References on the Administration of Calcitonin and Calcitriol Using ALZET® Osmotic Pumps

1. Calcitonin

**Q7040:** S. P. Yoon, *et al.* Exogenous CGRP upregulates profibrogenic growth factors through PKC/JNK signaling pathway in kidney proximal tubular cells. Cell Biology and Toxicology 2018;34(4):251-262

**Agents:** Calcitonin gene-related peptide, SP600125, chelerythrine, CGRP8-37 receptor antagonist **Vehicle:** Saline; DMSO;

**Route:** IP; Kidney (cortical region); **Species:** Mice; **Pump:** Not Stated; **Duration:** Not Stated;

**ALZET Comments:** Dose (30 ng/kg/d); 10% DMSO used; Controls received mp w/ vehicle; animal info (Male C57BL/6 mice aged 8 to 10 weeks); CGRP is a 37-amino acid neuropeptide; enzyme inhibitor (SP600125 is a c-Jun Nterminal protein kinase (JNK), and chelerythrine is a specific protein kinase C (PKC) inhibitor); CGRP infused to the cortical region of the denervated kidney via an ALZET intrathecal catheter. The catheter was anchored to the obstructed ureter, and osmotic pump placed SC; some mice were given CGRP8-37 (120 μg/kg/d), SP600125 (30 mg/kg/d), chelerythrine (5 mg/kg/d) or vehicle (0.9% saline or 10% DMSO in 0.9% saline) via IP pump.

**Q7052:** I. Rossetti, *et al.* Calcitonin gene-related peptide decreases IL-1beta, IL-6 as well as Ym1, Arg1, CD163 expression in a brain tissue context-dependent manner while ameliorating experimental autoimmune encephalomyelitis. J Neuroimmunol 2018;323(94-104

**Agents:** Calcitonin gene-related peptide **Vehicle:** CSF, artificial; **Route:** CSF/CNS (intrathecal); **Species:** Mice; **Pump:** 2002; **Duration:** 2 weeks;

**ALZET Comments:** Controls received mp w/ vehicle; animal info (7-8 week old C57BL/6 female mice); peptides;


**Agents:** Calcitonin gene-related peptide antagonist (8-37) **Vehicle:** Saline; **Route:** SC; **Species:** Mice; **Pump:** Not Stated; **Duration:** 7 days;

**ALZET Comments:** animal info (Nine-week-old male C57BL/6 mice as wild type mice);


**Agents:** Neuroendocrine peptide aminoprocacalcitonin, anti- **Vehicle:** PBS; **Route:** Not Stated; **Species:** Mice (transgenic); **Pump:** 1004; **Duration:** 28 days;

**ALZET Comments:** Dose (500 μg/kg/day); animal info (Adult male C57BL/6 and APP/PS1 mice); neurodegenerative (Alzheimer Disease);

**Q4655:** J. G. Yan, *et al.* CALCITONIN PUMP IMPROVES NERVE REGENERATION AFTER TRANSECTION INJURY AND REPAIR. MUSCLE & NERVE 2015;51(229-234

**Agents:** Calcitonin **Vehicle:** Water, distilled sterile; **Route:** CSF/CNS (sciatic nerve); **Species:** Rat; **Pump:** 2006; **Duration:** 12 weeks;

**ALZET Comments:** Animal info (Sprague Dawley, 250-300g, 3 months old); half-life (p.233); long-term study; “To achieve a continuous and gradual mode of delivery, a mini-osmotic pump was implanted to deliver medication at a constant 0.15 ul/h” pg 233; “Calcitonin has short absorption and elimination half-lives of 10–15 minutes and 50–80 minutes, respectively; however, using an osmotic pump allows for gradual and prolonged release.” pg233; pg230 diagram of pump implantation;


**Agents:** Calcitonin gene-related peptide, alpha; calcitonin gene-related peptide (8-37), alpha **Vehicle:** PBS; **Route:** SC; **Species:** Mice; **Pump:** Not Stated; **Duration:** 7 days; 14 days;

**ALZET Comments:** Controls received mp w/ vehicle; animal info (CGRP -/-); immunology;
Agents: Calcitonin gene-related peptide (8-37) Vehicle: Not Stated; Route: CSF/CNS (intrathecal); Species: Rat; Pump: 2001; Duration: 7 days;
ALZET Comments: Controls received mp w/ vehicle; animal info (female, Lewis, 180-200g); behavioral testing (Von Frey filaments; Plantar test); immunology; peptides;

Agents: Antibody, anti-aminoprocalcitonin Vehicle: Saline; Route: IP; Species: Rat; Pump: 2001D; Duration: 18 hours;
ALZET Comments: Controls received mp w/ control antibody; animal info (male, Wistar, 280-300g); tissue perfusion (peritoneum); immunology; peptides; Catheter used to cannulate peritoneum;

Agents: Calcitonin gene-related peptide Vehicle: CSF, artificial; Route: CSF/CNS (intrathecal); Species: Mice; Pump: 2002; Duration: 14 days;
ALZET Comments: Controls received mp w/ vehicle; good methods (pg. 20); stress/adverse reaction: animal death (2/31 total) (see pg. 20); ALZET mouse intrathecal catheter used; encephalomyelitis model; Dose (50 pmol/hour CGRP);

Agents: Calcitonin gene related peptide (8-37) Vehicle: Not Stated; Route: Not Stated; Species: Mice; Pump: Not Stated; Duration: 6 weeks;
ALZET Comments: Animal info (male, C57BL6J, 22 months old); functionality of mp verified by serum levels; peptides; diabetes;

Agents: Parathormone (1-84), rat; calcitonin, rat Vehicle: Acetate buffer; Route: SC; Species: Rat; Pump: 2001; Duration: 22 days;
ALZET Comments: Animal info (female, Wistar, E0); replacement therapy (thyroidectomy); teratology;

Agents: Nifedipine; calcitonin Vehicle: Not Stated; Route: CSF/CNS (sciatic nerve); Species: Rat; Pump: 2006; Duration: 4 weeks;
ALZET Comments: Controls received mp w/ saline or sham only; animal info (3 month old, male, Sprague-Dawley 250-300g); functionality of mp verified by decrease in calcium levels; peptides; Picture of MP p56, Fig1A. MP Pump setup p56, Fig1B;

Agents: hADM22-52; calcitonin gene-related peptide (8-37) Vehicle: Saline; Route: SC; Species: Rat (pregnant); Pump: 1003D; Duration: 3 days;
ALZET Comments: Controls received mp w/ vehicle; animal info (Sprague Dawley, female, mature, 12-13 wks old); hADM22-52 is a ADM receptor blocker; hCALCA8-37 is a CALCA receptor antagonist

Agents: Calcitonin Vehicle: Not Stated; Route: Not Stated; Species: Rat; Pump: Not Stated; Duration: 4, 12, 24 hours;
ALZET Comments: Animal info (Brattleboro rats); functionality of mp verified by measuring urine output and concentration; ALZET osmotic pumps used to infuse calcitonin to increase trafficking of aquaporin 2 vesicles in collecting duct. Effect is short lived, despite continued calcitonin delivery; might need dose adjustment; diabetes;

**Agents:** Calcitonin gene related peptide; calcitonin gene related peptide (8-37)  
**Vehicle:** Not Stated;  
**Route:** SC;  
**Species:** Rat;  
**Pump:** Not Stated;  
**Duration:** 10 days;  
**ALZET Comments:** Controls received mp w/ saline; animal info (Sprague Dawley, male, 292-305 g, 67 days); CGRP (8-37) is a CGRP1 receptor antagonist; peptides


**Agents:** Calcitonin  
**Vehicle:** Saline;  
**Route:** SC;  
**Species:** Rat;  
**Pump:** Not Stated;  
**Duration:** 7 days;  
**ALZET Comments:** Controls received mp w/ vehicle; pumps removed and new pump implanted after 10 days; animal info (Brattleboro, VP-deficient, adult, male)

Q0079: B. Morte, et al. Thyroid Hormone Regulation of Gene Expression in the Developing Rat Fetal Cerebral Cortex: Prominent Role of the Ca\textsuperscript{2+}/Calmodulin-Dependent Protein Kinase IV Pathway. Endocrinology 2010;151(2):810-820

**Agents:** Parathyroid hormone (1-84); calcitonin, rat  
**Vehicle:** Acetate buffer;  
**Route:** SC;  
**Species:** Rat;  
**Pump:** 2001;  
**Duration:** Not Stated;  
**ALZET Comments:** Controls received mp w/ vehicle; animal info (female, Wistar, adult, 250-300 g); replacement therapy (parathyroidectomy)


**Agents:** Parathyroid hormone, rat (1-84); calcitonin, rat; thyroxine  
**Vehicle:** Acetate buffer;  
**Route:** SC;  
**Species:** Rat (pregnant);  
**Pump:** 2001;  
**Duration:** 4-8 days;  
**ALZET Comments:** Teratology; peptides; animal info (Female, Wistar, 250-300 g); replacement therapy (parathyroidectomy)


**Agents:** Calcitonin, salmon  
**Vehicle:** Not Stated;  
**Route:** SC;  
**Species:** Mice;  
**Pump:** 1002;  
**Duration:** 14 days;  
**ALZET Comments:** Controls received mp w/ vehicle; functionality of mp verified by residual volume; dose-response (Fig. 2); animal info (female, C57BL/6, 6 wks old)


**Agents:** Calcitonin gene-related peptide (8-37)  
**Vehicle:** Not Stated;  
**Route:** SC;  
**Species:** Mice;  
**Pump:** Not Stated;  
**Duration:** 3, 7 days;  
**ALZET Comments:** Controls received mp w/ saline; animal info (male, C57BL/6, wt, a-CGRP, -/-, 20-25g., Bile duct ligation); peptides


**Agents:** Adrenomedullin, human; calcitonin gene-related peptide (8-37)  
**Vehicle:** Saline;  
**Route:** SC;  
**Species:** Rat;  
**Pump:** Not Stated;  
**Duration:** 7, 14 days;  
**ALZET Comments:** Controls received mp w/ vehicle; cardiovascular; peptides; ischemia (cerebral); animal info (male, Sprague-Dawley 220 g);"continuous and longterm infusion of AM starting at day 1 after I/; MCAO


**Agents:** Parathyroid hormone, bovine 1-34; calcitonin, salmon  
**Vehicle:** Saline, physiological; cysteine hydrochloride;  
**Route:** SC;  
**Species:** Mice;  
**Pump:** 1003D;  
**Duration:** 12 hours;  
**ALZET Comments:** Controls received mp w/ vehicle

**Agents:** Calcitonin gene-related peptide (8-37)  **Vehicle:** Not Stated;  **Route:** IP;  **Species:** Rat;  **Pump:** Not Stated;  **Duration:** Not Stated;

**ALZET Comments:** Cardiovascular; peptides; ischemia (cardiac)


**Agents:** Calcitonin gene-related peptide (1-8), alpha-Rat; Calcitonin gene-related peptide (1-13), alpha-Rat; Calcitonin gene-related peptide (1-14), alpha-Rat  **Vehicle:** Saline; Bacitracin;  **Route:** IV (jugular);  **Species:** Rat;  **Pump:** 2002;  **Duration:** 14 days;

**ALZET Comments:** Controls received mp w/ vehicle; antihypertensive; peptides; N-terminal rat alpha-CGRP (calcitonin gene-related peptide) was dissolved in saline with 1 mg/ml of Bacitracin & infused in either a 2002 (lower doses) or a 2ML2 (higher doses)


**Agents:** Calcitonin, salmon  **Vehicle:** Acetic acid; Sodium acetic; Sodium chloride; water, distilled;  **Route:** SC;  **Species:** Rat;

**Pump:** 2ML1;  **Duration:** 7 days; 24 hours;

**ALZET Comments:** Functionality of mp verified by calcitonin plasma levels


**Agents:** Calcitonin gene-related peptide (8-37)  **Vehicle:** Not Stated;  **Route:** IP;  **Species:** Mice;  **Pump:** 1002;  **Duration:** 28 days;

**ALZET Comments:** Paper incorrectly states the release rate and duration of Model 1002 which delivers at 0.25 ul/hr for 14 days; CGRP is an adrenomedullin receptor antagonist; peptides


**Agents:** Calcitonin gene-related peptide  **Vehicle:** Saline;  **Route:** SC;  **Species:** Rat;  **Pump:** 2ML1;  **Duration:** 4 days;

**ALZET Comments:** Blood pressure taken; peptides


**Agents:** Calcitonin gene-related peptide (8-37)  **Vehicle:** saline;  **Route:** SC;  **Species:** Rat (pregnant);  **Pump:** 2ML2;  **Duration:** Not Stated;

**ALZET Comments:** controls received mp with saline; stability verified by mass spectrometry for 14 days (p. 625); peptides; agent is a potent vasodilator


**Agents:** Parathyroid hormone, human 1-34; calcitonin, salmon  **Vehicle:** Acetate buffer; saline;  **Route:** SC;  **Species:** Rat;  **Pump:** 2004;  **Duration:** 28 days;

**ALZET Comments:** Controls received mp w/ vehicle or sham ovx + mp w/ vehicle; functionality of mp verified by serum levels; replacement therapy (ovariectomy); comparison of daily PTH sc injections vs. mp; peptides

P4895: D. Q. Chu, et al. The calcitonin gene-related peptide (CGRP) antagonist CGRP(8-37) blocks vasodilatation in inflamed rat skin: involvement of adrenomedullin in addition to CGRP. Neuroscience Letters 2001;310(169-172

**Agents:** Calcitonin gene-related peptide (8-37)  **Vehicle:** Saline; BSA;  **Route:** SC;  **Species:** Rat;  **Pump:** Not Stated;  **Duration:** Not Stated;

**ALZET Comments:** controls received mp w/ vehicle; cardiovascular; peptides; CGRP (8-37) is a calcitonin gene related peptide antagonist.  BSA was .01% in saline to prevent adsorption.
**Agents:** Calcitonin gene-related peptide  
**Vehicle:** Saline;  
**Route:** Intramuscular (soleus);  
**Species:** Rat;  
**Pump:** 2002;  
**Duration:** Not Stated;  
**ALZET Comments:** controls received mp w/ vehicle; tissue perfusion (soleus muscle extrajunctional surface); peptides

**Agents:** Tetrodotoxin; potassium chloride; calcitonin gene-related peptide  
**Vehicle:** Saline; Dye, methylene blue;  
**Route:** Intramuscular (soleus);  
**Species:** Rat;  
**Pump:** 2001; 2002;  
**Duration:** 3 - 8 days;  
**ALZET Comments:** Controls received mp w/ vehicle; tissue perfusion (soleus muscle); detailed methods for catheter construction and surgical procedures (p. 114 - 115); Lynch coil technique used; pump filled w/ saline and agents loaded in modified PE-100 tubing; solutions separated by oil drop; pumps w/ various flow rates were tested but model 2002 was chosen because its small flow rate permits to avoid excessive fluid accumulation on muscle surface; diagram of pump-catheter assembly and location (p. 114); peptides

**Agents:** L-NAME; Calcitonin gene-related peptide  
**Vehicle:** Saline, sterile;  
**Route:** SC;  
**Species:** Rat (pregnant);  
**Pump:** 2ML1;  
**Duration:** 1 week;  
**ALZET Comments:** controls received mp w/ vehicle; peptides; CGRP and L-NAME or vehicle delivered from separate pumps concomitantly.

**Agents:** Parathyroid hormone; Calcitonin; Vitamin D, 1,25-dihydroxy  
**Vehicle:** NaCl; HCl; Cysteine; Saline, isotonic;  
**Route:** SC;  
**Species:** Rat;  
**Pump:** 2002;  
**Duration:** 13 days;  
**ALZET Comments:** controls received sham tubing; functionality of mp verified by hormone assays; replacement therapy (thyroidectomy, thyroparathyroidectomy); dose-response; stress/adverse reaction: high doses led to animal death; peptides; agents given singly and in combination

**Agents:** Calcitonin gene-related peptide (8-37)  
**Vehicle:** Saline;  
**Route:** SC;  
**Species:** Rat;  
**Pump:** 2001;  
**Duration:** 7 days;  
**ALZET Comments:** controls received mp w/ vehicle; CGRP (8-37) is a calcitonin receptor antagonist; peptides

**Agents:** Calcitonin gene-related peptide  
**Vehicle:** Not Stated;  
**Route:** scrotal;  
**Species:** pig (neonate);  
**Pump:** 2002;  
**Duration:** 2 weeks;  
**ALZET Comments:** controls received mp w/PBS; tissue perfusion (undescended testes); replacement therapy (cryptorchidism); dose-response; peptides; "Injection of excess CGRP into the scrotum may delay descent...the pig model has overcome some of these difficulties as the o

**Agents:** L-NAME; Calcitonin gene-related peptide  
**Vehicle:** Saline, sterile;  
**Route:** SC;  
**Species:** Rat (pregnant);  
**Pump:** 2ML2;  
**Duration:** no duration posted;  
**ALZET Comments:** controls received mp w/ saline; peptides; agents given singly and concomitantly in the same pump; cardiovascular
Agents: Calcitonin, salmon Vehicle: Saline; Benzyl alcohol; Gelatin; Route: Not Stated; Species: Rat; Pump: Not Stated; Duration: 6 weeks;
ALZET Comments: controls received mp w/vehicle; functionality of mp verified by calcitonin plasma levels by radioimmunoassay; comparison of s.c. injections vs. mp; pumps replaced after 3 weeks; peptides

Agents: Calcitonin, salmon Vehicle: Aminocaproic acid; HCl; Route: Not Stated; Species: Rat; Pump: 2ML1; Duration: 4 days;
ALZET Comments: controls received mp with vehicle

Agents: Calcitonin, human Vehicle: Not Stated; Route: intrauterine; SC; Species: Rat; Pump: 2001; Duration: 14 days;
ALZET Comments: tissue perfusion (uterus); dose-response (graph, p.832); comparison of polyurethane matrices vs mp

Agents: Calcitonin analog; Calcitonin, synthetic salmon Vehicle: Gelatin; Saline; Route: SC; Species: mice; Pump: 2001; Duration: 48 hours;
ALZET Comments: pumps were reimplanted in a second animal; analog is RG-12851

Agents: Parathyroid hormone, bovine; calcitonin Vehicle: Saline, cysteine hydrochloride; Route: SC; Species: mice; Pump: 2001; Duration: 7 days;
ALZET Comments: Controls received mp w/ vehicle; animal info (4-5 wk old); Dose: calcitonin (2 IU/ul), PTH (1 IU/ul);

Agents: Calcitonin, Parathyroid hormone Vehicle: Saline, Physiologic; Route: SC; Species: Mice; Pump: 2001; Duration: 3, 6, 12, 18, 24, 48 and 72 hrs;
ALZET Comments: Controls received mp w/ vehicle; dose-response (0.1, 0.375, 0.5, 0.75, 1.0, or 1.25 IU/hr); Multiple pumps per animal (2); Animals receiving both hormones were implanted through separate skin incisions with two pumps. “To control the uniformity of the applied stimulus, we used surgically implantable Alzet osmotic minipumps... for continuous subcutaneous infusion of calcitonin.” pg 15; Dose (0.5 IU/hr);

Agents: Calcitonin, salmon Vehicle: Aminocaproic acid; Route: SC; Species: Rat; Pump: 2002; Duration: 1, 4, 7 days;
ALZET Comments: controls received mp w/vehicle; dose-response; mp primed w/saline 12 hours before implantation; stability; peptides

Agents: Calcitonin Vehicle: Not Stated; Route: SC; Species: Rat; Pump: 2002; Duration: 6-12 days;
ALZET Comments: controls received sham implantation; replacement therapy (thyroparathyroidectomy); peptides

Agents: Calcitonin, salmon  
Vehicle: Albumin, bovine serum; PBS  
Route: SC  
Species: Rat (pregnant)  
Pump: Not Stated  
Duration: 5 days  
**ALZET Comments**: pump model not stated; replacement therapy (thyroparathyroidectomy); peptides

Agents: Calcitonin; Parathyroid hormone, bovine  
Vehicle: Cysteine HCl; HCl; Saline  
Route: SC  
Species: Rat  
Pump: Not Stated  
Duration: 7 days  
**ALZET Comments**: organ replacement therapy (thyroparathyroidectomy); peptides

Agents: Calcitonin, salmon  
Vehicle: Not Stated  
Route: SC  
Species: Rat  
Pump: Not Stated  
Duration: 1-14 days  
**ALZET Comments**: comparison of human vs. animal data; intermittent vs. mp infusion; peptides

2. Calcitriol

Agents: Calcitriol  
Vehicle: Saline  
Route: SC  
Species: Rat  
Pump: 2004  
Duration: 4 week  
**ALZET Comments**: Dose (6 ng/day); 0.9% NaCl used; Controls received mp w/ vehicle; animal info (Female and male Wistar rats); Blood pressure measured via tail cuff method;120.6 mmHg - 132.9 mmHg;cardiovascular;

Agents: Calcitriol  
Vehicle: PEG  
Route: SC  
Species: Rat  
Pump: 2002  
Duration: 4 weeks  
**ALZET Comments**: Animal info (male, Wistar-Kyoto, 200-230g); functionality of mp verified by residual volume; pumps replaced every 2 weeks; post op. care (penicillin SC injection 5000 U/kg); bp measured using tail cuff;

Agents: Calcitriol  
Vehicle: Not Stated  
Route: SC  
Species: Rat  
Pump: Not Stated  
Duration: 6 weeks  
**ALZET Comments**: Controls received mp w/ vehicle; animal info (male, Spontaneously hypertensive rats, stroke-prone, 8 weeks old); cardiovascular; long-term study;

Agents: Calcitriol  
Vehicle: Ethanol; propylene glycol  
Route: SC  
Species: Rat  
Pump: 2004  
Duration: Not Stated  
**ALZET Comments**: Control animals received mp w/ vehicle; animal info (Sprague Dawley, P9); 50% ethanol used; 50% propylene glycol used

Agents: Calcitriol  
Vehicle: DMSO  
Route: SC  
Species: Mice  
Pump: Not Stated  
Duration: 6 weeks  
**ALZET Comments**: Controls received mp w/ vehicle; animal info (Sprague-Dawley, 8 wks old)

Agents: Aldosterone; calcitriol  
Vehicle: Not Stated  
Route: SC  
Species: Rat  
Pump: 2004; 2ML4  
Duration: 4 weeks  
**ALZET Comments**: Controls received no treatment; replacement therapy (uninephrectomy); cardiovascular; animal info (male, Sprague-Dawley, 8 wks old)

Agents: Parathyroid hormone (1-34); Calcitriol; Vehicle: Cysteine; Propylene glycol; Saline; Route: Not Stated; Species: Rat; Pump: 2002; Duration: 14 days;

ALZET Comments: controls received mp w/vehicle; replacement therapy (parathyroidectomy; nephrectomy); peptides


Agents: Calcitriol; Parathyroid hormone (1-34) Vehicle: Cysteine; HCl; Saline; Route: Not Stated; Species: rabbit; Pump: Not Stated; Duration: 7 days;

ALZET Comments: functionality of mp verified by serum levels; peptides


Agents: Oxacalcitriol, 22-; Vitamin D3, 24,25-dihydroxy-; Vitamin D3, 1,25-dihydroxy- Vehicle: Propylene glycol; Route: SC; Species: Rat; Pump: 2002; Duration: 14 days;

ALZET Comments: no comment posted


Agents: Oxacalcitriol, 22-; Vitamin D3, 24,25-dihydroxy- Vehicle: Propylene glycol; Route: SC; Species: Rat; Pump: Not Stated; Duration: 3 days;

ALZET Comments: functionality of mp verified by serum levels; toxicology


Agents: Calcitriol Vehicle: Propylene glycol; Route: SC; Species: rabbit; Pump: 2002; Duration: 7, 28 days;

ALZET Comments: functionality of mp verified by plasma levels; toxicology


Agents: Calcitriol Vehicle: Propylene glycol; Route: SC; Species: rabbit; Pump: Not Stated; Duration: 2 weeks;

ALZET Comments: dose-response; functionality of mp verified by serum levels


Agents: Calcitriol; Vitamin D3, 1,25-dihydroxy- Vehicle: Ethanol; Propylene glycol; Route: SC; Species: Rat; Pump: 2001; Duration: 1 week;

ALZET Comments: functionality of mp verified by measuring residual volume


Agents: Calcitriol Vehicle: Propylene glycol; Route: SC; Species: rabbit; Pump: 2ML1; 2ML4; Duration: 7, 28 days;

ALZET Comments: functionality of mp verified by serum calcitriol levels; dose-response (graphs); 3 doses of calcitriol infused


Agents: Calcitriol Vehicle: Propylene glycol; Route: SC; Species: Rat; Pump: 2002; Duration: 40 days;

ALZET Comments: comparison of calcitriol or OHD2 po 3x/wk vs. mp infusion; intermittent oral dosing; mp replaced every 2 weeks; no stress implied by the normal growth of the animals