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Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan,
and Aero Medical Laboratories, Wright Air Development Division, Dayton, Ohio

MITOCHONDRIA AND MITOCHONDRIAL ENZYMES
A COMPARATIVE STUDY OF LOCALIZATION IN THE CAT'S BRAIN STEM

By

REINHARD L. FRIEDE and RALPH A. PAX

With 5 Figures in the Text

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The histological demonstration of mitochondria in brain tissue dates back to BETHE (1903), HELD (1909), NICHOLSON (1916), and COWDRY (1916), but only the last decades have brought about a better understanding of their metabolic significance. Mitochondria are complex morphological carriers of organized enzyme systems which are indispensable for the cell's metabolism, for example the citric acid cycle or biological oxidations. In the light of this knowledge, functional significance must be attributed to gradients of the number of mitochondria among regions of the brain and its various cell types. Data on normal architectonic differences of the distribution of mitochondria in the nuclei of the brain are scarce (THURLOW 1917; RASMUSSEN 1919).

The availability of histochemical techniques for the demonstration of certain mitochondrial enzymes stimulated the desire to compare the histochemical distribution of these enzymes with that of mitochondria. The present article summarizes the results of such a comparison, pursued in support of a histochemical atlas of the distribution of succinic dehydrogenase, cytochrome oxidase, DPN- and TPN-diaphorase, and vascularization in the brain stem of the cat (FRIEDE 1961). The first two enzymes are known to be localized almost exclusively in the mitochondria, probably in the cristae mitochondriales (PALADE 1952).

Material and Methods

Succinic dehydrogenase was demonstrated with Nitro BT (NACHLAS and Coworkers 1957) using the modifications suggested by one of the authors (FRIEDE 1959). Cytochrome oxidase was demonstrated with BURSTONE's technique (1959). Material for mitochondrial stains was fixed by perfusion with REGAUD's fluid, embedded in paraffin, and sectioned at 4 micra thickness. Sections were stained with ALTMANN's anilin-fuchsin.

Results

The distribution of mitochondria in the nuclei of the cat's brain stem showed considerable gradations both in the perikarya (THURLOW 1917) and the neuropil (COWDRY 1916). Such gradations were consistently paralleled by gradations of the histochemical enzyme reactions. Some of the most characteristic regions are described in the following; the histochemical atlas should be consulted for further information on enzyme distribution.

Cerebellar cortex

The upper part of the molecular layer of the cerebellar cortex contained fewer mitochondria than its deep part. This gradient of mitochondria was paralleled by

an increase of enzyme activity in the deep part of the molecular layer; the width of these two zones was about equal for both mitochondria and enzyme activity. The lamina of the Purkinje cells exhibited many mitochondria in the perikarya and about the membranes of Purkinje cells (RASMUSSEN 1919), while fewer mitochondria were found in the glial cells between them. Succinic dehydrogenase and cytochrome oxidase showed the same distribution. Most conspicuous was the distribution of mitochondria (ORTIC-PICON and PEREZ-LISTA 1929; SCHARRER

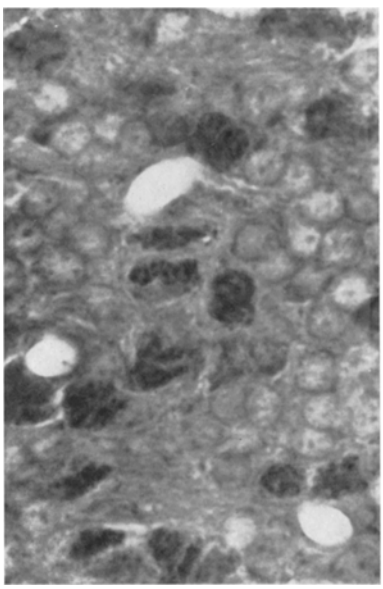

Fig. 1

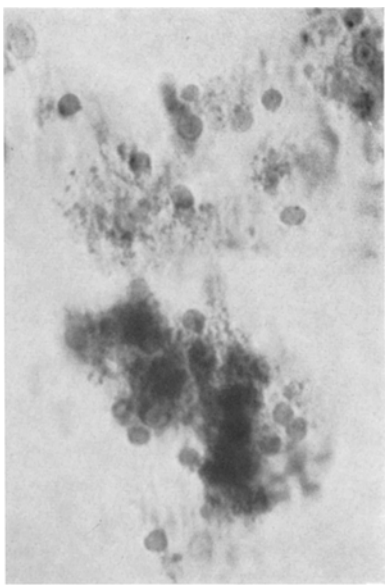

Fig. 2

Fig. 1. Mitochondria in the cerebellar granular layer. The glomerula cerebellaria stand out heavily stained by an abundance of mitochondria, while the perikarya of the granular cells contain very few of them. The nuclei are counterstained. 760 ×

Fig. 2. Succinic dehydrogenase activity (tetrazolium reaction) in a tissue fragment from the cerebellar layer. Several spherical glomerula cerebellaria contain very strong activity while little activity is found in the perikarya. The nuclei are counterstained with chromalum gallocyanin. 320 ×

1954) and enzymes in the granular layer, where both were found almost exclusively in the glomerula cerebellaria which represent the synaptic areas of the mossy fibers and the dendrites of the granular cells (Fig. 1). In sections stained with conventional stains, the glomerula appear as optically empty areas between the densely packed perikarya of the granular cells. The latter contained very little-if any-of either mitochondria or enzyme activity. In preparations of disintegrated tissue it was possible to isolate glomerula cerebellaria as spherical corpuscles containing strong enzyme activity (Fig. 2), while the perikarya of granular cells had very little activity (FRIEDE 1960).

Lower medulla oblongata

The distribution of mitochondria and enzymes in a section through the caudal termination of the fourth ventricle is described in the following (Fig. 3) because of several histochemical peculiarities of this region. The ependyma showed many mitochondria in the superficial portion of the cells. The enzyme activity, likewise,

was strongest underneath the cuticula which borders the ventricle. At the borders of the area postrema there was a sudden decrease of both mitochondria and enzyme activity in the ependyma; the cells which covered the ventricular

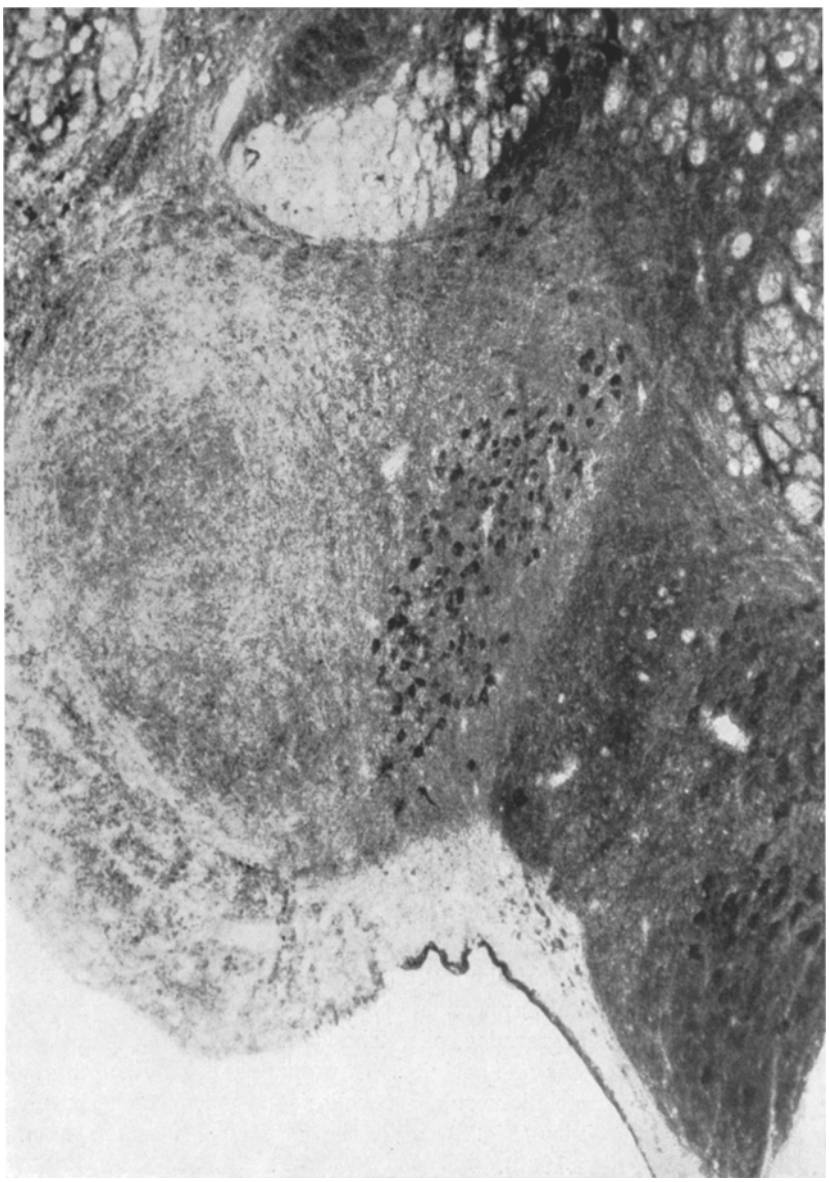

Fig. 3. Distribution of succinic dehydrogenase at the caudal termination of the fourth ventricle. From top to bottom: Area postrema and Nucl. tractus solitarii (light staining); dorsal vagal nucleus (activity in cells only); Nucl. intercalatus (diffuse activity); and Nucl. nervi hypoglossi (activity in the large motor cells). The light oval area at the right margin of the picture is the solitary tract. 120 ×

surface of the area postrema contained very few mitochondria and, likewise, lacked enzymic activity (Figs. 4, 5). The tissue of the area postrema also showed few mitochondria and little enzyme activity except for a few scattered, minute cells which were rich in both. The area postrema borders the nucleus of the solitary tract and the dorsal vagal nucleus. Both nuclei were characterized by having

less mitochondria than other nuclei of the brain stem; THURLOW (1917), among nine nuclei of the medulla oblongata, recorded for the dorsal vagal nucleus the lowest count of mitochondria. Histochemically, these nuclei exhibited exceptionally weak enzyme activity (Fig. 3), both in their perikarya and dendrites. The scarcity of mitochondria in the nerve cells of the dorsal vagal nucleus appeared to be of particular significance, since this nucleus has weak activity of succinic dehydrogenase and cytochrome oxidase but very strong activity of DPN-diaphorase. These three enzymes usually show gradations proportional to each other,

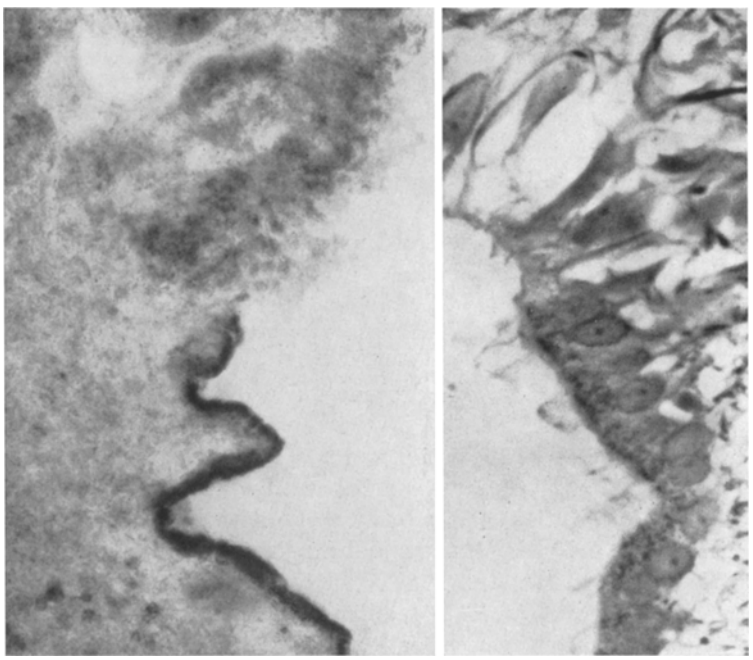

Fig. 4 Fig. 5

Fig. 4 and 5. Sharp termination of succinic dehydrogenase activity (Fig. 4, 400 ×) and mitochondria (Fig. 5, 760 ×) in the ependyma at the borders of the area postrema

except in the dorsal vagal nucleus and some others where their patterns are strikingly disproportional. The first two enzymes are almost exclusively mitochondrial while DPN-diaphorase is considered to be localized, in part, in microsomes. It seems significant, therefore, that the scarcity of mitochondria in the dorsal vagal nucleus agrees with the weak activity of succinic dehydrogenase and cytochrome oxidase rather than the strong activity of DPN-diaphorase. Comparing the dorsal vagal nucleus with the adjacent hypoglossal nucleus, considerably more mitochondria and enzyme activity were found in the latter. The enzyme activity was somewhat stronger in the cell bodies, but was still stronger in the neuropil between them. A similar gradient in cell bodies and neuropil was found for the mitochondria. The mitochondria in the neuropil were identical with the mitochondria in the „Grundnetz" described by HELD (1912) half a century ago; a sharper cytological localization in dendrites or synapses was not possible.

In the reticular formation, particularly in its medial part, the enzyme activity prevailed in the perikarya over that in the scanty neuropil; a similar pattern was

found for the mitochondria. Many mitochondria were found attached to the surface of the cells, apparently outside of the membrane in the terminal buttons. In histochemical preparations, the discernment of the reaction at the cell's surface and the reaction in the cytoplasm was difficult. During experimental chromatolysis, however, the enzyme activity of the cytoplasm decreased while strong activity persisted at the cell's membrane (FRIEDE 1960). This distribution was similar to the normal arrangement of mitochondria at the membrane.

Nucleus coeruleus and nucleus of the mesencephalic trigeminal root

These two nuclei deserve particular attention because of their conspicuous enzyme patterns. The nucl. coeruleus was characterized by its very weak activity of succinic dehydrogenase and cytochrome oxidase and also by few mitochondria. The nucleus of the mesencephalic root of the trigeminal nerve, on the other hand, showed all its enzymic activity in the perikarya and none in a neuropil. This pattern was unique, since any other nucleus of the brain showed at least a little neuropil with enzyme activity. The unique enzyme distribution in this nucleus was paralleled by numerous mitochondria in the perikarya (THURLOW 1917), but none in the intervening tissue.

Discussion

The localization of succinic dehydrogenase and cytochrome oxidase in mitochondria has been established through numerous biochemical investigations; in the rat's brain, about 78% of the activity of succinic dehydrogenase was found in the mitochondria (ABOOD, GERARD, BANKS and TSCHIRGI 1952). Evidence for the histochemical localization of these enzymes in mitochondria was obtained by electron-microscopical studies (PEARSE and SCARPELLI 1959). The present comparison of the distribution patterns of enzyme activity and mitochondria is in accordance with this concept. The cytological localization of the enzyme reactions studied was found to be very accurate as judged by the identical distribution of mitochondria.

The activity of oxidative enzymes in a given region may be considered an approximative parameter of the intensity of the oxidative metabolism. SCHARRER (1954) showed a relationship between the frequency of mitochondria and the capillarization of several nuclei of the brain. A relationship between the activity of succinic dehydrogenase and capillarization was established by measurements in 64 nuclei of the cat's brain stem (FRIEDE 1961). The present investigation showed that *the distribution of mitochondria is identical with that of enzyme activity.* The capillarization, the distribution of mitochondria, and the distribution succinic dehydrogenase and cytochrome oxidase in a given region, thus, appear related to each other quantitatively. This can be expected: The mitochondria are carriers of oxidative enzymes; and the supply with capillaries likely adjusts to the local demands of the oxidative metabolism. A comparison of all these patterns provides a quite reliable picture of the functional activity of a given region.

Summary

The cytological distribution and the frequency of mitochondria in certain nuclei of the cat's brain stem was compared with the histochemical reactions for

succinic dehydrogenase and cytochrome oxidase, which are known to be mitochondrial enzymes. The distribution of mitochondria was found to be identical with that of the above oxidative enzymes. The significance of these findings for the understanding of the regional chemistry and metabolism of the brain is indicated.

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REINHARD L. FRIEDE, M. D. Mental Health Research Institute
University of Michigan, Ann Arbor, Michigan, USA