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Renal Clearance Studies in the Pig

Surgical Procedures, Performance of Investigations and Physiological Data

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Summary. The pig, especially the miniature pig, has in recent years been used increasingly frequently as a laboratory animal and experience shows that as such it is very useful in many circumstances. The aim of the present investigation was to study the renal function in unsedated and unrestrained pigs. Techniques for the implantation of central venous catheters, for cystostomy, for thyroidectomy and for open kidney biopsy are described. Implantation of central venous catheters and cystostomy are necessary procedures for making clearance studies in unsedated and unrestrained pigs possible. The following mean values for the renal function were obtained:
Cl_{In} 4.11 ml/min/kg, Cl ^{51}Cr-EDTA-p 3.82 ml/min/kg, Cl ^{51}Cr-EDTA-w.b. 3.89 ml/min/kg, Cl_{PAH} 21.4 ml/min/kg, Cl ^{125}I-hippuran 17.4 ml/min/kg, Tm_{PAH} 4.31 mg/min/kg and TRP over 80%.

Key words: Clearance studies, serum electrolytes, pigs.

Introduction

The pig, especially the miniature pig, has become more and more important as a laboratory animal in medical research because of its physiological resemblances to man (4, 10, 16).

Hitherto studies on renal physiology have been performed mainly in the dog, despite essential differences in anatomy and renal function between the dog and man. Only a few studies on renal function in the pig have been published (3, 7, 8, 18, 26).

Since we planned an experimental study in animals, in which renal function analyses were of great importance and for which the pig was considered to be the most suitable laboratory animal, we had to work out an appropriate technique for clearance studies in unsedated and unrestrained pigs and to establish normal values for their renal function as well as normal serum electrolyte values.

Material and Methods

Laboratory Animals

The investigations were performed in female pigs of the Swedish Land race, Swedish Yorkshire race and crosses between these races, aged 10-14 weeks, weighing from 16.5 to 28 kg at the beginning of the investigation.

Housing

The pigs were housed in pens 2.5 m x 2 m, one pig per pen. Before the clearance investigation the pig was transferred to a smaller, mobile crate, 0.5 m x 1.25 m, in which it could stand up or lie down without restraint.

Food

The pigs were given commercial pig food (Piggfor^{R}). They had free access to drinking water.

Anaesthesia

The pigs were kept without food for 12-18 h before the surgical procedures and clearance investigations. No premedication was given before the anaesthesia. At the first surgical intervention in each animal, anaesthesia was induced with halothane-oxygen-nitrous-oxide, which was also

used for the maintenance anaesthesia. At later operations anaesthesia was induced with thiopentone sodium (Pentothal sodium, Abbott), injected into a central venous catheter (see Surgical procedures). The inhalation anaesthesia was very easily controlled and the pigs recovered from it very quickly.

40 pigs were anaesthetized on 132 different occasions. In one case there was a temporary cessation of breathing and in another cardiac arrest occurred during the first anaesthesia in a very agitated pig. No other anaesthetic complications were noted.

Endotracheal intubation in the pig is rather difficult (5, 17, 23). All surgery undertaken could be carried out with the pig in a lateral recumbent position and the inhalation anaesthetics could be given by means of a simple mask over the snout.

Surgical Procedures

Two central venous catheters (size 8 Ch infant feeding tubes) were implanted, one in the superior vena cava and one in the right atrium, via the external or the internal jugular vein and were pulled out behind the ear of the pig (2, 16). The catheters were flushed daily with heparinized saline (50 mg of heparin/100 ml of isotonic saline) and remained patent throughout the investigation, i.e. for 10-12 days.

Cystostomy was performed through a suprapubic mid-line incision. A size 16-20 Ch catheter was implanted through a cystotomy and the incision in the bladder was closed with invaginating purse string sutures (16). The urethra was ligated and then divided. The catheter was directed towards the perineum through a subcutaneous tunnel. Pezzer or Couvelaire catheters were used; of the two the latter type proved to be the best choice.

Thyroidectomy was performed in 19 pigs. Exploration of the thyroid was undertaken in another 12 pigs. These operations were easily performed through a mid-ventral incision (22), even with the animal in a lateral recumbent position.

Kidney biopsies were carried out in 33 pigs, each on 2 different occasions. The kidneys were easily accessible through a transversal incision below the last rib.

Technique for Clearance Studies: Food but not water was withheld for 12-18 h before the investigation.

To ensure a high diuresis isotonic saline at a volume of 0.2-0.3 ml/min per kg body weight (ml/min/kg), was given intravenously through one of the 2 central venous catheters. This catheter was also used for blood sampling during the clearance investigation. The other was used for infusion of the test substances which were administered by means of a constant speed pump.

Blood, plasma and urine blanks were taken before the priming doses were given. Blood and plasma samples were then taken in the middle of each clearance period. The infusion of the test substances was interrupted (for less than 1 min) while the blood samples were taken. At the beginning and end of each clearance period the urinary bladder was irrigated with approx. 100 ml of isotonic saline and was emptied by means of air inflation and abdominal compression.

The clearance periods were about 30 min each and the equilibration period about 45 min.

For the clearance determinations the priming doses were: for the clearance of inulin (Cl_{In}) 90 mg/kg, for the clearance of para-amino-hippuric acid (Cl_{PAH}) 20 mg/kg and for the transfer maximum of PAH (Tm_{PAH}) 300 mg/kg, and the constant infusion doses were: for Cl_{In} 0.90 mg/min/kg, for Cl_{PAH} 0.40 mg/min/kg and for Tm_{PAH} 5 mg/min/kg. Beside these test substances ^{51}chromium complexed with ethylene-diamine-tetra-acetic acid (^{51}Cr-EDTA) and ^{125}iodine-hippuran (^{125}I-hippuran) were infused for determination of the glomerular filtration rate (GFR) and the renal plasma flow (RPF). The investigation consisted of 2 periods for determination of Cl ^{125}I-hippuran and Cl_{PAH} and 2 for determin tion of Tm_{PAH}. Cl ^{51}Cr-EDTA, calculated from the activity in plasma (Cl ^{51}Cr-EDTA-p) and in whole blood, corrected by the haematocrit value (Cl ^{51}Cr-EDTA-w.b.) were used to determine GFR in all 4 periods. In 6 cases Cl_{In} was determined at the same time. In 11 cases the tubular reabsorption of phosphates (TRP) was determined. The whole investigation took about 4 hours.

Inulin was determined according to Heyrovsky (9) and PAH according to Brun (1).

Tm_{PAH} was calculated from the formula

$$U_{PAH} \times V - 0.83 \times Cl\ ^{51}Cr\text{-}EDTA\text{-}p \times P_{PAH}$$

where U = urine concentration, V = volume of urine in ml/min and P = plasma concentration. 0.83 is a constant given by Reubi (21) to correct for the PAH bound to protein. Tm_{PAH} was calculated only in cases where the plasma concentration of PAH was at least 40 mg/100 ml.

The isotope concentrations in the blood, plasma and urine were determined in 2 ml samples with a well-crystal scintillation detector (Landis & Gühr, Switzerland) to a statistical precision of $\pm$ 1%.

A microhaematocrit centrifuge (No. 490, International Equipment Co, USA) was used for the haematocrit determinations.

TRP was calculated from the formula

$$1 - \frac{U_{Ph} \times V}{P_{Ph} \times Cl\ ^{51}Cr\text{-}EDTA\text{-}p}$$

where Ph = phosphates.

Blood Sampling

Blood samples were taken daily for the determination of serum electrolytes and calcium ion

activity (Ca^{++}). Sodium, potassium, chlorides, bicarbonate, serum proteins, creatinine, urea-N and phosphates were determined by means of standard clinical chemistry laboratory methods adapted to a multichannel auto analyzer (Technicon auto analyzer SMA 6/60). The total serum calcium and serum magnesium were determined using an atomic absorption spectrophotometer (Unicam SP 90 B). The blood samples were collected in glass test tubes, left to coagulate at room temperature and centrifuged. Serum was separated and stored at $-25^{\circ}C$.

Ca^{++} was determined using an ion specific flow-through electrode (Orion Research Corp, USA). The blood samples were taken anaerobically, left to coagulate at room temperature and then centrifuged. Serum was collected in plastic syringes which were hermetically sealed and stored at $-25^{\circ}C$ (20).

All blood samples from each pig were analyzed on the same occasion using the above mentioned methods.

Statistical Methods

Standard statistical methods have been used for the calculation of mean values, linear regressions and correlation coefficients (Pearson's product moment correlation coefficient).

Results

Blood Analyses

The values for the serum electrolytes and Ca^{++} are shown in Table 1. The values for sodium, potassium, calcium, bicarbonate and serum proteins are in all essentials consistent with previously reported normal values for pigs (14, 16). The values for phosphates, creatinine and urea-N agree with the values for miniature pigs of the same age published earlier by McClellan et al. (14).
The mean for the haematocrit was 33%. This agrees with the findings reported by Marshall et al. (16), but is lower than those reported by Tegeris et al. (27).

The value for Ca^{++} was equivalent to 40.5% of the total serum calcium.

Renal Clearance Studies

The results of the clearance studies in 18 normal pigs are shown in Table 2.

For 4 pigs with normal serum creatinine but in which studies on the maximum urine concentration capacity were performed the day before the clearance investigations, the values are shown in Table 3.

The correlations between Cl_{In} and $Cl\ ^{51}Cr$-

Table 1. Serum electrolytes and calcium ion activity.
The values are given as the mean ± the 95% confidence limits of the mean. MS_B measures the dispersion between the animals and MS_W the dispersion within the animals

		X ±	MS_B	MS_W
Na	mEq/l	147.3 ± 1.3	17.23	3.05
K	mEq/l	4.37 ± 0.11	0.1298	0.0611
Ca	mEq/l	4.97 ± 0.14	0.1937	0.0493
Mg	mEq/l	1.76 ± 0.05	0.0280	0.0144
Cl	mEq/l	103.2 ± 1.5	22.45	4.41
CO_2	mEq/l	26.8 ± 1.17	13.91	0.68
P	mg/100 ml	8.41 ± 0.48	2.36	0.31
Protein	g/100 ml	5.63 ± 0.26	0.4166	0.0304
Creatinine	mg/100 ml	0.87 ± 0.10	0.0782	0.0061
Urea-N	mg/100 ml	11.8 ± 2.44	48.31	2.97
Ca^{++}	mMol/l	1.01 ± 0.04	0.0070	0.0050

Table 2. Clearance values in 18 normal pigs.
The values are given in ml/min/kg (mg/min/kg for Tm_{PAH}).
The plasma concentrations (P_{In} and P_{PAH}) are given in mg/100 ml

Cl_{Inulin}	P_{Inulin}	Cl ^{51}Cr-EDTA-p	Cl ^{51}Cr-EDTA-w.b.	Cl ^{125}I-hippuran	Cl_{PAH}	P_{PAH}	Tm_{PAH}	P_{PAH}
		3.41	3.49	15.6	18.4	1.69	3.35	42.1
		4.28	4.41	27.0	41.0	1.00	-	34.6
		3.51	3.57	13.2	13.0	3.17	4.15	68.4
4.19	2.40	3.85	3.93	13.8	14.0	1.00	3.80	46.1
4.34	3.85	4.60	3.72	14.4	19.1	1.85	3.69	50.6
4.20	3.74	3.83	4.07	14.2	23.9	1.67	4.74	54.5
		3.35	3.54	12.4	16.0	1.65	3.63	62.3
		4.50	4.51	18.6	24.0	1.24	5.45	43.9
		3.79	3.68	19.0	24.4	1.02	4.08	63.7
		4.16	-	21.5	27.4	1.96	6.09	56.5
		3.66	3.91	17.5	19.4	2.45	4.56	47.6
		3.29	3.53	15.0	15.0	1.88	3.70	81.3
		4.53	4.51	20.1	24.4	1.44	-	26.2
		4.06	4.11	13.8	13.2	4.30	4.63	46.9
3.88	5.11	4.27	4.40	20.2	25.6	1.69	4.21	49.2
		3.20	3.18	18.2	21.8	1.75	4.01	70.3
		4.21	4.33	25.1	28.0	1.26	3.96	56.3
		3.31	3.31	14.3	16.2	2.64	4.91	71.3
Mean 4.15		3.82	3.89	17.4	21.4		4.31	
S.D.		0.42	0.41	4.16	6.95		0.73	
S:E.M.		0.10	0.10	0.98	1.64		0.18	

EDTA-p and between Cl_{In} and Cl ^{51}Cr-EDTA-w.b. are shown in Figs. 1 and 2. The correlation between Cl ^{125}I-hippuran and Cl $_{PAH}$ is shown in Fig. 3.

TRP was over 80% (80.1-96.5%) in the 11 cases in which it was calculated.

Discussion

Only a few studies on renal function in the pig have been published (Table 4).

In pigs blood sampling presents some difficulties. To make repeated blood sampling easy and completely painless for the animal it is necessary to insert a central venous catheter, which makes it

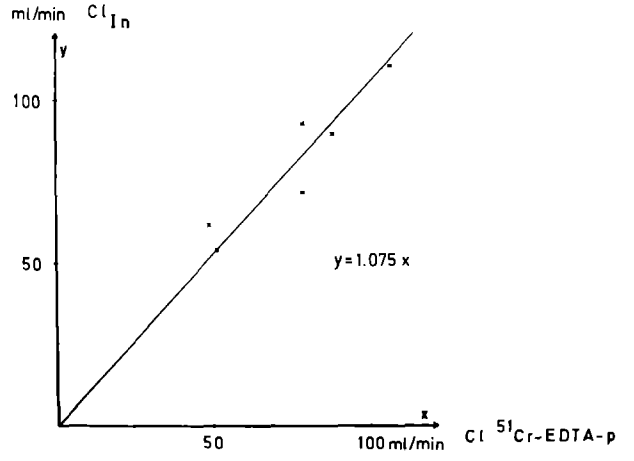

Fig. 1. Correlation between simultaneous clearances of ^{51}Cr-EDTA-p and inulin in 6 pigs

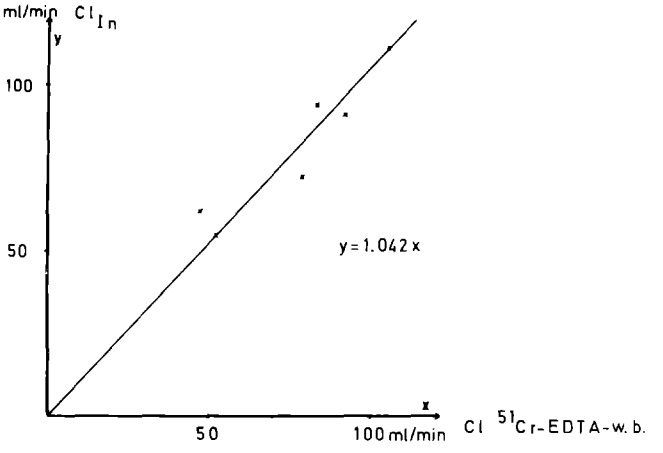

Fig. 2. Correlation between simultaneous clearances of ^{51}Cr-EDTA-w.b. and inulin in 6 pigs

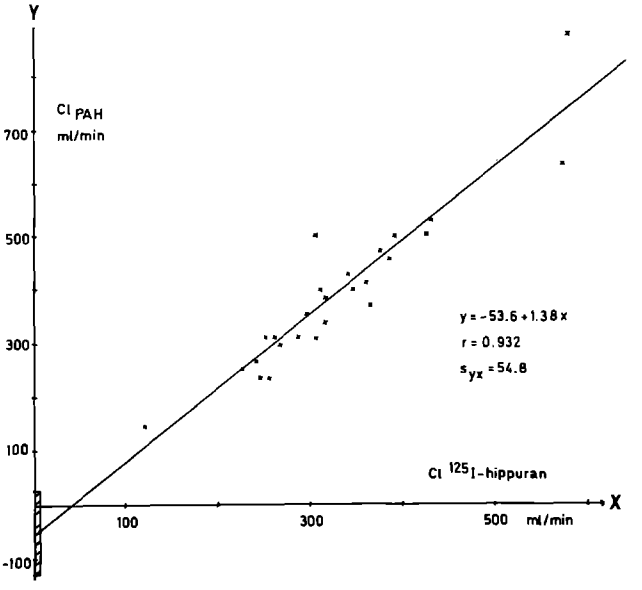

Fig. 3. Correlation between simultaneous clearances of ^{125}I-hippuran and PAH in 26 pigs. The intercept (-131, 24. 1) is marked on the Y-axis

easy also to administer intravenous fluid to an animal without the need to restrain it.

To be able to perform clearance studies it is necessary to obtain complete collection of urine throughout accurately timed periods. Catheterization of the urinary bladder is thus necessary. Such a catheterization can be performed in the female pig without difficulties (11). However, the pigs may urinate, bypassing the catheter, and even if a suprapubic cystostomy has been carried out they may urinate normally (16). To ensure a correct and complete urine collection it was thus necessary to perform a cystostomy and to ligate and divide the urethra.

To ensure a high diuresis it is necessary to give the animal an ample volume of water. This can be given through a stomach tube or as an intravenous infusion. As we planned to operate on the animals immediately after the clearance studies we had to give the fluid intravenously. Intravenous infusion of isotonic saline at a volume of less than 0.3 ml/min/kg has no effect on GFR and RPF in normal man (29).

In this investigation Cl ^{51}Cr-EDTA-p was chosen as the expression of GFR. Several investigations (6, 12, 13, 25) have shown that Cl ^{51}Cr-EDTA values are very close to and slightly lower than Cl_{In} values.

In this study simultaneous determinations of Cl ^{51}Cr-EDTA and Cl_{In} were made in only 6 cases, to confirm that the correlations between these clearance values in the pig are the same as in man (Figs. 1 and 2).

The mean for Cl ^{51}Cr-EDTA-p in normal pigs was 3.82 ml/min/kg which equals a Cl_{In} value of 4.11 ml/min/kg. This value is lower than that reported by Munsick et al. (18) but higher than those found by Dalgaard-Mikkelsen et al. (3), Gyrd-Hansen (7, 8) and Suarez et al. (26). Dalgaard-Mikkelsen et al. (3) and Suarez et al. (26) carried out their investigations under urethane sedation and thiopentone anaesthesia respectively and several studies have shown that anaesthesia is as a rule associated with a reduction in GFR and RPF. However, Gyrd-Hansen (8) compared Cl_{In} and Cl_{PAH} in conscious pigs with Cl_{In} and Cl_{PAH} in urethane sedated or pentobarbitone-anaesthetized pigs and did not find any differences between these 3 groups.

The mean for Cl_{PAH} was 21.4 ml/min/kg. This value is slightly higher than that found by Munsick et al. (18), but considerably higher than that found by Gyrd-Hansen (7, 8).

Table 3. Clearance values in 4 pigs subjected to dehydration the day before the clarance investigation

Cl_{Inulin}	P_{Inulin}	Cl ^{51}Cr-EDTA-p	Cl ^{51}Cr-EDTA-w.b.	Cl ^{125}I-hippuran	Cl_{PAH}	P_{PAH}	Tm_{PAH}	P_{PAH}
		2.46	2.54	13.8	16.4	1.64	3.90	42.0
		2.95	2.92	11.9	13.3	1.84	3.39	41.9
2.61	1.10	2.47	2.51	14.9	18.6	1.40	3.48	44.1
		2.96	3.00	13.7	16.9	2.50	6.27	57.5
Mean		2.71	2.74	13.6	16.3		4.26	

Table 4. The values are given in ml(mg)/min/kg. n = the maximal number of animals investigated

	n	Cl_{In}	Cl_{PAH}	Tm_{PAH}
Dalgaard-Mikkelsen et al. (3) [a]	8	3.65		
Munsick et al. (18)	4	5.00	19.5	3.10
Suarez et al. (26) [a]	9	3.50		
Gyrd-Hansen (7)	14	2.10	6.4	2.30
Gyrd-Hansen (8) [b]	5	3.20	8.7	

a Sedated or anaesthetized animals
b Animals from the same farm as those used by Dalgaard-Mikkelsen et al. (3).

The clearance of an isotope-labelled I-hippuran was studied at the same time as Cl_{PAH}. ^{125}I-hippuran was chosen since this substance is more stable and has a longer half-life than ^{131}I-hippuran. Previously published investigations (13, 15, 19) have shown Cl ^{125}I-hippuran to be lower than Cl_{PAH}. In the present study in the pig Cl ^{125}I-hippuran was found to be 17.4 ml/min/kg and the correlation between Cl_{PAH} and Cl ^{125}I-hippuran is consistent with previous observations in man (Fig. 3).

The mean for Tm_{PAH} was 4.31 mg/min/kg. Calculations of Tm_{PAH} were only made in cases where the plasma concentration of PAH was 40 mg/100 ml or higher. The value thus obtained is higher than those reported by Munsick et al. (18) and Gyrd-Hansen (7). These authors did not, however, give any information about the plasma concentration of PAH at their determinations of Tm_{PAH}.

The variations in the values for Cl_{In}, Cl_{PAH} and Tm_{PAH} between Dalgaard-Mikkelsen et al. (3), Suarez et al. (26), Munsick et al. (18), Gyrd-Hansen (7, 8) and the present study may be due to racial differences and to differences in hydration during the clearance investigations (8).

We originally planned also to determine the maximum urine concentration capacity in the pig.

However, when the animals had been deprived of water for 18 h before this investigation, there was a marked rise in serum creatinine in 4 pigs, with a maximum value of 6.2 mg/100 ml, and clearance studies the following day resulted in values well below those in the animals not deprived of water. In another 4 pigs, also deprived of water for 18 h for this investigation, no rise in the serum creatinine was observed but the clearance values were lower than those in the pigs not deprived of water. This might be due to immature kidney function in these young pigs, although according to Vogh & Cassin (28) mature kidney function seems to exist in pigs by the age of 2-3 months.

To avoid sources of error when using growing pigs as laboratory animals, it is thus of great importance to make sure that the pigs are well hydrated, as they seem to be very sensitive to dehydration.

Our experience shows that, with appropriate investigation methods, the pig is an excellent laboratory animal for studies on renal physiology and that probably it can well replace the dog which has hitherto been the animal of choice.

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