HISTOCHEMICAL DEMONSTRATION OF THE Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE ADRENAL GLANDS OF THE DOMESTIC FOWL AND JAPANESE QUAIL

HISTOCHEMICAL DEMONSTRATION OF THE Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE ADRENAL GLANDS OF THE DOMESTIC FOWL AND JAPANESE QUAIL

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Introduction

Δ^5-3β-hydroxysteroid dehydrogenase catalize an important reaction in the biosynthesis of the steroid hormones. The enzyme converts Δ^5-3β-hydroxysteroid into Δ^4-3-ketosteroids in the presence of nicotinamide adenine dinucleotide (NAD or DPN) as a coenzyme[1]. In the adrenal glands, it converts pregnenolone or 17α-hydroxy-pregnenolone into progesterone, and dehydroepiandrosterone into Δ^4-androstene-3, 17-dione. Histochemically, the Δ^5-3β-hydroxysteroid dehydrogenase is demonstrable by the use of tetrazoles, specially of Nitro-Blue tetrazolium[2]. According to Rubin and her co-workers[3], the enzyme reaction is most intense in the outer fasiculata, but weak in zona reticularis of various mammalian adrenal glands. In the mouse, however, the reaction is limited in the outer zona fasciculata, when dehydroepiandrosterone is used as a substrate. A similar result was obtained by Allen[4] with the adrenal glands of mice of various postnatal ages.

In spite of the increasing literature on the mammalian adrenal glands, no report is available on the presence of the Δ^5-3β-hydroxysteroid dehydrogenase activity in the avian adrenal glands. The present study was planned, therefore, to provide informations on the histochemistry of this enzyme in the avian adrenal tissues. Special attention was paid to the zonal difference of the enzyme activity in the cortical cells, since the presence of a functional zonation of the avian adrenal cortex was recently suggested[5].

Two species, the domestic fowl and the Japanese quail, were used. In addition, three mammalian species, the rat, ox, and goat were employed for comparison.

Materials and Methods

Nine male birds of the Japanese quail (Coturnix coturnix japonica), purchased

from one hatchery in Toyohashi City and reared under continous lighting, were used in this study. Their body weight was between 61 and 80g. Three cockerels, purchased from one hatchery in Sendai were also used. They were approximately 150 days old and weighed about 2.5kg. The adrenal glands of these birds were removed immediately after decapitation. The glands were then embedded in fresh albumen in a chilled isopentane bath following the technique of Rahman and Luttrel[6]. Fresh frozen sections of eight to 10μ thick were prepared using a cryostat maintained at/18°C. The sections were mounted on cover slips, and dried at room temperature for about five minutes. The dried sections were then treated in the same manner as that employed by Mori et al.[7], though the incubation medium was slightly modified. The composition of the medium was as follows:

Δ^5-3β-hydroxysteroid	1 mg in 5ml acetone
Propyreneglycol	1 ml
0.2M Phosphate buffer, pH 7.4	8 ml
Nitro-Blue tetrazolium chloride, 0.1% aq. soln.	2 ml
NAD (Sigma Chemical Co.)	6 mg in 2 ml distilled water

The steroids employed as substrates were, dehydroepiandrosterone (DHA), pregnenolone, 17α-hydroxy-pregnenolone, and testosterone propionate. The substrates were dissolved in acetone, and the acetone was evaporated off in a 37°C oven, followed by the addition of the other constituents. Control media lacked steroid substrates or NAD.

Adrenal glands were also collected from four adult male rats, one male goat, weighing about 47kg, and an ox of 22 months old. The glands were treated in the same manner as those described above.

Results

When dehydroepiandrosterone was used as a substrate, the cortical cells (interrenal cells) of the adrenal glands in the two avian species showed a strong reaction to the Δ^5-3β-hydroxysteroid dehydrogenase (5-3β-enzyme) after two hours of incubation at 37°C. The medullary or suprarenal cells and the capsular connective tissue cells showed no enzymatic reaction. The blue deposits of the formed formazons were found in fine granule form in the cytoplasm of the cortical cells (Table 1 and Figs. 1–2). The whole cortex of the adrenal glands showed a uniform reaction to the enzyme, and both the subcapsular and mid-cortical zones of the cortex exhibited an equally intense reaction.

In the mammalian adrenal glands examined, the 5-3β-enzyme reaction was also positive in the cortical cells as reported by the preceding workers (2–4, 8). In the rat, zona fasiculata and zona reticularis showed a most intense reaction after 30 minutes of incubation. The cells in zona glomerulosa showed only a moderate reaction after one hour of incubation (Fig. 3), as already reported by Mori et

Table 1. Histochemical demonstration of the 5-3β-hydroxysteroid dehydrogenase activity in the adrenal glands of domestic fowl and Japanese quail. (incubation time, 120 minutes at 37°C).

Animal	Substrate	Tissue*		
		Cortex		Medulla and capsule
		Subcapsular zone	Mid-cortical zone	
Japanese quail	Dehydroepi-androsterone	++	++	−
	Pregnenolone	+~++	+~++	−
	17α-hydroxy-pregnenolone	+	+	−
	Testosterone propionate	−	−	−
Domestic fowl	Dehydroepi-androsterone	++	++	−
	Pregnenolone	++	++	−
	17α-hydroxy-pregnenolone	±~−	±~−	−
	Testosterone propionate	−	−	−

* No reaction was found in the tissues incubated in the media lacking the steroid substrates or NAD.

al.[7]. Similar results were obtained in the adrenal glands of the goat and ox (Figs. 4, 6). In the goat, islands of cortical cells were frequently present in the capsular connective tissues (Fig. 4). The 5-3β-enzyme reaction of these islands was rather intense, indicating that they were different from the adjacent glomerulosa cells as far as this enzyme activity was concerned. The capsular connective tissue cells and the medullary cells showed no reaction to the enzyme after prolonged incubation (Table 1).

No reaction of the 5-3β-enzyme was found in the control sections of the adrenal glands in all species tested, when the sections were incubated in the media lacking either dehydroepiandrosterone or NAD (Fig. 5).

Substrate specifity (Table 2). When testosterone propionate was employed as a substrate, no reaction of the 5-3β-enzyme was found in the adrenal cortical cells of avian as well as of the mammalian species. When pregnenolone was employed instead of dehydroepiandrosterone, the enzyme reaction in the glands of domestic fowl and Japanese quail was uniformly positive in the cortex, in both the subcapsular and mid-cortical zones. The reaction was almost as intense as that observed with dehydroepiandrosterone. In a few cases, the reaction appeared somewhat

Table 2. Histochemical demonstration of the 5-3β-hydroxysteroid dehydrogenase activity in the adrenal glands of the rat, ox and goat (incubation time, 30 minutes at 37°C).

Animal	Substrate	Tisse*			
		Cortex			Medulla and capsule
		Z. glomerulosa	Z. Fasciculata	Z. reticularis	
rat	Dehydroepi-androsterone	+	⫲	⫲～⫲	−
	pregnenolone	+	−	±～⫲	−
	17α-hydroxy-pregnenolone	−	−	−	−
	Testosterone propionate	−	−	−	−
ox	Dehydroepi-androsterone	+	⫲	⫲～⫲	−
	pregnenolone	±～+	−	±～+	−
	17α-hydroxy-pregnenolone	−	−	−	−
	Testosterone propionate	−	−	−	−
goat	Dehydroepi-androsterone	+	⫲	⫲～⫲	−
	pregnenolone	±～+	−	±～+	−
	17α-hydroxy-pregnenolone	−	−	−	−
	Testosterone propionate	−	−	−	−

* No reaction was found in the tissues incubated in the media lacking the steroid substrates or NAD.

stronger in the subcapsular zone, but this was not consistent (Figs. 7–8). In the rat, goat, and ox, the 5-3β-enzyme activity was only slightly positive in the zona fasciculata when pregnenolone was employed as a substrate (Figs. 9–10).

The intracapsular islands of the cortical cells in the goat adrenal glands, mentioned above, showed a moderate but sometimes stronger reaction to the 5-3β-enzyme with pregnenolone as a substrate. This indicated that the cells in the islands were a peculiar type of cortical cells which could utilize dehydroepiandrosterone and pregnenolone as substrates of the enzyme (Fig. 10).

When 17α-hydroxy-pregnenolone was employed as a substrate, a weak but positive reaction of the 5-3β-enzyme was found in the cortical cells of the avian adrenal glands. The reaction was nearly uniform throughout the cortex, though

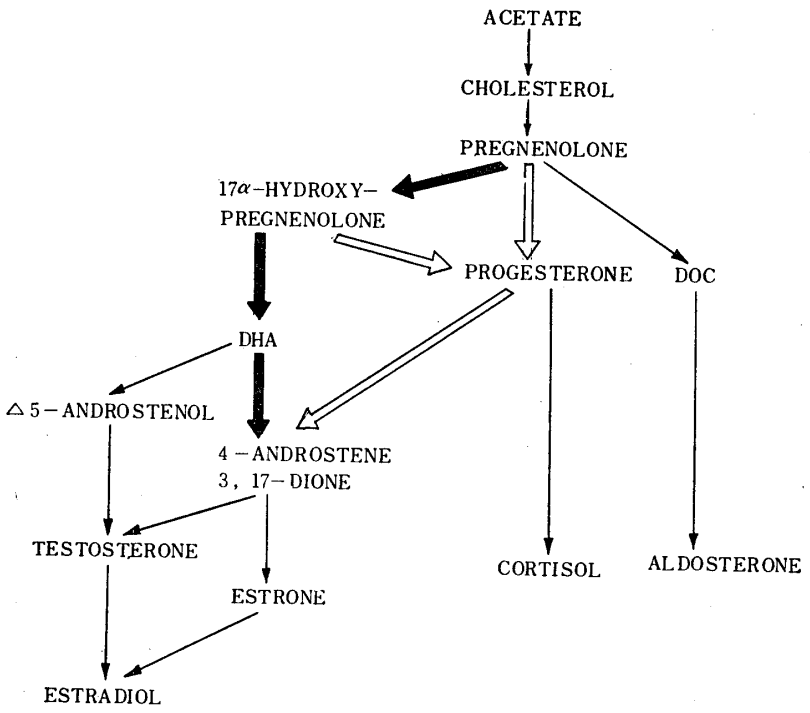
occasionally the subcapsular zone showed a stronger reaction (Figs. 11–12). The adrenal cortex of the rat, goat, and ox failed to show any positive reaction of the 5-3β-enzyme after two hours of incubation.

The medullary or suprarenal cells of the adrenal glands in the five species tested in this study did not show any positive reaction to the 5-3β-enzyme, with any of the steroids mentioned above.

Discussion

According to Bhattacharyya and Ghosh[5], the outermost zone of the pigeon adrenal cortex is functionally different from the inner zone. The cortical cells in the outermost or subcapsular zone slightly hypertrophied after thiourea treatment, while those in the inner or mid-cortical zone grossly atrophied. From these results and also from that of Sinha[9], the authors suggested that the mid-crotical zone is responsible for the secretion of the mineral-corticoids in the adrenal gland of pigeon.

In the present study, the activity of the 5-3β-hydroxysteroid dehydrogenase was histochemically demonstrated in the cortical cells of the adrenal glands in the domestic fowl and the Japanese quail. The reaction was uniformly positive throughout the cortex, though occasionally and with pregnenolone as substrate was slightly stronger in the subcapsular zone. This did not mean that, however,



Text-Figure 1. Biosynthesis of the steroid hormones in the adrenal cortex (Modified from Samuels 1960).

the zonal differences of the cortex were absent in the adrenal glands of these avian species. Namely, the 5-3β-enzyme is not directly related to the biosynthesis of the aldosterone (Text-Fig. 1). The result of the present study may be interpreted to mean that the proposed production of the mineralo-corticoids in the mid-cortical zone[5], if limited in this zone, did not intervene in the biosynthesis of the steroid sex hormones which were necessarily catalized by the 5-3β-enzyme.

It was noted that, in the mammalian species, the activity of the 5-3β-enzyme was low in zona glomerulosa. Although this was repeatedly reported by the preceding workers, no interpretation was given of this phenomenon by them (2–4, 7, 8). It has been well established that zona glomerulosa is the site of the aldosterone secretion in the mammalian adrenal glands[10]. The results that dehydroepiandrosterone was not effectively utilized in the zone, and the pregenolone was better utilized by the 5-3β-enzyme indicated that the cells in zona glomerulosa were differentiated, at least functionally and histologically, towards the secretion of steroids other than the sex hormones.

In the avian adrenal glands, pregnenolone and 17α-hydroxy-pregnenolone were utilized as the substrates for the 5-3β-enzyme. From this data and from those mentioned above, the present authors were inclined to suppose that there existed two main pathways towards Δ^4-androstenedione in the avian adrenal cortex, as indicated by the white and black arrows in Text-Figure 1. On the contrary, only one of them, i.e., via dehydroepiandrosterone (indicated by the black arrow in Text-Figure 1), was present in the mammalian adrenal glands.

It was found that the intracapsular islands of the cortical cells in the goat adrenal glands could catalize pregnenolone as well as dehydroepiandrosterone. This suggested that the cells in the islands differed from zona glomerulosa and also from zona fasciculata as far as the 5-3β-enzyme was concerned, and that they might be capable of synthesizing Δ^4-androsteneidione via at least two different pathways, already observed in the avian tissues.

The work of Wattenberg[2] has opened a new approach to the histochemistry of the steroid biosynthesis. It is hoped that the application of a group of suitable steroid substrates and cofactors together with a more advanced type of the hydrogen receptors will make the determination of the various steps involved in the biosynthesis of the steroid hormone possible. A further investigation along this line is planned.

Summary

The activity of the 5-3β-hydroxysteroid dehydrogenase (5-3β-enzyme) was demonstrated histochemically in the cortical cells of the adrenal glands in the domestic fowl and the Japanese quail. Four 3β-hydroxysteroids, dehydroepiandrosterone, pregnenolone, 17α-hydroxy-pregnenolone, and testosterone propionate were employed as substrates. The results are summarized as follows.

1. The activity of the 5-3β-enzyme was almost uniformly positive in the cortex of the avian species. Both the subcapsular and the mid-cortical zones of the cortex exhibited an intense reaction of the enzyme, with the steroids employed except testosterone propionate.

2. In the mammalian adrenal glands, the reaction of the 5-3β-enzyme was most intense in zona fasciculata, moderate in zona reticularis, and weak in zona glomerulosa with dehydroepiandrosterone as a substate. When pregnenolone was employed, the cells in zona glomerulosa and zona reticularis showed a weak but positive reaction to the enzyme. The reaction was negative in the cortex when 17α-hydroxy-pregnenolone or testosterone propionate was employed as substrates.

3. The cortical cells in the intracapsular islands in the goat adrenal gland exhibited positive reactions of the 5–3β-enzyme with dehydroepiandrosterone and pregnenolone as substrates. This suggested that the cells might be different from adjacent zona glomerulosa and also from the upper zona fasciculata in steroid secretion.

4. The significance of the above data was briefly discussed in relation to the possible existence of a more complicated pathways towards the synthesis of steroid hormones in the avian adrenal cortex than that in the mammalian species, and to the zonation of the avian adrenal cortex suggested by Bhattacharyya and Ghosh[5].

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Plate 1

Explanations of the Figures

All microphotographs were taken with the 8–10μ fresh frozen sections of the adrenal glands cut in a cryostat, and fixed in 10 percent neutral formalin after incubation. The sections were stained by Kernechtrot solution for nuclear differentiation. C: cortex, F: Zona fasciculata, G: Zona glomerulosa, I: intracapsular cortical cells, M: medulla, R: Zona reticularis.

Fig. 1. 5–3β–enzyme reaction in the adrenal gland of domestic fowl. Heavy deposition of the formazan is limited in the cortex. × 15.

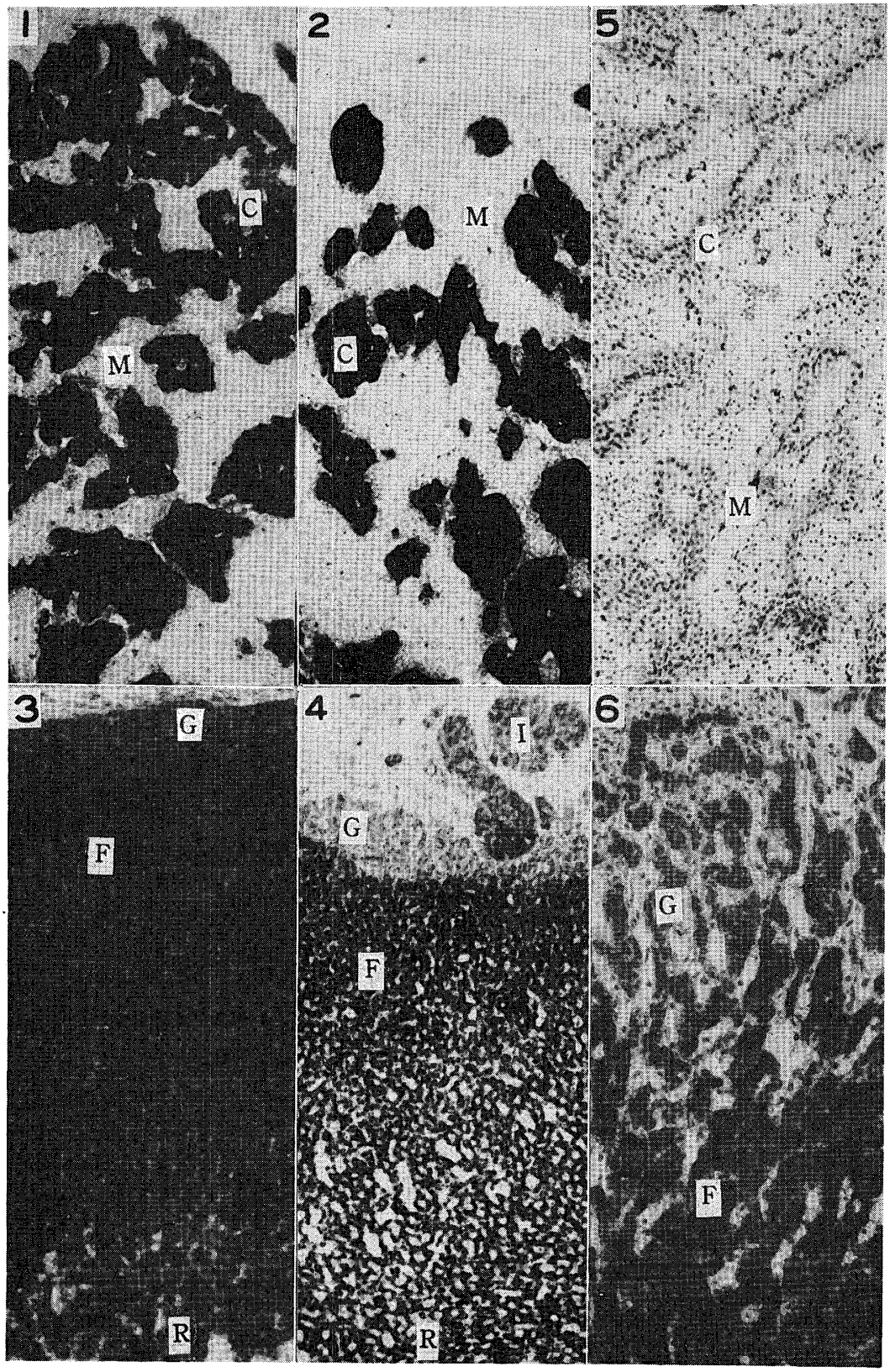
Fig. 2. 5–3β–enzyme reaction in the adrenal gland of the Japanese quail. The reaction of the enzyme is uniformly positive throughout the cortex. × 15

Fig. 3. The rat adrenal glands. The enzyme reaction is weaker in zona glomerulosa. × 36

Fig. 4. The goat adrenal glands. Strong reaction in Zonae fasciculata and reticularis, and a weak one in Zona glomerulosa. An intermediate reaction is noted in the intracapsular cortical cells. × 15

Fig. 5. The adrenal gland of the domestic fowl. Control section from the same tissues as in Fig. 1. No enzyme reaction is found. The nuclei and cytoplasm are lightly stained by Kernechtrot. × 20

Fig. 6. The bovine adrenal gland. The 5–3β–enzyme reaction is weak in Zona glomerulosa. × 54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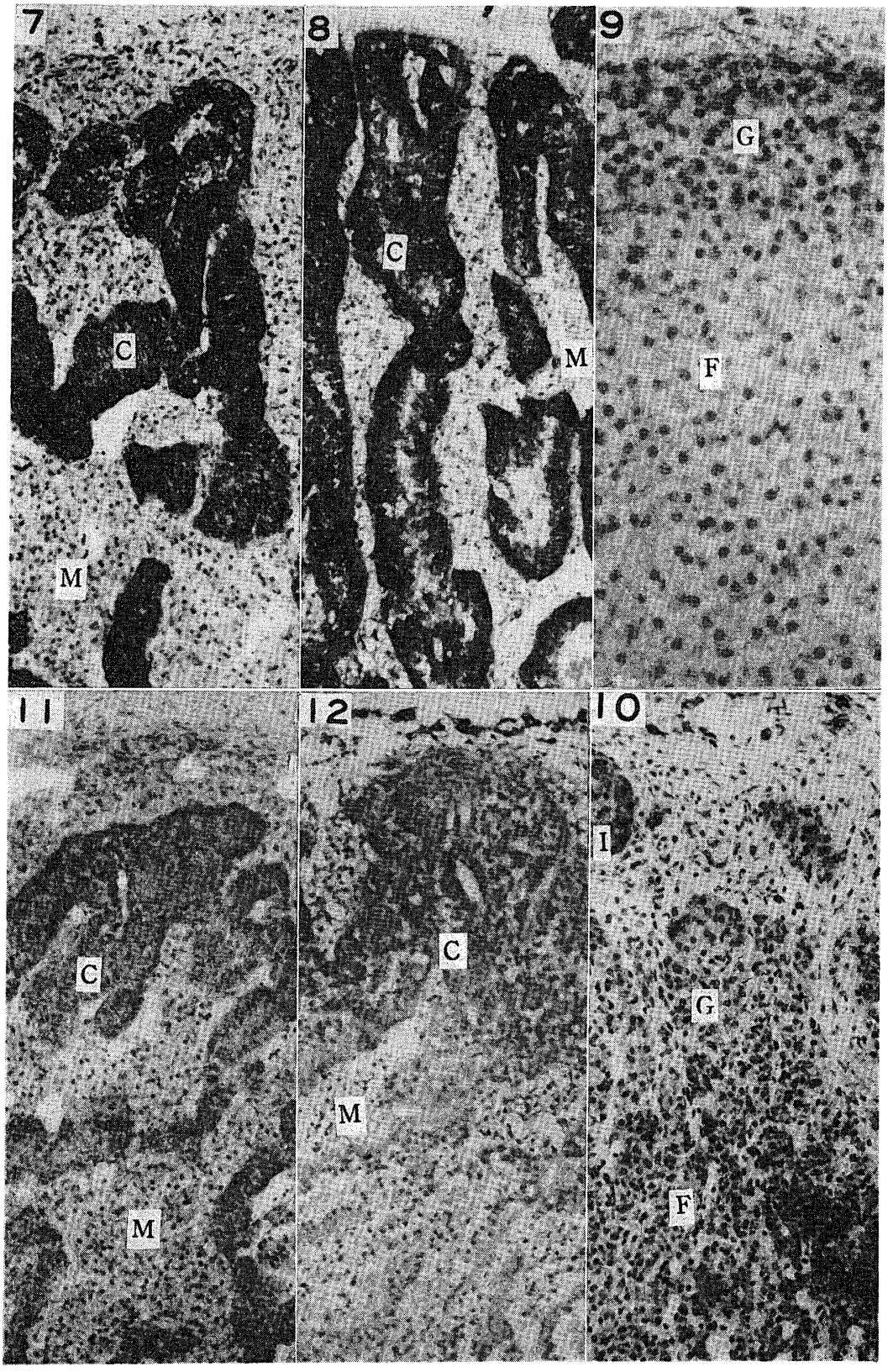
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Plate 2

Explanation of the Figures

Figs. 7–8. Pregnenolone is employed as a substrate. An intense reaction of the 5–3β–enzyme in the cortex. Fig. 7: domestic fowl, Fig. 8: Japanese quail. × 30

Fig. 9. The rat. Pregenolone is employed as a substrate. A weak reaction is noted in Zona glomerulosa, but negative in Zona fasciculata. × 75.

Fig. 10. The goat adrenal gland. Pregnenolone is employed as a substrate. The enzyme reaction is moderately positive in zona glomerulosa and in the intercapsular cortical cells. × 33

Figs. 11–12. 17-α-hydroxy-pregnenolone is employed as a substrate. A weak reaction of the 5-3β–enzyme in the cortex. Fig. 11: domestic fowl, Fig. 12: Japanese quail. × 30.