A Histochemical Study of Various Enzymes in the Adrenal Gland of Mammals

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Introduction

Morphological findings were obtained from mammalian adrenal glands revealing that there were considerable macroscopic and microscopic differences in structure depending upon the species of animals.^{1,2)}

Recent studies have come to shed more light in the explanation of possible relations between endocrinological functions and fat distribution in the adrenal gland. But a few reports seem to have been made of pursuance of hydrolytic and oxidative enzymes in the adrenal glands. The related work so far performed has led to a concept that further study of the role in steroid hormone synthesis using enzyme localizing techniques may prove interesting. The present study deals with the enzyme histochemistry in various zones of mammalian adrenal glands.

Materials and Methods

Adrenal glands from dogs, guinea pigs, rabbits, cats, cattle, monkeys and men were used. Human glands were obtained from surgical materials. All specimens were immediately frozen in dry-ice, and fresh frozen sections were cut at 12μ in a cryostat maintained at -20°C using a sliding microtome. For the histochemical demonstration of hydrolytic enzymes, the fixed tissue sections were used (about 10-20 minutes in 10 % formalin). But for the detection of oxidative and reducing enzymes unfixed sections were employed.

The following enzymes were detected histochemically :

Hydrolytic Enzymes ; Alkaline phosphatase (azo coupling method), acid phosphatase (azo coupling method), non-specific esterase (azo coupling method), β -glucuronidase (post coupling method), aminopeptidase (copper chelating method using L-leucyl- β -naphthylamide).

Oxidative and Reducing Enzymes ; Succinic dehydrogenase, DPN-dependent dehydrogenase (Lactic, malic, glutamic, α -glycerophosphate, β -hydroxybutyric), TPN-dependent dehydrogenase (isocitric, glucose-6-phosphate), 3 β -ol dehydrogenase. For the demonstration of oxidative enzymes Nitro BT was applied as an electron acceptor.

Results

1. *Hydrolytic enzymes.*

The distribution of alkaline phosphatase, acid phosphatase, non-specific esterase and β -glucuronidase in the adrenal gland was summarized as Table 1. Alkaline phosphatase activity was remarkably strong in the z. glomerulosa of the dog, and no enzymatic activity was found in the medulla of all species. Acid phosphatase was characteristically stained in the medulla of the rat. Esterase activity in the medulla of mammals except for man was the highest, while stainability was variable in the cortex for all species. β -glucuronidase revealed a significant reaction in the adrenal cortex, but that of the medulla was absent.

Table 1 Hydrolytic Enzymes

Species	Zones	Hydrol.	Alk Pase	Ac pase	β -Glucur.	β -Est.
Dog	Z. glomerul.		2	trace	1	1
	Z. fascicul.		1	trace	1	1
	Z. reticul.		trace	—	trace	1
	medulla		—	—	—	3
Guinea Pig	Z. glomerul.		1	1	trace	1
	Z. fascicul.		1	1	1	trace
	Z. reticul.		trace	—	1	trace
	medulla		—	trace	—	2
Rat	Z. glomerul.			trace	1	
	Z. fascicul.			trace	2	
	Z. reticul.			trace	1	
	medulla			2	trace	
Cat	Z. glomerul.		trace	trace		1
	Z. fascicul.		trace	1		2
	Z. reticul.		trace	1		1
	medulla		1	—		2
Man	Z. glomerul.		1	trace		1
	Z. fascicul.		1	trace		1
	Z. reticul.		trace	trace		1
	medulla		—	trace		trace

— Negative

2 Moderate Positive

1 Slight Positive

3 Strong Positive

2. *Dehydrogenases.*

Succinic dehydrogenase. The enzymatic activity was comparatively low in the zona fasciculata, but high in the medulla of the dog. A trace of the activity was seen in the zona fasciculata of the guinea pig. Succinic dehydrogenase some stainability in the zona fasciculata of the rabbit, rat, cat, monkey and man. The cortical zone of the cow proved high in enzyme activity.

Table 2 Dehydrogenases

Species	Dehydroge. Zones	Succ.	Lact.	Mal.	Glut.	α - Glyc.	β - Hydr.	Isocit.	G-6-P.	3 β -Ol.
Dog	Z. glomerul.	+	+	+	±	±	+	+	##	
	Z. fascicul.	±	±	±	-	-	±	-	±	
	Z. reticul.	+	±	±	-	±	±	+	+	
	medulla	++	++	++	++	+	++	±	-	
Guinea Pig	Z. glomerul.	+	±	±	-	-	+	+	±	+
	Z. fascicul.	±	±	±	-	±	±	++	##	±
	Z. reticul.	-	±	-	-	±	-	+	++	±
	medulla	-	±	+	±	-	±	-	-	-
Rabbit	Z. glomerul.	+	±	±	+	-	+	±	+	
	Z. fascicul.	+	±	±	±	-	±	+	++	
	Z. reticul.	+	±	±	±	-	±	+	+	
	medulla	±	+	±	±	-	+	-	-	
Rat	Z. glomerul.	±	±	±	-	-	±	±	+	+
	Z. fascicul.	+	±	+	±	±	±	+	##	±
	Z. reticul.	+	±	-	±	-	+	+	##	-
	medulla	±	±	+	±	±	-	-	±	-
Cat	Z. glomerul.	+	+	±	-	-	+	±	+	
	Z. fascicul.	+	++	±	±	-	±	++	##	
	Z. reticul.	±	++	±	±	-	±	-	++	
	medulla	±	+	±	-	-	-	-	-	
Cow	Z. glomerul.	++	-	±	-	-	-	++	##	-
	Z. fascicul.	++	±	+	±	±	±	+	++	±
	Z. reticul.	++	±	+	±	±	±	+	++	±
	medulla	-	-	±	±	±	±	-	-	-
Monkey	Z. glomerul.	±	-	-	±	-	±	++	++	±
	Z. fascicul.	+	±	±	±	±	±	+	+	-
	Z. reticul.	±	-	-	-	-	-	++	++	-
	medulla	-	±	±	±	±	±	-	-	-
Man	Z. glomerul.	+	+	+	±	±	+	±	+	-
	Z. fascicul.	+	++	+	+	±	+	+	++	+
	Z. reticul.	+	+	+	±	-	±	±	+	±
	medulla	-	-	-	-	-	-	-	-	-

- Negative + Slight Positive
 ++ Moderate Positive ## Strong Positive

Lactic dehydrogenase. The cells of the zona fasciculata of the dog showed a low activity, while the zona glomerulosa and medulla showed a high activity. On the contrary, the cortex and medulla of the cat and the cortex of man showed a high activity. The other mammals hardly disclosed specific distribution, depending upon the zone of the enzymatic activity, which was low or nil. Lactic dehydrogenase showed a relatively low activity in zona fasciculata except for the cat and man.

Malic dehydrogenase. The activity in the medulla was high, and was relatively high in the zona glomerulosa of the dog. But, cow and man showed in the cortical zone a comparatively high activity.

Glutamic dehydrogenase: A high enzymatic activity was present in the cortical inner zone of the dog. The zona fasciculata of man showed a relatively high activity.

α -Glycerophosphate dehydrogenase. The activity of medulla of the dog was rather high, and negative or trace in the other mammals.

β -Hydroxybutyric dehydrogenase. The medulla of the dog showed a high activity; and the zona glomerulosa of mammals revealed a high activity, too. All mammals except for cow and monkey exhibited a relatively high activity in the zona glomerulosa.

Isocitric dehydrogenase. Enzymatic activity in the zona fasciculata of the dog was positive, and that of rabbit, rat, cow, monkey and man was slightly high. The zona fasciculata of guinea pig and cat and the zona glomerulosa and reticularis of the monkey showed a high activity.

Glucose-6-phosphate dehydrogenase. The various zones of all the mammals showed a considerably intense activity as compared with the intensity of the other dehydrogenases. In the dog, the zona glomerulosa showed a very high activity, and the medulla negative. In the guinea pig, however, the zona glomerulosa had a trace degree of stainability and the cortical inner portion a markedly high stainability. In guinea pig, rabbit, rat, cat and man the activity was slightly positive in the zona glomerulosa and strongly positive in the cortical inner portion. The activity was similar to that of guinea pig and rabbit. The medulla of the rat only showed a low activity.

3 β -ol dehydrogenase. The zona glomerulosa of guinea pig and rat, and the cortical inner portion of man showed relatively high activity.

Discussion

There are comparatively a few reports concerned with the histochemical patterns of enzymes in the adrenal glands on mammals. In those studies, alkaline phosphatase activity was detected in the adrenal gland of certain species described by many workers.^{3,4,5} Eränkő⁶ and other Workers⁷ reported that the adrenal medulla of the rat contains an acid phosphatase. A histochemical demonstration of estrase was reported by Holmes⁴ and Allen⁸. According to Allen⁸, the enzymatic activity of both aliesterase and DPN-diaphorase reached their maximum in the X-zone in a 14 day mouse, followed by a gradual decrease. He reported that 90 day-old mice showed a marked sex-difference in DPN-diaphorase activity, that is, the outer zona faciculata of the female was rather higher but this zona showed a higher activity than in the male.

During the present study these were obtained rather characteristic localizations in each zone of the species on the basis of a histochemical demonstration of oxidative enzymes. In the adrenal medulla of the dog, the enzymatic activity of many dehydrogenase except for isocitrate and glucose-5-phosphate dehydrogenases was high. Glucose-6-phosphate dehydrogenase activity in the adrenal

Fig. 1

Fig. 2

Fig. 3

Fig. 4

glands of the mammals used was usually the highest, and the localization was divided into the following two groups: 1) High enzymatic activity demonstrated in the z. fasciculata and reticularis of guinea pig, rabbit, rat, cat and 2) high enzymatic activity demonstrated in the z. glomerulosa and reticularis of dog, cow and monkey. The zonal distribution of other oxidative and reducing enzymes showed patterns different from glucose-6-phosphate dehydrogenase.

It is unknown if the biosynthesis of steroids in the adrenal cortex resulted from various dehydrogenase activities. But it seems reasonable to consider that the reaction of glucose-6-phosphate¹⁰⁾ and 3 β -ol dehydrogenase¹¹⁻¹⁵⁾ was

Fig. 5

Fig. 6

associated the steroid metabolism. It is reported that the zonal distribution of glucose-6-phosphate dehydrogenase is correspondent with the presence of steroid hydroxylation in the adrenal cortex. That report is well reflected on the results of the present study with an encouraging suggestion. The localization of succinic, DPN-dependent dehydrogenases and isocitric dehydrogenase as well as glucose-6-phosphate and 3 β -ol dehydrogenase seems to afford some significant suggestion not only enzymatically but also endocrinologically.

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Explanation of figures

Fig. 1 Malic dehydrogenase in the dog adrenal gland. Medulla shows intense activity and moderate in the zona glomerulosa. $\times 40$

- Fig. 2 β -Hydroxybutyric dehydrogenase in the dog adrenal gland. Moderate to high activity is observed in the medulla. $\times 40$
- Fig. 3 Glucose-6-phosphate dehydrogenase in the dog adrenal gland. A marked staining is confined to the zona glomerulosa. $\times 40$
- Fig. 4 Isocitric dehydrogenase in the dog adrenal gland. Moderate staining is observed in the zona glomerulosa, but no reaction in the medulla. $\times 40$
- Fig. 5 Glucose-6-phosphate dehydrogenase in the guinea pig adrenal gland. Negative or only faint staining is present in the zona glomerulosa and the cortical inner portion a markedly high stainability.
- Fig. 6 Isocitric dehydrogenase in the monkey adrenal gland. The zona glomerulosa and reticularis showed a high activity.

Discussion

Dr. Mizutani : In the histochemical findings of your experiments on the various dehydrogenases, in general the reactions due to enzymic activities with the exception of G-6-P dehydrogenase seem light.

Do these findings depend on the conditions of the preparations themselves, not on the techniques ?

To my experiences, in effect, the reactions of dehydrogenases, you have shown in the present report, are usually strong on the whole.

Dr. T. Koizumi : The staining reaction of G-6-P dehydrogenase is considerably intense in comparison with those of the other dehydrogenases, showing only dim histochemical pictures. The tissue sections used in this experiment are unfixed cryostat sections. It is a subjective matter to some extent to determine whether the histochemical staining reaction is intense or not and in this experiment I have examined every section one by one.

Dr. Ohkawa : Whenever we try to demonstrate the activity of the so-called "endogenous activity" in the tissue sections of the adrenal gland, we usually get a strong reaction in the cortex. But, on the contrary, only a weak reaction can be seen in the medulla. The slides you showed seem to present such a non-specific activity to a considerable extent, so I think you have to do a control test with a solution containing no substrate.

Dr. T. Koizumi : There is a tendency that the reaction intensities of various dehydrogenases are relatively strong in the cortex and weak in the medulla. The number of the cases, however, of these experiments is small, so I would like to continue the work for getting a solution to the problem.

Dr. Morii :

- 1 In the experiments making use of adrenal cortex one must always treat the experimental animals carefully to remove every influence of "stressor" as possible as we can.

How have you done your experiments to avoid the influences of the "stressor" on the adrenal cortex ?

- 2 In the problem of animal species it is interesting that the mammalian adrenal cortex can be classified into two groups on the ground of endocrinological differences. The one of the two groups secretes "Compound F" and the other secretes "Compound B." Could you get any interesting differences of enzymic activities between the two ?

Dr. T. Koizumi : I have selected the normal animals without any treatments and used the adrenal glands of them for the histochemical purposes.

In the experiments making use of adrenal glands, as you, have, stated, we have to carefully remove the influences of stress. But as to the present reports of mine it is not necessary to mind the influences of stress.

I have used only one specimen of human adrenal gland obtained from the autopsied subject several hours after death. And the conditions of the human specimen are obviously different from those of the animals.