the ^{125}I-HCG in view of the %/g for the kidneys. A comparable target organ for ^{125}I-HCG was not found in male mice as evidenced by the %/g for the testis (0.17; see Table). The radioisotopic uptake levels of both hormones were unremarkable in all other tissues.

Autoradiographic analysis revealed the presence of isotopic foci in the thecal and interstitial cells of the mouse ovary in confirmation of previous studies[2,3]. Extremely heavy deposits localized in the thecal envelopes but not in the granulosal cells of Graafian follicles. More uniform distributions of radiohormone were apparent among the interstitial cells especially those near developing follicles. Corresponding tissue sections from control animals and from the liver of experimental animals were unremarkable.

As evidenced in the Figure, newly-formed corpora lutea of the intact mouse ovary demonstrated strikingly less isotopic foci than the surrounding theca and interstitium. The lutein cells, which comprised most of the mass of the luteal body, showed little affinity for the ^{125}I-HCG hormone. In contrast, the luteal bodies bearing signs of atresia showed heavy infiltration of radioisotope distributed throughout the luteal body. Thus, the accumulation of isotopic granules in the corpora lutea appeared to increase as vascularization progressed.

Present studies have demonstrated that ^{125}I-HCG concentrated in the ovary at 3 h postinjection. Radioactivity counts in the ovary of intact mice were 9 to 30 times grater than in any other tissue except the kidney. A comparable target organ for ^{125}I-HCG was not detected in the male including the testis and sex accessory glands. It may be noted that uptake of ^{125}I-HCG has only been demonstrated in the rodent testis by tissue silces maintained in vitro[14,1]. ^{125}I-HGH, used as a trophic hormone control, did not concentrate in any of the female organs and tissues.

Isotopic localization of ^{125}I-HCG occurred in the thecal and interstitial tissues in juxtaposition to developing follicles. Histologic localization of the radiohormone was less evident in the corpora lutea comprised predominantly of lutein cells. However, heavy deposits of isotope were observed in the atretic or older luteal bodies. Radio-foci were not observed in control tissues and in the livers of mice injected with ^{125}I-HCG.

The differential uptake of ^{125}I-HCG in the corpora lutea of the intact mouse ovary is of interest from several standpoints. First, in lieu of heavy uptake of ^{125}I-HCG in the corpora lutea of superovulated and intact rat ovaries, the present results regarding uptake in the mouse is somewhat surprizing, if not, contradictory. Secondly, as a target organ the mouse ovary provides a different model for cell receptor studies involving HCG. Thirdly, additional mechanism of steroid biosynthesis and regulation in the mouse may be implied when comparisons to the rat ovary are made.

The lack of HCG receptivity in the highly luteinized corpora lutea of the mouse may be explained in terms of corpora luteal development. The lutein cells of the mouse are derived almost exclusively from the granulosa cell population[15]. The cells of the granulosal layer did not display an affinity for ^{125}I-labeled HCG[2]; thus, the newly-formed luteal body is populated by cells whose predecessors did not localize HCG to any great extent. However, the thecal cells, which showed a great avidity for ^{125}I-HCG did not immediately infiltrate the corpus luteum and remain situated on the periphery as shown in the Figure. As the luteal body ages toward atresia, the thecal-derived cells infiltrated the mass of the luteal body and came to line the blood spaces and sinuses therein[15,16]. The thecal-derived cells of the corpus lutea retained their avid receptivity for HCG resulting in increased localization in the aging corpus lutea. Thus, the concentration of ^{125}I-HCG in the intact mouse luteal body was dependent on the 1, vascularization, 2, age, and 3, cell distribution in the luteal body.

Summary. The tissue localization of ^{125}I-HCG was studied in intact mice. ^{125}I-HCG concentrated in the thecal and interstitial tissues of the ovary. Differential uptake occurred in the corpora lutea which was dependent on the age and vascularization of the luteal body.

G. J. MIZEJEWSKI[17]

Veterans Administration Hospital, University of Michigan Medial Center, Ann Arbor (Michigan, USA), 10 June 1975.

[14] D. M. DEKRETSER, K. J. CATT and C. A. PAULSEN, Endocrinology *88*, 332 (1971).
[15] F. W. R. BRAMBELL in *Physiology of Reproduction* (Ed. A. S. PARKES; Longrans, Green, & Co., New York 1956), vol. 1, chapt. 5, p. 397.
[16] D. L. BASSETT, Am. J. Anat. *73*, 251 (1943).
[17] Present address: Division of Laboratories & Research, New York State Dept. of Health, Albany, New York 12201, USA.

PRO EXPERIMENTIS

A Simple Method of Preparing a Cell Suspension for Scanning Electron Microscopy

As part of a study on the behaviour of cell suspensions, it was decided to investigate the surface topography of 7-day neural retina cells with the scanning electron microscope[1]. For the sake of direct comparison with other investigative techniques it was most important that the cells were prepared from a monodispersed cell suspension.

Cell preparations have been obtained for scanning electron microscopy (SEM) by many methods[2], involving either plating out prior to critical point drying (CPD)[3], air drying[4] freeze drying[5] or spray freezing[6,7] from suspension.

The few methods described for handling cell suspensions for CPD were not suited to our needs for reasons discussed later. We, therefore, devised a versatile method for the processing of suspended cells for SEM, which ensures excellent preservation of surface structure and allows for comparisons with other data on cell suspensions.

Materials and methods. Neural retina cells from 7-day chick embryos were disaggregated following a previously described technique[8]. The cell suspension was spun at 300 *g* for 5 min, the supernatant discarded, and the cell

[1] G. E. JONES, J. Cell Sci. *17*, 371 (1975).
[2] K. R. PORTER, D. KELLEY and P. M. ANDREWS, Proc. of the 5th annual Stereoscan Scanning Electron Microscope Colloquium. Kent Cambridge Scientific, Inc., Morton Grove, ILL. (1972).
[3] L. P. EVERHART JR. and R. W. RUBIN, J. Cell Biol. *60*, 442 (1974).
[4] I. CARR, J. A. CLARKE and A. J. SALISBURY, J. Microsc. *89*, 105 (1969).
[5] R. A. HAMMOND, J. Microsc. *90*, 273 (1969).
[6] L. BACHMANN and W. W. SCHMITT, Proc. natn. Acad. Sci., USA *68*, 2149 (1971).
[7] H. PLATTNER, W. M. FISCHER, W. W. SCHMITT and L. BACHMANN, J. Cell Biol. *53*, 116 (1972).

pellet resuspended in 1% Osmium tetroxide in 0.1 mol dm^{-3} phosphate buffer pH 7.45. The fixation was for 20 min at room temperature. Dehydration was through an acetone series, 30 min at each step, to absolute acetone where 3 changes were given, the last just before CPD.

For CPD a cone was made from a 4 cm diameter disc of Whatman 542 filter paper (hardened, ashless). This was fitted into one of the wire thimbles of a Polaron E3000 CPD apparatus. The filter paper was then soaked with acetone and placed in the trough of the apparatus in its wire basket. The cone and trough were part-filled with acetone and the cell suspension dispensed into the cone. The trough was then transferred to the drying apparatus and the specimen was taken through the critical temperature in the normal manner.

On removing the sample, the cells can be seen as a fine brown powder in the tip of the cone. This powder

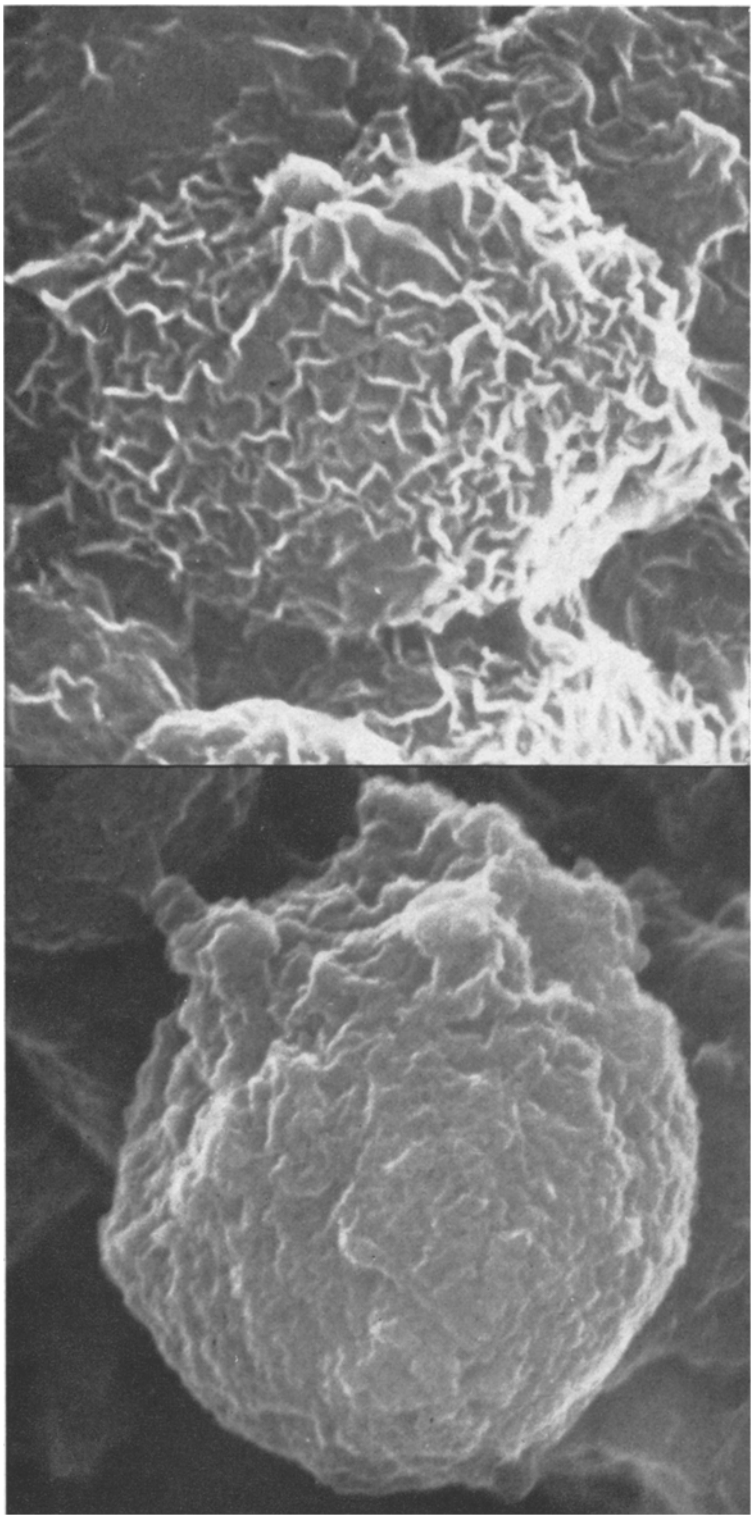

Fig. 1. SEM of neural retina cells air-dried from a monodispersed suspension. The cells appear grossly flattened. × 20,000.

Fig. 2. SEM of a neural retina cell critical point dried from a monodispersed suspension. The cell is spheroid and exhibits a complex morphology. × 20,000.

was tapped out onto a stub coated with colloidal silver, which had dried to the tacky stage.

The stubs were then coated in the usual way with gold/palladium (60:40) on an orbital rotary specimen holder in an Edwards Vacuum Coating unit. The pictures were taken using a Cambridge Stereoscan S4-10 model.

Results. Excellent surface preservation was obtained from cells prepared in the above manner. With air-dried cell suspensions, however, the cells appeared grossly flattened and many individual cells had a discoid shape. With CPD, the cells appeared roughly spherical, showing well preserved surface detail of some complexity, which was absent from the air-dried material (see Figures 1 and 2).

Discussion. If SEM is to be used in conjunction with other studies on cells in suspension (e.g. cell aggregation or cell electrophoresis) or performed with cells which radically alter their morphology when plated onto a substrate, then a reliable method of drying cells from a liquid suspension is required. Few workers doubt the advantage of the critical point drying method of ANDERSON[9] in safeguarding delicate surface structures when drying, and it is deemed preferable to freeze-drying and spray freezing[2] in preserving fine topology.

Our method is above all a simple and inexpensive way of surmounting the handling difficulties in keeping cells in suspension right up to the time of drying by the critical point method. Recent, more sophisticated, systems in addition to their complexity, seem unsuitable for small delicate cells[10] and often the cells must be applied to a surface before drying[11]. ROATH and NEWELL'S[12] modified embedding capsule method offers too great a hinderance to solvent exchange to be successful and the methods of SCOTT, THURSTON and MCKEE[13] and HORRIDGE and TAMM[14] are suitable only for large single cells (150 µm and larger). In addition to the greater complexity of the spray-cryofixation method[6,7], it engenders subsequent mounting difficulties and may well subject fragile microvilli of animal cells to mechanical damage.

Our method is simple, cheap and very versatile. As the process results in little loss of sample, only small numbers of cells need be collected, a great advantage where the harvesting of large numbers of cells is difficult or tedious.

Summary. A method is described for the preparation of suspensions of small single cells for scanning electron microscopy by the critical point drying technique. This procedure offers the advantages of reliability, cheapness, versatility and simplicity and may therefore be useful to many workers in varied fields.

G. E. JONES and R. GILLETT[15]

Biology Department, Queen Elizabeth College, Atkins Building, Campden Hill, London W8 7AH (England), 4 March 1975.

[8] G. E. JONES, J. Membrane Biol. *16*, 297 (1974).
[9] T. F. ANDERSON, Trans. N.Y. Acad. Sci. Ser. 11, *13*, 130 (1951).
[10] L. H. PRINCEN and F. L. BAKER, Paper Communication to 'Micro '74' London (1974).
[11] H. J. MARCHANT, J. Microsc. *97*, 369 (1973).
[12] O. S. ROATH and D. G. NEWELL, Paper Communication to 'Micro '74' London (1974).
[13] J. R. SCOTT, E. L. THURSTON and T. R. MCKEE, J. Microsc. *99*, 359 (1973).
[14] G. A. HORRIDGE and S. L. TAMM, Science *163*, 817 (1969).
[15] We are indebted to Miss V. COWPER for the operation of the scanning electron microscope.

A Simple Titration Assay for Anti-Concanavalin A Sera

Agglutinins forms plant sources, expecially concanavalin A (Con A), are carbohydrate-binding reagents widely used in biology[1]. Antibodies directed against Con A (and other lectins) are easily obtained and often very useful. However, the titration of anti-Con A sera is more difficult. Haemagglutination inhibition assays, analogous to the ones used to titrate antisera against viral agglutinins or whole viruses, fail because Con A adsorbs to some serum glycoproteins leading to nonspecific inhibition. Ouchterlony microimmune diffusion tests, on the other hand, are relatively insensitive and time consuming.

We now describe a simple and rapid 'indirect' haemagglutination assay which is based on the previous observation[2] that human erythrocytes do not agglutinate with Con A if they are kept in suspension by shaking. The principle is to coat the erythrocytes with Con A and to remove the excess of lectin without clumping of the cells; subsequent addition of anti-Con A antibodies immediately results in massive agglutination. Due to its excellent reproducibility, the assay is well suited for the standardization of the specific activities of anti-Con A sera.

Anti-Con A sera and IgG. Rabbit anti-Con A serum was prepared by 5 weekly i.m. injections of 1 mg Con A in 2 ml of phosphate buffered saline (PBS) with complete Freund's adjuvant. The animals were bled 14 days after the last injection. The sera were inactivated (56 °C, 30 min) and exhaustively absorbed with washed human erythrocytes.

For the preparation of IgG, the rabbit sera were precipitated in 50% ammonium sulfate, dialyzed with 0.015 *M* phosphate buffer pH 8 and chromatographed on DEAE cellulose using an ionic strength gradient in the same buffer (0.015–0.25 *M*). The first main fraction eluted at 0.015–0.02 *M* contained pure IgG as determined by immune electrophoresis.

For controls, rabbit sera of unrelated specificity (anti-Sendai virus) and IgG fractions prepared therefrom, were used.

Coating of erythrocytes with Con A. Human erythrocytes from fresh, heparinized blood (group O, Rh +) were washed 4 times in phosphate-buffered saline (PBS) pH 7.4 and adjusted to 2×10^8 cells per ml. An equal volume of a Con A solution (1 mg/ml) was then added. During 10 min incubation at room temperature, the cells were kept in suspension by occasional twirls on a Vortex mixer. After 10 min the suspension was diluted 20-fold with cold PBS and centrifuged for 3 min at 2,000 rpm in a clinical centrifuge. The supernatant was discarded, the original volume of PBS replaced and the cells (now slightly clumped) were quickly resuspended by vigorous shaking on the Vortex mixer for 10 to 20 sec. This suspension, while agitated, was stable for at least 20 min.

[1] G. L. NICHOLSON, Int. Rev. Cytol. *39*, 89 (1974).
[2] H. P. SCHNEBLI and TH. BÄCHI, Expl Cell Res. *91*, 175 (1975).