## Oxydative Enzymes in Maturing Hippocampus and Cerebellum of Hamsters

Succinic dehydrogenase (SDH) and DPN diaphorase (DPN-D) are oxidative enzymes in the citric acid cycle and are usually concentrated at the site of high energy production in the cell. Biochemical studies on liver and brain tissue homogenates have demonstrated that the majority of oxydative enzymes are concentrated in the mitochondria[1-3]. Electron microscopic and biochemical studies on the central nervous system have further shown that the presynaptic *bouton* is characterized by numerous mitochondria together with the accumulation of presynaptic vesicles[4]. According to this evidence, histochemical studies of oxydative enzymes in the developing brain may serve as a measure of evaluation of the synaptic maturation.

*Materials and methods.* One-, 3-, 5-, 7-, 10-, 15-, 17-, 20-, 30- and 50-day-old golden hamsters were decapitated without anesthesia. Brains were excised immediately and frozen by immersion in isopentane at $-70\,^{\circ}C$. Coronal sections through the infundibulum and sagittal sections through the thalamus were cut at 8 $\mu$ on the cryostat. SDH and DPN-D activities were studied histochemically according to the method described by NACHLAS et al.[5,6], using Nitro BT. Both for SDH and DPN-D, the sections were incubated in the respective substrate solutions for 30–60 min at $37\,^{\circ}C$. Following incubation, the slides were rinsed briefly in saline, fixed in formalin for 10 min, dehydrated by grading alcohol and mounted.

*Results.* In the hippocampal area before 10 days of age, SDH and DPN-D activities were strong in the stratum pyramidale (Figure 1). On the 15th day of age, however, the activity of these enzymes in the stratum pyramidale was considerably reduced, whereas in the stratum lacunosum and moleculare there was relatively strong activity. After 20 days of age SDH and DPN-D activity in the stratum pyramidale was weakly demonstrated. On the contrary, the stratum lacunosum and moleculare were shown to have intense activity (Figure 2), but the activity of these enzymes in the stratum radiatum and oriens were very weak at any stage of development.

In the developing cerebellum, strong activity of these enzymes appeared first in the perikaria of Purkinje cells. Before 10 days of age, the future molecular layer was occupied almost completely by cells of the external granular layer, and the internal granular layer was narrow and scanty in cellularity. SDH and DPN-D activity in these layers was negligible, and was found only in the perikaria of the Purkinje cells (Figure 3). Between the 15th and 17th day of age, the inner part of the developing molecular layer showed moderate enzyme activity. By day 20 the cells of the external granular layer had migrated inward to become granule cells and the molecular layer showed strong SDH and DPN-D activity. The activity was stronger in the deeper zone than the outer. In the granular layer, the glomeruli showed intense activity but the activity of the granule cells was negligible (Figure 4).

*Discussion.* Characteristic structures in the presynaptic endings are the synaptic vesicles and accumulated mitochondria. Maturation of the synapse could therefore be followed by studying the biochemical activities known to occur in these structures.

In the hippocampus, the major part of the pyramidal cell dendrites terminate in the narrow zone of the stratum lacnosum and moleculare, where they make synaptic connection with presynaptic endings of axons arising in the cells of the spheno-occipital ganglion and subiculum[7]. In the present study, these restricted zones show gradual changes in SDH and DPN-D according to the growth of animals. Strong activity of these enzymes at the stratum pyramidale of the younger animals may be related to high concentration of neurons and their structural maturation accompanied by the higher consumption of energy.

After 15 days of age, SDH and DPN-D increase rapidly in the glomeruli and the deeper part of the molecular layer to reach adult levels by 20 days of age. The glomerulus of the granular layer is composed of mossy fiber endings, the dendritic endings of granule cells, and the axon terminals of the Golgi type II cells[8]. The mossy fiber endings are large presynaptic bags containing masses of synaptic vesicles and numerous mitochondria[9]. In the deeper part of the molecular layer, arborized dendrites of the basket

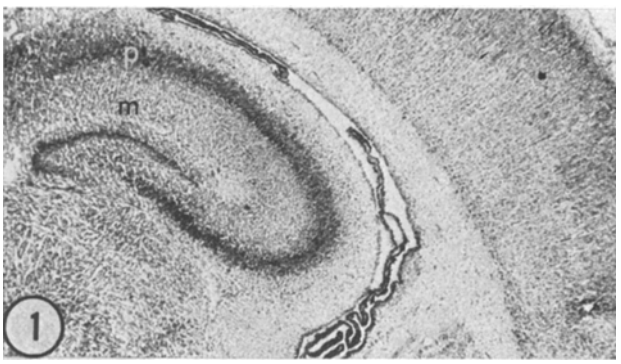


Fig. 1. Hippocampal area of 7-day-old hamster. The stratum pyramidale (p) shows strong SDH activity. × 25.

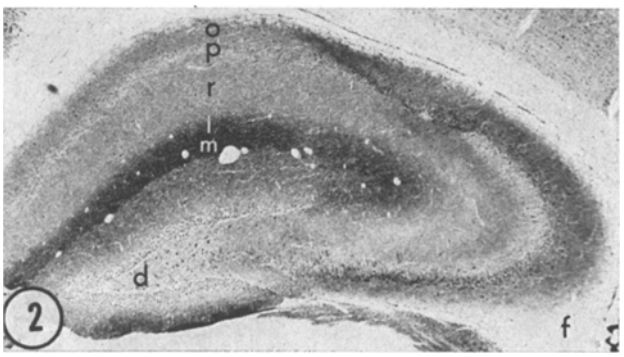


Fig. 2. 20-day-old hamster. The stratum lacunosum and moleculare (l and m) show intense DPN-D activity, whereas the activity in the stratum pyramidale (p) is negligible. o, Stratum oriens; r, stratum radiatum; d, fascia dentata; f, fimbria. × 25.

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cells, recurrent collaterals of the Purkinje cells, granular cell axons and climbing fiber endings increase the synaptic volume more than in the outer part[10,11].

Since SDH in the animal brain appears to be most concentrated in the mitochondria, it is apparent that very strong SDH and DPN-D activity in the glomeruli of the granular layer and the deeper part of the molecular layer of the mature cerebellum, and in the stratum lacunosum and moleculare of the hippocampus is due to abundant mitochondria-containing synapses in these regions. Strong activity of these enzymes in the Purkinje cells of the mature brain may be explained by the numerous axon terminals of the basket cells surrounding the cell body terminating on the axon hillock[10,11].

The development of the synapse can thus be evaluated histochemically by demonstrating these enzymes in the developing brain[12].

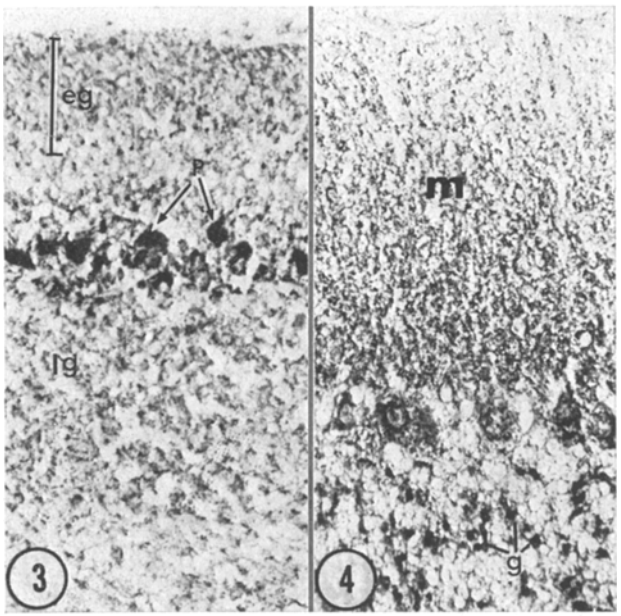


Fig. 3. Cerebellar cortex of a 10-day-old hamster. Immature Purkinje cells (p) show strong DPN-D activity. The external granular layer (eg) and the internal granular layer (ig) are weakly reactive. × 250.

Fig. 4. 20-day-old hamster. Perikaria of the Purkinje cell, deeper part of the molecular layer (m) and glomerulus (g) in the granular layer show intense DPN-D activity. × 250.

*Riassunto.* La deidrogenasi dell'acido succinico (SDH) e la diaforasi diposforpiridin-nucleotide (DPN-D) hanno dimonstrato attività intensa nei glomeruli delo strato granulare cerebellare, nella parte più profonda dello strato molecolare e nelle lamine lacunosa e molecolare dell'ippocampo. Tutte queste zone sono caratterizzate da numerose connessioni sinaptiche. L'attività degei enzimi ossidanti in queste zone aumenta rapidamente con la crescita degli animali.

M. SHIMADA[13]

*Department of Anatomy,*
*School of Medicine, University of Virginia,*
*Charlottesville (Virginia 22901, USA), 1 September 1969.*

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[13] Present address: Department of Pediatrics, Maizuru Red Cross Hospital, Maizuru, Kyoto Prefecture (Japan).

## Increased Plasma Fibrinogen and the Release of a Fibrinogen Enhancing Factor in Tumour-Bearing Rats

It is a common observation that fibrinogen is increased in patients suffering from cancer[1,2]. This high level is said to be the cause of the elevated sedimentation rate of cancer patients[3]. Increased fibrinogen levels are also found in acute and chronic inflammatory diseases[4]. In experimental animals an elevated plasma fibrinogen has been described during the growth of a transplanted rat sarcoma[5] and the V2 carcinoma of the rabbit[6]. Systematic fibrinogen determinations in animals bearing autochtonous tumours have, to our knowledge, not yet been published.

In our experimental study in rats fibrinogen determinations were made according to the method of RATNOFF and MENZIE[7]. Blood samples were taken by aortic puncture. For experimental tumours we used the Yoshida sarcoma and the benzypyrene sarcoma.

Forty-five female rats (Wistar strain, 120–150 g) were injected with $10 \times 10^6$ carefully washed Yoshida ascites sarcoma cells into the upper thigh. 15 min and every 24 h after the tumour cell injection, 5 animals were bled and fibrinogen determinations were made over a period of 8 days. The results are shown in Figure 1. There is a continuous rise of the plasma fibrinogen level during the 8 days of tumour growth.

In 30 rats of both sexes (250–300 g) with benzpyrene sarcoma the plasma fibrinogen level was correlated with the tumour volume. The tumour volume was estimated by measuring the 2 major diameters of the tumour ($d_1$ and $d_2$) parallel to the surface of the animal and the greatest width ($d_3$) perpendicular to the surface with a Vernier calliper. Based on the assumption that the tumours were hemiellipsoids the volume was calculated

$$V = \frac{(4n/3) \times (d_1/2) \times (d_2/2) \times d_3}{2} = 0.5236 \times d_1 \times d_2 \times d_3.$$

Fibrinogen determinations were performed in tumours of a volume from 1,197 mm³ to 80,028 mm³. Figure 2 demonstrates the spread of the fibinogen values in correlation

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