THE ACTIVITY OF SUCCINIC DEHYDROGENASE IN GLIAL TUMORS*

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Histochemical analysis of oxidation-reduction processes in tissues became possible following the introduction of substances which, in their reduced form, precipitate at the site of dehydrogenase activity as insoluble colored compounds. The tetrazolium salts possess such properties. In the oxidized form they are yellow or colorless, while, on reduction, they form the dark blue colored formazan. The formazans are insoluble in water and in many organic solvents used in histological procedures. Tetrazolium compounds were first used in histochemical investigations of oxidative cellular enzymes by Seligman and Rutenburg (1, 2). At present, many forms of these compounds are used, but Nitro-BT, [(2,2′-P-Nitrophenyl) 5,5′-Diphenyl-2,2′ (3,3′ Dimethoxy-4,4′ Biphenylen)-Ditetrazolium chloride] appears the best so far (3, 4). The tetrazolium compounds were used initially to demonstrate succinic dehydrogenase activity; however, they have been utilized in studies of other dehydrogenase activities. Further study (5 to 10) has disclosed that tetrazolium salts are reduced not directly by the dehydrogenases but through intermediate flavoproteins or pyridine nucleotides. Even though the reduction of tetrazolium salts is mediated through flavoproteins, such reactions occur at the site of the activity of the particular dehydrogenases under study. The specificity of the reaction is secured by the addition of the appropriate substrate to the incubating medium containing the tetrazolium salt (7, 8). Oxelrod and Ball and Cooper, quoted in Sumner and Myrbäck (11), established that a flavoprotein intermediary was an essential element in the succinic dehydrogenase system.

Succinic dehydrogenase is an important enzyme in tissue oxidative metabolism and may be considered as an indicator of the sites of these processes and their relative activity. It was, therefore, considered of interest to study a series of glial tumors of varying degrees of anaplasia with respect to their succinic dehydrogenase activity (SDA). The astrocytic group formed the majority of cases because of its frequency and variability in histopathological grading. Samples of these tumors were compared with normal and neoplastic oligodendroglia (because of their reported high degree of oxidative activity (12 to 17)) and with normal and reactive astroglia in respect of their SDA.

Investigations of succinic dehydrogenase activity in nervous tissue have been carried out by many authors. Because fresh tissue is necessary, most experiments have been carried out on animals (2, 18 to 26) or on tissue cultured *in vitro* (27). Few experiments have been done with human brain tissue (21, 28, 29). The majority of authors regards SDA as occurring in high activity within

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the neurons and the neuropil, while the glial cell bodies have a very low or no SDA. However, Potanos, Wolf and Cowen (29) observed high levels of SDA in the cytoplasm of glial cells, especially in oligodendroglia. Friede (28) has also observed a high degree of SDA in the reacting glia surrounding brain tumors. There are few publications concerning SDA in glial tumors. Ogawa and Zimmerman (30) have examined SDA in an ependymoma induced in C3H mice by implantation of carcinogens. Friede (28) has examined various human brain tumors including gliomas. Potanos, Wolf, and Cowen (29) added a glial tumor to their studies of nonneoplastic brain tissue.

MATERIAL AND METHOD

The tissue examined comprised biopsy specimens of 20 glial and 2 non-glial brain tumors obtained at operation. In addition to tumor tissue, most specimens included brain tissue from the area surrounding the tumor. Additional control material was obtained from a case of non-neoplastic temporal lobe epilepsy. The specimens may be classified as follows:

1. Pilocytic astrocytoma—4 cases.
2. Gemistocytic astrocytoma—3 cases.
3. Malignant glioma—3 cases.
4. Glioblastoma multiforme—7 cases.
5. Oligodendroglioma—3 cases.
6. Metastatic bronchogenic carcinoma—1 case.
7. Epidermoid cyst of the posterior fossa—1 case.

The technique used was that of Potanos, Wolf, and Cowen (29), a modification of the original method devised by Seligman and Rutenburg. The specimen of tumor and surrounding brain was sectioned, immediately on operative removal, into blocks of 0.5 mm. thickness by means of a Stadie-Riggs microtome, the procedure being carried out in a humid chamber. The blocks were then immersed in the incubating medium (vide infra) for 24 hours at 6°C. After incubation, they were fixed for 24 hours in 10 per cent neutral isotonic formalin and embedded in paraffin. Serial sections of 10 microns thickness were cut and samples from different depths of the blocks mounted in the usual manner on microscope slide. Half of the sections were counterstained with eosin while the other half were not.

The incubating medium was made up with equal parts of the following two solutions: *a:* 5 ml. 0.2M phosphate buffer (pH 7.6), and 5 ml. 0.2M aqueous sodium succinate; *b:* 0.1 per cent aqueous Nitro-BT (Dejac, Philadelphia). In addition, in 6 cases, extra tissue slices were incubated in a solution (described by Ogawa and Zimmerman), consisting of equal parts of (a) 0.1 per cent Nitro-BT in 0.1M phosphate buffer (pH 7.6); (b) 0.1M sodium succinate in buffer; (c) 0.03 per cent phenazine methosulfate, and (d) 0.1M phosphate buffer (pH 7.6). They were otherwise treated in the same way as the first group. A control sample in each case was heated in Elliott's solution, prior to incubation, to a temperature of 80°C. for 5 minutes. In 6 cases, additional tissue samples were incubated in a modified medium from which the sodium succinate substrate had been omitted.

The method of Potanos, Wolf and Cowen, used in this study, avoids freezing the tissue with the possibility of damaging mitochondria. The tissue was not injected with medium as in the original description of the method as we found penetration of the tissue satisfactory. However, in those cases in which additional samples were incubated in the phenazine methosulfate medium of Ogawa and Zimmerman, penetration was obviously inadequate. Samples incubated for 6 to 8 hours did not consistently differ from those treated for the full 24 hours, although in all cases samples were continued for the longer period. Swelling of the specimen was usually considerable and resulted in rarefaction of the tissue and diminution in cellular packing density when compared with ordinary histological material.

RESULTS

Pilocytic Astrocytoma: Tumors of this category showed the lowest degree of SDA among those studied. However, in all cases, most tumor cells contained fine, deeply stained blue formazan granules lying in their cytoplasm and processes. These cytoplasmic granules preferentially formed thin dense rings surrounding the nuclei which remained unstained. A few tumor cells were entirely devoid of formazan granules. We did not observe any consistent difference in SDA between the elongated piloid cells and the small fibrillary astrocytes. In contrast, the cytoplasm of occasional gemistocytes scattered throughout these tumors showed relatively high SDA (fig. 1). The cytoplasm of vascular endothelium contained fine formazan granules.

Gemistocytic Astrocytoma: All tumors in this category gave a positive reaction. Again the cytoplasm of both cell body and processes contained formazan granules, while the nuclei were devoid of them (fig. 5). The distribution of formazan granules within the cytoplasm varied a great deal. In many instances they densely filled the whole cell body (fig. 5b); in other cells there was a prominent perinuclear ring of granules. The intensity of SDA varied and appeared to be proportional to the volume of cytoplasm of the cell. The larger gemistocytes, sometimes with 2 or 3 nuclei, showed a much stronger reaction. In addition to gemistocytes, other astrocytic forms and spongioblasts occurring in these tumors also gave a positive tetrazolium reaction (fig. 2). The intensity of the tetrazolium reaction was often stronger in the perivascular processes and in the sucker feet which sometimes were present in the plane of section. In general, those tumor cells lying close to blood vessels seemed to possess a more strongly positive reaction.

Malignant Glioma and Glioblastoma Multiforme:* These categories of tumor will be discussed together because of the similarity of results obtained. In both categories there was a high degree of SDA (figs. 3, 4 and 7). Variability in the intensity of the tetrazolium reaction from cell to cell was a prominent feature. As with gemistocytic astrocytoma, there was a striking parallelism between the intensity of the tetrazolium reaction and the apparent volume of cytoplasm. Coarse, spherical or rod-shaped, formazan granules were particularly abundant in the cytoplasm of giant multinucleate cells (fig. 8); the density of the formazan deposits within the cytoplasm of these cells prevented assessment of any particular distribution of activity within the cell body other than the observation that the nuclei did not partake in it. There were also dense granular deposits in the cellular processes and again these were more pronounced in the perivascular zones and in the sucker feet (fig. 9). The cytoplasm of endothelial cells contained fine blue granules (fig. 7), while necrotic areas were entirely negative.

Oligodendroglioma: None of the 3 examples of this category were pure oligodendrogliomas, as all contained areas of astrocytic tissue and in one case

* Anaplastic astrocytic tumors which, in ordinary histological preparations, did not show all the customary criteria of glioblastoma multiforme were classified as "malignant glioma".

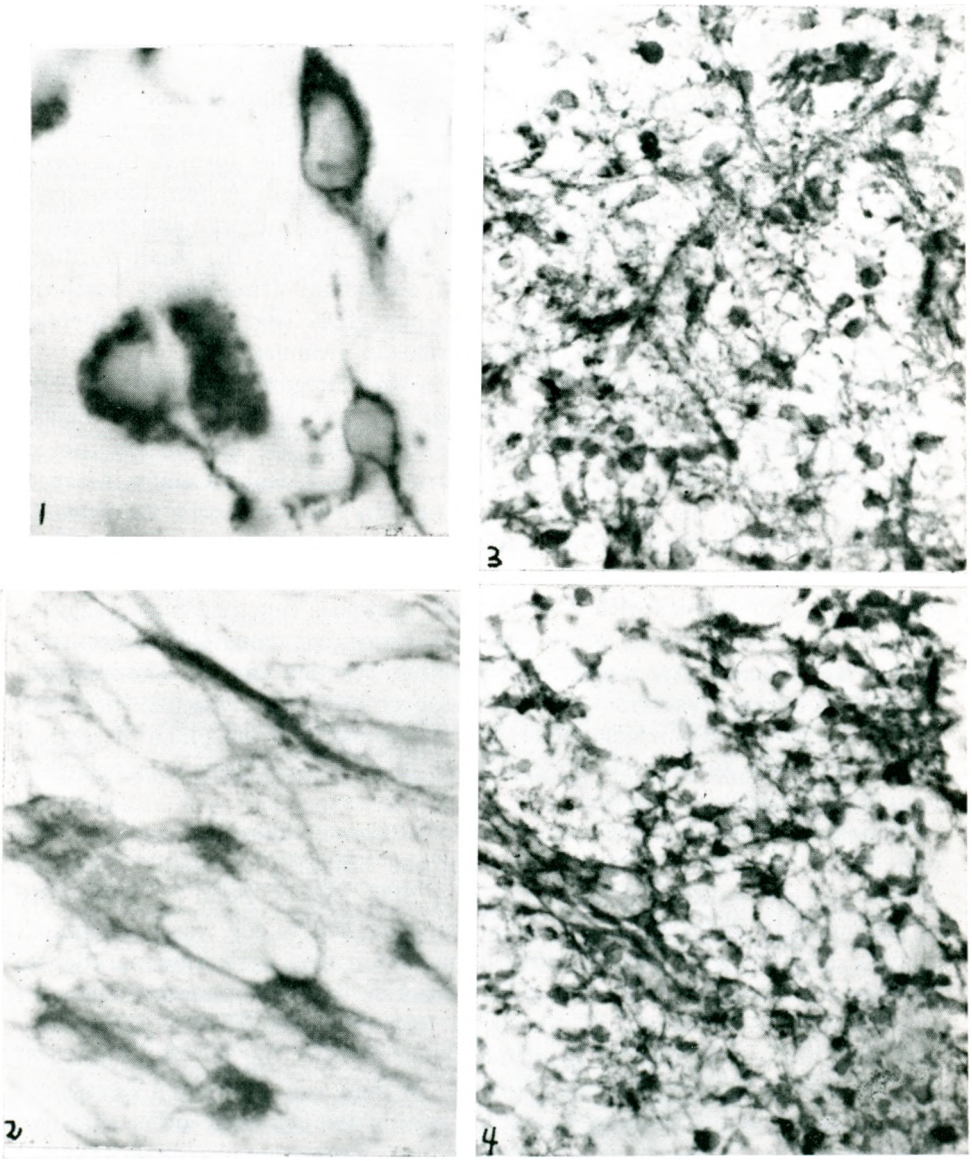


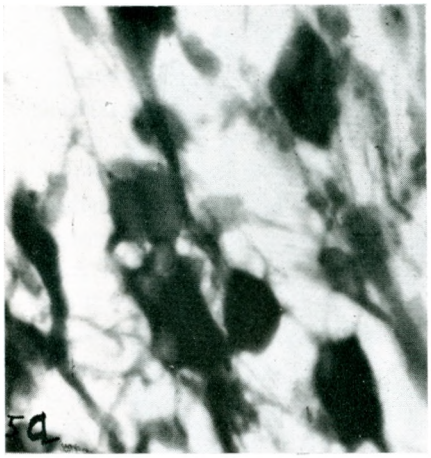
FIG. 1. Pylocytic astrocytoma. Nitro-BT (method of Potanos, Wolf and Cowen) without counterstain. Formazan granules, seen in the cytoplasm of tumor cells, vary in amount with the volume of the cytoplasm; × 1300.

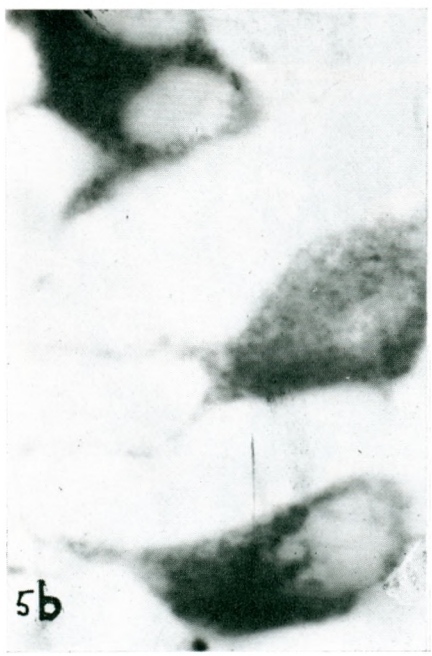
FIG. 2. Gemistocytic astrocytoma: all forms of cells give tetrazolium reaction; × 800.

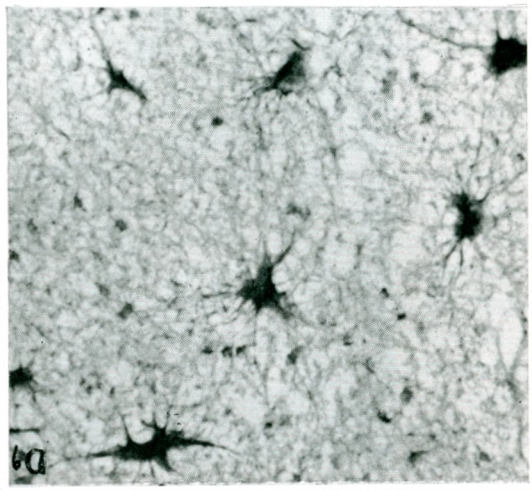
FIG. 3. Malignant glioma: general view showing activity in all cell types; × 450.

FIG. 4. Glioblastoma multiforme: all tumor cells contain formazan granules; granules are also present in vascular endothelium; × 450.

there had been a previous operation and X-ray therapy resulting in areas of dense fibrous tissue within the tumor. Variation in the intensity of the tetrazolium reaction in different portions of the tumor was the most striking feature of this category of tumors. There were some areas in which cells were densely







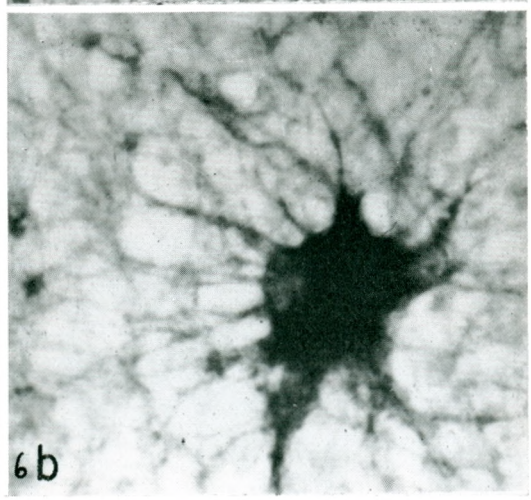
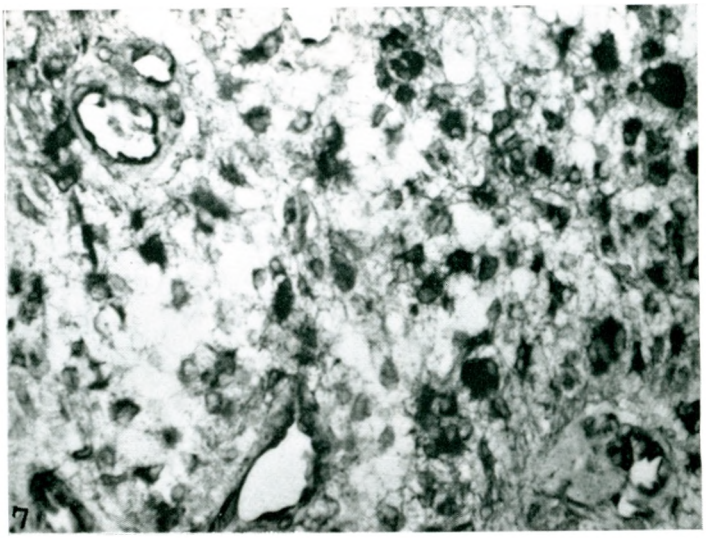


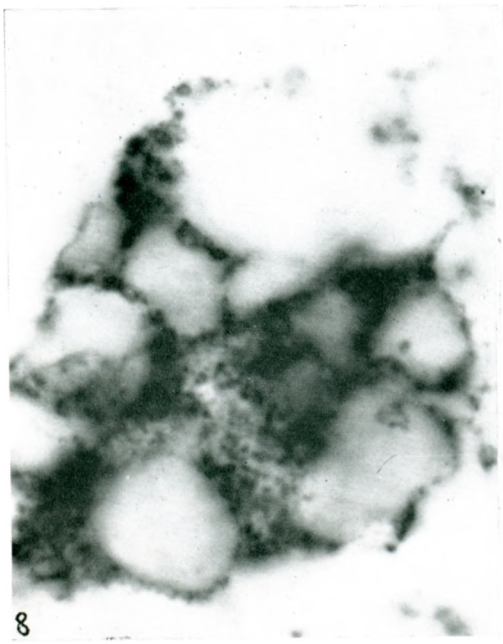
FIG. 5. Gemistocytic astrocytoma: method of Potanos et al. without counterstain. *A*. Strongly positive formazan reaction within tumor cells. × 800 — *B*. Higher magnification showing cytoplasm crammed with granules; nucleus remains unstrained. × 2000.

FIG. 6. Reactive astrocytes in white matter beyond margin of tumor: method of Potanos et al., counterstained with eosin; formazan granules present in both cell body and processes. A, × 380. B, × 1400.

filled with formazan granules while other areas were composed of cells with uniformly scanty granular content situated mainly at the periphery of the cell. Short, thin processes of oligodendrocytes were observed in areas where the tumor tissue showed a slightly less dense pattern, and here and there were abundant formazan deposits lying among the tumor cells.

Metastatic Bronchogenic Carcinoma (1 case): The epithelial cells forming





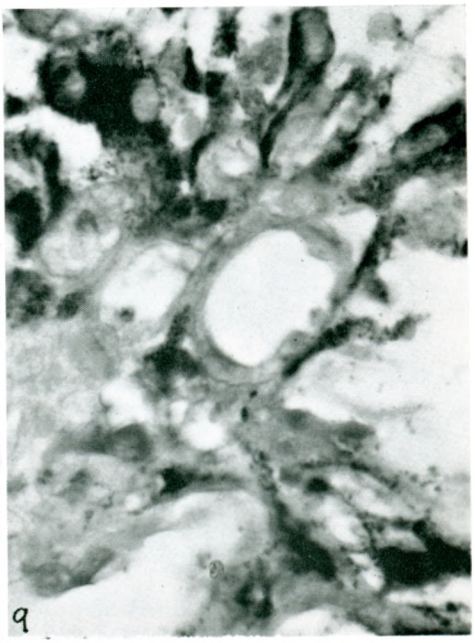


FIG. 7. Glioblastoma multiforme: abundant formazan deposits in the large tumor cells; deposits also abundant in endothelial cells of blood vessels. × 450.

FIG. 8. Glioblastoma multiforme: high power view of tumor giant cell showing coarse granulation in interstices between multiple nuclei. × 2500.

FIG. 9. Glioblastoma multiforme: detail of perivascular arrangement showing formazan granules in vascular processes and sucker feet; connective tissue elements of blood vessel wall remain unstained. × 650.

the acini of the tumor were densely filled with fine, deep blue formazan granules. The large vesicular nuclei were entirely negative.

Epidermoid Cyst of the Posterior Fossa (1 case): Here, only scanty granules of formazan were seen in the epithelial cells of the basal layer, and in the endothelial cells of blood vessels.

Non-Neoplastic Brain Tissue: The neurons of the undamaged cerebral and cerebellar cortex were densely loaded with coarse, blue formazan deposits. These granules filled both cytoplasm and dendrites, but the nuclei were free of formazan. The amount of formazan deposition was greater in larger neurons than in smaller ones and this was especially striking in the cerebellar cortex. The presence of very dense, deeply stained formazan deposits in the neuropil was a prominent feature of the gray matter, and this was responsible for the deep coloration of the cortex seen even on naked eye examination of the blocks. Extracellular deposits in the white matter were very scanty. The astrocytes and oligodendrocytes of the white matter contained formazan granules in their cytoplasm and processes, but these were both finer and fewer than those occurring in neurons. Oligodendroglia showed more activity than astroglia.

Rather constant features were present in the brain surrounding tumors, where hypertrophied astrocytes with abundant cytoplasm and thin processes contained numerous fine formazan granules in both cell body and processes (fig. 6a and b).

DISCUSSION

According to Warburg, the activity of oxidative metabolism in neoplastic tissue is lower than that in normal tissue from which the tumor has originated. The results of some studies, however, do not fit this generalization. Pearson and Defendi (31) did not observe any difference between the SDA of biliary duct epithelium and papillary adenocarcinoma of the liver experimentally induced in rats. SDA in skin carcinoma of mice is relatively high while in normal skin it is rather low (11). Oxidative metabolism of human glial tumors as assayed by oxygen uptake was shown by Heller and Elliott (14) to be low when compared with neuronal and glial metabolism on a *per* cell basis. Pope and his colleagues (12, 15) concluded that glial tumors of all types had a lower cytochrome oxidase activity than their tissue of origin. Allen (13) found that the cytochrome oxidase activity of well differentiated astrocytomas was 10 per cent of that of cortex while oligodendroglioma was about 70 per cent, and glioblastoma lay in between.

Zimmerman and Ogawa (30), investigating the succinic dehydrogenase activity of an experimentally induced ependymoma in mice by biochemical and histochemical methods, found that the activity of this enzyme was lower in the tumor than in normal brain tissue. This is in accord with the low SDA found histochemically in human glial tumors by Friede (28). However Potanos, Wolf and Cowen (29) again using histochemical methods, did not find any significant difference in activity between oligodendrocytes of normal and neoplastic tissue. Our results differ considerably from those of Friede. SDA was readily found

in all tumors examined. The more anaplastic the tumor the greater was its SDA. The lowest SDA was found in pilocytic astrocytoma and higher activity occurred in gemistocytic astrocytoma; malignant glioma and glioblastoma multiforme exhibited the highest degree of activity. Cells of pilocytic astrocytoma have an SDA roughly comparable with normal astrocytes of the white matter, but consistently lower than that of reactive astrocytes as seen around tumors. Anaplastic categories of astrocytic tumors show greater SDA than do oligodendrogliomas; SDA in the latter, although variable, corresponds in average to that of gemistocytic astrocytoma. The amount and intensity of positive tetrazolium reaction in individual tumor cells seems to be related to the apparent cytoplasmic volume of the cell body. A similar suggestion has been made by Pearson and Defendi (31) in connection with hepatic carcinoma. With an increase in cytoplasmic volume there is probably an increase in mitochondria; the mitochondrial localization of SDA is well established (17, 32–33). In glial tumors, then, the total SDA depends on the predominant cell type and the cellular packing density.

We feel that qualitative histochemical investigations showing the presence and distribution of succinic dehydrogenase in different glial cells, both neoplastic and normal, together with rough comparisons of their amounts, should be supplemented by quantitative microchemical examinations which would determine the enzymatic activity as measured *per* unit of cytoplasmic volume or mitochondrial fraction. The value of such investigation for the proper evaluation of the degree of activity of cellular oxidative processes, was shown by Hydén and his colleagues (17) in their experiments on the enzymatic activity of oligodendroglia in comparison with that of neurons.

SUMMARY

1. Succinic dehydrogenase activity of astrocytic tumors of varying degrees of anaplasia was assessed by means of the tetrazolium reaction and compared with the activity in oligodendrogliomas and in the various elements of normal brain.

2. Succinic dehydrogenase activity was present in all glial tumors examined. It increased with increasing anaplasia of the tumors. The SDA of oligodendroglioma, while high, was less than that of glioblastoma multiforme.

3. The SDA in the cells of piloid astrocytoma appeared to be similar to that of normal astrocytes in white matter. SDA in the cells of gemistocytic astrocytoma, malignant glioma and glioblastoma multiforme exceeded considerably that in normal glia. Reactive glia also showed greater SDA than normal glia.

4. There appears to be a relationship between the volume of cytoplasm constituting the cell body and the amount of SDA exhibited by the cell.

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