the supernatant after absorption. It was found that more than 98 percent of all concanavalin A had been removed by the absorption procedure. The immunological suppressive capacity of each concanavalin A supernatant was confirmed by measuring its ability to block pokeweed mitogen–stimulated immunoglobulin M (IgM) synthesis of normal mouse splenocytes, as assessed by cytoplasmic immunofluorescence at the termination of a 7-day culture period. Since each of these supernatants has a soluble immune response suppressor activity and is prepared according to the method used by Rich and Pierce to produce SIRS they are referred to by this acronym. It should be noted, however, that these are crude supernatants that contain other active factors. The SIRS was stored frozen in portions at −20°C until utilized. These frozen portions retained activity for at least 3 months under these storage conditions. The animals were treated twice weekly with 0.5 ml of the absorbed supernatants, thus representing one-half of the SIRS produced by 2×10^6 spleen cells exposed to concanavalin A and then cultured in 1 ml of media.

6. The serum concentrations of immunoglobulins M, G, and A (IgM, IgG, and IgA) were determined on all killed mice by a single radial immunodiffusion in agar on commercially obtained agar plates and with mouse Ig standards.
7. J. E. Claymaet and R. M. Nakamura, *Am. J. Clin. Pathol.* **58**, 338 (1972). Human HEP_2 cells were incubated for 30 minutes with a 1 : 10 dilution of the mouse serum being tested. The slides were then washed with phosphate-buffered saline and then incubated with a 1 : 20 dilution of fluorescein isothiocyanate–conjugated rabbit antiserum to mouse IgG (Miles Research, Elkhart, Indiana). The coated slides were coded and read as −, +/−, or +. Serum from 24-week-old NZB/W mice and from normal BALB/c mice were used as positive and negative controls, respectively.
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
9. I. Zan-Bar, D. Nachtigal, M. Feldman, *Cell. Immunol.* **17**, 202 (1975).
10. M. J. Dauphinee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2637 (1974).
11. M. E. Gershwin, A. Ahmed, A. D. Steinberg, G. B. Thurman, A. L. Goldstein, *J. Immunol.* **113**, 1068 (1974).
12. M. E. Gershwin and A. D. Steinberg, *Proc. Soc. Exp. Biol. Med.* **147**, 425 (1974).
13. S. Kysela and A. D. Steinberg, *Clin. Immunol. Immunopathol.* **2**, 133 (1975).
14. L. M. Parker, T. M. Chused, A. D. Steinberg, *J. Immunol.* **112**, 285 (1974).

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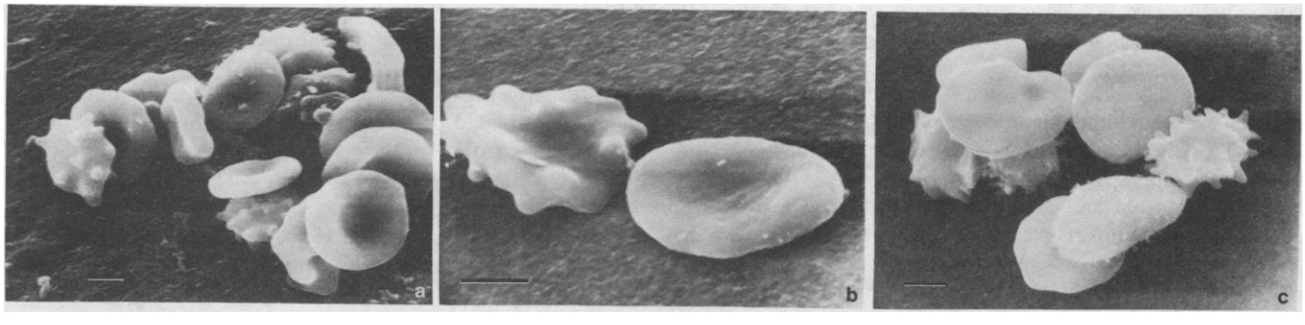
Morphologies of Cells from 1-Day Chick Embryos

Abstract. *Cells disaggregated from 1-day chick embryos show three morphologies, two reminiscent of mammalian erythrocytes and one epithelial. The presence of intermediate forms suggests that the three morphologies are interconvertible.*

During experiments in which we have been investigating the control of morphogenetic movements in early chick embryos by extracellular adenosine 3′,5′-monophosphate (cyclic AMP) signals, it became necessary for us to determine the shapes and sizes of cells disaggregated from the embryos (*1*). We examined the cells by light microscopy and scanning electron microscopy (SEM). We found that most cells were 3 to 5 μm in diameter and had one of three morphologies: biconcave, like mammalian erythrocytes; knobbed or hedgehog-like, a form also reported for mammalian erythrocytes (*2*); and finally epithelial-like, with projecting microvilli (see Fig. 1). They occurred in the following proportions: biconcave, 69 percent; knobbed, 18 percent; and epithelial-like, 13 percent. Intermediate forms were also seen, suggesting that the morphologies are interconvertible.

Scanning electron micrographs were taken of the cells and cell aggregates from disaggregated chick embryos after approximately 1 day's incubation [Hamburger-Hamilton, stage 5 (*3*, *4*)]. The entire blastodisks were removed and disaggregated by suspension in 0.1 m*M* EDTA in Ringer solution (*3*). After disaggregation, the cells were centrifuged and resuspended in Ringer solution twice at 4°C to remove all traces of EDTA. The cell suspensions were then plated onto 2 percent Difco purified agar made up in Ringer solution and allowed to settle for 2 hours at 37°C.

The cells on agar blocks (5 by 5 by 2.5 mm) were then prepared for SEM in a Coates & Welter model 50-4 scanning electron microscope. They were passed through a series of seven ethanol-H_2O mixtures ranging from 10 percent ethanol to absolute ethanol. Next they were transferred from absolute ethanol to amyl acetate in seven steps ranging from 10 percent amyl acetate in ethanol to 100 percent amyl acetate. Finally, they were dried by the critical-point method and



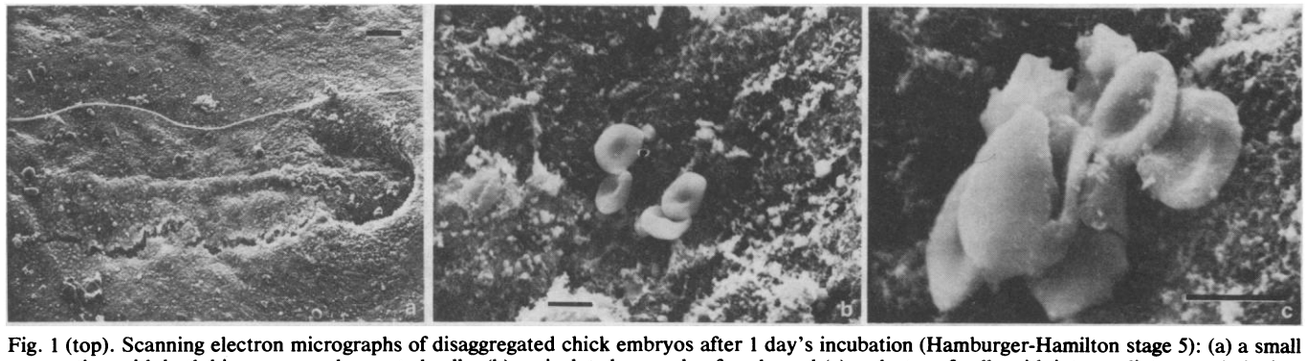


Fig. 1 (top). Scanning electron micrographs of disaggregated chick embryos after 1 day's incubation (Hamburger-Hamilton stage 5): (a) a small aggregation with both biconcave and crenated cells; (b) an isolated example of each; and (c) a cluster of cells with intermediate morphologies. Scale bars, 2 μm. Fig. 2 (bottom). Scanning electron micrographs of cell aggregates in whole embryo (Hamburger-Hamilton stage 7-8): (a) entire embryo (scale bar, 100 μm) and (b and c) cell aggregates in the area opaca with biconcave and crenated morphologies (scale bars, 6 μm).

coated with carbon (200 Å) and Au-Pd (600 Å).

The morphologies of most cells were found to be remarkably similar to those of mammalian red blood cells. Typical photographs of cells and cell aggregates are shown in Fig. 1. They exhibit the biconcave, cup-shaped, crenated, and intermediate morphologies characteristic of mammalian red blood cells. They are, however, smaller than blood cells, being only 2.5 to 5.5 μm in size, and they can easily be distinguished from chick erythrocytes, which are oval.

The resemblance between cultured chick embryonic cells and mammalian red blood cells was noticed long ago by Shipley (*5*). He observed cultured cells explanted from the area opaca of an 18-hour embryo and noted that many appeared remarkably like young mammalian red blood cells, being about 4 μm in diameter. Our scanning electron micrographs are of cells fixed within 2 hours of disaggregation, without culturing, and from embryos at a stage at which no blood corpuscles or hemoglobin are present. They exhibit the same resemblance to mammalian blood cells, and our observations with the light microscope confirm Shipley's.

We also examined a whole embryo (Hamburger-Hamilton stage 7-8) by SEM and observed cells with all three morphologies throughout its ventral surface (Fig. 2). It should be noted that the embryo was explanted on its vitelline membrane and incubated (37°C) for 20 hours before fixing. During the incubation a time-lapse film of its development was taken. The embryo was on a 2 percent agar substrate made up in Ringer solution and thin albumen and was covered with Klearol mineral oil. After the filming the embryo was removed from its vitelline membrane and fixed by the procedure outlined above, except that ethanol–Ringer solution was substituted for ethanol-H_2O. The time-lapse film recorded normal development during the 20-hour period.

Sheetz *et al.* (*6*) have shown that shape changes in mammalian blood cells are produced through the bilayer coupling in the cell membrane. They find shape changes occurring within 1 minute at 4°C. If, as seems likely, a similar mechanism is responsible for the diverse shapes observed in early embryonic cells, then it may provide one of the basic mechanisms of early cell differentiation. The resulting range of cell morphologies could serve diverse embryonic functions; the biconcave shape being suitable for active transport or diffusion of metabolites and the crenated for cell aggregation and adhesion. Furthermore, the possession of two such similar ranges of morphology by mammalian erythrocytes and early embryonic chick cells suggests that the shapes observed, including the biconcave, may be more widespread than has been assumed. It is likely that all the morphologies seen represent the action of very fundamental processes controlling cell shape.

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References and Notes

1. A. R. Gingle, J. F. Grutsch, R. K. Raman, A. Robertson, unpublished experiments.
2. D. W. Thompson, *Growth and Form* (Macmillan, New York, 1945), p. 436.
3. D. A. T. New, *The Culture of Vertebrate Embryos* (Logos, London, 1962), p. 245.
4. V. Hamburger and H. L. Hamilton, *J. Morphol.* **88**, 49 (1951).
5. P. G. Shipley, *Anat. Rec.* **10**, 347 (1916).
6. M. P. Sheetz, R. G. Painter, S. J. Singer, *J. Cell Biol.* **70**, 193 (1976).
7. Supported by a grant-in-aid from the Sloan Foundation to A.R., who is also grateful for support from R. Haselkorn. The authors wish to acknowledge the SEM Users' Laboratory at the University of Chicago, in which part of these results were obtained. The laboratory is supported by an NIH resource grant. A.R.G. is supported by NIH National Research Service Award GN 7190.

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Characterization of a New Human Diploid Cell Strain, IMR-90

Abstract. *A new human diploid fibroblast-like cell line has been established from lung tissue of a female fetus. This has been frozen away in large quantity and characterized for use in research and related purposes. This is the first of a planned series of human cell lines to be established, characterized, and banked in large quantity in support of the National Institute on Aging research and general cell biology.*

A new human diploid cell line designated IMR-90 has been established and banked for research and related purposes. The National Institute on Aging (NIA), National Institutes of Health (NIH), through contract to the Institute for Medical Research (IMR), Camden, New Jersey, initiated IMR-90 to be available as a replacement for WI-38 (*1, 2*) as the NIH stock of low-passage WI-38 cells has become relatively limited for purposes other than vaccine manufacture. Cell line IMR-90 is the first of several planned human cell lines to be established, characterized, and stored in large quantity in support of NIA research programs, general cell biology, and related activities. Cultures are available from the IMR and other sources of distribution. The NIA-supported somatic cell genetics resource has been described (*3*), and the related NIA cellular aging program has been discussed (*4*). IMR-90 was derived from a human female embryo, and otherwise parallels WI-38 to minimize variables of replacing WI-38 within ongoing laboratory programs. The extensive frozen stock of IMR-90, combined with the management protocol for distribution, enhances extensive long-term availability of the line.

The culture was established from a human fetus obtained 7 July 1975 from a therapeutic abortion performed on a 38-year-old white female (para 6, gravida 8). The gestational age of the fetus was 16 weeks; its length was 7 cm; there were no apparent physical abnormalities. Laboratory work completed on the mother consisted of a normal complete blood count; blood type was A positive; serology tests were negative; calcium, phosphorus, glucose, blood urea nitrogen, uric acid, cholesterol, total protein, creatinine, lactate dehydrogenase, and urinalysis were all within normal limits. Both lungs of the fetus were aseptically removed in a laminar flow hood and transferred to two sterile petri dishes. The lungs were washed with three changes of medium containing 100 units of penicillin per milliliter and 100 μg of streptomycin per milliliter. All tissue was minced into pieces (with two sterile scalpel blades) approximately 1 to 2 mm in size (the Maitland method) (*5*). Eight to ten fragments of lung tissue were transferred with a sterile Pasteur pipette to plastic T25 flasks. The flasks were then inverted, with the tissue pieces adhering to the top of the vessel. The growth medium was McCoy's 5A (Flow Laboratories, Inc., Rockville, Maryland) plus 20 percent fetal calf serum; it contained penicillin (100 unit/ml) and streptomycin (100 μg/ml). At the time of the first passage, antibiotics were removed from the medium and were not used again. Four 500-ml bottles of this McCoy's medium and two 500-ml bottles of serum from the same lot used to establish the culture were frozen for future reference. The serum used was not inactivated by heat, nor was it dialyzed.

After 1 hour, the inverted flasks were turned so that culture medium gently flowed over the tissue explant, which was then incubated at 37°C in an atmosphere of 5 percent CO_2 in air. Forty-five flasks were established by this method.