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ACTIVITY OF REDOX ENZYMES IN GLIAL TISSUE CULTURED *IN VITRO* UNDER VARIED GAS ATMOSPHERE *

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The effect of various proportions of oxygen in the atmosphere on the behavior of redox enzyme activities in glial cultures was studied.

The activity of these enzymes is dependent on the oxygen content in the atmosphere, the solubility of oxygen in the medium, the age of the culture and the type of glial cells.

The greatest decrease in enzymatic activity was observed in complete hyperoxia and anoxia.

Intermediate states of hypoxia and hyperoxia did not noticeably affect the activity of dehydrogenases.

Young, 2—3-weel-old cultures, exhibited the greatest drop in enzymatic activities.

The pattern of the enzymatic reactions and the distribution of activities in different types of glial cells were the same as in normal gas atmosphere.

Succinic, NAD, glutamic, α-glycerophosphate, glucose-6-phosphate and lactic dehydrogenases showed the greatest decrease in activity.

The enzymatic activity of all the dehydrogenases studied was directly dependent on the functional state and the morphology of the glial cells.

The successive step in investigations on the adtivity of redox enzymes in glial cells cultured *in vitro* (*Mossakowski et al.* 1965, *Renkawek* and *Mossakowski* 1966) involved the follow-up of the behavior of these dehydrogenases in glial cultures growing in an atmosphere with varying oxygen content.

The aim of the investigation was also to compare the redox enzyme activity in glia cultured under pathological conditions and that in sections taken from brains subjected to anoxia and hypoxia; moreover, a functional interpretation is given of the changes known in classical neuropathology and induced in glial tissue by a decrease or an increase in the oxygen content and by enzymatic activity.

EXPERIMENTAL

The investigation was performed on tissue cultures of cerebellar glia of 6—24-hour-old white Wistar rats. The cultures were grown in Carrel flasks, according to *Kraśnicka* and *Mossakowski* (1965).

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The method of changing the gas atmosphere and the pathogenic conditions of culture growth were the same, as those reported by *Kraśnicka et al.*, 1967 (see Table I in the foregoing publication).

Histochemical studies were carried out with cultures aged 1—2, 3—5, 7, 10—14, 21—23, 25—35 days. The following redox enzymes were investigated: succinic dehydrogenase, NAD dehydrogenase and the associated lactic, glutamic and α-glycerophosphate dehydrogenases as well as glucose-6-phosphate dehydrogenase associated with coenzyme II.

A detailed description of the histochemical technique is given in the publications dealing with the activity of the foregoing enzymes under normal conditions (*Mossakowski et al.* 1965, *Renkawek* and *Mossakowski* 1966).

RESULTS

I. Anoxia

The activity of all the dehydrogenases was lower than normal. This finding especially concerns succinic, NAD and glutamic dehydrogenase, the activity of glucose-6-phosphate, α-glycerophosphate and lactic dehydrogenase remaining almost unchanged.

The activity of NAD dehydrogenase decreases most in the undifferentiated spongioblasts derived from 2—4-day cultures. After 2 weeks of growth the activity is slightly lower than normal, and predominates in the oligoglia. The cell processes show very low activity.

The activity of succinic dehydrogenase is very low up to 2 weeks of growth; in older cultures it increases slightly, without ever reaching the normal level. In gemistocytes and regressive forms of the changed glia as well as in the macrophages the activity is somewhat better preserved.

The activity of glucose-6-phosphate and α-glycerophosphate dehydrogenase is slightly lowered only in very young cultures and returns to normal during the later stages of growth. Regressively and progressively changed glia shows high activity. Lactic dehydrogenase activity is slightly decreased and rather irregularly distributed in the cell cytoplasm only in 2—4-day cultures; during the later stages it is normal. On the other hand, glutamic dehydrogenase activity remains considerably lower, as compared with the normal level, up to 3 weeks of growth. In the later stages and in gemistocytes the activity is fairly well preserved.

II. Hypoxia

Succinic and NAD dehydrogenase show a considerable decrease in activity up to 2 and 3 weeks of growth, this being followed by a slight increase. The activity in the cell processes is greatly lowered. Gemistocytes, occurring in

great numbers in hypoxia, exhibit a rather irregular and non-uniform activity in the cytoplasm, with a tendency to concentration in its part.

The activity of glucose-6-phosphate and α-glycerophosphate dehydrogenase is lower than normal only in very young cultures; gemistocytes and the regressively changed astroglia show fairly high activity.

Lactic dehydrogenase activity is normal both in young and older cultures. The activity of glutamic dehydrogenase is decreased up to 2 weeks of growth and then increases gradually.

III. Hyperoxia (50—70% of oxygen)

In this group succinic and NAD dehydrogenase exhibit a drop in activity, especially in young cultures, whereas the remaining dehydrogenases preserve better their activity. Succinic dehydrogenase activity is low up to 3 weeks of growth and declines most in the processes. The activity is well preserved in the numerous regressive and progressive forms. The distribution of the intensity of enzymatic activity relative to the type of glial cells, the stage of growth and the kind of enzyme are the same as under normal conditions.

IV. Extreme hyperoxia (100% of oxygen)

The activity of succinic and NAD dehydrogenase is markedly decreased and nearly absent in young cultures. After 2 weeks of growth NAD dehydrogenase shows an increase in activity, while succinic dehydrogenase activity continues to be low and is concentrated exclusively in the cell cytoplasm.

The activity of glucose-6-phosphate and α-glycerophosphate dehydrogenase is low in young cultures. After 2 weeks of growth numerous forms of regressively changed glia exhibit fairly distinct activity.

Lactic and glutamic dehydrogenase activity is reduced in young cultures; it gradually and slightly increases after 3 weeks of growth and predominates considerably in regressive glia.

The results are compiled in Tables I, II and III, together with the normal levels determined in our previous investigations.

DISCUSSION

The results indicate that the activity of the individual redox enzymes is dependent on the gas atmosphere as well as on the age of the culture and the type of glial cells. The basic pattern of distribution of enzymatic activity is common for all dehydrogenases and resembles the pattern described for cultures grown under standard conditions. The significant differences between the enzymatic activity pattern of the astrocytes and the oligodendrocytes persist in all the cultures, irrespective of the changed environmental conditions, with slight deviations dependent on the experimental conditions. In all the stages

Table I

Succinic dehydrogenase activity

Days	Oxygen content				
	0%	±5—10%	±30 Normal	±50—70%	100%
1—3	0/+	0/+	+	+	0/+
5—7	+	+	++	++	0/+
10—14	+/+	+	+++	++	+
21—23	++	++	++++	+++	+
25—35	+	++	++++	+++	+

Table II

NAD and glutamic dehydrogenase activity

Days	Oxygen content				
	0%	±5—10%	±30% Normal	±50—70%	100%
1—3	+	+	++	+	0/+
5—7	+	+	++	++	+
10—14	++	++	+++	+++	++
21—23	+++	+++	+++++	++++	+++
25—35	+++	+++	+++++	+++	++

Table III

Glucose-6-phosphate, α-glycerophosphate and lactic dehydrogenase activity

Days	Oxygen content				
	0%	±5—10%	±30% Normal	±50—70%	100%
1—3	++	++	+++	++	0/+
5—7	++	++	+++	+++	+
10—14	+++	+++	++++	++++	++
21—23	++++	++++	+++++	+++++	+++
25—35	++++	++++	++++	++++	++

of growth, the activity in the oligoglia predominates over that in the astroglia, this being confirmed by the investigations of *Pope* and *Hess* (1957) on brain sections. The results of the present investigation as well as those obtained with the use of tissue sections suggest the oxygen metabolism to be more intensive in the oligoglia than in the astroglia. In the present investigation a close correlation was observed between enzymatic activity of the individual enzymes and both the morphology and functional condition of glial cells. On the basis of evaluation of glial cell morphology, conclusions can be reached on the enzymatic activity, resulting from the intracellular changes at the given moment. In degeneratively changed glial as well as in polynuclear cells and those devoid of processes, the activity of all the dehydrogenases examined is always high, irrespective of the changes in the gas atmosphere. Progressively changed glia, gemistocytic in type, showing abundant, lobular protoplasm, also exhibits increased enzymatic activity, as compared with morphologically normal glia. The macrophages exhibit maximal enzyme activity.

The present results are in good agreement with those obtained by *Colmant* and *Peters* (1965) as well as by *Zeman* (1963) on tissue sections of the brain undergoing anoxia and hypoxia. According to those authors, hypertrophic glia and astrocytes which have become reactive show an increase in dehydrogenase activity.

In the present investigation enzymatic activity is markedly decreased in young cultures aged up to 2 weeks; in older cultures enzyme activity is more pronounced. Anoxia induces a considerable drop in succinic, NAD and glutamic dehydrogenase, associated with a relatively high glucose-6-phosphate, lactic and α-glycerophosphate dehydrogenase activities. In this group the occurrence is observed of a fairly large number of gemistocytes with the activity distributed throughout the cell protoplasm. The activity of all the dehydrogenases investigated declines pronouncedly in cell processes. Apparently, the preservation of glucose-6-phosphate and α-glycerophosphate dehydrogenase activity indicates that the complete absence of oxygen in anoxia induces a compensating intensification of the anaerobic glucose metabolism; consequently, the activity of the above enzymes does not decrease. The function of the remaining dehydrogenases is blocked by complete absence of oxygen; possibly, their low activity results from damage to the mitochondrial apparatus of the cell (*Becker* 1963).

In hypoxia the drop in activity is lower than in anoxia and involves the same enzymes as in the latter condition, i.e. succinic, NAD and glutamic dehydrogenase; it is most pronounced in young cultures. The activity of glucose-6-phosphate dehydrogenase is fairly low and that of lactic dehydrogenase is best preserved. After 2 weeks of growth the activity of the dehydrogenases examined is normal. In hypoxia *Zeman* (1963), *Becher* (1963) as well as *Colmant* and *Peters* (1965) fiund in tissue section material a drop in succinic and NAD de-

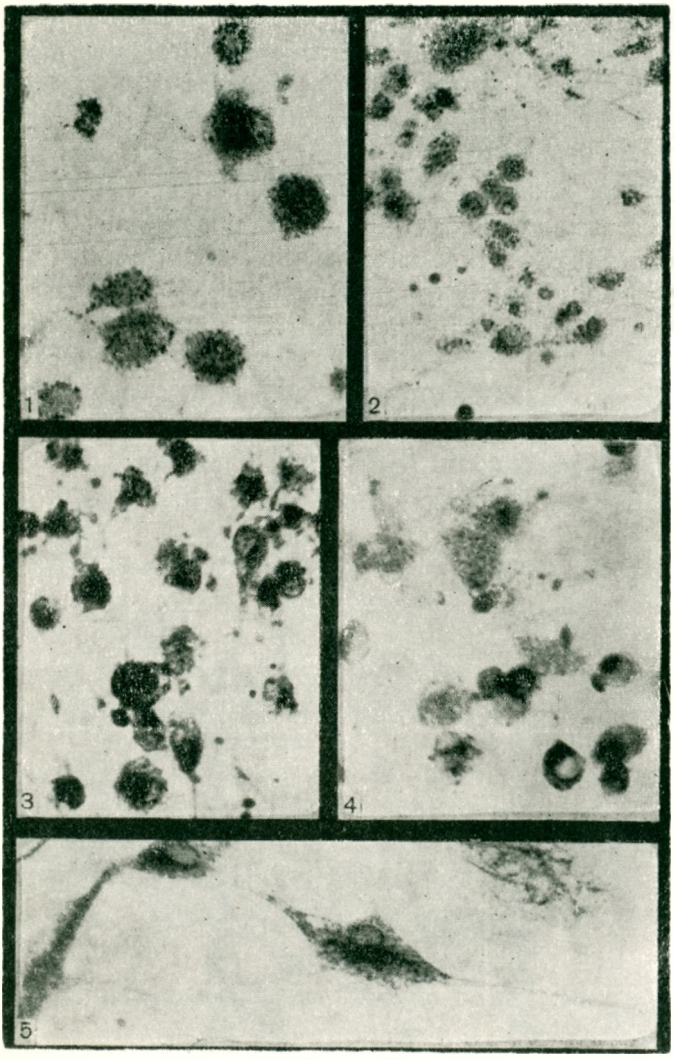

Fig. 1—5

Fig. 1. Fourteen-day culture. NAD dehydrogenase. Anoxia. Very slight activity in the processes. Cells loosing their processes exhibit higher activity than normal oligoglia.

Fig. 2. Fourteen-day culture. Succinic dehydrogenase. Anoxia. Low activity, often in the form of intraplasmic agglomerations.

Fig. 3. Seven-day culture. Glucose-6-phosphate dehydrogenase. Anoxia. Numerous forms without processes with considerable activity; preserved activity in the processes.

Fig. 4. Seven-day culture. Glutamic dehydrogenase. Anoxia. Slight activity in the astrocytes, marked activity in degenerating cells.

Fig. 5. Thirty-day culture. Lacting dehydrogenase. Anoxia. Astrocyte with fairly high activity in the neighborhood of the nucleus. Magn. oc. × 15, obj. × 40.

hydrogenase activity. These authors reported a considerable decline in activity or its total disappearance in brain tissue undergoing hypoxemic necrobiosis. According to the electron microscopic results of *Hager* (1960) obtained on brain tissue subjected to hypoxydosis, after a few minutes of anoxia, swelling of mitochondria and disappearance of the cytoplasmic reticulum take place; this finding might be an additional argument for the explanation of the low activity of the dehydrogenases associated with the above named intracellular structures. *Zeman* postulates that the drop in enzymatic activity results from early mitochondrial damage, i.e. mainly from the inhibition of or decrease in the rate of protein synthesis. The presently reported finding on dehydrogenase activity in older cultures being normal, is in good agreement with the results obtained on tissue sections by *Hamberger* and *Hyden* (1963); these authors observed that in slight hypoxia enzymatic activity in mature glia remains within the normal range. Apparently, this finding can be explained by the well known inertia of the glia and its capacity to endure a decrease in the tissue oxygen content.

In hyperoxia, dehydrogenase activity is relatively well preserved, notwithstanding the numerous morphological changes. In general, it would seem that in hyperoxia the activity is more intensive than in hypoxia. Succinic dehydrogenase shows the highest sensitivity towards an excess of oxygen, lasting up to 3 weeks of growth.

The mechanism of the toxic action of oxygen on the nervous tissue still remains obscure. The effect of oxygen on the nervous system has been investigated by *Wolman* (1963). According to this author, brain tissue is highly sensitive to the toxic action of oxygen; pure oxygen inhibits respiratory activity and probably blocks the enzyme action. This author postulates that an excess of oxygen injuries the cell wall and disrupts its lipid structure.

Fig. 6. Three-week culture. NAD dehydrogenase. Hypoxia. Gemistocytes with activity non-uniformly distributed in the cell cytoplasm.

Fig. 7. Two-week culture. Succinic dehydrogenase. Hypoxia. Low activity and absence of activity in the processes.

Fig. 8. Two-week culture. Glucose-6-phosphate dehydrogenase. Hypoxia. Marked, non-uniform and very slight activity in the processes.

Fig. 9. Two-week culture. NAD cehydrogenase. Hyperoxia. Numerous degenerated forms and those without processes, exhibited low activity.

Fig. 10. Two-week culture. Glucos.-6-phosphate dehydrogenase. Hyperoxia. Numerous forms without processes with fairly high activity.

Fig. 11. Two-week culture. α-Glycerophosphate dehydrogenase. Hyperoxia. Astrocyte with liquefied protoplasm and low activity, forms without processe exhibiting marked activity.

Fig. 12. Three-week culture. Lactic dehydrogenase. Numerous regressive forms of the glia exhibiting high activity. Magn. oc. × 15, obj. × 40.

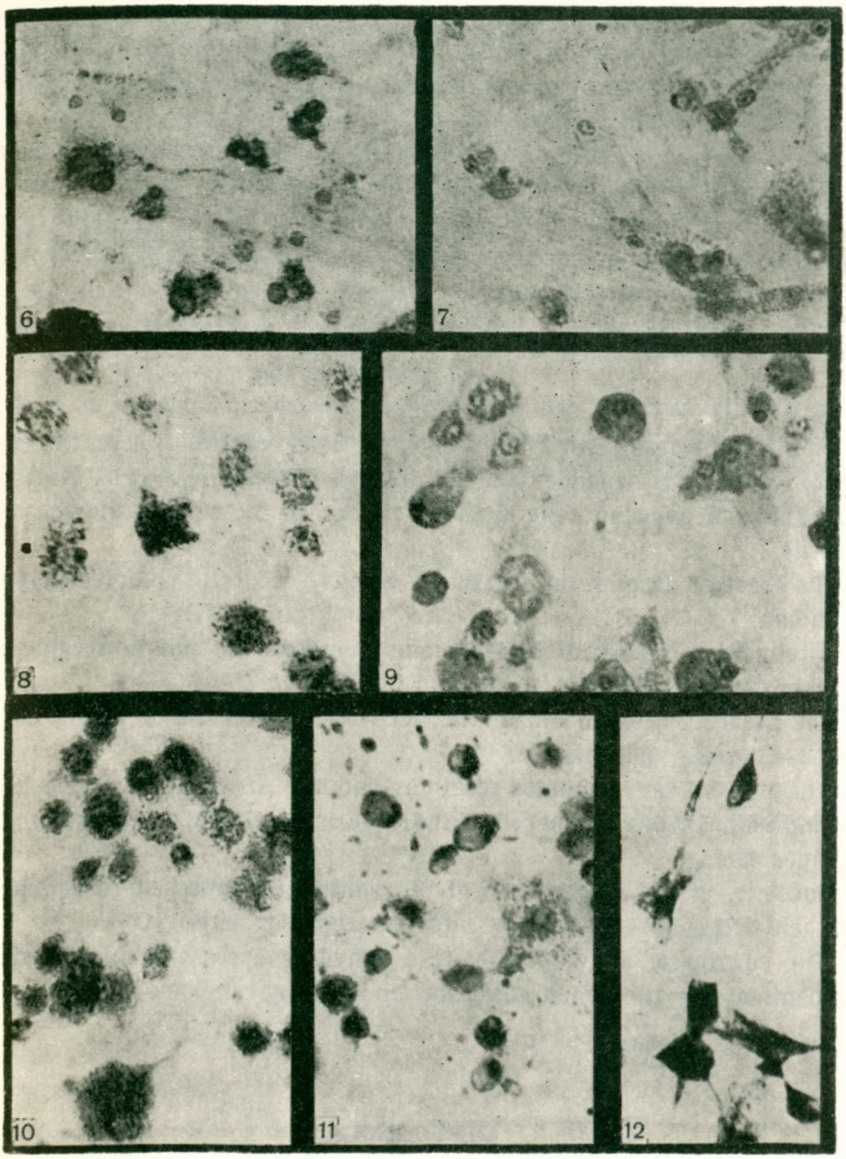

Fig. 6—12

In the presently investigated material, the activity of succinic, NAD, glucose-6-phosphate and α-glycerophosphate dehydrogenase in tissue cultures grown under pure oxygen was very low or nearly absent; the activity is only preserved in cell plasma an single formasan grains. Dehydrogenase activity becomes somewhat more pronounced, although still low, only after 3 weeks of growth. The activity of glutamic and lactic dehydrogenase is somewhat better preserved,

as compared with the foregoing dehydrogenases. In maximal hyperoxia, numerous degenerative forms of the glia cover the picture of morphologically unchanged glia with its very low enzyme activity, since all the degenerative forms show a relatively higher activity.

In the evaluation of these results, it should be remembered that the cultured cells are nourished directly by the medium; consequently, the effect of the gas atmosphere over the medium is apparent only in so far, as the gases (oxygen) dissolves in the medium. For technical reasons, the differences were not taken into account in the present experiments.

CONCLUSIONS

1. In glial cultures grown *in vitro* under various gas atmosphere, the activity of redox enzymes is dependent on the oxygen content in the gas atmosphere, the solubility of oxygen in the medium, the age of the culture and the type of glial cells:

a) the greatest decrease in enzymatic activity occurs in complete hyperoxia and anoxia;

b) intermediate stages of hypoxia and hyperoxia do not noticeably affect the dehydrogenase activity;

c) the greatest drop in enzymatic activity is observed in young cultures, up to 2—3 weeks of growth.

2. The pattern of enzymatic reactions and the distribution of the activity in the individual types of glial cells is the same as in cultures grown under normal gas atmosphere.

3. Succinic, NAD, glutamic, α-glycerophosphate, glucose-6-phosphate and lactic dehydrogenase show the greatest decrease in activity.

4. The enzymatic activity of all the dehydrogenases examined is directly dependent on the functional condition and the morphology of glial cells.

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REFERENCES

1. *Becker, N. H.*: Cytochemical studies in cerebral hypoxia. Selective vulnerability of the brain in hypoxaemia. Blackwell Sc. Publ., Oxford 1963, 317—327.
2. *Colmant, H. J., Peters, G.*: Zerebrale Hypoxie, zwanglose Abhandlungen aus dem Gebiet der normalen und patologischen Anatomie. Heft 16, Georg Thieme Verlag, Stuttgart 1965.
3. *Hager, H., Hirschberger, W., Scholtz, W.*: Electron microscopic changes in the brain tissue of Syrian hamster following acute hypoxia. Quoted after *Zeman*.
4. *Hamberger, A., Hyden, H.*: Inverse enzymatic changes in neurons and glia during increasing function and hypoxia. J. Cell. Biol., 1963, 16, 521—527.

5. *Kraśnicka, Z., Mossakowski, M. J.*: Zagadnienie zmienności morfologicznej tkanki glejowej hodowanej *in vitro* (Problems of morphological variability of glial tissue cultured *in vitro*). Neuropat. Pol., 1965, 3, 397—408.
6. *Kraśnicka, Z., Renkawek, K., Liwnicz, B. H.*: Obraz morfologiczny tkanki glejowej hodowanej *in vitro* w zmiennej atmosferze gazowej (The morphology of glial tissue cultured *in vitro* under varied gas atmosphere). Neuropat. Pol., 1967, V, 1, 115.
7. *Mossakowski, M. J., Kraśnicka, Z., Renkawek, K.*: Aktywność enzymów oksydacyjnych w tkance glejowej hodowanej *in vitro* (Oxidative enzyme activity in glial tissue cultured *in vitro*). Neuropatol. Pol., 1965, 3, 245—258.
8. *Pope, A., Hess, H. H.*: Cytochemistry of neurons and neuroglia in metabolism of the nervous system. Ed. D. Richter Pergamon Press, London 1957, 72—86.
9. *Renkawek, K., Mossakowski, M. J.*: Aktywność enzymów oksydacyjnych w tkance glejowej hodowanej *in vitro* (Oxidative enzyme activity in glial tissue cultured *in vitro*). Part II. Neuropat. Pol., 1966, 4, 53—62.
10. *Wolman, M.*: Study of the effects of hyperoxia on CNS. Selective vulnerability of the brain hypoxaemia. Blackwell Sc. Publ., Oxford 1963, 317—327.
11. *Zeman, W.*: Histochemical and metabolic changes in brain tissue after hypoxaemia. Selective vulnerability of the brain in hypoxaemia. Blackwell Sc. Publ., Oxford 1963, 317—327.