

# TECHNIQUES FOR THE SERIAL COLLECTION OF BLOOD SAMPLES AND INSPECTION OF GONADS IN FREE-LIVING ALBATROSSES

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**ABSTRACT.** Methods for obtaining serial blood samples (daily and three-hourly) and for examining and measuring reproductive organs in free-living albatrosses are described. Simple cannulation techniques were highly efficient and over 800 plasma samples were obtained in a single season with very little disruption of breeding activity. The use of Ketamine and Saffan as anaesthetics is evaluated, the former being easier to administer, the latter giving quicker and better recovery. Laparoscopy techniques for gonad examination are described; only 10% of 160 birds treated did not immediately resume normal breeding activities and all were seen later in the season.

## INTRODUCTION

At Bird Island, South Georgia, (54°S, 38°W) three species of *Diomedea* albatrosses (the wandering albatross *D. exulans*, the black-browed albatross *D. melanophris* and the grey-headed albatross *D. chrysostoma*) breed in large numbers in separate and accessible colonies. Extensive information is available on their ecology at this site (Croxall and Ricketts, 1983; Prince, 1980, in press; Prince and others, 1981; Tickell, 1968), hence the suitability of the species for endocrinological and general physiological research in the field.

The present study was concerned with the endocrine control of the timing and frequency of breeding, which involved measuring changes in the secretion of reproductive hormones throughout albatross breeding cycles. This required the frequent taking of blood samples from the same individual and inspection of the state of reproductive organs. It was necessary to devise ways of doing this while minimizing disturbance to the birds and ensuring that they continued to incubate, brood or rear chicks successfully. This paper describes the techniques developed.

## MATERIALS AND METHODS

### *Blood sampling*

*Single sampling.* Blood was drawn into a 5-ml heparinized syringe (Sarstedt Monovette) from the intertarsal vein using a 20 Gauge by 26 mm needle. The needle was bent by about 70° to facilitate entry into the vein. The smaller species (*D. melanophris* and *D. chrysostoma*) could be readily sampled by one person (Fig. 1), but with *D. exulans* assistance in restraining the bird was beneficial (Fig. 2). Over 800 samples were taken in these ways and the methods proved to be reliable and efficient. If the bird was captured while active (i.e. not incubating), or if the weather was warm (i.e. direct radiation from the sun) the intertarsal vein was very dilated and the blood sample took only about one minute to collect. However, if the bird was incubating or had been inactive for many hours in cold conditions, the constricted vein required warming in the hand for about five minutes in order to collect the sample efficiently.



Fig. 1. Handling position for a single operator to take a blood sample from *D. exulans*, *D. melanophris* and *D. chrysostoma*.

*Repeated blood collection by cannulation.* A 20 Gauge by 32 mm intravenous catheter placement unit (Teflon/Radiopaque) was inserted into the intertarsal vein and an intermittent injection cap inserted onto the hub of the catheter (Fig. 3). Using adhesive tape a 'butterfly' attachment was made to secure the cannula unit to the leg (Fig. 4). Blood was then drawn into the unheparinized syringe, via a 20 Gauge needle through the injection cap and, after the sample had been taken, the interior of the unit was filled with heparin saline ( $1000 \text{ units ml}^{-1}$ ) to prevent the catheter lumen filling with clotted blood. The cannula was left in place for up to 26 consecutive days of daily collection and on other individuals was used over 36 hours for three-hourly sampling. On a few occasions swelling of the tissues around the tarsus reduced the function of the unit (e.g. by blocking the lumen), but in no instance did the cannula appear to cause irritation and albatrosses never attempted to remove them. On removal of the cannula a small absorbent gauze was held to the tarsus and attached with adhesive tape to reduce the risk of infection and external bleeding.

*Processing for storage.* The pre-heparinized syringes were such that the plunger and the needle could be removed after taking the sample, and the barrel centrifuged directly. Hence for routine collection no decanting of blood was required in the field. The samples were stored either in a water-tight container at ambient temperature ( $1-4^{\circ}\text{C}$ ) or in a vacuum flask containing crushed ice. All samples were centrifuged as soon as possible, always within four hours of collection, at 2500 rpm for 15 min on a



Fig. 2. Handling position for restraining *D. exulans* both to take blood samples and to deploy a cannula.

MSE student centrifuge (powered by mains 250 V, 50 Hz or by a portable Honda 1000 petrol generator). The plasma was drawn off, 0.1% v/v sodium azide added as a preservative, then stored in a sealed tube at  $-20^{\circ}\text{C}$ .

#### *Anaesthetics and laparoscopy*

*Anaesthetic and recovery from anaesthesia.* Ketamine (Borner Lambert: Vetalar; active components Ketamine  $100\text{ mg ml}^{-1}$ ) and Saffan (Glaxo: active components alphaxalone  $9\text{ mg ml}^{-1}$  and alphadolone acetate  $3\text{ mg ml}^{-1}$ ) were the anaesthetics used, being administered by intramuscular (IM) and intravenous (IV) routes respectively. Table I summarizes the dosages that gave 10–15 min good surgical anaesthesia with an acceptable recovery time. IV Ketamine trials showed that dilution of the drug would be necessary if the duration of anaesthesia was to be kept short. This, accompanied by the long and traumatic recovery (characteristic of

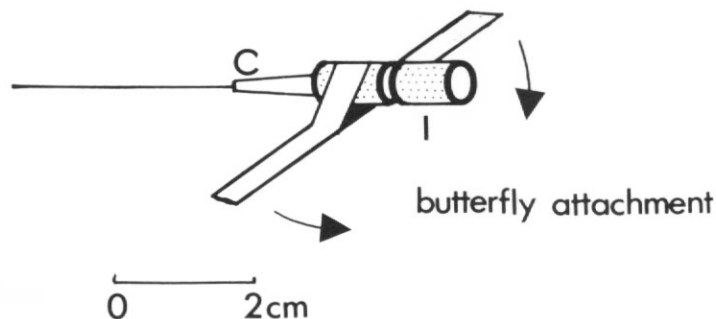


Fig. 3. Cannula (C) with intermittent injection cap (I) with attached 'butterfly' strap.



Fig. 4. Cannula in position with the intertarsal vein and the complete unit attached to the tarsus.

Ketamine) and the higher risks of infection (if diluted from manufacturer's solution) increased the complexity of field operations. Its administration was therefore restricted to IM routes. Saffan gave excellent anaesthesia and relaxation. Ketamine gave excellent anaesthesia with moderately poor relaxation; supplementary doses were occasionally required for *D. exulans* but never for *D. melanophris* and *D. chrysostoma*. When Ketamine was used, full consciousness was regained after one to one and a half hours by birds of all species. The birds needed to be restrained in a bag for this period if they were to resume incubation or brooding. For birds in the chick-rearing period of their cycle it was found better to leave them in deep tussock grass *Poa flabellata* to prevent the risk of over-heating or vomiting in the confines of the bag. Nearly 90% of 85 birds treated resumed their normal behaviour afterwards. The remaining 10% failed to incubate or brood satisfactorily immediately after treatment and many eventually deserted their nests. All were seen in the colony at least once later in the season.

Table I. Doses of Ketamine and Saffan required to give 10–15 min good surgical anaesthesia in *Diomedea* albatrosses. IM = intramuscular; IV = intravenous.

Species	Ketamine (IM)		Saffan (IV)	
	Main (mg kg <sup>-1</sup> )	Dose Supplementary (mg)	Main (mg kg <sup>-1</sup> )	Dose Supplementary (mg)
<i>D. exulans</i>	8	9 (IV)	3.6	18
<i>D. melanophris</i>	8	–	3.4	2.5
<i>D. chrysostoma</i>	8	–	3.4	2.5

Using Saffan, recovery was usually within 20–40 min and there was little or no effect on the birds' ability to resume incubating, brooding or feeding young immediately. Generally, the use of Saffan was restricted to *D. melanophris* and *D. chrysostoma* since the volume of drug needed was small enough to be given quickly, a single injection sufficing for the whole operation. Because of the large size of *D. exulans*, adequate doses (3–4 ml) were difficult to give in a single IV injection and supplementary doses were often required. Ketamine was preferable for this species despite the slower recovery rate and the risk that the bird would be more reluctant to resume normal behaviour. When it was essential to guarantee quick and complete recovery Saffan was used; either a cannula was inserted to provide a very easy

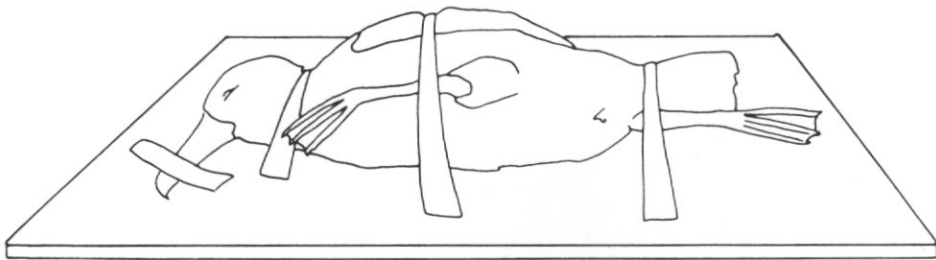


Fig. 5. Laparotomy board with four adjustable straps.

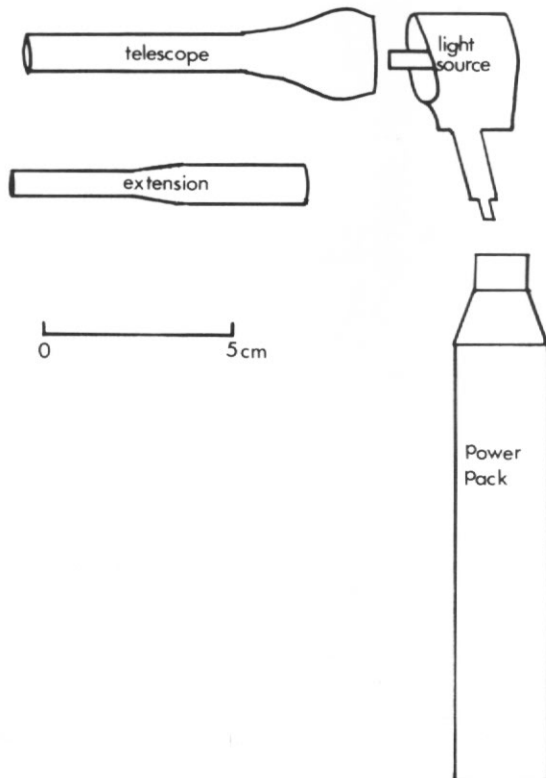


Fig. 6. Laparoscope: a modified veterinary otoscope.

method of giving supplementary doses or a single injection was used when the operation could be completed in 6–7 min duration.

*Laparotomy and laparoscopy.* After anaesthesia the bird was restrained for laparotomy on a board with adjustable straps, with its left side uppermost (Fig. 5). The area of down between the pelvis, last rib and edge of the brood patch was removed and the skin cleaned with surgical spirit. A skin incision 1 cm long was made between the pelvis and the last rib and 1 cm dorsal to the characteristic line of body feathers bordering the brood patch. (This feather tract is also visible in birds lacking developed brood patches.) The body wall musculature was punctured with round nosed scissors through, but slightly dorsal to, the incision and the fibres teased apart. The laparoscope, a veterinary otoscope with enlarged extension tube (Fig. 6), was sterilized (with liquid antiseptic) and passed into the abdominal cavity viewing in a

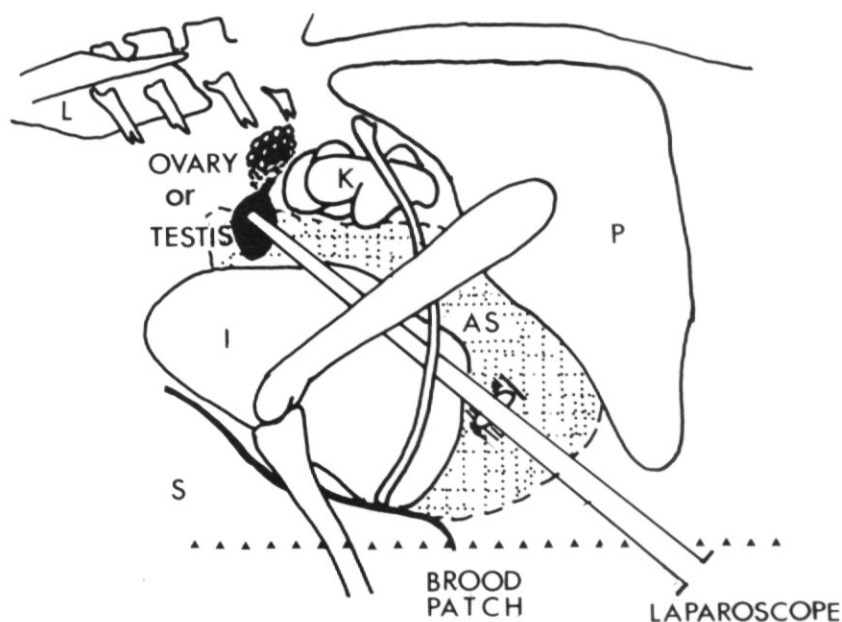


Fig. 7. Diagrammatic representation of the gonad location and position of the skin incision. L: lung, P: pelvis, K: kidney, I: intestine, AS: posterior abdominal air sac, S: sternum.

dorsal and anterior direction. It was usually possible to avoid damaging the abdominal air sac in the process. The gonads were situated on the dorsal side of the cavity and anterior to the large, red, lobulated kidney (Fig. 7). Testes were sometimes hidden in folds of the small intestine. After removing the laparoscope the skin wound was sutured with 2/0 USP cat gut. Providing the body wall musculature had been perforated slightly dorsal to the incision, then only the skin was sutured. Procaine penicillin  $30 \text{ mg kg}^{-1}$  and benthamine penicillin  $28 \text{ mg kg}^{-1}$  were given as antibiotics before the birds were released. The laparoscope offered good visibility and the size of the testis and the diameter of most ovary follicles were accurately estimated. Ovary follicles from females at the copulatory phase of their breeding cycle were too large for easy examination with this instrument.

## DISCUSSION

Blood sampling and laparotomies have been employed in field studies of small passerine birds (Wingfield and others, 1978; Dawson, 1982; Silverin, 1983), but the albatrosses in this study posed new problems that required specific techniques. No birds were to be killed, so blood had to be collected regularly and efficiently via vein puncture. Because of the birds' large size and thick down and the prevailing inclement weather, laparotomies often take 5–10 min and the bird therefore needs to be properly anaesthetized and restrained. Thus there was a need to use an anaesthetic and a dose that would immobilize the bird but allow a rapid recovery so that incubation, brooding or chick-rearing could be resumed as soon as possible after the operation. Albatrosses needed one-tenth of the dose (per unit weight) of Ketamine or Saffan recommended for cats and primates. If larger doses were given, the duration of anaesthesia increased and the period of time before the bird could be safely released was several hours longer. Tolerance to overdoses was not investigated.

With all birds laparotomized, full surgical recovery was within 14 days. The skin wound had fully healed and down was re-growing over the exposed skin. The naked skin had never been fully exposed since no feathers other than down were removed at any stage. Within 24 hours about 80% of the birds were seen in the colony. In birds previously incubating or brooding over 95% of birds resumed normal behaviour.

An advantage of the large size of the albatross is that repeated blood samples can be taken from the same individual, both daily and at three hourly intervals over 36 hours. The development of the cannulation technique was essential to avoid repeated vein puncture and removal from the nest. In this respect the cannula described was very simple and efficient to use.

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