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HISTOCHEMICAL DISTRIBUTION OF THE SECONDARY ALCOHOL DEHYDROGENASE IN THE RAT, ESPECIALLY IN THE ADRENAL CORTEX

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In the site of steroid production, the histochemical activity of secondary alcohol dehydrogenase on isopropanol as its substrate with NAD as its coenzyme will reflect the inversion of 20α-22R-dihydroxycholesterol in the biosynthesis of steroid hormone, We applied this method on organs from all parts of the body of Wistar strain rats and observed positive reaction in the adrenal cortex, ovary and testis. In the adrenal cortex of mature rats, the zona glomerulosa exhibited a weakly positive activity, the outer to middle portions of the zona fasciculata an intensely positive activity, the inner portion of the zona fasciculata a weakly positive or negative activity, and the zona reticularis a positive activity.

A proportional relationship between secondary alcohol dehydrogenase activity and the amount of lipid was present in the adrenal cortex of mature rats. In addition, the specific distribution of young and old rats was described.

The secondary alcohol dehydrogenase is the enzyme of dehydration on the secondary alcohol.

In 1965, Hardonk[1] noted the similarity between the enzymes participating in the oxidative cleavage of the side chain of cholesterol as a precursor of steroid and secondary alcohol dehydrogenase. Using various secondary alcohols, the distribution of activity of the dehydrogenase in the human body was studied. Among them an enzyme acting on isopropanol as its substrate with nicotinamide-adenine dinucleotide (NAD) as its coenzyme had a distribution of activity in the human body in agreement with the site of steroid production such as the adrenal cortex, ovary and testis, according to the description of Hardonk[1]. The histochemical detection of various enzymes participating in the biosynthesis and metabolism of steroid hormones in the adrenal cortex and other tissues has been carried out on steroid-3β-ol dehydrogenase[6], glucose-6-phosphate dehydrogenase and other enzymes[2),3),4)]. However, when no specific significance has yet been established, the secondary alcohol dehydrogenase activity will be important, too.

We applied this method on organs from all parts of the body, particulary the site of steroid production of male and female Wistar rats, considering the relationship between this enzyme activity and lipid stain.

MATERIAL AND METHOD

As test animals, male and female rats of Wistar strain weighing 200 g (female) or 300 g (male) were used at 4-5 months of age.

In addition, young rats at 40-50 days after birth and old rats at 12-18 months were also used.

The method of histochemical demonstration of the enzyme activity is described by Hardonk[1] as below. Fresh tissues were frozen and serial sections 10μ thick were cut in a cryostat and stored at −25°C. Prior to incubation the sections were placed in acetone at −20°C for 30 minutes in order to dissolve lipid droplets, thus preventing absorption for formazan to these droplets. Next the sections were air-dried and incubated for 2 hours at 37°C in the incubating medium. After staining the sections were fixed in formalin and embeded in glycerol-gelatin. The incubating medium contained : 0.5 ml isopropanol, 1.0 ml nitro-BT (1 mg per ml), 0.7 ml nicotinamide (1.6 mg per ml), 0.5 ml NAD (5 mg per ml), 2.0 ml phosphate buffer (pH 7.4, 0.2 *M*) and 2.3 ml distilled water

The observation of the sections were carried out immediately after the reaction. Secondary alcohol dehydrogenase is abbreviated as SA-DH in this paper.

RESULT

A. Distribution of the secondary alcohol dehydrogenase activity in the various tissues are shown as follows :

Adrenal cortex	##
Adrenal medulla	−
Ovary, follicular theca cells	##
Ovary, theca lutein cells	##
Ovary, granulosa lutein cells	##～#
Testis, Leidig cells	##
Testis, Germ cells	−
Adenohypophysis	+
Neurohypophysis	−
Thyroid, follicular epithelium	+
Pancreas, acinic cells	+
Pancreas, islet cells	−
Liver cells	##
Renal tubular epithelium	#～−
Esophageal squamous cells	+
Pulmonary alveolar epithelium	−
Striated muscles	−

(##, intensely positive, #, positive, +, weakly positive, −, negative)

B. The secondary alcohol dehydrogenase activity in the organs of steroid production, particulary adrenal cortex

In the adrenal cortex of mature rats, the zona glomerulosa exhibited a weakly or moderately positive reaction for SA-DH activity, distinctly demarcated with the outermost portion of the zona fasciculata which showed an intensely positive reaction. This contrast is characteristic in this enzyme histochemistry. In the zona fasciculata, the SA-DH activity was intensely positive in the outer to the middle portions. Formazan granules which were mostly coarse and granular were interspersed with minute granules in the cytoplasm except the nucleus. In the

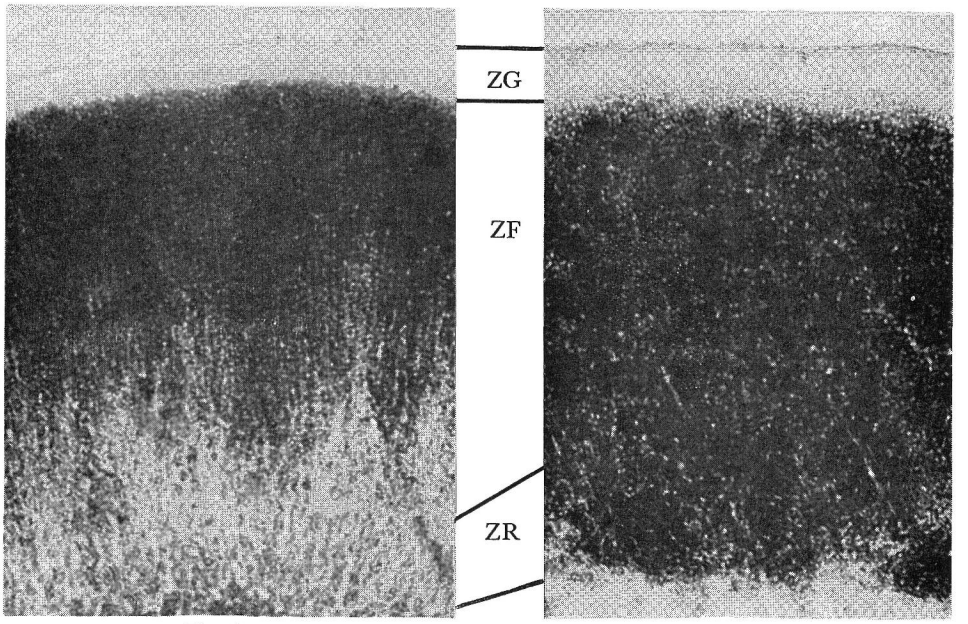

Fig. 1. Fig. 2.

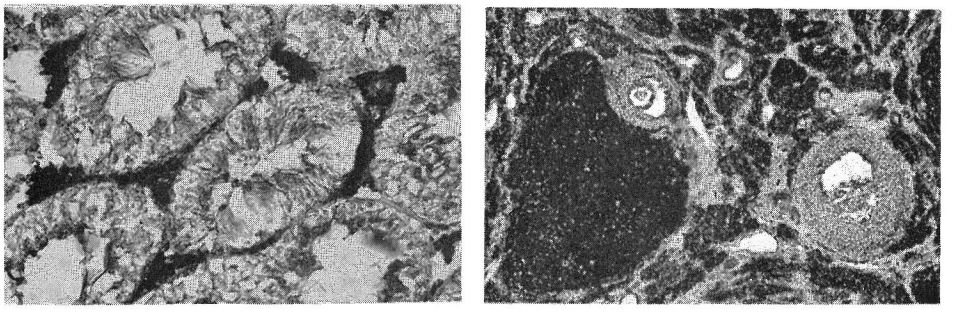

Fig. 3. Fig. 4.

Fig. 1. Secondary alcohol dehydrogenase activity of the adrenal cortex in the mature rat. (4 mon.) Note negative reaction in the zona glomerulosa (ZG), intensely positive in the zona fasciculata (ZF) and weakly positive in the inner portion of ZF and the zona reticularis (ZR). X60.

Fig. 2. Adrenal cortex of the young rat (40 days). Note intensely positive reaction in ZF and ZR. X60.

Fig. 3. Testis of the mature rat. Positive reaction only in the Leydig cells. X150.

Fig. 4. Ovary of the mature rat. Positive reaction in the granulosa lutein cells and the theca lutein cells. X60.

inner portion of the zona fasciculata, especially in the innermost portion, the SA-DH activity was weakly positive to negative. The zona reticularis exhibited a positive SA-DH activity which was more intense toward the inside.

In the adrenal cortex of rats at 40-50 days of age, the distribution was characterized by a weakly positive reaction in the zona glomerulosa with no clear borderline

with the outermost portion of the zona fasciculata, and gradual increase in activity was seen from the outermost portion of the zona fasciculata toward the inner layer. The outer and middle portion of the zona fasciculata had a moderately to an intensely positive reaction. The inner portion of the zona fasciculata and zona reticulairs had an intensely positive reaction. Such distribution was the reverse of the distribution between the inside and outside as far as the zona fasciculata and zona reticularis were concerned, being in disagreement with the finding in mature rats. This so-called juvenile type of the distribution was seen in the half of rats 40-50 days of age. Besides, a so-called intermediate type of the distribution with an intensely positive reaction in the middle to the inner portion of the zona fasciculata, with a decrease in activity toward the outer portion of the zona fasciculata and toward the zona reticularis forming a *mountain-like* distribution, and mature type of the distribution were seen in other half of young rats. Such a distribution of SA-DH activity in young rats might explain the tendency of changes from the juvenile type to the mature type in responce to the growth.

One of the characteristic findings in rats of 40-50 days of age was the presence of a round or oval nodular part in the zona reticularis. In this part, the SA-DH activity was intensely positive. The number of these nodular part was 1-8 per tissue slice, with an average of 3-4. This nodular parts of a small number were also seen in rats of 4-5 months.

In the old rats of 12-18 months, the distribution was almost the same as that in mature rats of 4-5 months of age, except more intense reaction was obtained in the zona reticularis and the inner portion of the zona fasciculata. In addition, various type of nodular cell groups were frequently noted in the zona fasciculata and zona reticularis, but rarely in the zona glomerulosa, showing intensely positive reaction or negative reaction of the SA-DH activity.

DISCUSSION

In 1965, Hardonk[1] noted the similarity between the enzymes participating in the oxidative cleavage of the side chain of cholesterol as a precursor of steroid and secondary alcohol dehydrogenase. Using various secondary alcohols, the distribution of activity of dehydrogenase in the human body was studied. Among them, an enzyme acting on isopropanol as its substrate with NAD as its coenzyme had a distribution of activity in the human body in agreement with the sites of steroid production such as the adrenal cortex, ovary and testis, according to the description of Hardonk[1] and our experiment. Hardonk[1] noted that SA-DH is related with dehydrogenase activity participating in the change of 20α-22 R-dihydroxycholesterol, one of secondary alcohol, to pregnenolone.

The present authors applied this method on organs from all parts of the body of Wistar strain rats and also on the human body. In the human adrenal cortex, the zona reticularis exhibited positive reaction for SA-DH activity and other zones were negative, but in the rats, the zona fasciculata was intensely positive and zona reticularis was weakly positive. In the adrenal cortex of rats, the relationship between the SA-DH activity and lipid with oil red O stain and the finding in the cells is interesting. In the outer portion of zona fasciculata, an intensely positive SA-DH activity was associated with a large amount of coarse lipid granules and cytologically

large clear cells. In the inner portion of zona fasciculata and zona reticularis, a weakly positive to negative SA-DH activity was seen with a small amount of finely granular lipid and small compact cells in H-E stain. In the zona glomerulosa, usually the SA-DH activity was weakly positive and lipid appeared in drops and clear type cells were present. In this zone of rats of 12-18 months when the SA-DH activity was negative to weakly positive, lipid content was decreased and many small compact type cells were present. Considering these findings, a proportional relationship between SA-DH activity and the amount of lipid was present in the adrenal cortex not only in the zona fasciculata and reticularis but also in the zona glomerulosa of mature rats. This tendency was observed in the nodular part of young and old rats. But, the relationship of these findings is inversely proportional in some nodular part of old rats.

In the young rats, SA-DH activity in the zona fasciculata and zona reticularis was entirely reversed to the mode of the distribution as that in adult rats. This may show the undeveloped biosynthetic function of corticosteroid in these zone.

This enzyme histochemical method will be important with steroid-3β-ol dehydrogenase[6] because no specific significance has yet been established on the histochemical detection of various enzymes[2),3),4)] participating in the biosynthesis and metabolism of steroid hormones in the adrenal cortex.

The changes of this enzyme activity on the influence of SU-4885 and ACTH will be showed on other paper[5] in press.

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