PRO EXPERIMENTIS

A bio-implantable solid-state camera for long-term video-recording of abdominal organs in situ[1]

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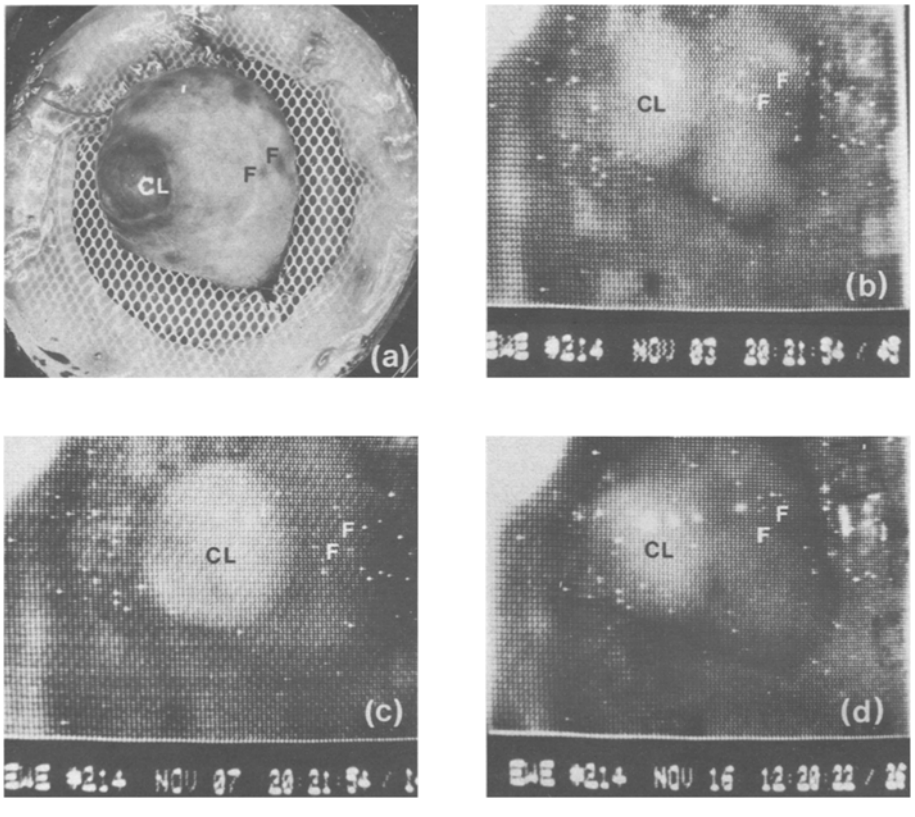
Summary. A new solid-state camera was constructed and implanted in sheep. It permits continuous or selective video-recording of the ovary in the unrestrained animal during an entire oestrous cycle (16 days).

Arrays of light-sensitive silicon sensors interconnected to form charge-coupled devices (CCDs) were first demonstrated as area image sensors in 1973[2]. We have built a CCD-based camera, implanted it surgically in ewes, and obtained video-recorded images of the ovary surface for periods of up to 14 days without restraining the animals. Compactness and light weight as well as low power and voltage demands, reliability, and excellent light sensitivity of CCD cameras combine to offer many advantages over traditional (open surgery and laparoscopy) techniques for the observation of abdominal organs in situ. The CCD is a spatially organized, light-sensitive semiconductor structure overlaid with a conducting electrode grid which allows the transport of a minority charge by moving its storage site. The charge created by light energy impinging on photosites is first accumulated, and then is passed sequentially under the electrodes to an output that converts the arriving charge to a voltage proportional to the integrated light energy transduced at the photosite. Object images formed in this manner, by a CCD camera with a lens system focussed on the object, can be stored on video-tape for later study.

The need for such a camera arises in reproduction research from problems encountered in the ongoing search for better indicators and predictors of ovulation, which can provide alternatives in human fertility regulation, and which can improve efficiency in livestock breeding. Whereas there are several peripheral variables (e.g electric potential, temperature, properties of body fluids) and ovarian variables (e.g intra-ovarian pressure, transmembrane potential, electric impedance) which can be followed by means of transducers and investigated as to their suitability as ovulation indicators, there is the fundamental problem of how to verify whether a transduced variable produces a reliable ovulation signal. Claims that it does should preferably be supported by direct and concurrent visual evidence of ovulation to establish the precise chronological relationship. However, such investigations are hampered by the lack of ready visual access to the ovary, for the ovary is completely and closely surrounded by abdominal viscera which both obscure its surface from view and interfere with transducer attachments. To resolve these problems, we[3] designed ovary-isolating chambers for implantation in the abdominal cavity of ewes. Throughout one or several oestrus cycles of 15–16 days, the chambers provided frequent, random, and unobstructed visual access to the ovary. We now report on a further development.

The present chamber is made of acrylic tubing (10 cm long, 5 cm in outside diameter) and has 2 compartments: one is designed to enclose an ovary in situ, the other is hermetically sealed and houses a CCD with lens system



Photograph of a sheep ovary just before camera implantation (*a*), and video-recorded views (*b–d*) obtained by an implanted solid-state (CCD) camera and photographed from the television monitor screen. The selected images shown were recorded: *b* 1 day, *c* 5 days, *d* 14 days after implantation. Corpus luteum (CL) and follicles (F) have gray-scale intensities unrelated to their visual appearanc under normal illumination because of the IR absorption properties of arterial and venous blood and the high sensitivity of silicon sensors (CCDs) in the near IR. Scale: height of letters (CL, F) represents 2 mm.

and light source. Total weight, including drainage tubes and cables, is 500 g. Maximum power dissipated by incandescent light source and camera is 1.2 W, a level which produces no noticeable thermal effects on abdominal organs. Flexible ribbon cables, which pass through an incision in the flank of the animal, connect the camera to the processing circuitry in a pack mounted on the back of the animal, allowing the ewes full freedom of movement in their pens. The output image is annotated, monitored, and stored on time-lapse recorded video-tape using modified commercial television equipment.

Chambers were fixed in the abdominal cavity of 3 ewes for periods of up to 14 days before removing the chamber for observation. During this time, the animals behaved normally, moved freely within their pens, and followed their usual sleeping and eating patterns. Small amounts of clear serous fluid accumulated inside the heparinized chamber, and 5–10 ml were drained daily. Some oedema developed and was apparently responsible in part for an increase in ovarian volume evident 5 days after placement. At the time of removal after 14 days, the ovary was somewhat reddened and swollen, and the chamber was walled off by loose omental adhesions. The photograph and the selected video-taped images in the figure show an ovary on the day of implantation, and 1, 5, and 14 days later.

The video-taped pictures were obtained with a CCD array of 100×100 photosites which imaged an object field of 36×48 mm. Arrays having a greater number of photosites are now available and will add considerable detail to images, but the array used is adequate to demonstrate the effectiveness of CCD cameras in monitoring an abdominal organ.

Before this development, continuous observation of abdominal organs was not feasible without applying considerable restraint and usually anaesthesia to the animal; long-term continuous observation was impractical. Now, future applications of bio-implantable CCD cameras seem to be limited only by the requirements: a) that the tissue of interest be isolated for viewing, and b) that the axial orientation of tissue and camera be maintained (a restriction useful in itself in following morphologic changes). Research areas involving studies of ovulation are likely to benefit most from the promise of regular and continuous observation allowing to isolate particular short-term events such as rupture of the graafian follicle.

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Possibility of using ^{131}I-albumin as a marker for the estimations of microbial protein synthesis rates in the rumen

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Summary. Total microbial protein synthesis rates in the rumen of buffaloes were estimated by isotope dilution technique, using ^{131}I-albumin treated with tannic acid as a marker. The animals were fed groundnut cake treated with formaldehyde to meet 50% of their digestible crude protein (DCP) requirement and 2.5% urea molasses mixture was given to meet the remaining requirement of DCP. Wheat straw was fed as the basal roughage. The total average microbial protein synthesis was 58.14 g/day.

Methods for the in vivo measurements of either bacteria or protozoa production rates in the rumen using isotope dilution technique have been described earlier[1-7]. In this communication, ^{131}I-albumin treated with tannic acid to protect its degradation in the rumen was used as a marker for the estimation of total microbial proteins (both bacterial and protozoal) synthesis rates.

Materials and methods. 2 male Murrah buffaloes (*Bos bubalis*), about 3 years old, fitted with permanent rumen cannulae were used in the present experiments. Animals were fed groundnut cake treated with formaldehyde[8] (5% w/w of crude protein content) to meet 50% of their digestible crude protein (DCP) requirement and 2.5% urea molasses mixture was given to meet the remaining requirement of DCP. Wheat straw was fed as the basal roughage. Mineral mixture and vitamins were added according to their requirements. The animals were kept on pre-experimental feeding period for 4 weeks, and thereafter the animals received their ration in 12 equal parts at 2-h-intervals for a period of 3 weeks. The residue, if any, at the end of each 2-h-interval was removed and weighed. ^{131}I-albumin procured from Bhabha Atomic Research Centre, Trombay, was treated with 10% tannic acid solution for 18 h to protect its degradation in the rumen and was resuspended in 100 ml iso-osmotic saline with the help of all glass Potter Elvehjem homogenizer. An aliquot was taken for the estimation of radioactivity and the albumin solution was injected into the rumen in a single dose. The contents of rumen were mixed by hand simultaneously. Samples (16 ml) from the rumen were drawn at various time intervals upto 10 h from 4 different sites and were processed for estimating the radioactivity. Total rumen liquor was taken in equal volume of 20% TCA to make the final concentration 10% and centrifuged at $27,000 \times g$ for 15 min in a Sorvall Superspeed Refrigerated Centrifuge model RC 2B. The pellet was digested twice in 10% TCA at 80 °C for 15 min, and centrifuged. The pellet was further extracted with ethanol, acetone and finally with

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