started. Indeed the cortical resistance did not increase markedly during this time. In eight other animals the cortex was frozen after the quick resistance change had taken place and the rate of resistance change was definitely falling off. Table 2*A* shows the interval between severing the aorta and freezing the cortex in these eight experiments. The mean is 5 minutes and the interval between the two groups of experiments was thus decreased to 4 minutes. The sudden resistance change including its onset and decline takes 2–3 minutes of this period.

In these two groups of cortices the di-

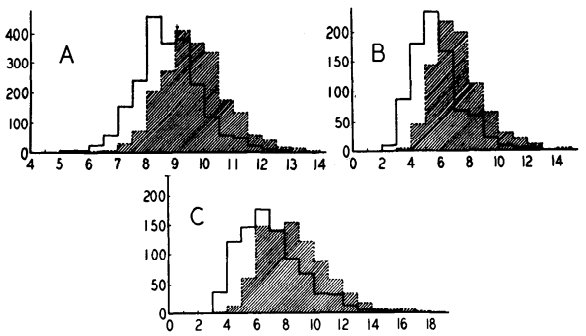

FIG. 2. Histograms of diameters of cells and apical dendrites. On the ordinates are plotted the number of cells resp. apical dendrites, on the abscissa the diameter in mm on the screen of the camera lucida. *A* shows 2 histograms. One, heavily outlined, is of the diameters of cells in rabbit cortex before the resistance change; the striped histogram is of cell diameters after this change has taken place. *B* and *C* show histograms of the diameters of apical dendrites before and after the resistance change in rabbit cortex (*B*) and cortex of the cat *C*.

TABLE 2. DIAMETERS OF APICAL DENDRITES IN RABBITS (SECOND SERIES), IN CATS AND IN MONKEYS

No.	Before Resistance Change	After Resistance Change	Time of Freezing
	A. Rabbits (second series)		
21	5.3 ± 0.14		
22		7.1 ± 0.14	5′50″
23		7.2 ± 0.16	5′30″
24	5.6 ± 0.15		
25	5.8 ± 0.17		
26		7.3 ± 0.16	4′20″
27		7.1 ± 0.14	4′50″
28	6.0 ± 0.19		
29		7.0 ± 0.15	5′10″
30	5.8 ± 0.15		
31		7.1 ± 0.17	4′10″
32	5.8 ± 0.17		
33	5.6 ± 0.15		
34		7.7 ± 0.17	5′—
35		7.8 ± 0.21	5′30″
36	6.0 ± 0.17		
Means	5.7 ± 0.06	7.3 ± 0.06	5′—
	B. Cats		
37		8.1 ± 0.24	11′50″
38	6.7 ± 0.20		
39		8.0 ± 0.19	10′30″
40	6.4 ± 0.17		
41		8.1 ± 0.20	8′40″
42	6.5 ± 0.21		
43	7.3 ± 0.23		
44		9.0 ± 0.20	10′—
45		9.2 ± 0.26	8′30″
46	7.3 ± 0.21		
47	7.1 ± 0.22		
48		8.9 ± 0.24	8′40″
49		8.6 ± 0.22	8′—
50	7.2 ± 0.21		
51	7.3 ± 0.22		
52		9.2 ± 0.22	7′50″
Means	7.0 ± 0.07	8.6 ± 0.08	9′30″
	C. Monkeys		
53	7.5 ± 0.22		
54		9.7 ± 0.25	6′50″
55	8.0 ± 0.23		
56		9.1 ± 0.24	7′—
57	7.8 ± 0.19		
58		9.4 ± 0.26	9′20″

ameters of apical dendrites were measured in the way described above. The results are shown in table *2A* which is arranged in the same way as table 1. The mean dendritic diameter in all cortices which were frozen 1 minute after circulatory arrest was 5.7 mm (1.9 μ), with a standard error of 0.06 mm (computed as described for the apical dendrites in the first rabbit series). The mean diameter of the dendrites in cortices frozen after the conclusion of the quick resistance change was 7.3 mm (2.4 μ), with a standard error of 0.06 mm. The difference in mean dendritic diameter of the two groups was 27%. It is statistically highly significant (t = 20). These values are not greatly different from the figures of the first series of rabbits (table 1) in which the two groups of cortices were frozen with a time difference of 15 minutes. The results of this series of rabbits therefore increases the likelihood that the differences in diameter of the apical dendrites observed are correlated with the sudden resistance change.

Histograms of the dendritic diameters of the two groups of cortices of the second series of rabbits (fig. *3B*) shows the distribution to be slightly skewed. It shows furthermore the variability of the dendritic diameters. The histogram of the diameters in cortices frozen after the resistance change is similar to that in cortices frozen before the change, but is moved to the right.

A comparison of the apical dendrites in preparations from cortices frozen before and after the rapid resistance change shows them to be of a different appearance. The dendrites in the preparations frozen after the sudden resistance change appear not only thicker but also seem more lightly stained than the dendrites in preparations fixed before the change. These differences were often so great that a glance at the apical dendrites sufficed to identify the origin of the preparation. A more thorough study made it possible to make a positive identification in all cases by taking into account only the diameter and general appearance of the apical dendrites.

Diameter of Apical Dendrites in the Cat. In a series of 16 cats the cortex was frozen either 1 minute after circulatory arrest by cutting the abdominal aorta, or after the conclusion of the sudden resistance change. Figure 3 shows the changes in conductance (1/R) and the asphyxial potential in one of these experiments (no. *44*, table *2B*). The general course of both phenomena resembles closely that of the rabbit (2). Table *2B* shows the period of time between severing the aorta and freezing of the cortex. The mean of this period in the cat was 9 minutes 30 seconds whereas in the rabbit it was 5 minutes. This is partly due

to the longer latency of the sudden resistance change in the cat. The mean latency in the eight experiments on cats was 4 minutes 30 seconds ± 30 seconds versus a mean of 2 minutes 50 seconds ± 10 seconds in a comparable group of rabbits (2).

A slice of brain tissue was taken from the middle of the suprasylvian gyrus and stained with silver. The preparations obtained were in general less suitable for the measurement of neuronal elements than those of the rabbit because of a more pronounced ice formation in the cortex. For this reason no attempt was made to compare cell diameters which differ considerably less than the diameters of apical dendrites in cortices before and after the resistance change. The branching of the dendrites in the cortex of the cat often started at a deeper level and was not as regular as in the rabbit. This made the place where the dendritic diameter was measured less distinct and certain. Table 2*B* shows the result of the measurements. The standard error of each group of about 100 measurements was larger than in comparable measurements in the rabbit. The mean diameter of the apical dendrites measured in cortex frozen 1 minute after circulatory arrest was 7.0 mm (2.3 μ). The standard error, computed as described for the apical dendrites in the first rabbit series, was 0.07 mm. The dendritic diameter in cortices in which the sudden resistance change had taken place was 8.6 mm (2.9 μ) with a standard error of 0.08 mm. The difference of 23% between the two groups of experiments is statistically highly significant ($t = 15$).

A histogram of the dendritic diameters (fig. 2*C*) showed a slightly skewed distribution. The histograms of the measurements before and after the sudden resistance change are similar in general appearance, but the latter is moved to the right with respect to the former.

In the cat the apical dendrites in preparations from cortices before and after the resistance change also showed often an obvious difference in diameter and general appearance. A more thorough examination of such cortices made it possible to determine the origin of the preparation in all cases.

Diameter of Apical Dendrites in the Monkey. In a series of six rhesus monkeys the diameters of apical dendrites were examined. The weights

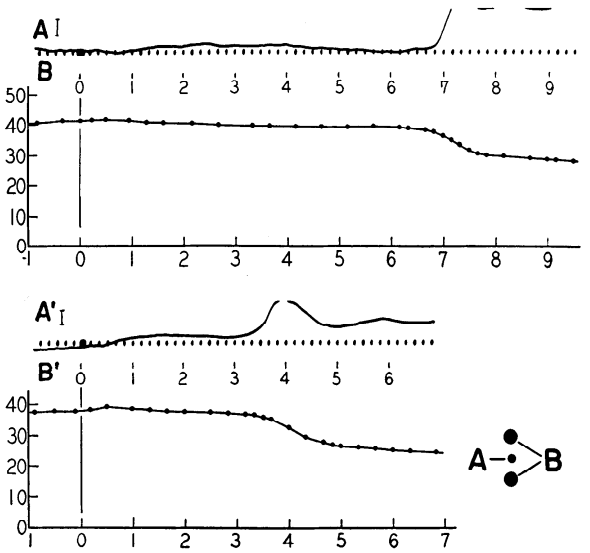

FIG. 3. *A* and *A'* show the asphyxial potentials, *B* and *B'* the conductivity changes after circulatory arrest in the cortex (at time 0). *AB* shows these changes in the cat, *A'B'* in the monkey. On the abscissa time in minutes, on the ordinate conductivity in mhos × 10^5. The vertical calibration lines indicate 5 mv for the asphyxial potential. The electrode placement for the recording of asphyxial potentials (*A*) and for the resistance changes (*B*) is indicated in the insert.

of the animals differed considerably (from 2.5 to 6 kg). They were matched as well as possible in pairs according to weight. In one animal of each pair the cortex was frozen 1 minute after the cerebral circulation was stopped by severing the aorta. In the other monkey the cortex was frozen after the rapid resistance change had taken place. Figure 3 shows the conductance changes and the asphyxial potential in one of the experiments (no. 56, table 2*C*). These phenomena are not materially different from those observed in the rabbit. The latent period of the rapid resistance change is longer than in the rabbit and approaches the latent period found in the cat. A slice of brain tissue for silver staining was taken from comparable places of the postcentral gyrus in all six experiments. The preparations showed ice formation even in the superficial regions of the cortex. As in the cat the branching of the apical dendrites started some distance from the molecular layer. In table 2*C* are given the means of about 100 measurements of apical dendrites in each cortex. They are expressed again in millimeters as measured on the screen of the camera lucida. By multiplication by one-third the diameter in μ is found. The number of experiments is too small to allow statistical evaluation. However,

the results indicate that the same observation as made in rabbits and cats also holds for the monkey, namely that the mean diameter of the apical dendrites after the sudden resistance change is greater than before this phenomenon has taken place.

DISCUSSION

The cells and apical dendrites in cortices frozen after the sudden asphyxial resistance change had consistently a larger diameter than those in control cortices frozen before this change occurred. The question arises as to how closely such differences, observed in histological preparations, represent actual differences in cell and fiber diameters at the time of freezing. Usually no obvious signs of ice formation were observed in the upper 0.2–0.3 mm of rabbit cortex. The deeper layers had a vesicular appearance which probably indicates the formation of relatively large ice crystals in the tissue during freezing. Although the freezing point of alcohol (± -115°C) is above the temperature at which vitreous freezing of water takes place (-130 to -140°C), it is below the vitrification temperature of tissue which is given as -100°C (10). It seems unlikely, however, that even in the superficial cortical layers freezing was vitreous. Freezing in this region was probably so quick that the ice crystals formed were too small to be observed with the light microscope. There can be no doubt, however, that freezing of the superficial cortical regions occurred at high speed. This is of importance, since ice formation occurs simultaneously in the intra- and extracellular compartment only when tissue is cooled at high rate (11). When frozen slowly, ice formation starts in the extracellular fluid, which is concentrated by the removal of water as ice crystals. By osmotic action this concentrated extracellular fluid will cause a water movement from the intra- into the extracellular compartment. When ice formation takes place simultaneously in cells and fibers and in the extracellular fluid, the ice crystals in both compartments will compete for the available water and the original water distribution will be better maintained. In the superficial 0.2–0.3 mm of rabbit cortex in which there were usually no signs of ice crystals the fluid distribution between the extra- and intracellular compartments may therefore have been preserved during freezing. The cortices of cats and monkeys showed usually ice crystals in the superficial regions. The fluid distribution was therefore probably not preserved as well in these species as in the rabbit during freezing.

The more or less wrinkled appearance of the apical dendrites indicates that fixation of the frozen cortex in alcohol at -17 to -20°C causes shrinking of the tissue, although this may have been less severe than that which occurs during alcohol fixation at room temperature. It seems unlikely that shrinking accentuates the differences between perikaryal and dendritic diameters in cortices before and after the sudden resistance change. It is more likely that these differences are decreased since a structure containing a high proportion of water tends to shrink more than one of smaller water content. The differences in diameter of the neuronal structures in the two groups of cortices may therefore well have been greater than is indicated by the figures.

The nerve cells in the rabbit were found to increase 11.2% in diameter during the first 15 minutes after circulatory arrest. Considering the cells as spheres this would imply a volume increase of 40%. The apical dendrites of rabbit cortex increases 34% in diameter under these conditions. In a second series of experiments on rabbits an increase of 27% was found. If the apical dendrites are considered as cylindrical bodies which increase their diameter by an average of 30% during the sudden resistance change, then the increase in volume of these structures would be 69%. These figures for the volume increase of cells and apical dendrites could form the basis for an estimate of the amount of fluid stored by the cortical neurons during the sudden resistance change, if the total perikaryal and dendritic volume in the cortex were known.

A number of estimates has recently been made of the proportion of cortical tissue occupied by perikarya. Using the statistical procedure described by Chalkley (12), Peters and Flexner (13) estimated this quantity as about 12% in the frontal cortex of the adult guinea pig while Eayrs and Taylor (14) arrived at a similar figure for the same cortical area in the young rat. By the same technique, Shariff (15) estimated the perikaryal volume in the striate area of Tarsius as 23.8% of the cortical volume, in area 6 as 9.1% and in the temporal and

parietal regions as 16.3%. In man the values for the same cortical areas proved to be 8.7, 4.5 and 4.7%, respectively. On the other hand, employing a different mode of computation, Sholl (14) reported the somewhat surprisingly high figure of 25–35% for the striate area of the cat. Sholl furthermore estimated the total dendritic volume as 20–30% of the cortical volume in the same area of the cat (16). There are obviously species differences in the perikaryal volume, the smallest mammals having the highest packing density of cortical nerve cells (17) and the largest perikaryal volume. Assuming a figure of 15% for the perikaryal volume of the striate area, which was used in the present experiments on rabbits, seems a conservative first approximation. This figure is considerably below Sholl's estimate for the striate cortex of the cat but is above the estimate for man. Assuming furthermore on the basis of Sholl's figures (14) that the total dendritic volume more or less equals the perikaryal volume, one can estimate roughly the amount of fluid which is transferred from the extra- into the intracellular compartment during the resistance change. This figure is about 16% of the cortical volume. Since, as was discussed above, the volume increase of cells and fibers is likely to be greater than the figures used and the estimate of perikaryal and dendritic volume may have been on the low side, it seems quite possible that the transport of fluid from the extra- into the intracellular compartment is in reality greater than this.

Since an extracellular volume of 25% was estimated from the chloride space (2) which is 30–35% of the cortical volume (18, 19), it is obvious that a large part of the extracellular fluid is transported into the neuronal elements of the cortex during the sudden resistance change. This transport of extracellular ions and water is so large that it seems ample to account for the drop in conductivity (30–35%, 2), and there does not seem to be any necessity to assume that the glial elements of the cortex are involved in this process. Although no actual measurements were made, a comparison of the diameter of axons stained with silver in cortices before and after the sudden resistance change showed them not to be obviously different. It seems therefore either that the cortical axons do not participate in the ionic transport or that they do this only to a small extent.

It is possible to calculate the transport of ions across the membrane during the period of the sudden resistance change and to compare this with the ionic flow during activity of an excitable membrane. In the second series of rabbit experiments (table 2*A*) the mean radius of the apical dendrites before the resistance change was .95 μ, after the resistance change 1.2 μ. The volume of a dendrite of mean radius and of length L before and after the resistance change is thus $L\pi(0.95)^2\ 10^{-8}$ cc and $L\pi(1.2)^2\ 10^{-8}$ cc. The surface of this dendritic portion is $2L\pi\ (0.95)\ 10^{-4}$ cc. The volume increase per square centimeter of the membrane then is

$$\frac{L\pi(1.2)^2\ 10^{-8} - L\pi(0.95)^2 \times 10^{-8}}{2L\pi\ 0.95 \times 10^{-4}} = 2.8 \times 10^{-5}\ \text{cc/cm}^2$$

The body fluids have a molarity of about 0.154 so 1 cc of intercellular fluid contains approximately 1.54×10^{-4} M. of solute. The salt transport across the membrane during the sudden resistance change is thus $2.8 \cdot 10^{-5} \times 1.54 \cdot 10^{-4} = 4.3 \times 10^{-9}$ M/cm^2. Most of this transport takes place during the steepest portion of the sudden resistance change which may last 1.5 minutes or roughtly 10^5 msec. The transport per millisecond is thus 4.3×10^{-14} M/cm^2/msec. or 0.043 $\mu\mu$M/cm^2/msec. Hodgkin (20) computed for the squid giant axon a minimum transport of 1.6 $\mu\mu$M of sodium/cm^2/impulse. The sodium entry actually determined in squid axon was of the order of 4 $\mu\mu$M/cm^2/impulse (21–23). A comparison of the entry of sodium during an impulse which lasts at the most a few milliseconds with the ion transport per millisecond during the sudden resistance change shows the latter to occur at a considerably lower rate. The impressive volume increase of the neuronal elements is possible at this low rate only because of the relatively long time (2–3 min.) available for it.

A striking feature of electron microscope pictures of the cerebral cortex is the close proximity of nerve cell bodies, nerve fibers, glial cells and glial processes, leaving space for a minimum of intercellular material only (24–27). Such findings are hard to reconcile with the relatively low specific resistance of cortical tissue (between 200 and 300 ohms, 2, 8, 28). Cole (6) concluded from his work on the resistance of cell suspensions that the measuring current is transported mainly by ions in the intercellular fluid. A rough calculation of

the cortical resistance on the basis of the principles laid down by Cole and assuming an intercellular space of 25% gave a value of 360 ohms, in fair agreement with the figures actually determined (2). If cortical tissue were of the structure suggested by electron microscopy, it should have a greater specific resistance than the values actually found.

It would seem possible that the lack of intercellular material in electron microscope pictures is a secondary phenomenon caused by dehydration and by redistribution of water between the extra- and intracellular compartments during the preparation of the tissue for electron microscopy. The process of fluid redistribution resulting in a cortical resistance increase and a swelling of the neuronal elements discussed in this paper could be one of the mechanisms, but not necessarily the only one, which produces the apparent scarcity of intercellular material in electron microscope pictures.

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