necessary for the immune response are phagocytic and that these cells, by interacting with antigen, process antigen in some manner, then clearly x-irradiation does not injure macrophages primarily or any processing mechanism of these cells for sheep erythrocyte antigens.

Many elegant experiments in which cells are used to reconstitute heavily x-irradiated animals have identified radiosensitive populations required for the immune response, but it is obviously impossible to evaluate any requirement for radioresistant populations with such a model. This first demonstration, that a step or cell required for the antibody response is relatively x-ray resistant, may permit further dissection of the complex cell interactions required for the immune response.

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References and Notes

1. F. J. Dixon, D. W. Talmage, P. H. Maurer, *J. Immunol.* **68**, 693 (1952). A good general evaluation of the effect of x-irradiation in the immune response is presented by W. H. Taliaferro, L. G. Taliaferro, B. N. Jaroslow [*Radiation and the Immune Mechanisms* (Academic Press, New York, 1964)]. The effect of x-irradiation on the mouse spleen plaque-forming cell response has been reported by J. C. Kennedy, J. E. Till, L. Siminovitch, E. A. McCulloch [*J. Immunol.* **94**, 715 (1965)].
2. R. Gallily and M. Feldman, *Immunology* **12**, 197 (1967); J. F. Pribnow and M. S. Silverman, *J. Immunol.* **98**, 225 (1967).
3. R. I. Mishell and R. W. Dutton, *Science* **153**, 1004 (1966); *J. Exp. Med.* **126**, 423 (1967).
4. D. E. Mosier, *Science* **158**, 1573 (1967).
5. All antibody responses are measured as the number of plaque-forming cells by using a slide modification (*2*) of the assay system described by N. K. Jerne and A. A. Nordin [*ibid.* **140**, 405 (1963)].
6. All x-irradiation was done with Maxitron General Electric 250 [250 kv(peak), 30 ma]. The mice, placed in Lusteroid tubes on a Lucite turntable (3½ turns per minute), were exposed to total body x-irradiation at a target distance of 78 cm (average output, 58.5 r/min) with 0.25 mm Cu and 1 mm Al filter (half-value layer, 1.05 mm Cu).
7. All incubations involved maintaining the cultures at 37°C on a rocker table at 13 oscillations per minute in an atmosphere of 7 percent O_2, 10 percent CO_2, and the balance N_2.
8. D. E. Mosier and L. Coppleson, *Proc. Nat. Acad. Sci. U.S.* **61**, 542 (1968).
9. D. E. Mosier, F. W. Fitch, D. A. Rowley, A. J. S. Davies, *Fed. Proc.* **28**, 375 (1969).
10. G. F. Mitchell and J. F. A. P. Miller, *J. Exp. Med.* **128**, 821 (1968).
11. Attempts to substitute other phagocytic cells (for example, mouse Kupffer cells or rat spleen adherent cells) for mouse spleen adherent cells have been unsuccessful.
12. D. A. Rowley, unpublished data.
13. M. Fishman, *Nature* **183**, 1200 (1959); —— and F. L. Adler, *J. Exp. Med.* **117**, 595 (1963); M. D. Schoengerg, V. R. Mumaw, R. D. Moore, A. S. Weisberger, *Science* **143**, 964 (1964); G. Harris, *J. Immunol.* **9**, 529 (1965).
14. Supported by PHS grants AI-96-08 and HE-05667, and the Argonne Cancer Research Hospital, operated by the University of Chicago for AEC. The advice of Drs. D. A. Rowley and F. W. Fitch, and the technical assistance of Miss Helga Tremmel, are sincerely acknowledged.

29 May 1969

Mammary Alveolar Epithelial Cells: Effect of Hydrocortisone on Ultrastructure

Abstract. *Hydrocortisone is necessary for the formation of rough endoplasmic reticulum in mammary alveolar epithelial cells. This membrane system is required for the synthesis of the milk protein, casein, but it is not required for the synthesis of a nonmilk protein fraction.*

Insulin, hydrocortisone, and prolactin are necessary for the induction of casein synthesis by mouse mammary gland explants (*1*). After 4 days of culture with insulin and hydrocortisone (*2*), explants from mice midway through pregnancy do not make detectable casein; however, within 12 hours after the addition of prolactin these explants do make casein. In contrast, explants that have been cultured in the absence of hydrocortisone (medium containing

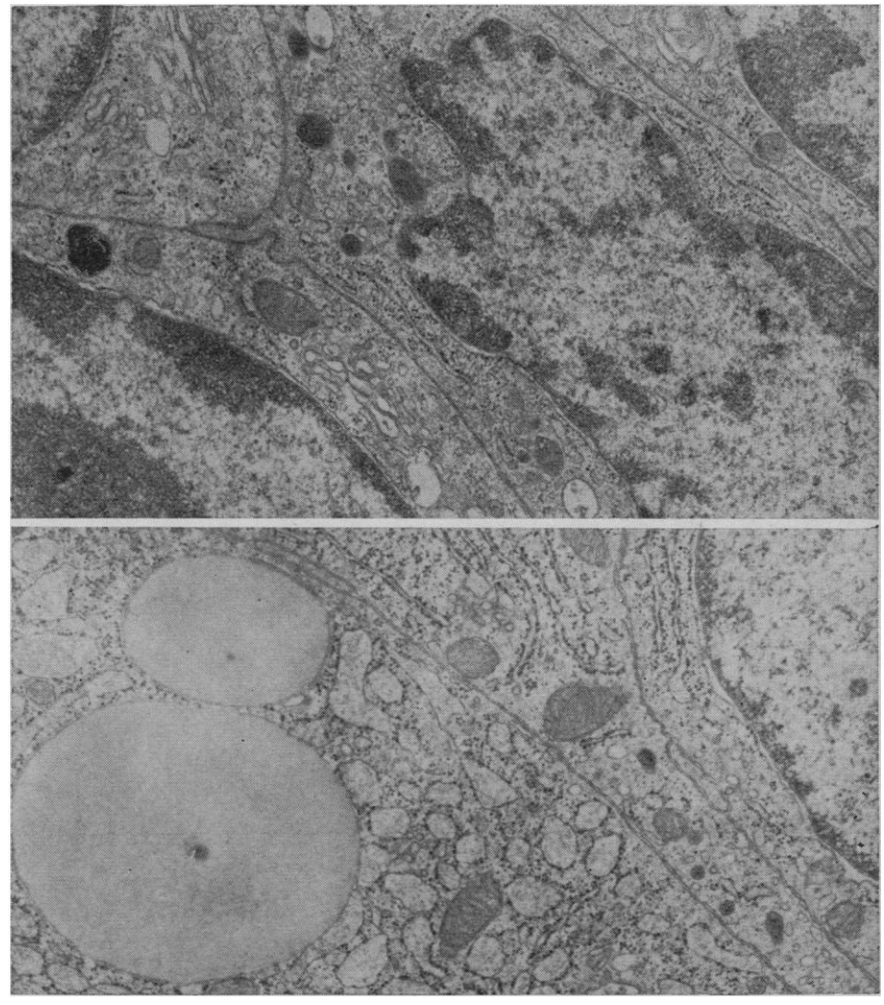

Fig. 1 (top). Ultrastructure of alveolar epithelium from mammary gland of C3H/HeN mouse midway through pregnancy. This micrograph shows portions of four alveolar epithelial cells from explants cultured for 96 hours in Medium 199 (*1*), containing 5 μg/ml of crystalline beef insulin (Lilly). The explants were fixed in Karnovsky's fixative (*7*) and embedded in Maraglas (*8*). Sections were cut on a Porter-Blum MT-1 ultramicrotome, stained with lead citrate and uranyl acetate (*9*), and examined in an RCA EMU-3G electron microscope. In most respects, these cells resemble the alveolar epithelial cells seen in the uncultured tissue. The cytoplasm contains very little rough endoplasmic reticulum. The epithelium contains no visible secretory products (approximately × 20,400). Fig. 2 (bottom). Ultrastructure of alveolar epithelium in explants derived from the same animals as in Fig. 1, cultured for 96 hours in Medium 199 containing 5 μg/ml of crystalline beef insulin (Lilly) and 5 μg/ml of hydrocortisone. The tissue was fixed and prepared as in Fig. 1. Rough endoplasmic reticulum (RER) is present throughout the cytoplasm. It often forms parallel nondilated cisternae and sometimes whorls. In some cells the RER is dilated. Lipid droplets (left side of micrograph) are often present in the cytoplasm (approximately × 21,250).

only insulin) do not respond similarly to the addition of prolactin (*3*). The action of hydrocortisone that endows mammary epithelial cells with the capacity to respond to prolactin in terms of casein synthesis has been heretofore unknown. This report describes an effect of hydrocortisone on the ultrastructure of the alveolar epithelial cells.

Alveolar epithelial cells in explants that have been cultured in the presence of insulin for 4 days have only a small amount of rough endoplasmic reticulum (RER) (Fig. 1). This ultrastructural appearance was very constant in cultured tissue from eight animals, and is also representative of the morphology of the majority of alveolar cells in uncultured tissue from C3H/HeN mice midway through pregnancy. When explants from the same animals were cultured for 4 days in the presence of both insulin and hydrocortisone a dramatic alteration was observed. The alveolar epithelial cells developed an extensive RER system (Fig. 2).

This marked difference in the structure of these cells is not reflected in their ability to synthesize some nonsecretory proteins, for it has been demonstrated (*4*) that insulin causes a fourfold increase in the formation of a nonmilk protein fraction, and that hydrocortisone exerts no effect on such synthesis in the presence of insulin. It appears, however, that the presence of the hydrocortisone-induced RER is a prerequisite for the formation of secretory protein, such as casein, under the influence of prolactin.

Work by others has implicated steroids in the formation of smooth membranes in a variety of systems (*5*). Rough endoplasmic reticulum has been shown to be associated with secretory proteins in a number of tissues (*6*). The observations presented here suggest that the formation of RER, under the influence of hydrocortisone, in mammary alveolar cells is necessary for full expression of the genetic potential of these cells. Prolactin, a terminal agent in the differentiation of mammary epithelium, elicits milk protein synthesis only in cells which possess this membrane system.

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References

1. W. G. Juergens, F. E. Stockdale, Y. J. Topper, J. J. Elias, *Proc. Nat. Acad. Sci. U.S.* **54**, 629 (1965).
2. R. W. Turkington, D. H. Lockwood, Y. J. Topper, *Biochim. Biophys. Acta* **148**, 475 (1967).
3. D. H. Lockwood, F. E. Stockdale, Y. J. Topper, *Science* **156**, 945 (1967).
4. D. H. Lockwood, R. W. Turkington, Y. J. Topper, *Biochim. Biophys. Acta* **130**, 493 (1966).
5. A. H. Conney, K. Schneidman, M. Jacobson, R. Kuntzman, *Ann. N.Y. Acad. Sci.* **123**, 98 (1965); A. L. Jones and D. W. Fawcett, *J. Histochem. Cytochem.* **14**, 215 (1966); M. W. Rancourt and G. Litwack, *Exp. Cell Res.* **51**, 413 (1968).
6. L. G. Caro and G. E. Palade, *J. Cell Biol.* **20**, 4 (1964); S. Ito and R. J. Winchester, *ibid.* **16**, 541 (19(S. L. Wissig, *J. Biophys. Biochem. Cytol.* **7**, 419 (1960); *J. Cell Biol.* **16**, 93 (1963).
7. M. J. Karnovsky, *J. Cell Biol.* **27**, 137A (1965).
B. O. Spurlock, V. C. Kattine, J. A. Freeman, *ibid.* **17**, 203 (1963).
E. S. Reynolds, *ibid.*, p. 208.

23 May 1969; revised 30 June 1969

Uptake of Isolated Chloroplasts by Mammalian Cells

Abstract. *Mouse fibroblasts (L cells) in suspension culture incorporated isolated chloroplasts of spinach and African violets and isolated mitochondria of chicken liver. The organelles resided in the cell cytoplasm and were not contained in vacuoles or digestion vesicles. Green cells divided like normal cells. Green chloroplasts were followed for five cell generations or 5 days, at which time hybrid cells were greatly outnumbered by nongreen progeny cells. The ingested chloroplasts retained their structural integrity as determined by electron microscopy of organelles and hybrid cells and by analysis of photochemical activity and DNA in chloroplasts reisolated from cells after 1 or 2 days in culture.*

The introduction of exogenous genetic material or DNA-containing structures into animal cells can be achieved by diverse techniques. These include infection of cells with bacteria and viruses, formation of hybrids of two cell types by fusion (*1*), transplantation of isolated single nuclei in eggs and embryos (*2*), microinjection of nuclear and mitochondrial fractions (*3*), and pinocytic uptake of purified DNA (*4*). We have been interested in the problem of the degree of independence which can be achieved by various cytoplasmic organelles that contain DNA, such as mitochondria and chloroplasts.

In order to introduce isolated heterospecific cytoplasmic organelles into mammalian cells with technical facility and high yield of hybrid cells, advantage was taken of the phagocytic properties of mouse fibroblasts (L cells) grown in suspension culture. These cells incorporated isolated chloroplasts and mitochondria within 30 minutes after addition of the organelles to the cell culture. We have first concentrated on chloroplasts because they are easier to follow than mitochondria. Unlike the fate of DNA-containing gelatin particles, which were hydrolyzed in digestion vacuoles within an hour after uptake by L cells (*5*), green chloroplasts were seen in L cells for at least 5 days or five cell generations after ingestion. Green cells divided like normal cells, randomly segregating chloroplasts into progeny cells. After six divisions, green cells were greatly outnumbered by cells of subsequent generations, limiting further observations on their fate.

The ingested chloroplasts retained a considerable degree of structural integrity. With electron microscopy it was seen that the chloroplasts residing in the cytoplasm were only partially surrounded by a membrane. No phagocytosis vesicles surrounded the chloroplasts. The organization of chloroplast lamellae was similar to that in freshly isolated organelles. Chloroplasts reisolated after 1 or 2 days in cell culture showed light-stimulated Hill reaction activity (reduction of dichlorophenolindophenol) and CO_2 fixation; the chloroplast DNA was found to be in macromolecular form with a molecular weight in the range of 10^7 to 10^8 daltons.

Chloroplasts were isolated from leaves of fresh spinach (*Spinacia oleracea*) and African violets (*Saintpaulias*) by grinding the thoroughly washed leaves at 4°C in a mortar with a modified Honda (*6*) medium (0.25*M* sucrose, 2.5 percent Ficoll, 5 percent Dextran-40, 0.01 percent bovine serum albumin, 6 m*M* mercaptoethanol, 25 m*M* tris-(hydroxymethyl)aminomethane-HCl, *p*H 7.8). The solutions and glassware were sterile. The suspension was filtered through cheesecloth, centrifuged twice at 400*g*, and the bulk of chloroplasts was sedimented at 1000*g*. The pellet was washed twice in the same medium. Electron microscopy of the isolated organelles showed that approximately 40 to 60 percent of spinach chloroplasts and 10 to 30 percent of African violet chloroplasts retained at least part of their surface membrane. Further