Asphyxial Changes in the Cerebellar Cortex[1]

A. VAN HARREVELD
Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California

The changes produced in the cerebral cortex by asphyxiation and by spreading depression are qualitatively quite similar. Both cause surface negativity of the cortex (Leão, '47, '51) and an increase in cortical impedance (Leão and Ferreira, '53; Freygang and Landau, '55; Van Harreveld and Ochs, '56, '57). Furthermore both result in a transport of water (Van Harreveld, '57, '58) and electrolytes (Van Harreveld and Schadé, '59) from an extraneuronal space into apical dendrites. These similarities suggested a basic identity of the processes underlying the asphyxial changes and spreading depression (Leão, '51; Van Harreveld and Stamm, '53; Van Harreveld, '58; Van Harreveld and Schadé, '59; Bureš and Burešová, '60). Spreading depression has not been described in the cerebellum and attempts in this laboratory to elicit this phenomenon in the cerebellar cortex have met with failure. Leão had a similar experience (personal communication). In view of the inability of the cerebellar cortex to elaborate spreading depression it was of interest to investigate the effect of asphyxiation on the cerebellum and to look in particular for impedance changes and the transport of water and electrolytes in its cortex.

METHODS

The physiological experiments were performed on rabbits. The cerebellar cortex was exposed in ether narcosis, but the measurements and recordings were carried out under Intocostrin (E. R. Squibb and Sons, New York) immobilization. Potentials were led off between an electrode placed on the vermis and an indifferent one on the ear. The electrodes for the impedance measurements were silver discs 1½ to 2 mm in diameter which were placed 3–4 mm apart on the same folia of the vermis. In attempts to elicit spreading depression a third, stimulating electrode was placed on these same folia. The impedance bridge described previously was used (Van Harreveld and Ochs, '56). The measuring current was a 1000-cycle sinusoidal current attenuated below the threshold of stimulation. Asphyxiation was caused by severing the abdominal aorta.

The water and electrolyte transport was investigated in rabbits and rats. The histochemical technique used, which shows either the location of chloride alone, or in addition to this, phosphate, carbonate and an unidentified (probably organic) anion, has been described in detail (Van Harreveld and Potter, '61). The treatment started with freezing the exposed vermis by pouring isopentane cooled to its fusion point into a cup of skin made by sewing the edges of the wound exposing the cerebellum to a steel ring. The frozen tissue was then kept in 90% alcohol saturated with silver nitrate (about 2.5%) at a temperature of $-25°C$. During the ensuing substitution fixation silver ions diffused into the tissue precipitating the anions mentioned above. After one week the tissue was washed for 4 to 5 hours in a repeatedly changed molar sodium nitrate solution at 0°C, then for an additional 12–18 hours in the same solution at room temperature. The tissue blocks were dehydrated, embedded in paraffin and sectioned (10 μ). To observe the position of all the electrolytes precipitated by silver ions the sections were mounted without further treatment and exposed to sunlight. To study the location of the chloride alone, the preparations were deparaffinized and passed for a few minutes through a 1% nitric acid (volume) solution which dissolved all silver salts but the

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chloride. After washing, the sections were mounted and exposed to light.

RESULTS

Physiological experiments

Spreading depression. In agreement with previous attempts no spreading depression was observed in the cerebellar cortex even when stimulating with strong direct currents or with concentrated potassium chloride solutions. Both are very effective in producing spreading depression in the cerebral cortex of the rabbit. Since the impedance change is a reliable sign of spreading depression this has been looked for mostly. In a few experiments an unsuccessful search for slow potential changes was made.

Some procedures have been described which enhance the tendency of the cerebral cortex to react with spreading depression on adequate stimulation. Effective in this respect are dehydration (Marshall, '50), long exposure of the cortex (Marshall and Essig, '51), cooling (Marshall, Essig and Dubroff, '51), treatment of the cortex with isotonic sucrose solutions (Essig and Marshall, '50) or with solutions containing abnormally high potassium concentrations (Marshall, Essig, and Witkin, '51). With these procedures spreading depression can be produced in the retrosplenial area of the rabbit in which it cannot normally be elicited (Van Harreveld and Bogen, '56). It is possible that application of any of these treatments would also enable the cerebellar cortex to respond with spreading depression. This has not been tried in the present series of experiments, however.

Asphyxial changes. An impedance increase develops consistently in the cerebral cortex after asphyxiation. For the first minutes after circulatory arrest the impedance tends to rise slowly and gradually. After about three minutes, however, a sudden and large impedance increase develops during which, in the course of about two minutes, 30% or more of the conductivity of the cerebral cortex is lost (Van Harreveld and Ochs, '56). Although small potential variations may occur during the first minutes of asphyxiation (Van Harreveld and Stamm, '53), a pronounced surface negativity of the cerebral cortex develops at the start of the sudden impedance rise (Van Harreveld and Ochs, '56).

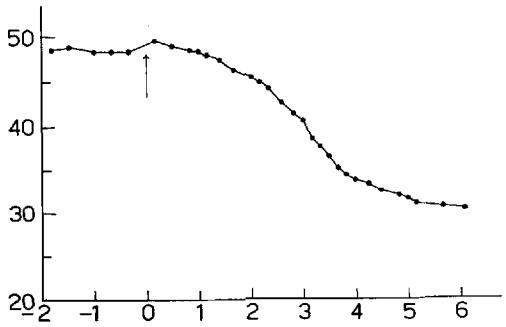

Fig. 1 Loss in conductivity of cerebellar cortex during asphyxiation. The conductivity, expressed in mhos $\times 10^5$, is plotted on the ordinate; time in minutes on the abcsissa. At the arrow the circulation is arrested.

The impedance of the cerebellar cortex also increased after asphyxiation. This increase was of the same magnitude as in the cerebral cortex; its time course was somewhat different, however. In the cerebellar cortex the impedance rose more gradually. Figure 1 shows the changes in asphyxial conductivity (the reciprocal of the impedance expressed in mhos $\times 10^5$) of the cerebellum after circulatory arrest. Although there is an acceleration of the loss in conductivity two to three minutes after cutting the aorta, the sudden drop which is so typical for the cerebral cortex is lacking.

As in the cerebral cortex a surface negativity developed in the cerebellum after asphyxiation. This potential change started from one to three minutes after cutting the aorta, and was of considerable magnitude (15 to 20 mv) at its maximum. It sometimes consisted of several waves, which could represent the asphyxial potentials picked up by the relatively large leading off electrode from two or three folia in which the potential developed with a slightly different time course.

Histological findings

The silver-substitution method was applied to cerebellar cortex frozen during normal oxygenation or a few seconds after circulatory arrest (controls), and after the asphyxial impedance increase had taken

place (experimental preparations). In rabbits the vermis was exposed and a skin cup prepared in ether narcosis then the preparation was immobilized with Intocostrin. In control rabbits the cerebellum was frozen while the circulation was intact and after it has been ascertained that the impedance was within the range for normal oxygenated cerebellar cortex. In the experimental rabbits the vermis was frozen after the asphyxial impedance increase had taken place and the rate of change was falling off, usually 6–7 minutes after cutting the aorta.

In rats the vermis was exposed in urethane narcosis. In all preparations the cortex was left exposed for 15–20 minutes, but care was taken to keep it moist. In the controls the head was cut off and transferred immediately to cold isopentane. In the experimental rats a period of 8 minutes was interposed between severing the head and freezing to allow the development of the asphyxial changes.

Figure 2A is a photomicrograph of an acid treated preparation which shows the distribution of chloride in rat cerebellar cortex frozen before, and figure 2B of a similar preparation frozen after the asphyxial changes had taken place. As was found in the cerebral cortex the chloride was rather uniformly distributed in the control preparation. In the asphyxiated cerebellar cortex a transport of chloride had taken place into certain cellular elements which are darker whereas the tissue in between them is lighter than in the control preparation. Some of the structures into which chloride had moved could be readily identified as dendrites of Purkinje cells. Since the cortex had been cut at right angle with the folia of the vermis the dendritic arborizations of these cells are in the plane of the section. It was especially in the main dendritic trunc(s) and the first branches that chloride had accumulated (fig. 2B, C). The more superficial part of the arborization which consists of smaller branches was not visible in these preparations. No consistent transport of chloride into the perikarya of the Purkinje cells was observed except at the origin of the dendritic trunc(s) (fig. 2B).

There are, however, other structures in which chloride is concentrated. They consist of relatively thick fibers which in favorable preparations (fig. 2D) can be seen to begin close to the granular layer and run all the way to the surface of the cortex. Some of these fibers branch but the great majority run undivided and straight through the entire molecular layer. Although the axons of the granular cells take a similar course, they divided in their two branches at all levels of the molecular layer. Furthermore these axons are very thin whereas the fibers taking up the electrolytes have a considerable diameter. Also dendrites of the Golgi cells or grand cellules étoilées (Cajal, '55), the perikarya of which are situated in the granular layer, have a course in the plexiform layer somewhat similar to that of the fibers which take up chloride. The dendrites of the Golgi cells branch rather profusely, however, and often do not reach the cerebellar surface. Furthermore close to the granular layer these dendrites often run parallel with the surface of that layer, which is never observed of the fibers taking up chloride after asphyxiation. These latter fibers can thus neither be readily identified with the axons of the granular cells, nor with the dendrites of the Golgi cells. However, these structures do resemble strikingly the fibers of the epithelial cells of Golgi (Cajal, '55) also called the "Stüzzellen" of Bergmann (Jansen and Brodal, '58), a glial component of the cerebellar cortex. Figure 2E shows these elements in rabbit cerebellum treated with a glia stain. The cells of origin of the fibers are relatively small and adjoin the molecular layer. The fibers which arise from these cells run with a minimum of branching straight through the molecular layer to the surface where they form end feet. The end feet form together the limiting membrane of the cerebellar cortex. A comparison of the fibers stained with the silver-substitution and with the gold sublimate methods as shown in figure 2D and E, demonstrates the similarity of their course.

No transport of chloride into the cell bodies of Bergmann's "Stützzellen" could be demonstrated. An inspection of the granular layer in preparations frozen before and after the asphyxial changes had

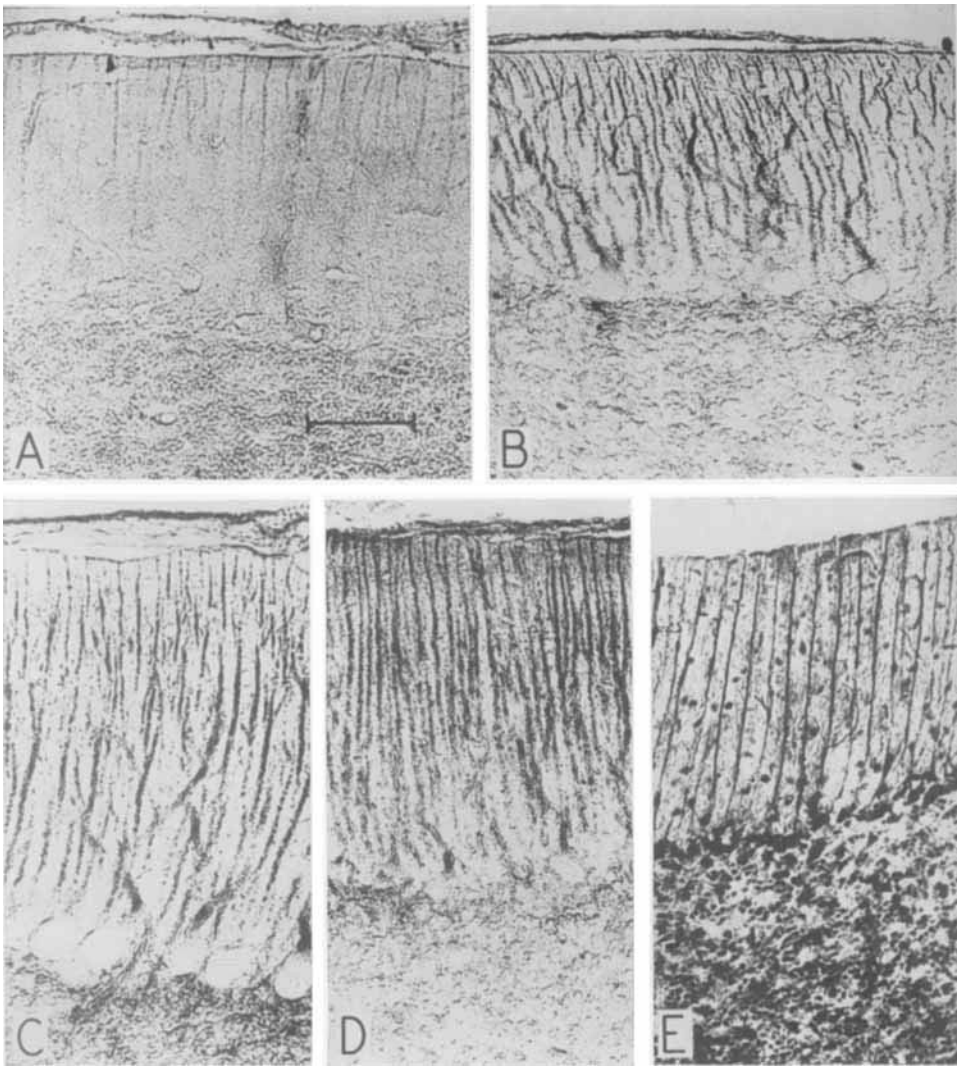

Fig. 2 Photomicrographs of cerebellar cortex treated with the silver-substitution method. The preparations shown in A and B are of rat cerebellum which had been treated with nitric acid. A is cortex frozen before, B after the asphyxial impedance increase had occurred. The photomicrographs C and D are asphyxiated cerebellar cortex of the rabbit treated with neutral solutions. To enhance the contrast a green filter was used in making the pictures A, B, C and D. E is rabbit cerebellum stained with Cajal's gold-sublimate method. The magnification of all the photomicrographs is the same. The horizontal calibration line in A indicates 100 μ.

taken place did not reveal consistent differences in chloride distribution.

As mentioned above the chloride was rather uniformly distributed in control preparations of the rat frozen a few seconds after circulatory arrest. However, even in these preparations the outlines of the large dendrites of the Purkinje cells and of the fibers of Bergmann could be faintly seen. In the control preparation shown in figure 2A some fibers of Bergmann are visible in the top of the plexiform layer.

Preparations of rat cortex treated with neutral solutions and thus showing besides chloride the other ions precipitated

by silver nitrate (carbonate, phosphate and an unidentified, organic anion) were somewhat darker than the acid treated preparations which show chloride alone. The distribution of these electrolytes in control and experimental preparations was very similar to that of acid treated preparations, however.

The cerebellar cortex of rabbits showed the same rather uniform distribution of electrolytes in the oxygenated controls and a concentration of these ions in the large dendrites of the Purkinje cells and in the fibers of Bergmann in the experimental preparation as described above for the rat. Figure 2C and D which are photographs of preparations of asphyxiated cerebellar cortex of the rabbit treated with neutral solutions demonstrate the electrolyte transport in this species.

In most preparations the electrolyte transport had taken place both into the fibers of Bergmann and into the dendrites of the Purkinje cells. In such preparations electrolytes had moved mainly into the dendrites in the bottom of the plexiform layer, whereas the transport had taken place only into the fibers of Bergmann in the top of that layer (fig. 2B and C). In some cerebellums, however, the electrolytes had moved almost exclusively into the Bergmann fibers (fig. 2D) which then can be followed easily from the granular layer to the surface of the cerebellums.

Ratio between Bergmann's fibers and Purkinje cells in the cerebellar cortex of rats and rabbits. On the basis of their anatomical features the fibers in the molecular layer of the cerebellar cortex which take up electrolytes after circulatory arrest were identified as the fibers of Bergmann's "Stützzellen." It was attempted to support this conclusion by determining the ratios between the Purkinje cells and these fibers in preparations stained with a glia stain in which the fibers of Bergmann and their cells of origin can be identified with certainty, and in preparations treated with the silver-substitution method. This ratio rather than the number of fibers in a known volume of cerebellar cortex was chosen because of the great differences in the treatment of the tissues which may cause different shrinkage. For the glia stain Cajal's gold-sublimate method (Conn, Darrow and Emmel, '60) was used (fig. 2E). The Purkinje cells were sufficiently stained in these preparations to be easily recognizable. The staining of asphyxiated cerebellar cortex with the silver-substitution method makes the fibers readily visible. These preparations were counterstained with methylene blue to facilitate the recognition of the Purkinje cells. In both kinds of preparations all the Purkinje cells and all the fibers were counted in well stained relatively straight stretches of cortex. The curved cortex at top and bottom of the folia was avoided because of the differences in the density of Purkinje cells described there. In table 1 the results of these cell and fiber counts are given. Four rat and 4 rabbit cerebellums were used for each of the staining methods. One to 4 folia were chosen of each cerebellum which were sufficiently long to make the counting of a reasonable number of cells and fibers possible. The table shows the number of cells and fibers and their ratio in each of these folia. In the preparations of rats treated with the silver-substitution method a total of 171 cells and 691 fibers were counted which gives a ratio of 4.0. In the preparations stained with the Cajal method these numbers were 199 and 870, which gives a ratio of 4.4. The means of the ratios in individual folia with the two staining methods were 4.1 and 4.4 respectively, both with a standard error of 0.1. The difference between these two mean ratios was statistically not significant ($t = 2.0$).

In the rabbit 147 cells and 935 fibers were counted in the preparations treated with the silver-substitution method, which gives a ratio of 6.4. In the cerebellums stained with the gold-sublimate method these figures were 185 and 1086 with a ratio of 5.9. The means of the ratios in individual folia with the two methods of staining were 6.4 and 5.8 with standard errors of 0.3 and 0.2 respectively. Also the difference between these mean ratios was not significant ($t = 1.7$).

The similarity of the ratios indicates that the number of the fibers stained with the silver-substitution method and with a recognized glia stain is the same in a given volume of cerebellar cortical tissue. This strongly supports the identification of

TABLE 1

Ratio between Bergmann's fibers and Purkinje cells in the cerebellar cortex of rats and rabbits

Silver substitution method				Cajal's gold-sublimate method			
Cerebellum	Cells	Fibers	Ratio	Cerebellum	Cells	Fibers	Ratio
Rat 1	17	61	3.6	Rat 5	25	102	4.0
	12	54	4.5		32	133	4.2
Rat 2	22	97	4.4	Rat 6	25	112	4.5
	20	85	4.3		24	103	4.3
	27	107	4.0				
Rat 3	22	91	4.1	Rat 7	19	86	4.5
	13	52	4.0		26	106	4.0
Rat 4	38	144	3.8	Rat 8	23	109	4.7
					25	119	4.8
Means	171	691	4.1		199	870	4.4
Rabbit 1	12	87	7.2	Rabbit 5	17	82	4.8
	11	78	7.1		17	89	5.2
	21	128	6.1		12	70	5.8
					16	87	5.4
Rabbit 2	16	111	7.0	Rabbit 6	25	156	6.2
	12	54	4.5		21	133	6.3
	16	115	7.2				
Rabbit 3	16	93	5.8	Rabbit 7	15	106	7.0
	14	85	6.1		21	105	5.0
Rabbit 4	18	109	6.1	Rabbit 8	26	162	6.2
	11	75	6.8		15	96	6.4
Means	147	935	6.4		185	1086	5.8

the fibers which take up electrolytes after circulatory arrest as Bergmann's fibers.

Changes in the diameter of Bergmann's fibers after circulatory arrest. The asphyxial transport of electrolytes into the apical dendrites of the cerebral cortex is accompanied by a marked swelling due to a simultaneous transport of water to maintain osmotic equilibrium (Van Harreveld, '57). A similar swelling can be expected to occur in the fibers of Bergmann after circulatory arrest. The diameters of these fibers were measured in preparations frozen before and after the asphyxial electrolyte transport had taken place. This did not pose any difficulties in asphyxiated cerebellums where the fibers were well stained with the silver-substitution method. In the control preparations in which the fibers were much more faint this was less easy but still possible, especially when the preparations had been treated with neutral solutions. The fiber diameters were determined in a plane midway between the surface of the cerebellar cortex and the granular layer. The measurements were made under oil immersion, using a "Zeiss" camera lucida. The apparent diameters of the fibers were marked on the paper acting as a screen of the camera lucida, and were later measured to the nearest 0.1 of a mm. These figures will be used in the present paper. Since the magnification of the optical system was about 1400 ×, multiplication with 0.72 gives the diameter in μ. The above method is the same as that employed for the measurement of apical dendrites in the cerebral cortex (Van Harreveld, '57).

Table 2 shows the diameters of Bergmann's fibers of rats. In 8 animals the cerebellum was frozen before the asphyxial changes could take place and in 8 preparations a period of 8 minutes was interposed between circulatory arrest and freezing. In each cerebellum all the fibers (about 100) in a well stained, straight stretch of cortex were measured. The mean diameters in the 8 control cortices varied between 2.6 and 3.3 mm (1.9 and

TABLE 2

Diameters of Bergmann's fibers in rats expressed in an arbitrary unit of length

No.	Frozen before asphyxial changes	No.	Frozen 8 min. after circulatory arrest
1	3.0 ± 0.05	9	4.9 ± 0.09
2	2.6 ± 0.05	10	5.7 ± 0.12
3	2.8 ± 0.05	11	5.3 ± 0.13
4	2.6 ± 0.04	12	4.9 ± 0.08
5	2.6 ± 0.05	13	5.5 ± 0.11
6	3.0 ± 0.05	14	5.2 ± 0.11
7	3.3 ± 0.07	15	5.2 ± 0.12
8	3.0 ± 0.06	16	4.9 ± 0.09
Means	2.9 ± 0.02		5.2 ± 0.04

2.4 μ), the standard errors were small (0.04 to 0.07 mm). The mean diameters after 8 minutes asphyxiation were considerably larger, ranging between 4.9 and 5.7 mm (3.5 and 4.1 μ) with larger standard errors (0.08 to 0.13 mm). The mean diameter of the 800 fibers in the control preparations was 2.9 mm (2.1 μ) ± 0.02 mm and in the asphyxiated cerebellar cortices 5.2 mm (3.7 μ) ± 0.04 mm. This difference is highly significant ($t = 55$). Figure 3B shows histograms based on these measurements. The diameters in the control preparations show little variability whereas the diameters of the experimental cortices vary considerably. Since these are the same populations of fibers the swelling was apparently not uniform.

Table 3 shows a similar series of experiments with rabbits. The diameters of Bergmann's fibers in 6 control experiments varied between 2.8 and 3.1 mm (2.0 and 2.2 μ). In 6 asphyxiated cerebellums the diameters were between 4.7 and 6.0 mm (3.4 and 4.3 μ). The mean of all 600 control measurements was 3.0 mm (2.2 μ) ± 0.02 mm. The mean diameter in all the experimental cerebellar cortices was 5.2 mm (3.7 μ) ± 0.04 mm. This difference is again highly significant ($t = 46$). Figure 3A shows histograms of all fiber diameters measured in control and experimental rabbit cerebellums. As in the rat the variability in the experimental preparations was greater than in the controls. The increase in diameter which is about the same in rats as in rabbits is very considerable (70 to 80%). This corresponds to a volume increase of Bergmann's fibers of about 300%.

Horstmann and Meves ('59) pointed out that differences in the intensity of silver impregnations in preparations treated with one of the routine methods for the nervous system could account for differences in diameter of fibers since silver is precipitated on the surface of the stained structures. In the present instance there is a considerable difference in the staining of Bergmann's fibers in the control and experimental preparations. However, the method employed shows the presence of electrolytes and is not based on the precipitation of silver compounds on surfaces. The increase in diameter observed, must therefore be considered as a true swelling of the fibers after circulatory arrest.

DISCUSSION

The asphyxial changes in the cerebellar cortex described above are similar to those in the cerebral cortex. In both structures the impedance increases considerably, and a transport of electrolytes and water into cellular elements takes place. There are differences, however. In the cerebral cor-

TABLE 3

Diameters of Bergmann's fibers in rabbits expressed in an arbitrary unit of length

No.	Oxygenated cerebellum	No.	Asphyxiated cerebellum	Interval between circulatory arrest and freezing
1	3.0 ± 0.05	7	5.0 ± 0.09	6′ 10″
2	2.8 ± 0.04	8	6.0 ± 0.09	6′ 30″
3	2.9 ± 0.04	9	5.5 ± 0.10	7′ 30″
4	3.1 ± 0.05	10	4.8 ± 0.10	6′ —
5	2.9 ± 0.04	11	4.7 ± 0.08	6′ 40″
6	3.1 ± 0.07	12	4.9 ± 0.11	6′ 50″
Means	3.0 ± 0.02		5.2 ± 0.04	

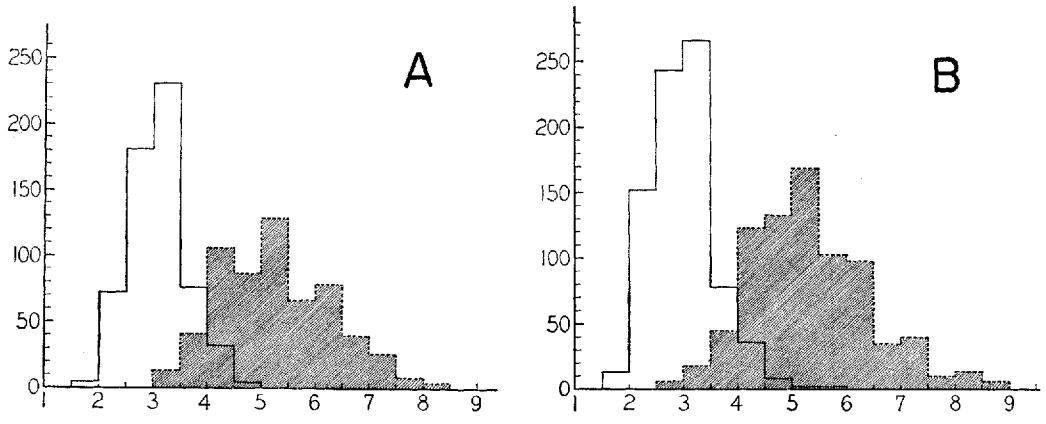

Fig. 3 Histograms of the diameters of Bergmann's fibers. On the ordinate are plotted the number of fibers, on the abscissa the diameter in an arbitrary unit of length. A shows two histograms, each based on 600 measurements in the rabbit. The non-hatched histogram of cerebellums frozen while the circulation was intact; the hatched histograms of cerebellums frozen after the circulation had been arrested for 6–7 minutes. In B two similar histograms each based on 800 measurements in rats are shown. The non-hatched histogram is of cerebellums frozen a few seconds after cutting off the head. A period of 8 minutes was interposed between severing the neck and freezing of the cerebellums used for the hatched histogram.

tex the electrolytes moved primarily into the apical dendrites. There was also some transport into the pyramidal cells, but no evidence of an electrolyte movement into glial elements was found. In the cerebellar cortex electrolytes not only moved into dendrites of the Purkinje cells but in addition to this into Bergmann's fibers, an element of the cerebellar cortex which is generally considered to be of glial nature. In some preparations the latter was even the most pronounced electrolyte movement.

As mentioned above the similarity between the features of spreading depression and of the asphyxial changes in the cerebral cortex is so great, that the processes underlying these phenomena are assumed to be identical (Leão, '51; Van Harreveld and Stamm, '53; Van Harreveld, '58; Van Harreveld and Schadé, '59; Bureš and Burešová, '60). It has been suggested that the basic mechanism underlying the features both of spreading depression and of the asphyxial changes is an increase of the ion permeability of the cell membrane (Van Harreveld and Ochs, '56, '57; Van Harreveld and Schadé, '59, '60). A similar membrane change can be expected to result in the asphyxial electrolyte movement into the nervous and glial elements of the cerebellum. Since the asphyxial potential is in all probability the result of these membrane changes it seems likely that both neural and glial elements are responsible for the surface negativity in the cerebellum after circulatory arrest.

It is of interest that the asphyxial electrolyte transport is observed in certain structures and not in other, similar ones. For instance electrolytes move regularly into the apical dendrites of pyramidal cells in the cerebral cortex but not into the basal dendrites. An explanation for this observation can be sought in the competition of various structures for a limited amount of chloride in the cortex (Van Harreveld and Schadé, '59). If during asphyxiation the membrane changes responsible for the ion transport start in the apical dendrites, then the available chloride may have been exhausted before such changes occur in the basal dendrites. Such reasoning can be used to explain the observed variations in the asphyxial electrolyte transport in the cerebellum. In some cerebellums the transport occurred exclusively into the fibers of Bergmann, in others the electrolytes moved also into the dendrites of Purkinje cells. According to

the above view the membrane changes would in some preparations have occurred first in the Bergmann fibers, in others they would have developed simultaneously in these fibers and in the dendrites of the Purkinje cells.

In the cerebral cortex the asphyxial impedance increase develops suddenly after a latency of about three minutes, accompanied by the asphyxial potential (Van Harreveld and Ochs, '56). In the cerebellum the impedance rose more slowly and gradually. The latter feature may be related to the inability of the cerebellar cortex to elaborate spreading depression. Leão ('47, '51) observed during asphyxiation of the cerebral cortex the development of foci of surface negativity with different latencies. From such foci negativity may spread by a process identical or similar to spreading depression, engulfing the entire cortex in a short time. Such a concept which is supported by Bureš and Burešová's ('60) finding that asphyxiation activates latent foci of spreading depression can account for the sudden impedance increase of the cerebral cortex. In the cerebellum asphyxial membrane changes starting in certain foci would not be able to spread since in this part of the nervous system the conditions for phenomena like spreading depression are not favorable. As asphyxiation proceeds membrane changes may develop at other spots until finally the entire cerebellar cortex is involved. Such a concept agrees with the more gradual development of the impedance rise in the cerebellum.

The observation that not only nervous elements but also glia cells can participate in the asphyxial water and electrolyte transport enhances the potential magnitude of intracortical shifts of material. Estimates of extracellular space based on the study of material fixed for light- or electronmicroscopy in which such shifts can be expected to have taken place should therefore be regarded with caution.

SUMMARY

As in the cerebral cortex asphyxiation produces surface negativity and an impedance increase in the cerebellar cortex. A histochemical method indicating the presence of choloride alone, or in combination with phosphate, carbonate and an unidentified (probably organic) anion was applied to cerebellar cortex of rabbits and rats either before, or after the asphyxial impedance increase had taken place. A rather uniform distribution of these electrolytes was observed in the plexiform layer of cortices in which the asphyxial changes had not taken place. In asphyxiated cerebellums the electrolytes were concentrated in the main dendrites of the Purkinje cells and in structures which by their anatomical features were identified as the fibers of Bergmann's "Stützzellen" (epithelial cells of Golgi), a glial component of the cerebellar cortex. This identification was supported by the demonstration that in preparations treated with the histochemical method the ratio between these fibers and the Purkinje cells, and in cerebellar cortex treated with a glia stain the ratio between the fibers of Bergmann and the cells of Purkinje are similar. A comparison of the diameter of the fibers of Bergmann in asphyxiated and non-asphyxiated cerebellar cortex indicates that a 70–80% increase in thickness takes place during the asphyxial impedance rise. This corresponds to an increase in the volume of these structures of about 300%.

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