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.

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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.

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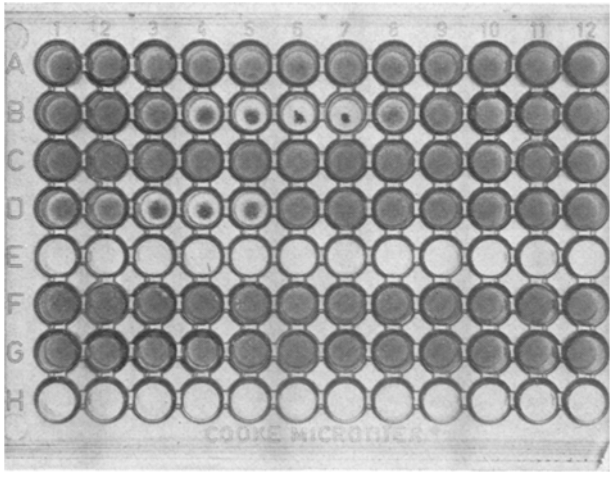

Titration of sera and IgG fractions against concanavalin A.
A) Control serum (anti-Sendai).
B) Anti-Con A serum.
C) Control IgG (anti-Sendai) (1.05 mg/ml in the first well).
D) Anti-Con A IgG (1.25 mg/ml in the first well).
F) and G) same as B) and D) respectively, expect that the erythrocytes were *not* coated with Con A.
Twofold serial dilutions in PBS from 1 to 11; controls without serum or IgG in 12.

Sensitivity of Ouchterlony diffusion test and 'indirect' haemagglutination assay

	Highest dilutions giving positive results	
	Ouchterlony	Agglutination
Anti-Con A serum	1:40	1:256
Anti-Con A IgG	320 µg/ml	66 µg/ml

Ouchterlony double diffusion tests were run overnight at room temperature. The distance between the wells was 3 mm and each well contained 9 µl of the Con A solution or serum- or IgG dilution, respectively. Immunoprecipitin lines were examined without prior staining of the gels. Agglutination assays were done as described in the text.

The conditions of the incubation (500 µg Con A/ml, 10 min, room temperature) ensure a reproducible preloading of approximately 90% of the available Con A receptor sites[2].

Reaction with antisera. Serial dilutions of antisera in PBS were prepared in microtiter plates (Cooke, Engineering) (25 µl in each well) in advance, and 25 µl of the Con A-coated erythrocytes were added to each well. The plate was shaken for 30 to 60 sec on a Tayo Bussan mixer and read within the next 2 to 3 min.

Results. As seen in the Figure, specific antisera and specific IgG fractions caused massive agglutination (rows B and D, respectively). No agglutination was observed with sera and IgG of unrelated specificity (rows A and C, respectively) or when erythrocytes not coated with Con A were reacted with anti-Con A sera or IgG (rows F and G, respectively).

The anti-Con A serum used here agglutinated coated cells up to a dilution of 1:256 (well B8; 4 assays) and the anti-Con A IgG fraction down to a concentration of 66 µg/ml (well D5). At high concentrations of serum and IgG agglutination was inhibited, suggesting a prozone effect.

As a comparison (Table) we observed immuno-precipitin lines in Ouchterlony plates between Con A (100 µg/ml) and rabbit anti-Con A serum diluted up to 1:40, and rabbit anti-Con A IgG diluted to 320 µg/ml, whereas no reactions were found with the unrelated IgG. A second weak precipitin line found between Con A and undiluted anti-Con A serum was identical with that obtained with undiluted anti-Sendai serum, indicating the presence of serum components different from IgG, but reacting with the lectin. Similarly, in the agglutination assays we observed some slowly developing non-specific agglutination after about 10 min; this, however, could easily be separated from the rapid, specific agglutination shown in the Figure.

Summary. A method for the titration of anti-Con A sera is described. The test is based on the fact that agglutination of Con A-coated human erythrocytes can be prevented by agitation; subsequent addition of Con A-specific antisera and IgG to Con A-coated cells leads to immediate clumping.

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Visualization of Chicken Red Blood Cells in Capillaries by Immunofluorescence

The measurement of regional blood flow distribution using radioactive labelled microspheres has been the object of intensive investigation[1-4]. Difficulties which arise from the rheological properties of these microspheres[2] can be partially avoided by employing radioactive labelled frog red blood cells[5]. Both methods however demand considerable medical preventive precautions and can only be used under closed laboratory conditions. Therefore the development of less difficult methods must be attempted. In the present report, a simple procedure is proposed consisting of labelling red blood cells from chicken by means of a specific antigen-antibody reaction and visualization of the erythrocytes in the capillary vessels.

Materials and methods. a) Preparation of antiserum against chicken red blood cells: Chicken red blood cells are washed with saline solution 6 times by serial centrifu-

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