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Correlations between the Electroencephalogram and Cortical Histochemical Changes in Experimental Brain Lesions

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The relationship of EEG findings, histochemical data on glycogen, and oxidative enzymes (DPN-diaphorase) were studied in experimental cortical lesions in the guinea pig. There was a correlation between more than 50 per cent decrease of oxidative enzyme reaction in cortical tissues, the deposition of glycogen in the tissue, and the appearance of slow-wave activity in the EEG. Slow-wave activity was related to the presence of tissue showing almost normal cell population but markedly decreased enzyme reaction; decrease of enzyme activity in necrotic cortical tissue was without significance for the appearance of slow waves. The significance of these data for the interpretation of a delta focus in the human EEG was discussed, based on histochemical and EEG data from forty-four neurosurgical human biopsies.

Introduction

Conventional histological techniques have failed to reveal correlations between specific tissue changes and types of alterations of the EEG, such as a delta focus, or spike activity. Little work has been done with histochemical techniques. Pope and others (11) found no significant correlation of histochemical data on cytochrome oxidase and the EEG in experimental and human epileptogenic foci. Enzyme-histochemical changes following experimental convulsions (8) are of questionable significance. It appeared more promising to investigate the histochemical correlates of focal slow-wave activity, as done in the present paper which reports on local correlations between the EEG frequency, changes of oxidative en-

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zymatic activity, and deposition of glycogen in experimental cortical injuries.

Material and Methods

An analysis of EEG and histochemical data was done on fourteen young adult guinea pigs (6 to 9 months); a large volume of histochemical material from both normal and experimentally injured brains was available for comparison.

Recording Technique. Symmetrical trephine holes were made in both parietal regions. The dura was left intact on the left side; it was opened on the right side and a controlled lesion was made as described below. The trephine holes were closed with prefitted polyacryl lids with built in silver-wire electrodes; the skin was sutured. Twenty-four and 48 hours after the surgery, monopolar recordings, using the ear as reference, were made with an Offner recorder from unanesthetized animals. The animals were killed by a blow to the neck after the last recording and the brains were removed as quickly as possible for histochemical investigation.

Histochemical Techniques. The brains were transected in a plane through both areas of recording; two-thirds of the affected area was used for enzyme histochemistry and one-third for the demonstration of glycogen. DPN-diaphorase was demonstrated with the technique of Farber, Sternberg, and Dunlap (2) using 30-μ frozen sections from formalin-fixed tissue (12). The intensity of the reaction was measured with a Welch Densichron densitometer using the method described previously (5). The optical density of the putamen was considered 100, and of white matter 10.

Succinic dehydrogenase and cytochrome oxidase were demonstrated in 60-μ frozen sections from unfixed tissue, using the techniques of Nachlas and co-workers (10) and Burstone (1).

Glycogen was demonstrated with the periodic acid-Schiff (PAS) reaction in paraffin sections from material fixed in Carnoy's fluid immediately upon removal. No counterstain of the PAS reaction was used, since counterstaining can impair the discernibility of fine granules. The specificity of the reaction was controlled with diastase.

The cell population was studied in alternate sections stained with chromalum-gallocyanin.

Methods for Cortical Injuries. These methods aimed at the production of a local decrease of oxidative enzymatic activity in the cortex without a major loss of nerve cells. Incisions into the cortex, single or multiple,

usually did not produce a major decrease of the enzyme reaction in the adjacent tissue, even if accompanied by compression or rough treatment. The tissue between the incisions showed either little decrease of the enzyme reaction or a complete necrosis. At the cut surface, surviving tissue showed a narrow, severely damaged zone with a marked decrease of enzyme activity.

Because of the relative inefficiency of incisions to produce a graded decrease of the enzyme reaction, another method was developed; injection of small volumes (0.1 to 0.2 ml) of distilled water into the cortex produced a marked decrease of enzyme activity, particularly in the neuropil, while the perikarya of the pyramidal cells in the deep layers showed little change. Multiple injections permitted one to produce large areas of greatly decreased enzyme activity. Injection of too much water resulted in tissue necrosis. Incision and injection techniques were not used in combination.

Human Material. Findings of human neurosurgical cortical biopsies were included in this report because of the similarities of the experimental and the human material. The biopsies were fixed in Carnoy's fluid immediately upon removal; glycogen was demonstrated in 10-μ paraffin sections using the periodic acid-Schiff reaction. Preoperative routine EEG recordings were available in each case so that the histochemical data could be compared with the EEG from the electrode nearest to the site of the biopsy.

Results

Oxidative Enzymes. The distribution of DPN-diaphorase in the cerebral cortex resembled closely that of succinic dehydrogenase. Since the latter has been mapped in detail (5), this mapping was used as a reference for normal enzyme distribution. The sizable, normal gradations of enzyme activity among areas of the cerebral cortex made it mandatory to have a strictly symmetrical arrangement of lesion and control. Densitometer measurements permitted a comparison of the intensity of the reaction in corresponding regions of the hemispheres; the decrease of enzyme in the lesion was expressed as a percentage of the densitometric data in the homologous region of the intact hemisphere. The technique for DPN-diaphorase was used for routine evaluation. A comparative study of DPN-diaphorase, succinic dehydrogenase, and cytochrome oxidase was made in only one of the animals because of the little volume of tissue available. These enzymes are known to show a very similar distribution in most nuclei of cat brain stem (6) and they also showed a similar

distribution in this material. Thus, the pattern of DPN-diaphorase was assumed to indicate general alterations of oxidative enzymatic activity.

Multiple incisions into the cortex usually produced either less than 50 per cent decrease of enzyme activity in the tissue between the incisions or a complete necrosis. The adjacent portions of the cortex showed a normal or an almost normal reaction in the neuropil,[2] except for a narrow layer of severely traumatized tissue at the cut surface.

Multiple injections of minute volumes of distilled water produced a widespread decrease of enzyme activity in the neuropil, while the large pyramidal cells of the fifth layer, which normally showed a strong reaction in their perikarya, showed little change. The alterations, thus, were accentuated in the neuropil of the upper four layers; the densitometric decrease (Table 1) refers to the findings in these layers.

Table 1 represents an attempt to quantitate the decrease of enzyme activity. Sections through the center of the lesion were projected on a screen and the areas of greatly decreased enzyme activity were outlined and measured planimetrically. The extent of the decrease in these areas was expressed as a percentage of the data of the homologous opposite region.

Orientation counts were made of the total cell population in regions with greatly decreased enzyme activity; such counts showed differences of less than 10 per cent which ruled out a false decrease of enzyme activity simulated by edema with increased volume of the tissue.

Glycogen. Under the conditions used, normal brain did not show histochemical deposition of glycogen granules. No glycogen was found at the site of the control recordings. The injured tissue, in contrast, showed a pathological deposition of glycogen granules, distributed perivascular or diffusely in the neuropil. These granules were selectively digested by diastase, proving glycogen. A rough correlation was found between decreased enzyme activity and the deposition of glycogen. Incisions into the cortex caused glycogen deposits in a layer of severely traumatized tissue adjacent to the cut. Injection of distilled water produced large areas of deposition of glycogen similar to the areas of decreased enzyme activity. These data agree with the findings of Shimizu

[2] The term neuropil is used descriptively, characterizing the homogeneously distributed enzyme activity between the perikarya. It is understood that dendrites, axon ramifications, synapses, and glial processes compose the neuropil. Histochemically, the neuropil behaves like an entity, quite independent from the behavior of the perikarya; for further discussion of the term neuropil, see (5).

TABLE 1

HISTOCHEMICAL DATA AND EEG CHANGES IN EXPERIMENTAL CORTICAL INJURIES

No.	Type of lesion	Decrease of DPN-diaphorase[a]		EEG recordings from lesions		
		Area (mm^2)	Maximal decrease (%)	1–3/sec	4–7/sec	β
36	Incision	0.7	60	—	Few	Normal
37	Incision	0.4	75	—	Few	Slightly reduced
38	Stitches	0.1		—	Few	Slightly reduced
39	Incision	0.3	60	—	Groups	Reduced
40	Incision	0.3	65	—	Few interspaced	Normal
41	Incision and pressure	3.7	80	Some	Predominant	Superposed on slow waves
42	Injection	0.7	75	—	Interspaced	Slightly reduced
43	Injection	1.6	60	Some	Groups	Superposed on slow waves
44	Injection	2.4	60	Some	Interspaced	Reduced
45	Injection	2.3	65	Many	Predominant	Few
46	Injection	2.6	75	Many	Many	Superposed on slow waves
47	Injection	1.0	65	Some	Predominant	Greatly reduced
48	Injection	6.0	60	Predominant	Predominant	None
49	Injection	3.4	65	Many	Predominant	Almost none

[a] For more than 50 per cent of control.

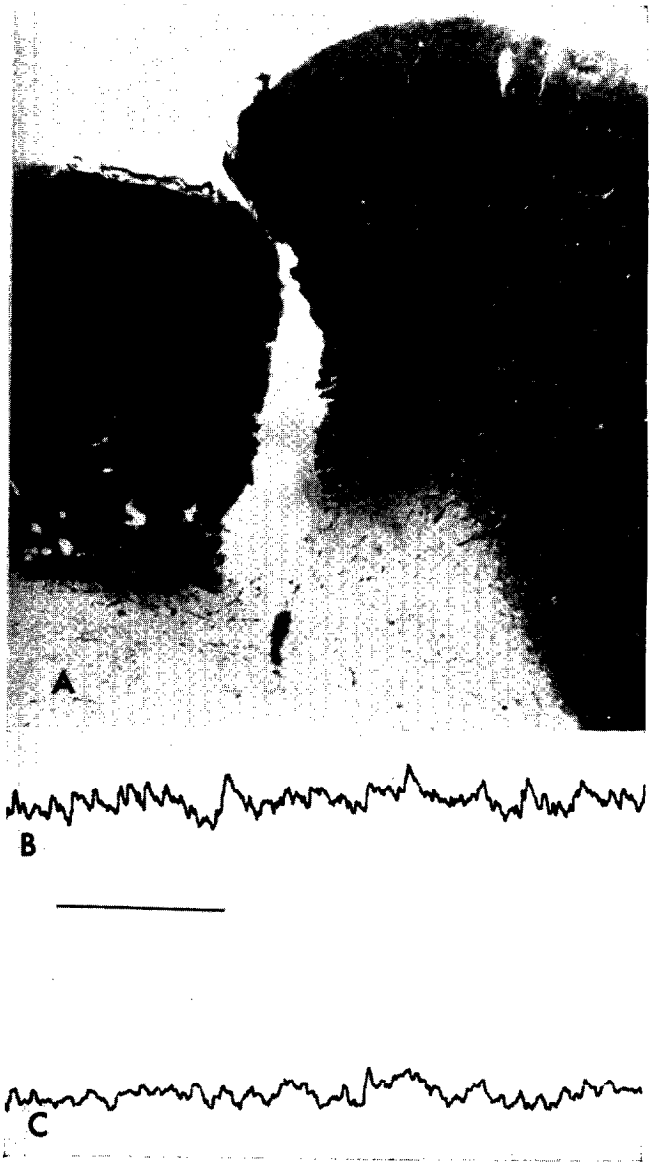

FIG. 1. A, very little decrease of the enzyme reaction adjacent to an incision into the cortex. The picture shows one of three parallel incisions. B, control recording from the homologous contralateral region. C, recording from A.

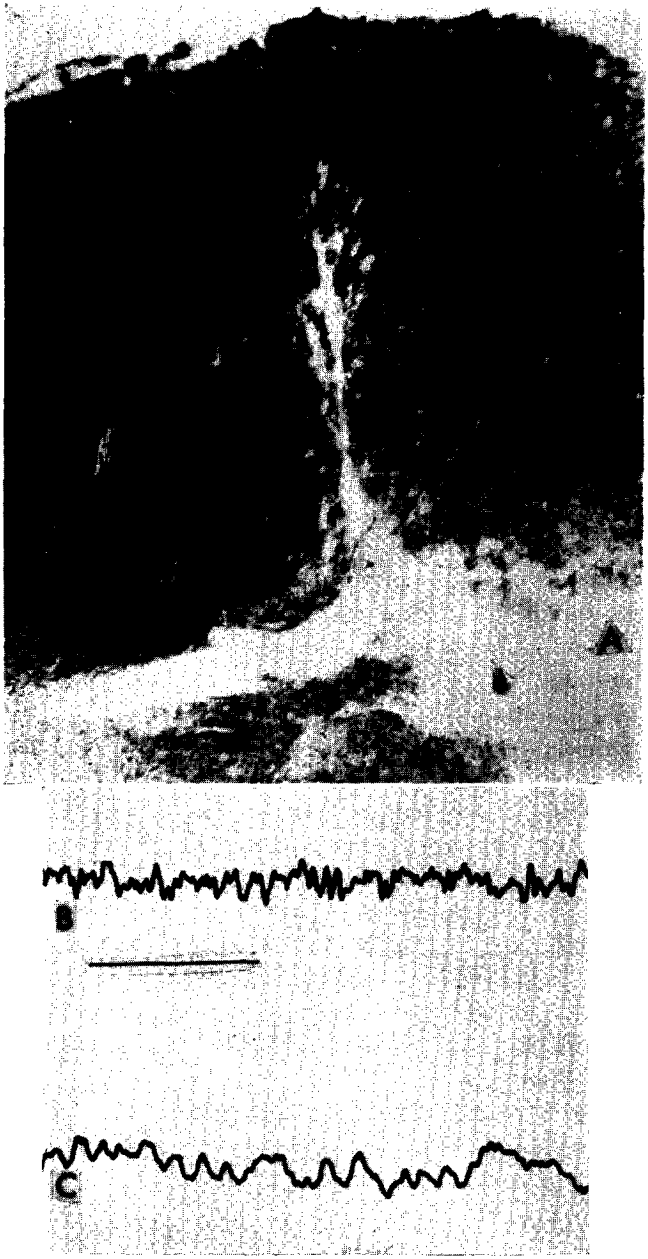

FIG. 2. A, region of transition between a normal enzyme reaction on the left and a large area of greatly decreased enzyme reaction, only partly visible, on the right. The center of the picture shows a needle tract. The separation of cortex and white matter is an artifact. B, control recording from the homologous contralateral region. C, recording from A.

and Hamuro (13), who described, in experimental brain wounds, decreased activity of succinic dehydrogenase, increased activity of glycogen synthesizing enzymes ("phosphorylase"), and deposition of glycogen correlated to each other in certain zones around the wound.

Changes in the Electroencephalogram. The EEG data were evaluated as to the appearances and prevalence of slow waves from the injured tissue. The correlation found between histochemical changes and slow-wave activity was so clear-cut, that one could predict the extent of histochemical changes from the EEG data (Figs. 1 and 2).

In all the animals the control side showed normal EEG recordings. The histochemical data at the control side were normal except for three animals which showed a slight damage of the upper part of the molecular layer with a slight (less than 50 per cent) decrease of the enzyme reaction which was not accompanied by EEG changes.

In the lesions, a slight decrease of the enzyme reaction, measuring less than 50 per cent, was without significance for the appearance of slow waves. The appearance of slow waves was related to a decrease of the enzyme reaction of more than 50 per cent of the reaction as compared with the contralateral reference region; it was also related to the size of the area with decreased activity, as shown in Table 1. Small areas of decreased activity showed only a few interspaced waves (4 to 7 per second); as the size of the areas increased, slow waves (1 to 3 per second) appeared and became predominant. As mentioned above, these changes were also roughly correlated to the deposition of glycogen. These data concerned nerve tissue with approximately normal cell population and a graded decrease in enzyme reaction. Necrotic tissue showed an excessive decrease, or loss, of enzyme activity, but such regions were carefully eliminated from the measurements. Although the water injection technique was more effective in producing decreased enzyme activity, it was not the type of injury but rather the amount of enzyme loss that was relevant (Table 1).

Implications of Human Electroencephalograms. The correlation between deposition of glycogen and decreased enzyme activity in injured tissue attributes significance to findings of a pathological deposition of glycogen in human cerebral cortex.

Table 2 compares the extent of pathological deposition of glycogen in forty-four neurosurgical biopsies from human cortex and the EEG data recorded from the same site before the operation. All the specimens were

fixed in Carnoy's fluid immediately upon removal and glycogen was demonstrated with the periodic acid-Schiff reaction, controlled by diastase. The amount of glycogen was quantitated in Table 2. Some of these data have been documented in detail (13), but Table 2 includes sixteen additional cases. The findings refer exclusively to cortical tissue (no tumor tissue or white matter); two-thirds of the specimens were taken from the border zone of brain tumors and about one-third from cortex adjacent to scar tissue.

TABLE 2

HISTOCHEMICAL GLYCOGEN FINDINGS AND EEG DATA IN FORTY-FOUR HUMAN BIOPSIES

EEG frequencies	Glycogen[a]			
	0	+	++	+++
8 to 12 per second	11	1	—	—
4 to 7 per second	2	5	9	1
1 to 3 per second	1	—	5	9

[a] Amount was quantitated: 0 = none; + = sparse, scattered granules; ++ = many granules; +++ = abundant granules.

These data evidenced a correlation between the extent of pathological deposition of glycogen in human cerebral cortex and the extent of slow-wave activity in the preoperative EEG, similar to that found in the experimental material. On the basis of the latter, one might expect decreased oxidative enzyme activity in the areas of deposition of glycogen. A decrease of succinic dehydrogenase activity has been demonstrated, in fact, in the same type of neurosurgical material from the border zone of brain tumors (4). A similar loss of DPN-diaphorase activity was found in current studies in the vicinity of arteriosclerotic brain lesions where one would expect presence of focal EEG changes.

Discussion

The above data indicated a correlation in the cerebral cortex between focal slow-wave activity in the EEG, deposition of glycogen, and a decreased activity of oxidative enzymes (DPN-diaphorase). A major decrease of oxidative enzyme activity in the cortex evidently impaired the utilization of carbohydrates in biological oxidations and the excess of unused carbohydrates resulted in the depositions of glycogen. Since the energy production of the brain is derived mainly from the oxidation of glucose in the citric acid cycle, any impairment of oxidation will affect the energy supply of the cells and thus the EEG. The correlation of

excessive deposition of glycogen, decreased activity of oxidative enzymes, and slowed frequencies in the EEG, therefore, is in keeping with our present concept of the energy metabolism of nerve tissue. Matsumoto and co-workers (9) attributed slowed EEG frequencies to a reduced redox potential of the cortex. Diminished activity of oxidative enzymes may likely produce slow-wave activity in analogy to a histotoxic anoxia produced by chemical blocking of oxidative enzymes (7). The present data suggest that only a major decrease of enzymatic activity, exceeding 50 per cent produces sufficient derangement of oxidative metabolism as to result in slow-wave activity; one should expect isoelectric activity from regions with complete loss of oxidative enzymes.

The above data suggest a correlation between impaired capacity for tissue oxidation and slow-wave activity in the EEG; the comparison with the similar effects of histotoxic anoxia represents only an analogy, since the full extent of metabolic derangement in the lesion is not known, and since many other mechanisms can produce slow-wave activity, such as other types of anoxia, lack of glucose, drugs, or functional changes. It was felt, however, that a greatly diminished capacity for biological oxidations represents a major factor, if not a cause of the delta focus which accompanies various types of human brain lesions.

It would seem that the above studies should have been done with biochemical assays rather than histochemical techniques. The latter, however, were of advantage since size and shape of the areas of decreased enzyme activity varied greatly. Because of their small size and normal cellularity, they were recognized only histochemically, and a selective macroscopical sampling for assays was practically impossible.

All the findings described indicate that changes in the neuropil are of much greater significance for the frequency of brain waves than the changes in the perikarya of nerve cells. A histochemical mapping of the cerebral cortex of the guinea pig showed differences among areas of the distribution and intensity of enzyme activity in the neuropil; comparison of these data with data from depth recordings in the various cortical layers opens a challenging field of correlations between neurophysiological and histochemical data (5). The neuropil should be considered as one possible source of alpha rhythm.

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