Acta vet. scand. 1975, *16*, 153—162.

From the Department of Pathology, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

ENZYME HISTOCHEMICAL STUDIES OF THE NORMAL PIG KIDNEY*

By

F. Elling and *T. Møller*

ELLING, F. and T. MØLLER: *Enzyme histochemical studies of the normal pig kidney.* Acta vet. scand. 1975, *16*, 153—162. — The activity and localization of NAD(P)H-tetrazolium reductase, lactate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase, glucose-6-phosphate dehydrogenase, α-glycerophosphate dehydrogenase, acid phosphatase, and alkaline phosphatase in the kidney of 11 female pigs were examined.

The pig kidney showed a higher activity of NAD(P)H-tetrazolium reductase in the distal tubules compared with the kidney of rat, mouse, rabbit, dog, cat, and man. The activity of succinate dehydrogenase and alkaline phosphatase was the same in the pig kidney as in the kidney of other examined species. In the pig kidney glucose-6-phosphate dehydrogenase precipitated in situ, while in rat and mouse this enzyme has proved to be highly diffusible.

enzyme histochemistry; kidney; pig.

Although enzyme histochemical techniques have been used for many years for demonstration of enzymatic activities in the normal kidney of many mammalians including the human, they have not, to the authors' knowledge, been applied to the pig kidney.

Wachstein (1955) demonstrated the distribution and activity of acid and alkaline phosphatase in the nephron of rat, mouse, rabbit, dog, cat, and human. *Sternberg et al.* (1956) were the first to demonstrate the localization and activity of NAD(P)H-tetrazolium reductase and succinate dehydrogenase in the kidney

* This study was supported by the Danish Agricultural and Veterinary Research Council.

of rat, guinea-pig, rabbit, dog, and cat. The effect of intracardial injection of serotonin on the activity of alkaline phosphatase and succinate dehydrogenase in the rat kidney was described by *Murray* (1965). *Jacobsen et al.* (1967) showed the localization and activity of acid and alkaline phosphatase in the proximal tubule and later (*Jacobsen* 1969) the lactate dehydrogenase activity and distribution in the rat kidney. It is the object of the present paper to describe the activity and distribution of some important enzymes in the normal pig kidney and to compare the enzyme profile of the pig kidney with the profile of other mammalians including the human.

MATERIAL AND METHODS

Eleven female pigs from 11 litters were purchased at about 8 weeks of age. They were fed a standard diet for bacon pigs. One pig was killed at the age of 10 weeks and 1 of 18 weeks. The remaining 9 pigs were killed when having achieved approx. 90 kg body weight, i.e. at the age of approx. 9 months. The animals were anesthesized with mebumal-sodium i.v., and the kidney tissue was removed during anesthesia. In order to clarify the effect of the anesthesia 1 animal was killed by cutting the carotid arteries, and the tissue was removed immediately after debleeding. The tissue utilized was removed from the cortex and the medulla and measured ½ × ½ × the distance between the capsule and the papilla. The tissue selected was frozen in a container with isopentane which was put in a freezing mixture consisting of acetone and dry ice and then stored at —80°C. Cryostat sections, 8 μm thick, were cut at —20°C with an American Optical Corporation "CRYOCUT". As cryostat sections may vary more than 10 % in thickness 2 incubation times, 20 and 30 min., were used in order to eliminate the thicker sections. The section incubated 30 min. should display a stronger reaction than the section incubated 20 min. The reaction was repeated whenever: a, the sections showed the same intensity, or b, the section incubated 20 min. showed a stronger reaction than the section incubated 30 min.

The sections were collected on cover slips, kept at room temperature until incubation, and then placed vertically in Columbia dishes. The reactions were interrupted by rinsing in demineralized water.

The tissues were examined for localization and activity of the following enzymes:

1. NADH-tetrazolium reductase
2. NADPH-tetrazolium reductase
3. LDH = lactate dehydrogenase
4. Isocitrate dehydrogenase
5. SDH = succinate dehydrogenase
6. G-6-DH = glucose-6-phosphate dehydrogenase
7. α-glycerophosphate dehydrogenase
8. Acid phosphatase
9. Alkaline phosphatase

In the reactions 1—7 the method of *Thomas & Pearse* (1961) was used as standard procedure. Incubation time was 20 and 30 min. at 37°C, and the sections were mounted in glycerol-gelatine.

The acid phosphatase reaction was made a.m. *Barka & Anderson* (1963), and the sections were afterwards stained with methyl green in 1 min., rinsed in demineralized water, dehydrated in alcohol, and mounted in dammar resin. In the alkaline phosphatase (a.m. *Burstone* 1958) the sections were rinsed in demineralized water prior to staining with Harris hematoxylin in 2 min., then rinsed in tap water for 5 min. and mounted in glycerol-gelatine.

Control measures

A. Diffusion of enzyme. In order to reveal whether the localization of the enzyme activity maintained in situ the maleimide inhibition method (*Høyer & Andersen* 1970) was employed. Additionally prefixation of tissue with 1 % methanol-free buffered (pH = 7.2) formaldehyde was employed prior to reaction for G-6-DH (*Andersen & Høyer* 1974).

B. "Nothing dehydrogenase" reaction. This was tested by the conventional incubation omitting the substrate.

C. Alcohol dehydrogenase. As alcohol dehydrogenase is able to reduce Nitro-BT, a section from each kidney was incubated with ethanol and NAD.

D. As controls in the acid and alkaline phosphatase reactions sections were incubated in substrate deficient media. In order to increase the acid phosphatase activity attempts to increase the permeability of the lysosome membrane were made by prefixating the tissue in formol-calcium in 2—12 hrs. at 4°C.

Evaluation of reactions

The enzyme histochemical reactions were evaluated qualitatively, i.e. the localization of the activity, as well as semi-quantitatively. The evaluation was made according to the scale described by *Jensen* (1973):

0: no reaction
1: weak reaction intimating the structure of the tissue
2: weak, although clearly positive reaction
3: strong, positive reaction

Abbreviations and nomenclature

NAD: nicotine-adenine-dinucleotide
NADP: nicotine-adenine-dinucleotide-3-phosphate
Nitro-BT: Nitro-blue-tetrazolium

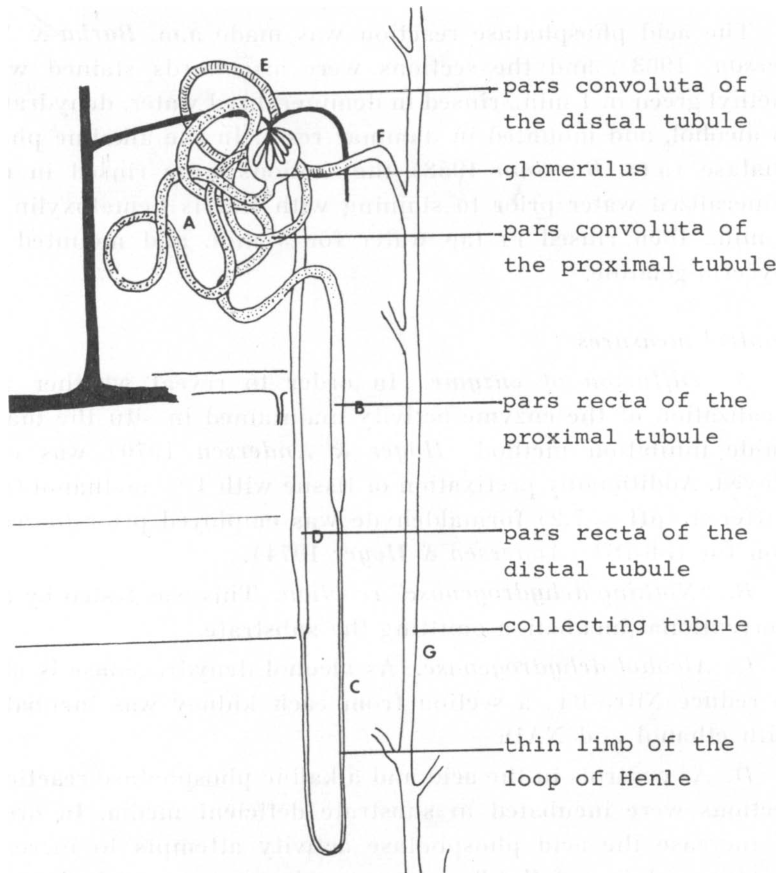


Figure 1. The nomenclature of the different segments of the pig kidney (*Dellman* 1971).

The various zones in the pig kidney are designated in the present paper by the terms used by *Dellman* (1971) (Fig. 1). The junction between the pars recta of the distal tubule and the pars convoluta of the distal tubule takes place at the macula densa. The collecting tubule consists of 3 portions: 1, the portion which connects the pars convoluta of the distal tubule with portion 2, which is the straight portion located in the medullary rays, and 3, the portion located in the papilla.

RESULTS

1. NADH-tetrazolium reductase

As indicated in Fig. 2 this enzyme demonstrated a weak activity in the glomerulus, a clearly positive reaction in the proximal tubules, and a weak reaction in the thin limb of Henle's loop. The pars recta of the distal tubule showed a strong positive activity, while there was a slightly weaker activity in the pars convoluta of the distal tubule and the collecting tubule. There was a weak reaction related to the endothelium of the vessels. No reaction was observed in the interstitial tissue. The localization of the activity in the proximal and the distal tubules was mainly in the basal part of the cells, where the mitochondria are abundant. In the other segments of the nephron the enzyme activity was uniformly distributed in the cells.

2. NADPH-tetrazolium reductase

This enzyme showed almost the same activity and localization as NADH-tetrazolium reductase (Fig. 2). However, a strong positive reaction was seen in the distal segment of the collecting tubules. Additionally, a clearly positive reaction was seen in the interstitial cells in the medulla.

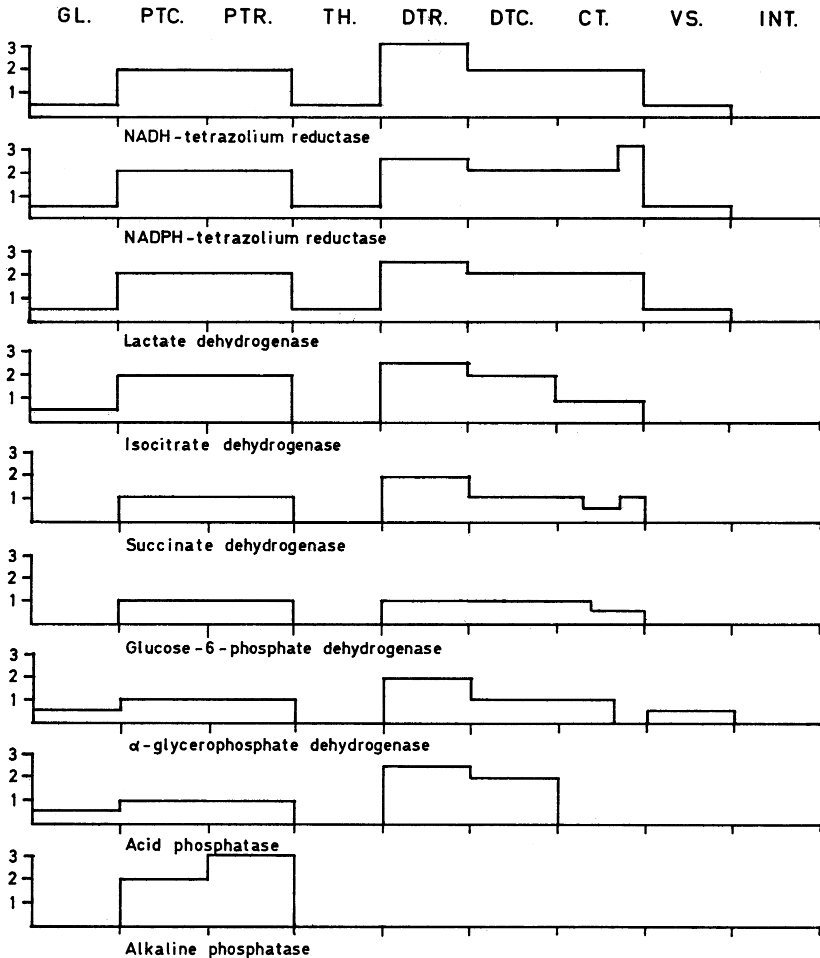
3. LDH = lactate dehydrogenase

LDH demonstrated the same activity, distribution, and localization in the cells as NADH-tetrazolium-reductase.

4. Isocitrate dehydrogenase

This enzyme showed a weak reaction in the glomerulus, a clearly positive reaction in the proximal tubules, no reaction in the thin limb of Henle's loop, while the strongest reaction was noted in the pars recta of the distal tubule. A clearly positive reaction was observed in the pars convoluta of the distal tubule

and a weak reaction in the collecting tubules. No reaction was seen in the vessels and the interstitial tissue (Fig. 2). The localization in the proximal and distal tubules was mainly in the basal part of the cells, while in the other portions of the nephron the activity was diffusely distributed in the cells.



GL :Glomerulus
PTC: Pars convoluta of the proximal tubule
PTR: Pars recta of the proximal tubule
TH :Thin limb of the loop of Henle
DTR: Pars recta of the distal tubule
DTC: Pars convoluta of the distal tubule
CT :Collecting tubule
VS :Vessels
INT: Interstitial tissue

Figure 2. The localization and activity of the enzymes in the pig kidney.

5. SDH = succinate dehydrogenase

SDH demonstrated a weak reaction in the proximal tubules, a strong positive reaction in the pars recta of the distal tubule and a weak reaction in the pars convoluta of the distal tubule and the proximal and distal portions of the collecting tubule, while the reaction in the portion of the collecting tubule located in the medullary rays was hardly cognizable. No reaction was noted in the glomerulus, the thin limb of Henle's loop, the vessels, or in the interstitial tissue (Fig. 2). The localization in the cells was the same as described above.

6. G-6-DH = glucose-6-phosphate dehydrogenase

G-6-DH showed a weak activity in the proximal and distal tubules and in the collecting tubules, located diffusely in the cells. No other elements of the kidney displayed activity (Fig. 2).

7. α-glycerophosphate dehydrogenase

This enzyme displayed a weak reaction in the glomerulus, the proximal and distal tubules, and the first and intermediate portions of the collecting tubule. An equally weak reaction was noted in the endothelial cells of the vessels, while no reaction was seen in the thin limb of Henle's loop or the ultimate portion of the collecting tubule or the interstitial tissue (Fig. 2). The activity was diffusely distributed in the cells.

8. Acid phosphatase

The glomerulus and the proximal tubule showed a weak reaction. A clearly positive reaction was noted in the distal tubule and the strongest in the pars recta. No reaction was seen in the thin limb of the loop of Henle, the collecting tubule, vessels, and the interstitial tissue. The localization was diffuse in the cells (Fig. 2).

9. Alkaline phosphatase

The activity of the alkaline phosphatase was seen in connection with the brush border of the proximal tubule with the strongest activity in the pars recta (Fig. 2).

LDH displayed diffusion in the maleimide blocked reactions, while G-6-DH showed no diffusion in neither the maleimide blocked reactions nor the prefixed sections.

The anesthesia did not show any effect on the enzymatic activity as there was no difference between the anesthesized pigs and the pig slaughtered by cutting the carotid arteries.

There was no activity of alcohol dehydrogenase in any of the examined reactions.

The prefixation of tissue in cold formol-calcium did not increase the activity of acid phosphatase.

No difference was observed between the subcapsular and the juxtamedullary nephrons in any of the enzymatic reactions.

The pigs slaughtered at the age of 10 and 18 weeks showed the same activity and localization of the examined enzymes as the pigs slaughtered at approx. 6 months of age.

DISCUSSION

The distribution and activity of NAD(P)H-tetrazolium reductase in the proximal tubules of the pig kidney were the same as in the kidney of rat, mouse, rabbit, dog, cat, and human, while the distal tubules of these species displayed a somewhat higher activity than the same portions of the pig kidney (*Wachstein* 1955).

In the rat *Sternberg et al.* (1956) demonstrated differences in the activity of NAD(P)H-tetrazolium reductase in the subcapsular nephrons compared with the juxtamedullary nephrons. Such a zonal difference was not seen in the pig kidney.

The activity and distribution of succinate dehydrogenase in the pig kidney was the same as in the rat (*Wachstein, Sternberg et al., Murray* 1965).

According to *Wachstein* the activity and distribution of acid phosphatase in the kidney of rat, mouse, rabbit, dog, cat, and human varied considerably in these species. Generally, however, these species showed the strongest activity in the proximal tubules, while the pig demonstrated the strongest activity in the pars recta of the distal tubule.

Based on the localization of the precipitate and the size of the granules in the acid phosphatase reaction, *Jacobsen et al.* (1967) demonstrated that the pars convoluta of the proximal tubule in the rat kidney could be differentiated in 2 segments. An equal segmental differentiation was demonstrated by the lactate dehydrogenase reaction (*Jacobsen* 1969). Such a segmental differentiation of the pars convoluta of the proximal tubule was not demonstrated in the pig kidney.

The distribution of alkaline phosphatase in the pig kidney, i.e. restricted to the proximal tubule with the highest activity in the pars recta, coincided with the findings in the rat (*Murray, Jacobsen et al.). Wachstein* demonstrated that mouse, dog, and cat only showed activity in the pars convoluta of the proximal tubule, while the rat, rabbit, and human showed equal activity in the pars convoluta of the proximal tubule. The remarkable observation that the capillaries in the pig kidney very rarely showed activity of alkaline phosphatase coincided with the findings in human biopsies (*Pedersen & Dalgaard* 1960). Glucose-6-phosphate dehydrogenase has for long been proved to be highly diffusible in mouse and rat (*Andersen & Høyer* 1974). However, in the pig kidney this enzyme appeared to precipitate in situ.

CONCLUSION

In the pig kidney succinate dehydrogenase displayed the same activity and distribution as in the other examined species. This also applied to alkaline phosphatase as it appeared in the latest publications. The pig kidney showed a higher activity of NAD(P)H-tetrazolium reductase in the distal tubules in comparison with the other examined species. The segmental differentiation of the pars convoluta of the proximal tubule of the rat kidney based on the activity of acid phosphatase and lactate dehydrogenase was not demonstrated in the pig.

REFERENCES

Andersen, H. & P. E. Høyer: Simplified control experiments in the histochemical study of co-enzyme-linked dehydrogenases. Histochem. 1974, *38,* 71—83.

Barka, T. & P. J. Anderson: Histochemistry. Theory, Practice and Bibliography. Hoeber Medical Division, New York, London 1963.

Burstone, M. S.: Histochemical comparison of naphtol AS-phosphates for demonstration of phosphatases. J. nat. Cancer Inst. 1958, *20,* 601.

Dellman, H.-D.: Veterinary Histology. An outline Textatlas. Lea & Febiger. Philadelphia 1971.

Høyer, P. E. & H. Andersen: Specificity in steroid histochemistry, with special reference to the use of steroid solvents. Distribution of 11 β-hydroxysteroiddehydrogenase in kidney and thymus from mouse. Histochem. 1970, *24,* 292—301.

Jacobsen, N. O.: The histochemical localization of lactic dehydrogenase isoenzymes in the rat nephron by means of an improved polyvinyl alcohol method. Histochem. 1969, *20,* 250—265.

Jacobsen, N. O., F. Jørgensen & Å. C. Thomsen: On the localization of some phosphatases in three segments of the proximal tubules in the rat kidney. J. Histochem. Cytochem. 1967, *15*, 456—469.

Jensen, H.: Enzymhistokemiske undersøgelser af fibroadenomatose og mammacarcinom. (Enzyme histochemical examinations of fibroadenomatosis and carcinoma of the mammary gland). Thesis. FADL's Forlag, København, Aarhus, Odense 1973.

Murray, S. M.: Pathogenesis of renal tubular necrosis induced by serotonin in the rat. J. Path. Bact. 1965, *90*, 409—417.

Pedersen, K. J. & O. Z. Dalgaard: The localization of non-specific esterases in human renal biopsies. Acta path. microbiol. scand. 1960, *50*, 228—236.

Sternberg, W. H., E. Farber & C. E. Dunlap: Histochemical localization of specific oxydative enzymes: II. Localization of diphosphopyridine nucleotide diaphorases and the succindehydrogenase system in the kidney. J. Histochem. Cytochem. 1956, *4*, 266—283.

Thomas, E. & A. G. E. Pearse: The fine localization of dehydrogenases in the nervous system. Histochem. 1961, *2*, 266.

Wachstein, M.: Histochemical staining reactions of the normally functioning and abnormal kidney. J. Histochem. Cytochem. 1955, *3*, 246—270.

SAMMENDRAG

Enzymhistokemiske undersøgelser af den normale grisenyre.

Aktiviteten og lokalisationen af NAD(P)H-tetrazolium reduktase, mælkesyredehydrogenase, isocitronsyredehydrogenase, ravsyredehydrogenase, glukose-6-fosfatdehydrogenase, α-glycerofosfatdehydrogenase, samt sur og alkalisk fosfatase blev undersøgt på 11 hungrise. Grisenyrens enzymprofil blev sammenlignet med andre pattedyrs. For NAD(P)H-tetrazolium reduktasens vedkommende viste det sig, at de distale tubulusafsnit i grisenyren viste en højere aktivitet end de samme afsnit i nyren hos rotte, mus, kanin, hund, kat og menneske. Der var god overensstemmelse mellem aktiviteten af ravsyredehydrogenase og alkalisk fosfatase i grisenyren sammenlignet med nyren fra andre undersøgte species. Ved reaktionen for glucose-6-fosfatdehydrogenasen fældede reaktionsproduktet ud in situ, medens dette enzym har vist en kraftig tendens til at diffundere hos mus og rotte.

(Received September 11, 1974).

Reprints may be requested from: Folmer Elling, the Department of Pathology, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen V, Denmark.