Abnormal Electrophoretic Patterns of Extractable Proteins and Enzymes (Isozymes) in Human Brain Tumors

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Extractable proteins and enzymes from 25 human brain tumors obtained at operation were separated in agar-gel micro-electrophoresis. 3 types of enzymes were investigated: lactate dehydrogenases (LDH), esterases, and

acid phosphatases. The findings were correlated to those of normal human brain tissue. The biochemical samples were controlled by alternating serial slices, that were subjected to histological examination. The protein fractions were characterized by their electrophoretic mobility and relative concentration.

All tumors showed alterations from normal protein distribution, especially in anodic pre-albumin, α , and γ -areas. 5 LDH isozymes were separated, numbered 1—5 from the anode, and evaluated by photometry.

In malign primary and secondary brain tumors dedifferentiation of the normal isozyme pattern was found, characterized by a relative decrease of fractions 1 and 2, accompanied by elevations of fractions 4 and 5. Benign gliomas showed relative decrease of fractions 4 and 5, oligodendrogliomas had higher values of fraction 1 than astrocytomas.

Practically, it was found that the biochemical examination of LDH patterns could support

Table 1.

Type of tumor	Distribution of LDH isozyme bands %				
Metastasis ad cerebrum anaplasticum	9	19	25	26	21
Metastasis ad cerebrum mucoide	9	13	29	26	23
Metastasis ad cerebrum mucoide	11	16	24	25	25
metastasis ad cerebellum adenomatosa	18	23	19	15	24
Glioblastoma, angioneurotic	17	18	27	20	17
Glioblastoma, angioneurotic	9	17	25	25	25
Glioblastoma, angioneurotic	12	18	23	25	22
Glioblastoma, angioneurotic	7	16	23	26	28
Glioblastoma or mal.ganglioglioma	17	18	27	20	17
Glioblastoma, big cells, lower malign.	29	25	24	14	8
Malign plexus papilloma, temporal	12	22	22	19	29
Astrocytoma, with few ependymal elem.	40	33	20	6	<1
Astrocytoma in cerebellum	30	32	32	6	<1
Oligodendroglioma, diffuse infiltrate	44	33	23	1	<1
Oligodendroglioma, clusters of oligo.	68	8	22	<1	<1
Normal brain, grey matter	27	27	29	15	2
Number of bands:	1	2	3	4	5

the histological differentiation between malign and benign brain tumors. Similar results were found in the LDH patterns of extracerebral tumors.

8—9 esterases were demonstrated with different substrates and characterized by differential inhibition with organophosphoric compounds, EDTA and eserine. At least one fraction of acid phosphatase was found.

Electrophoretic Properties of Atypical Human Serum Cholinesterase

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Atypical human serum cholinesterase^{1,2} differs from the normal in inhibitor specificity³. The two forms were separated by electrophoresis and ion exchange chromatography⁴ indicating a difference in molecular properties. To investigate whether the atypical form contains sialic acid, as does normal cholinesterase⁵, a serum sample containing only the atypical enzyme* was treated with neuraminidase and the electrophoretic mobility determined on paper⁶ at pH 4—12. Removal of sialic acid changed the mobility of the two forms of cholinesterase by the same amount, *i.e.* they contained about the same number of sialic acid residues per molecule. With the buffers used (acetate, barbital and glycine) the electrophoretic mobility of the two forms was identical (within $0.2 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1}$) both in the native and the sialic acid-free states.

1. Lehmann, H. and Ryan, E. *Lancet* **2** (1956) 124.
2. Kalow, W. and Staron, N. *Can. J. Biochem. Physiol.* **35** (1957) 1305.
3. Kalow, W. and Davies, R. O. *Biochem. Pharmacol.* **1** (1958) 183.
4. Liddell, J., Lehmann, H., Davies, D. and Sharih, A. *Lancet* **1** (1962) 463.
5. Svensmark, O. *Acta physiol. Scand.* **52** (1961) 267.
6. Svensmark, O. and Kristensen, P. *Danish Med. Bull.* **9** (1962) 16; *Biochim. Biophys. Acta.* **67** (1963) 441.

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Studies on the Interaction between Oximes and Organophosphorus Compounds or Phosphorylated Cholinesterases

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Phosphorylated cholinesterases obtained either after *in vitro* or *in vivo* reactions of the enzymes with organophosphorus compounds of

the general formula were reacti-

vated with N-methylpyridinium-2-aldoxime methane sulphonate¹ (P2S) or N,N'-trimethylene-bis (pyridinium-4-aldoxime) dibromide¹ (TMB-4). Reactivation and conversion to a non-reactivable form¹ (aging) *in vitro* and *in vivo* was measured. In the case of Tabun¹ P2S was shown to be a very poor reactivator, while TMB-4 had a good effect both *in vitro*² and *in vivo*³. After phosphorylation by the other organophosphorus compounds studied the effect of the two reactivators was more equal, though TMB-4 usually was the better reactivator.

Aging *in vitro* as well as *in vivo* was shown to differ with the nature of the groups R₁ and R₂ in the organophosphorus compound^{1,4} and with the nature and the source of the enzyme^{1,2}. It was also shown that chemical treatment, *e.g.* purification of the enzyme, can alter this reaction². Some phosphorylated oximes, corresponding to Tabun⁴ and Sarin^{4,5} were prepared and shown to be equally or even more toxic than the parent organophosphorus compound and to be inhibitors of cholinesterases. From studies on the phosphorylated oximes and on the reaction between oxime and organophosphorus compound in solution it was concluded that the four-substituted oxime yields a more stable phosphorylated compound than the two-substituted oxime.

1. O'Brien, R. D. *Toxic Phosphorus Esters*. Academic Press, New York and London 1960.
2. Heilbronn, E. *Biochemical Pharmacology*. *In press*.
3. Heilbronn, E. and Sundwall, A. *To be published*.
4. Heilbronn, E. *To be published*.
5. Hackley, B. E., Steinberg, G. M. and Lamb, J. C. *Arch. Biochem. Biophys.* **80** (1959) 211.