APPENDIX 1

Validity of endogenous creatinine and $Cr^{51}EDTA$ clearances as measures of GFR in sheep.

Historically, the clearance of inulin has been used as a measure of glomerular filtration rate (GFR). However the accurate determination of inulin in plasma and urine is relatively difficult. This, coupled with the need to accurately infuse an often pyrogenic inulin intravenously to achieve a constant plasma level, has led to the search for less labour intensive alternatives. Isotopic Cr^{51} ethylene diamine tetra acetate (Cr^{51}EDTA) is easily and accurately measured by gamma emission spectrometry. Creatinine, although reasonably difficult analytically has the added advantage that endogenous clearance may be used, nullifying the need for troublesome intravascular infusions.

GFR is normally measured during an infusion of a marker with due allowance for time to reach a steady state plasma level. This simple clearance technique has been validated for the use of Cr^{51}EDTA (Stacy & Thorburn 1966) and endogenous creatinine (Bishara & Bray 1978) in sheep. However the measurements of GFR made by these workers were over a very narrow range of GFR in normal animals. Valtonen *et al* (1982) found that endogenous creatinine clearance was similar to inulin clearance in goats with varying protein intakes.

The present study investigates the effects of varying protein intake, hypertonic NaCl infusion and acid-base status on GFR measurement using endogenous creatinine, Cr^{51}EDTA and inulin clearance.

Methods

Six merino ewes (2-3 years old) were housed in metabolism crates and fed their ration for 10 days prior to any experimentation.

On the morning of the day of experimentation one jugular vein and the bladder of each animal was catheterised.

The animals were fed either a low protein (LP - oaten chaff 5.1 % crude protein) or high protein (HP - lucerne chaff 15.6 % crude protein) diet and infused

via the jugular catheter with one of three infusates as follows:

Sheep No.	*Diet*	*Infusate*
1	HP	A
2	LP	A
3	HP	B
4	LP	B
5	HP	C
6	LP	C

Composition of the Infusates and Infusion Rates

Infusate	*Inulin*	*$Cr^{51}EDTA$*	*NaCl*	*HCl*	*Infusion Rate*
A	10%	0.04MBq/mL	0.8%	—	0.5mL/min
B	10%	0.04MBq/mL	0.8%	150mmol/L	0.5mL/min
C	5%	0.02MBq/mL	8.0%	—	1.0mL/min

The animals were infused for 6 hours. Four clearance periods, each of 1 hour duration, were completed in the latter 4 hours of infusion.

Cr^{51}EDTA was measured by gamma emission spectrometry (Gammamatic, Kontron, Sydney, Australia), inulin by the method of Bacon & Bell (1948) and creatinine using a Cobas-Bio centrifugal analyser (Hoffman La Roche, Switzerland).

Results

The values obtained from the six animals are shown in Fig. 1.

Discussion

There was good agreement between the three methods. The creatinine values from the animals given the acid tended to be on the higher side of the regression line. These increased values may be related to protein wasting due to acidosis (Hannaford *et al* 1982).

It is concluded that values obtained from the three methods are similar and hence endogenous creatinine clearance may be used where problems associated with intravascular infusions may be encountered.

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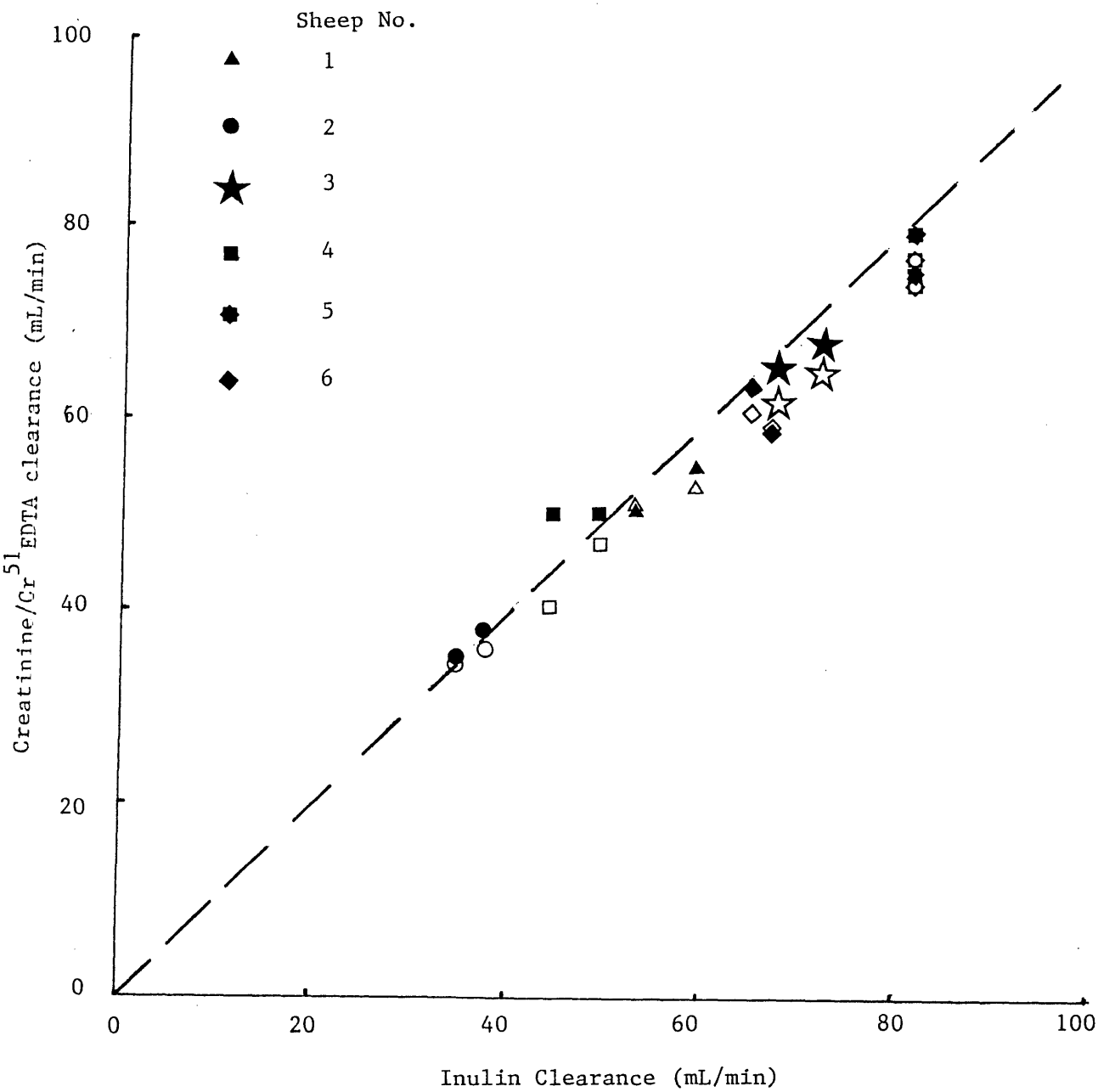

Fig. 1. Comparison of GFR markers. (Open symbols are $Cr^{51}EDTA$, Closed symbols are creatinine) Dashed line is y = x

APPENDIX 2

Extracellular Fluid Volume Determined from the Distribution Spaces of Inulin and $Cr^{51}EDTA$.

Extracellular fluid volume (ECV) is normally determined by serially sampling blood after a single intravenous injection or following the cessation of a continuous intravenous infusion of an appropriate marker. Mathematical analysis of the disappearance curve of the marker allows a reasonably accurate measure of the distribution space of that particular substance.

Cr^{51}EDTA and inulin are both considered to give distribution spaces equivalent to that of the ECV. However the true ECV at present cannot be measured and the tracers used only give an estimate of the true ECV.

Inulin space is regarded as the most accurate estimate of ECV. The analytical measurement of Cr^{51}EDTA is much less time consuming and more accurate than that of inulin.

The present study compares the distribution spaces of Cr^{51}EDTA and inulin in sheep.

Methods

20 mL of a solution containing 20 % inulin and 0.2 MBq/mL of Cr^{51}EDTA at approximately 38°C was injected into the jugular vein of 4 crossbred ewes. Blood samples were taken from the contralateral vein at approximately 3,7,20,35,50,65,80 and 100 min following injection. After centrifugation of the blood, plasma was removed and analysed for inulin by the method of Bacon & Bell (1948) and Cr^{51}EDTA using a gamma emission spectrometer (Gammamatic, Kontron, Sydney, Australia). Distribution volumes and glomerular filtration rates (GFR) were determined using the formulae given by Poulsen *et al* (1977).

Results

Results obtained are presented below.

Marker	$Cr^{51}EDTA$		*Inulin*	
	Space	*GFR*	*Space*	*GFR*
	9.58±0.67*	68.1±8.1	7.28±0.33*	72.1±6.4

*Difference is significant ($P < 0.01$).

The Cr^{51}EDTA space was 31.6 % larger than the inulin space, but a similar GFR was obtained with the two markers.

Discussion

Ladegaard-Pedersen & Engell (1972) found that the distribution space of Cr^{51}EDTA was 75 % and 95 % larger than that of inulin in nephrectomized man and dogs respectively. In their studies extrarenal clearance rate of the two compounds was similar. Stacy & Thorburn (1966) found that up to 2 % of Cr^{51}EDTA in plasma was protein bound and hence non-dialyzable. If Cr^{51}EDTA binds to red blood cells and tissue proteins more than inulin, then this may account for the greater volume of distribution found.

Comparison of ECV values, determined using different tracers can only be made if due allowance is made for the differences in distribution space of the markers used. Their comparison with the true ECV cannot be determined as even inulin space is only an estimate of the true ECV.

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APPENDIX 3

Simple Rapid Method of Rumen Cannulation

Previous methods of rumen cannulation of sheep have involved two stage operations where the rumen is sutured to the skin through dissected abdominal muscles and then several days later an incision is made through the skin and rumen wall to form a permanent fistula, to which a cannula may be fitted (Jarrett 1948).

Hecker (1969) adapted a method previously used for cattle (Balch & Cowie 1962) to sheep. This method involves the fitting of a metal bar clamp to a fold of rumen, which is exposed by laparotomy. After about 10 days the occluded fold sloughs away and a cannula may be inserted into the fistula.

These methods have been found to be both time consuming and somewhat traumatic to the animal. In addition the need for a relatively large incision in the rumen wall means that leakage of rumen contents around the cannula is a frequent problem.

Described below is a simple one stage operation requiring no suturing of the rumen wall using an incision smaller than the neck of the cannula ensuring no leakage of rumen contents.

Methods

A healthy sheep which has been fasted for 24 hours is anaesthetised with general anaesthetic (although the procedure could be carried out under local anaesthesia) and placed on its right side. The left side of the dorsal abdomen is clipped of wool and disinfected (Betadine Surgical Scrub, Faulding Medical Products, Lakemba, Australia.). After draping a vertical incision about 5 cm long is started about 3 cm posterior to the last rib and 3 cm ventral to the transverse process of the first lumbar vertebra.

The abdominal muscles and peritoneum are gently separated by blunt dissection. The rumen is withdrawn and a small incision (<2 cm) is made in a section free of any major blood vessels.

A rubber cannula (Starr Rubber Co. 52 Kincaid Ave, North Plymton, S.A.) is partially everted by pushing the flange through the neck of the cannula (see Fig 1A.). The folded cannula is then inserted through the incision and pushed inwardly until the neck of the cannula is tightly held by the tissue surrounding the incision. The cannula flange is then reverted to its initial shape.

Several swabs are placed in the cannula opening whilst the abdominal muscles (if necessary) and the skin is sutured closed. A PVC plate is placed over the cannula neck and a cut-off 20 mL syringe barrel and rubber stopper are held in the cannula neck with a cable tie. Two holes may be bored into the flanges of the syringe and wire tied over the stopper to eliminate the stopper becoming dislodged. A topical antibiotic and intramuscular penicillin as prophylactic measures are advised. After several days adhesions form between the rumen wall and the peritoneum. No peritonitis has been observed.

Discussion

To date this method has been used on 52 sheep for up to three years with excellent results. Occasional removal of the PVC plate and clipping of the wool is recommended to reduce tightness and the possibility of blowfly attack.

The operation can be completed in less than 20 minutes and the majority of animals eat their normal ration once the effects of anaesthesia have dissipated.

References

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Jarrett,I.G. (1948) *J.Coun.Sci.Ind.Res.Aust.* **21**, 311

Figure 1. **A**) *The rubber cannula (a) is everted as in (b), PVC plate (c) holds the cannula in position and a cable tie (d) holds the syringe barrel (e) and its stopper (f) in place.*

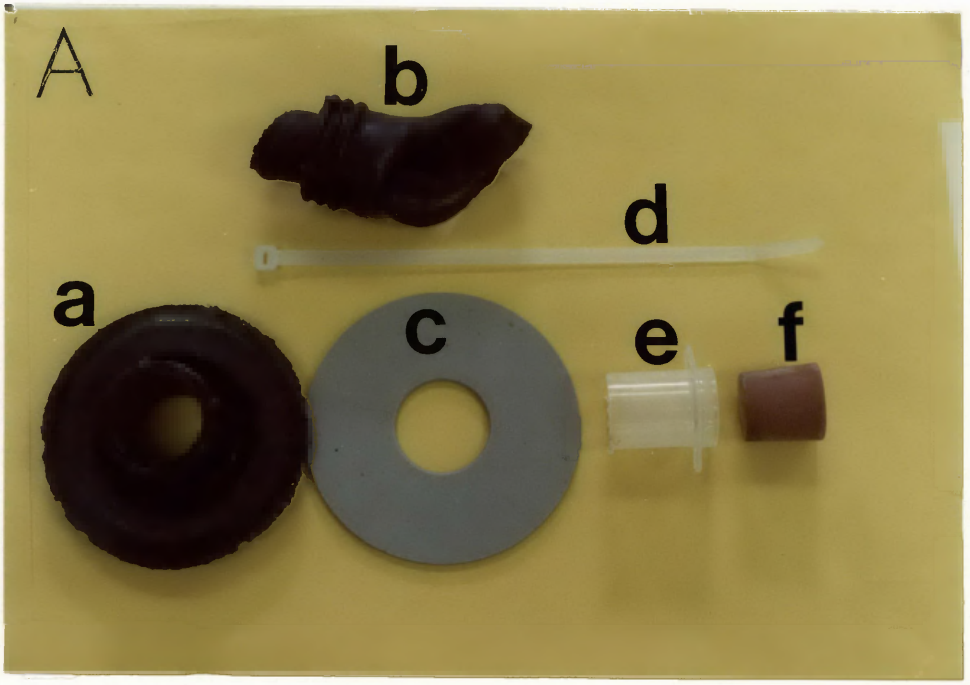
B) *The everted cannula ready for insertion through the incision in the rumen wall.*

C) *The everted cannula after insertion.*

D) *The cannula after reversion .*

E) *The cannula is held in place by the PVC plate.*

F) *A cannula 18 months after insertion.*

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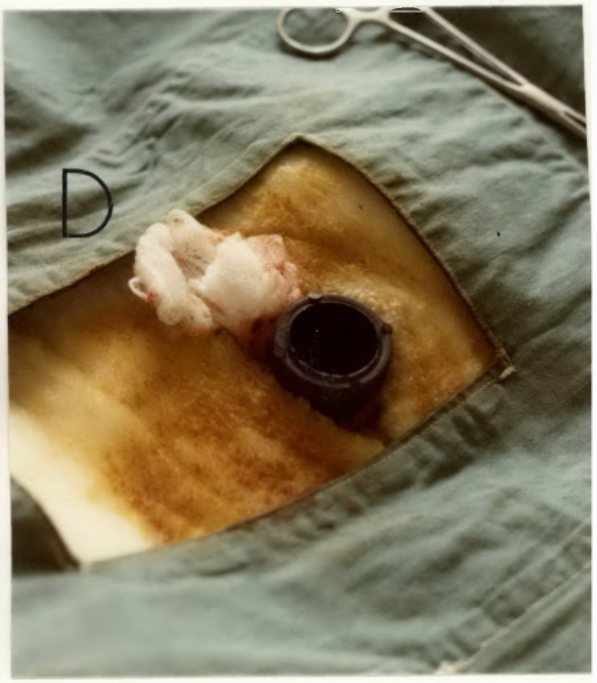
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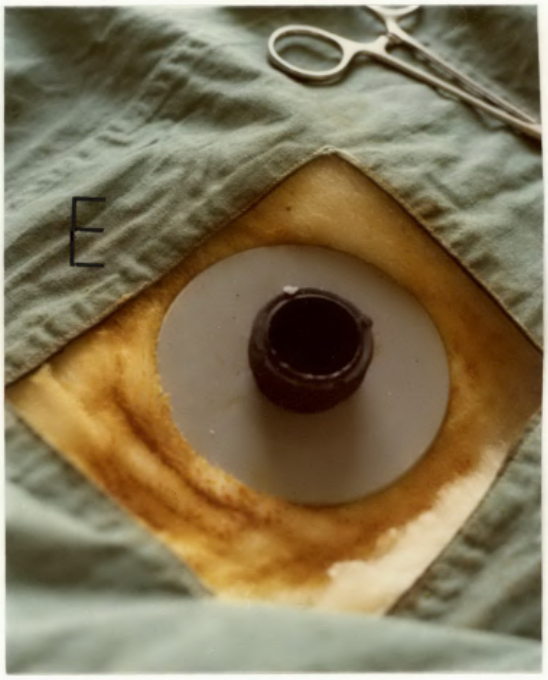
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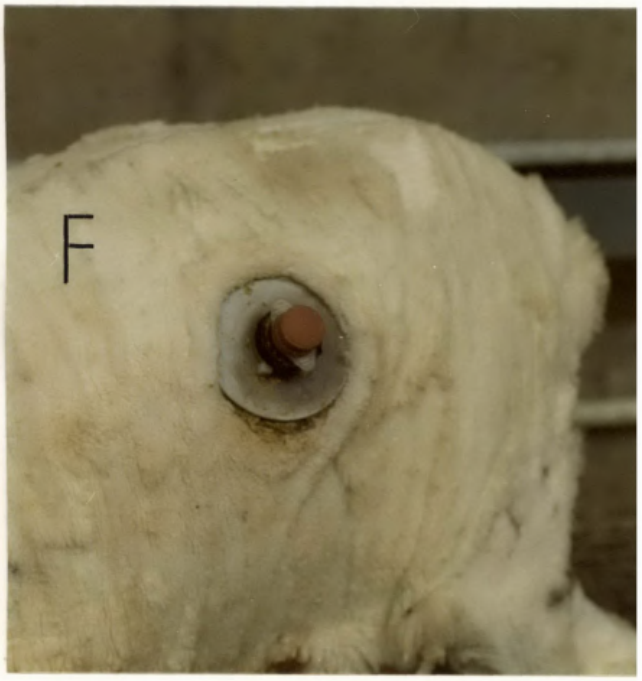
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