injured myocardium at intervals from 6 hours to one week were examined by various histochemical techniques as GOT, SDH and LAP. The earliest changes were recognized in infarcts within the first few hours as to a marked reduction of SDH and GOT of heart muscles. When a healing of necrotic myocardium by the proliferation of fibrous tissues has started, a prominent increase of LAP activity are observed within myocardial infarcts.

References


Explanation of Figures

Fig. 1-GOT activity in a 6 hour infarct. Small vacuolization are seen in the infarcted myocardial fibers. Diazonium method: × 100

Fig. 2-GOT activity in a 24 hour infarct. Note the weak diffuse reaction in groups of degenerating fibers in the lower infarcted portion. Diazonium method: × 40

Fig. 3-GOT activity in a 7 day infarct. There is a decrease in connective tissue in the myocardial infarct. The remained muscle fibers show normal enzymatic activity. Diazonium method: × 28

Fig. 4-GOT activity in proliferative connective tissue. Only nuclei of fibroblasts show enzymatic activity. Diazonium method: × 140

Histochemical Studies on Cultured Astrocytes

—discussing relationships morphology and function on astrocytes—

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Astrocytes in general are divided into two kinds of cell types, protoplasmic and fibrous. However, these cell morphological changes between both types are observed in reversible cell modulation within some extent, by findings with time lapse cine-equipment. Astrocytes react always in the alternative medium of ion-balance comparing in standard balanced salt solution
(BSS). But in most cases, these responses are reversible for replacement of standard BSS. Though it is similar alteration of ion-balance, in Mg free medium all astrocytes become to damage as time goes by, this change is irreversible even if cell environment is exchanged from tested medium to standard BSS. (Kasahara '63). Membranous processes of astrocytes show always prominent variation on cell environmental abnormality. It may be cell modulation to bioadaptable from for cell pathological media. It is supposed that in these conditions not only morphological aspects but metabolic functions show responses. To clarify this hypothetical problems we employed histochemical procedure for cultured astrocytes.

Hydrostatic pressures have been utilized in cultured astrocytes. Under these conditions cell morphological and cytochemical abnormality was exhibited. So pathological changes of astrocytes in vitro was compared with reactive astrocytes in vivo.

**Material and Method**

Tissue culture methods—all cultures were prepared from newborn cat cerebellum. Two culture methods were employed; RNS chamber explant preparations in fluid media (no plasma clot) and double coverslip method of RNS chamber using for pressure chamber. Technique for culturing neuroglia on glass without plasma clot was employed modifying method of Olmsted and Rose (1960). The medium used was 75% Gey's solution (600mg% glucose) containing 5% lactoalbumin hydrolysate, 20% calf serum and 5% chick embryonic extract.

Pressure Chamber—This equipment (Fig. 1,2) was designed by author and Sugiura. It has a strongest point of this pressure apparatus that is possible to observe cell morphological changes under high pressure using micro-

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Fig. 1 External aspect of pressure equipment.
Fig. 2 The cross section of Pressure Chamber.

scope of 1000-1500 magnifications. Time to spend in application or release of pressure was fixed at about 40 seconds, because the damages of cultured astrocytes undergoing mechanical attack were important for rising and falling gradients of pressure.

Staining methods—Protoplasmic and fibrous astrocytes obtained from short term culture to long term, undergone hydrostatic pressure as to make reversible changes was stained for cytochemical studies. Succinic dehydrogenase and DPN-diaphorase was demonstrated with nitro-BT. (Yonezawa 1962) Friede's method (1959) was used for phosphorylase. Lactic dehydrogenase was employed Matsunami's method (1959).

Observation

A. Enzymatic Characteristics of Astrocytes in Cultures of Cerebellum. The histochemical reactions of the 3 enzymes were demonstrated; succinic dehydrogenase (SD), DPN diaphorase and phosphorylase. DPN-diaphorase showed more intensity of reaction than SD did, but the patterns of both enzymes resembled each other closely. During early stage of cultivation, 1-2 weeks in vitro, protoplasmic astrocytes were found much more than fibrous type in numbers. After 4-5 weeks with continued cultivation, fibrous astrocytes became to remain constantly in culture field. Protoplasmic astrocytes showed...
little activity of the oxidative enzymes. But, according to elongate culture time, this type of astrocytes had a tendency to increase of these enzymatic reactions (Fig.3). There was much more enzymatic activity in hypertrophic
astrocytes than in normal. Activity of typical fibrous astrocytes was clearly and deeply demonstrated up to tips of processes (Fig. 4).

Phosphorylase showed a little reaction in protoplasmic astrocytes and no reaction in fibrous ones.

Fig. 6 Sequence of changes in astrocytes on application of 0.5kg/cm² at 36°C.
(A) Atmospheric pressure. (B) 17 minutes after application of pressure.
(C) 15 minutes after release of pressure in condition (B). Cell is spherical, membranous processes is now filament-like processes.

B. Morphological and Histochemical Changes of Astrocytes for Pressure Effect. Experiments were performed at a constant temperature (36°C) in order to establish a definitive morphological criterion for pressure effect. Generally the individual cell shape became somewhat more optically distinct from one another by pressure application. In these grade of pressure, remarkable changes did not occur after the pressure was released. According to elongate time or pressure application and apply higher pressure to cells, membranous processes and flattened cytoplasm became to show fiber-like appearances (Fig. 5). And then, astrocytes irreversibly affected after release of pressure. The results of these experiments are summarized in Fig. 7. It is a graph that shows the relation of pressure level and application time at a given pressure to ability of astrocytes to retain original shape or to change reversibly. Zone of time between 2 curves in this graph shows the borderland of reversible cell changes. After the astrocytes were applied the pressure effect below this borderland, 2 enzymatic activities in these cells were demonstrated. Comparing normal astrocytes to treated ones, activity of SD increased remarkably in the latter (Fig. 8). The other hand, LD had a little tendency of decrease in most of the case (Fig. 9).

Discussion

There is a little or little oxidative metabolism in cultured protoplasmic astrocytes as indicated by activity of SD and DPN-diaphorase in these. How-
Fig. 7 The relation of pressure and application time to ability of astrocytes to retain normal shape. Zone between 2 curves shows the borderland of reversible cell change.

Fig. 8 Succinic dehydrogenase, astrocytes 6 days in vitro. (A) control. (B) Astrocytes after pressure application (0.5kg/cm², minutes). Enzymatic activity increased remarkably in (B).

However, increased enzymatic activity in hypertrophic and fibrous astrocytes is demonstrated.

Phosphorylase activity is showed a little in protoplasmic and the absence of the activity is demonstrated in fibrous. Fibrous astrocytes discussed in this point, originate from normal protoplasmic ones. Cell modulation of astrocytes elongated culture time and morphological changes from protoplasmic to fibrous are seemed to change cell metabolic pattern.
Fig. 9 Lactic dehydrogenase, astrocytes 6 days in vitro. (A) Control.
(B) Astrocytes after pressure application (0.5kg/cm², 12 minutes).
LD activity decreases a little in (B). Staining granules have a tendency into increase in size.

In acute experiments using pressure chamber, morphological transformation form protoplasmic type to fibrous was revealed. And this transformation is accompanied by the same enzymatic transition. These findings, as Friede ('62) discussed, seem to indicate that astrocytes are capable of great adaptive changes, and also keep with their low vulnerability and high rate of survival under adverse neuropathological conditions in vivo.

Summary

In regard to morphology and function of cultured astrocytes, these were discussed from histochemical point of view. It was concluded that each type of astrocytes had different expression of metabolic patterns.

References