tosis of, or cell contact with, zymosan particles. These data are in accordance with those of Manganiello *et al.* (*15*) which illustrate that cyclic AMP concentrations in phagocytic cells do not increase on phagocytosis of particles. Similarly, Seyberth *et al.* (*16*) reported that the concentrations of cyclic AMP in phagocytic granulocytes do not increase during particle ingestion.

In view of these data, it is most tenable to consider that epinephrine inhibits the phagocytic release of lysosomal enzymes from human neutrophils via a mechanism involving an increase in intracellular cyclic AMP. The intracellular events that might link cyclic AMP to inhibition of enzyme release are not understood at the present time. Intracellular, rather than extracellular, actions of cyclic AMP, and therefore of epinephrine, appear to predominate since neither of these agents affect phagocytic uptake of particles when enzyme release is reduced. This failure of cyclic AMP to inhibit phagocytosis has been reported (*1*). One might suppose that cyclic AMP, as it does in other cells and tissues, activates a specific protein kinase, thus catalyzing the phosphorylation of important intracellular components. The consequent alteration of physical or functional properties (or both) of such intracellular components might produce changes in the degree to which granule constituents are released. Whether such intracellular mechanisms in the neutrophil exist and, if so, whether such events can explain adequately the inhibitory action of cyclic AMP on phagocytic enzyme release remain to be determined. Lysosome granules appear to be likely candidates for the intracellular effects of cyclic AMP, as cyclic AMP is capable of inhibiting leakage or release of contents from isolated lysosomes (*9*). In fact, epinephrine and other catecholamines also inhibit enzyme release from isolated lysosomes (*9*). Therefore, alterations in certain properties of the lysosome granule membrane by elevated intracellular cyclic AMP might explain the inhibitory action on lysosomal enzyme release from neutrophils by epinephrine. Such intracellular changes could interfere, conceivably, with certain normal functions of lysosome granules, such as peripheral migration and subsequent fusion of granules with heterophagic vacuoles or the plasma membrane, that lead to extrusion of granule contents.

Regardless of exact mechanism, inhibition of the phagocytic release of granule contents from human neutrophils by epinephrine and cyclic AMP, together with the concomitant elevation of neutrophil concentrations of cyclic AMP by epinephrine, suggests that the autonomic nervous system can regulate the inflammatory process by modulating intracellular neutrophil levels of cyclic nucleotides.

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Penetration of Somatic Mammalian Cells by Sperm

Abstract. *Penetration of somatic mammalian cells by spermatozoa occurred after simple admixture in culture. With sperm labeled in vivo, autoradiography revealed incorporation of DNA into nuclei of recipient cells, indicating release of DNA after entrance by sperm. This system provides a new approach to study the molecular biology of information transfer and of haploid gene expression.*

Mammalian cells can acquire genetic components by simple exposure to nucleic acids, viruses, microorganisms, other cells, and cellular organelles (*1*). A great variety of cultured mammalian cells can ingest not only these entities but also nonbiological substances such as asbestos and polystyrene spheres (*2*). Active participation by the target cells in processes analogous to phagocytosis or pinocytosis is required, since many of the ingested materials are either inert themselves or play only a passive role in the uptake process. Sperm, on the other hand, are specifically endowed with motility and enzymic mechanisms to enhance delivery of their genome to the egg. We have studied their interaction with somatic cells to see whether genetic information can be acquired from the sperm, as occurs in fertilization (*3*).

In orienting experiments, living sperm collected aseptically from Swiss white mouse epididymus and vas deferens were added to monolayer cultures of various malignant or diploid normal mammalian lines maintained in our laboratory. These included mouse, hamster, rat, and human lines. Periodic examination of living or fixed and stained specimens by phase contrast microscopy revealed that apparent penetration of cells in all the lines had occurred.

Mouse sperm and normal, diploid Chinese hamster (CH) fibroblast cells were used. In this pair of species heritable expression of mouse genes results after exposure of CH cells to either mouse tumor cells or the DNA isolated therefrom (*4*). Monolayer cultures of CH cells (*4*) were brought into suspension by routine trypsin treatment (0.125 percent trypsin and 0.01 percent ethylenediaminetetraacetate; 5 to 10 minutes; 37°C), and the rounded, washed cells were mixed with one to five times as many freshly collected sperm, which had been previously washed in Tyrode solution. The mixtures were suspended (final concentration, 2 to 4 × 10^5 CH cells per milliliter)

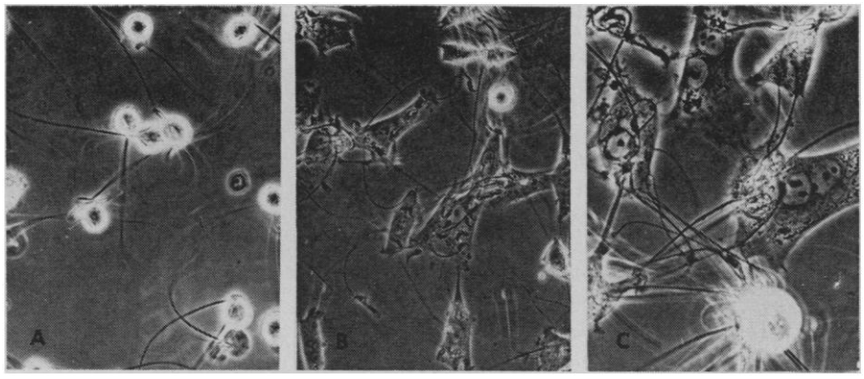

Fig. 1. Phase contrast micrographs of living cultures showing interactions of mouse spermatozoa and somatic Chinese hamster (CH) cells; the ratio of sperm to CH cells is 5 : 1. Sperm are seen within or intimately associated with CH cells. (A) Two hours after mixing in suspension (× 280); use of a television camera on the microscope permitted several people simultaneously to see the penetration on a monitoring screen. (B) After 24 hours, most CH cells have flattened and spread out (× 220). (C) After 40 hours, half of the CH cells are binucleate (× 350). The time for CH cells to double during the log phase of growth was 13 to 14 hours (*4*).

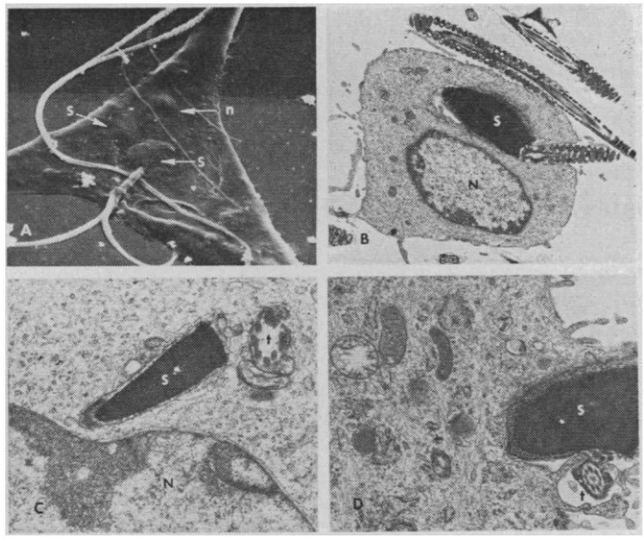

Fig. 2. Penetration of Chinese hamster cells by mouse sperm, 2 to 3 hours after mixing; *S*, sperm or sperm nucleus; *n*, nucleolus; *N*, nucleus of CH cell; and *t*, cross section of sperm tail. (A) Scanning electron micrograph (× 1,100). (B, C, and D) Transmission electron micrographs (×3,800, 11,100, and 11,200, respectively).

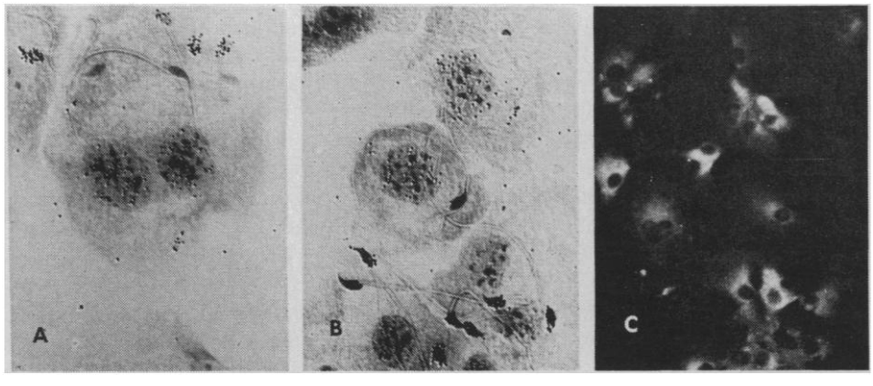

Fig. 3. Interaction of mouse spermatozoa and CH cells. (A) and (B) Autoradiographs showing nuclei of CH cells with label originating from tritiated mouse sperm which had been incubated for 3 days with CH cells; some apparently intact sperm still remain; Giemsa stain; partial Nomarski optics (× 440). (C) Culture of CH cells 21 days after mixed culture with mouse spermatozoa; fixed cells show positive indirect immunofluorescence reaction (*4*) after treatment with appropriately absorbed rabbit antiserum to mouse embryo and staining with fluorescein-conjugated goat antiserum to rabbit immunoglobulin G; similarly treated controls were negative; ultraviolet (× 160).

in Eagle minimum essential medium containing penicillin, streptomycin, and 10 percent fetal bovine serum and incubated in an atmosphere of 5 percent CO_2 and 95 percent air at 37°C in 60-mm dishes containing cover slips. To observe the progress of the interaction (*5*), we also cultivated the mixtures in microscope cover slip chambers and examined them in the living state with phase contrast optics or oblique illumination.

Within 2 hours after admixture, most of the still partially suspended CH cells had been penetrated by at least one spermatozoon when the initial ratio of sperm to CH cells was 5 (Fig. 1A) and about one-fifth were penetrated when the ratio was 1. The cells settled, became attached to the cover slips, and flattened out during the following 22 hours. The number of sperm or sperm heads which could be seen intimately associated with individual cells increased during this time (Fig. 1B). Few of the remaining sperm were still motile after the first day. At about 40 hours after mixing, about half of the CH cells appeared binucleate (Fig. 1C), but these contained only CH chromosomes as judged by karyological analyses. Cells with two nuclei were rarely seen in control cultures not treated with sperm. To learn whether sperm or sperm heads were only loosely associated with CH cells, we treated cultures with trypsin 2 to 4 days after admixture. Although release or dislocation of some attached sperm could be observed a few minutes after trypsin treatment, many sperm heads were unmistakenly retained within the rounding CH cells when the enzyme treatment was prolonged. The microscopic appearance of such preparations strongly resembled those of mouse spermatozoa that had penetrated Syrian hamster eggs from which the zona pellucida had been removed by trypsin treatment (*6*).

Cultures were washed 2 to 3 hours after admixture, centrifuged, and fixed in glutaraldehyde-acrolein for examination in a Cambridge scanning electron microscope; they were postfixed with OsO_4 and embedded in epoxy resin, and thin sections were stained with uranyl acetate and lead citrate for viewing in a Siemens electron microscope. Scanning electron micrographs showed cells, some of which were still largely in the rounded condition, into which sperm had apparently penetrated deeply; most of the still attached tails remained outside the cell. Although these micrographs did not unequivocally re-

veal the head within the CH cell, those of flattened cells (Fig. 2A) provided better evidence of both superficial and deep penetration. The transmission electron micrographs of thin sections left little doubt that actual penetration had occurred. Three illustrations probably representing different stages of entry are given (Fig. 2, B to D). Many of the sperm heads seen in the cytoplasm were not contained within vacuoles. These, as well as the observations on living specimens, suggest that many of the spermatozoa had not merely been drawn passively into the cytoplasm, but that their propulsion and movements contributed to the penetration. There is, however, the interesting instance of the nondestructive cellular ingestion of isolated nonmotile, but still viable, DNA-containing organelles such as spinach chloroplasts and chicken mitochondria by mammalian cells (*7*). The chloroplasts were taken directly into the cytoplasm and were not found in phagocytic vacuoles unless the organelles had previously been denatured by heat (*8*).

The microscopic data (for example, Fig. 2, B to D) show evidence of microvesiculation and blebbing within and between various membranes of the sperm head as well as between these and membrane or cytoplasmic components of the target cell. This kind of blebbing is reminiscent of some of the ultrastructural changes which have been described in the early stages of the interaction of sperm and mammalian eggs (*9*), and after the virus- and lysolecithin-induced fusion of rabbit sperm with mammalian somatic cells (*10*).

To study the fate of the DNA, we labeled sperm by three daily intraperitoneal injections of 20 μc of [^{3}H]thymidine (40 c/mmole) into adult Swiss mice. The spermatozoa were harvested at maturation 32 days later (*11*), and grown in mixed culture with equal numbers of CH cells as described above. The cultures were examined over a period of several days by autoradiography (*4, 5*), with a constant development time of 9 weeks. Under these conditions, about two-thirds of the sperm were labeled and showed about 14 ± 6 grains per labeled sperm. Except for the recognizable labeled sperm with which the CH cells were associated, label was not seen over the CH cells until the third day of mixed culture. At that time, label was seen only over nuclei (average count, 10 ± 5 grains) in 2 to 10 percent of the cells, and in most experiments about half of the cells labeled were binucleate with equal distribution of label over both nuclei (*12*) (Fig. 3, A and B). The labeling pattern is consistent with an intracellular or intranuclear release of DNA (or components thereof) from sperm only after penetration. If release had occurred before entrance of sperm, the label would have been greatly diluted and distributed over many CH cells (*1*).

Progeny cells have been examined to determine whether any functional information transfer can result from this kind of cell-cell union. For example, the characteristic appearance of embryonal gene products (such as antigens) in all mouse tumors examined (*13*) prompted a study to see whether synthesis of such fetal antigens could be initiated in CH cells by mouse sperm penetration. Cells from a mass culture 21 days after mixed culture of the mouse sperm and CH cells were examined by the indirect immunofluorescence technique (*4*) with rabbit antiserum to mouse embryo, which had been absorbed with mixed adult mouse (*13*) and CH internal organs. Some cells showed an immunofluorescence reaction (Fig. 3C) as if mouse-specific genes had been expressed in CH cells (*4*). Proof that transcription and translation of mouse spermatozoal information actually occurred, as suggested by this preliminary result, requires isolation and identification of the presumed gene products, or demonstration of mouse gene sequences in the CH cells. Other problems that may be amenable to study with this novel in vitro system include haploid gene expression (*14*), the possible establishment of haploid lines from target enucleate cells (*15*), and the repair of genetic disease at the cellular level.

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