Koelle, G. B. : J. Pharmacol. Exp. Therap., 103 : 153, 1951. 7) Toyoda, M. : Jap. J. Med. Prog., 40 : 559, 1953.

Discussion

Dr. Fujihara :

- 1) Can we apply the present method to the heart tissue ?
- 2) Did you make the comparison of the activities with biochemical experiment ?

Dr. Yasuda : We have also observed the double staining by Glenner's and Koelles's method and found the discrepancy of the localization of MAO activity. For example, the rete Malpighii of epidermis stained at the deepest portion by Glenner's method only, and at all portions by double staining.

The lamellae of Vater-Pacini stained in blue color by double staining, but not by single method. Then, in nerve fiber, brown and blue fibers were mixed.

Dr. Hashimoto : It seems to be necessary to reexamine the specificity of the reaction. The localization of MAO activity, stained by Glenner's method, can be compare with other staining patterns such as ChE-activity by means of microprojector through the pile of the sections.

Dr. Ueda : (to Dr. Fujihara)

We can surely apply this method to the heart tissue. We have no biochemical examination, but confirmed the coincidence of the localization of MAO activity through double and single staining.

(to Dr. Hashimoto)

By Takamatsu's method, weak endogenous staining can be sufficiently excluded.

Studies on Monoamine Oxidase in the Retina -changes in the photopic and scotopic conditions and changes at developmental stages-

Koku KOJIMA, Masaharu IIDA, Yoshinao MAJIMA, Shigeru OKADA,
Noriaki YOSHIDA, Koreyoshi KIRIBUCHI

*Department of Ophthalmology, Nagoya Univ., School of Medicine.
Showa ku, Nagoya shi.*

By histochemical studies of monoamine oxidase (MAO), we could suppose that tryptamine and serotonin exist also in the retina. In the present report we demonstrate some new findings about the monoamine oxidase of the chicken retina at the several developmental stages and about the influence of light upon the MAO activities in the frog retina.

Materials :

1) In the series of the developmental stages, for the histochemical study the eye-cups with retina were examined with the chick-embryos (incubated eggs) of several stages and new-born chickens and for the quantitative study

the homogenated retina and choroid with phosphate buffer solution was analysed.

2) In the study of photopic and scotopic conditions, the scotopic retinas used were dark-adapted for 24 hours in the dark room and photopic retinas were light-adapted by the exposure to 200W electric light for 6 hours and these eye-cups were histochemically studied. For quantitative analysis, the dark adapted retinas were homogenated with phosphate buffer solution and light adaptation was performed by exposing light to the materials as soon as the reaction started.

Methods :

1) For histochemical study we followed Glenner's method, but serotonin creatinin sulfate was applied instead of tryptamine hydrochloride, and nitro-BT (NBT) and neotetrazolium chloride (NT-salt) were used as a tetrazolium salt.

2) For the quantitative analysis, according to Blaschko's method the oxygen consumption was measured through Warburg manometry by using 3 mM tryptamine and 5 mM serotonin as substrate.

Results and Conclusions :

1) In 1959 Nakajima measured MAO activity by Warburg manometric method using the liver and brain tissues of a Guinea-pig with $3\mu\text{M}$ serotonin substrate. We measured the MAO activities of frog and chicken retinas with $5\mu\text{M}$ serotonin as substrate.

Comparing these results, the MAO activity in the frog retina showed about one sixth of that in the liver and one third of that in the brain, and the MAO activity in the chicken retina was proved to be about one fifteenth as much as in the liver and one ninth as much as in the brain of the respective animals. Thus, it can be said that the MAO activities are lower in the retina than in the liver and the brain, although there might be difference due to the organ specificity. (Table. 1)

Table 1. O_2 Uptake (MAO Activity measured by Warburg manometer at 37.0°C) unit- $\mu\text{l/h}$.

Liver	Brain	Frog Retina	Chick
51.6	29.0	9.58	3.72
material : 1ml. solubule MAO (supernatant) by Nakajima		material : 0.5ml. homogenate by Kojima et al	

2) Histochemical findings ;

a) Frog retina of several developmental stages, serotonin NT-method.

Fig. 1 (The 9th day chick embryo)

The MAO activity is observed diffusely all over and strong especially in the external layer of the origin of visual cells.

Fig. 2 (The 12th day chick embryo)

The strong MAO activity is observed in the three layers, ganglion cell layer, internal molecular cell layer and visual cell layer, and remarkable

especially in the external layer.

Fig. 3 (The 15th day chick embryo)

The MAO activity is weak throughout all the layers of the retina at this stage.

Fig. 4 (The 18th day chick embryo)

The MAO activity is still weaker and is slightly observed only in the visual cell layer.

Fig. 5 (Chicken newly born)

The activities are dimly observed in the layers of ganglion cells, inner molecular cells and visual cells.

b) Frog retina of several developmental stages, serotonin NBT method.

Fig. 6 (The 12th day chick embryo)

The MAO activity is highly recognized in all the layers and is particularly remarkable in the layers of ganglion cells, external molecular cells, inner plexiform layer and in the visual process of the visual cell layer.

Fig. 7 (The 15th day chick embryo)

The MAO activities are found in the nerve fiber layer, ganglion cell layer and internal plexiform layer, but are scarcely recognized in the external layers.

Fig. 8 (The 18th day chick embryo)

The MAO activities are poorly found only in the layer of ganglion cells and internal plexiform layer.

Fig. 9 (Chicken newly born)

The MAO activities are observed in internal layers, nerve fiber layer, ganglion cell layer, and internal plexiform layer.

From these histochemical findings mentioned above, it is concluded that the MAO activities become strong at the early stage of development (especially the 9th to the 12th day), and decrease at the late stage (about 18th day) when the development of the retina is almost complete. It might be due to the differentiation of the cells in the course of development.

c) A diagram of MAO activities in regard to developmental changes.

The MAO activities are the strongest on the 9th day and begin to decrease gradually and on the 15th day down to about one third as much as on the 9th

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10 Changes of MAO Activities with the Retinal Development of Chick Embryo (Blachko)

day, but slightly increase on the 18th day and maintain the level about a half of that on the 9th day until the embryonal development. (Fig. 10)

d) The changes of the MAO activities in the toad retina in the photopic and scotopic conditions by serotonin-NT method

The MAO activities are observed only in the supporting tissue in the scotopic condition (Fig. 11), but in the photopic condition they are recognized in the visual cell layer and external molecular layer as well as in the supporting tissue (Fig. 12).

e) The frog retina in the scotopic and photopic conditions by serotonin NBT method

The MAO activities are shown with the stained internal plate of the supporting tissues in the scotopic condition (Fig. 13), but are highly recognized in the ganglion layer and internal plexiform layer as well as the supporting tissue in the photopic condition (Fig. 14).

As shown above, not only by serotonin-NT but-NBT method, the MAO

activity is always stronger in the photopic than in the scotopic condition.

The quantitative analysis by Warburg manometric method with serotonin and tyramine as substrates also shows high activity of MAO and above all the difference of the MAO activities between in the scotopic and in the photopic condition is obviously recognized with the stronger activities in the photopic by serotonin (Fig. 15).

Fig. 15 Changes in MAO Activities of Frog Retina by Light and Dark Adaptation (Blaschko)

This means, we suppose, that serotonin might have a deep significance in the light stimulus or light condition in the retina.

Discussion

Dr. Uono :

How was the reason why the use of serotonin but not tryptamin ?

At the staining of retina, it needs to differentiate the specific reactions from the pigments.

Dr. Yoshida :

Serotonin was used to observe the significance of it as nerve transmitter in the retina.

Dr. Otori :

We confirmed the following two facts.

- 1) In the retina of albino rats, succinic dehydrogenase and cytochrome oxidase showed the similar localization.
- 2) In the lachrymal gland, succinic dehydrogenase and DPN-diaphorase showed the identical distribution pattern.

Histochemistry of the Human Lacrimal Gland

Toshifumi OTORI, Masakazu IGA, Masayuki HAGIHARA,
Jiro HARA, Nobuko FUJITA and Takashi MIZUKAWA

Department of Ophthalmology, Osaka University Medical School

Secretory mechanism of the lacrimal gland has been studied from a cytological point of view with special reference to secretion granules, mitochondria and Golgi apparatus¹⁾, and recent developments of submicroscopic morphology provided numerous bases for the methods of investigation of fine structure of the cells²⁾ in connection with cellular function. However, there are very few reports on the histochemistry of the lacrimal gland.^{3,4,5)}

This paper deals with a summary of histochemical studies made on nucleic acids, carbohydrates, lipids, proteins and enzymes of the human lacrimal gland.

1. Nucleic Acids

The pyronin methylgreen stain demonstrated that RNA protein was concentrated in the basal portion of the cells, with a confirmation on ribonuclease digestion test. This demonstration is quite compatible with the data concerning other gland cells. Special attention was directed to the possibility of staining the nucleus of the myoepithelial cells by Feulgen reaction with some promise, although its confirmation has been left unestablished.

2. Carbohydrates

It had been previously mentioned that PAS positive material was concentrated in the supranuclear portion of the cytoplasm. However, the use of freeze-substituted section revealed that there was no such eccentric accumulation of PAS positive material as shown in the alcohol containing fixatives. This suggests that there exist many factors for the preservation and fixation of carbohydrates within cells.

3. Lipids

As for the lipids of the lacrimal gland, there were observed a few droplets of neutral fats between gland cell acini. These droplets were stained metachromatically with Nile blue sulfate. Except for this observation, no characteristic findings were revealed by the present staining methods of lipids in the lacrimal gland.