muscle was filled with red and white Microfil through the femoral artery and a muscular branch, respectively. The tissue was processed in the same way as the heart tissue. Many capillaries at the border region between the two colors were double-filled with red and white Microfil. These results demonstrate that numerous capillary anastomoses are present in skeletal muscle, in contrast to the capillary beds in the heart, which are discrete. They further imply that our finding of end-capillary beds in the heart is not a technical artifact, for the same technique was employed for both myocardium and skeletal muscle; if capillary anastomoses existed in the heart they should have been observed with this method.

11. J. Caulfield and B. Klionsky, *Am. J. Pathol.* **35**, 489 (1959); A. H. Harken, C. H. Barlow, W. R. Harken III, B. Chance, *ibid.* **42**, 955 (1978); J. E. Lowe, K. A. Reimer, R. B. Jennings, *ibid.* **90**, 363 (1978); C. Mechthild, S. Pasyk, W. Schaper, *Circulation* **58** (Suppl. 2), II-37 (1978) (abstract).
12. We recently learned of papers by R. E. Brown [*Am. J. Anat.* **116**, 355 (1965)] and G. Ludwig [*Methods Achiev. Exp. Pathol.* **5**, 238 (1971)], describing capillary anatomoses in the myocardium; however, the techniques of vessel perfusion and analysis precluded an identification of the large coronary arteries supplying the microcirculation.
13. We thank H. Parker and W. Leon for expert technical assistance and T. Maio for secretarial help. Supported by NIH grant HL 23171-01 and contract HV 52997.

29 May 1979

Intestinal Absorption of Immune Complexes by Neonatal Rats: A Route of Antigen Transfer from Mother to Young

Abstract. *Horseradish peroxidase (HRP) in the presence of specific immunoglobulin G antibody to HRP is selectively absorbed from the gut lumen and transferred by intestinal epithelial cells to the lamina propria in newborn rats. The HRP is not transferred in detectable amounts in the absence of the antibody. Transport of maternally derived antigen via antigen-antibody complexes may have important influences on the developing immune system in young mammals.*

The transmission of maternal immunoglobulins across fetal and neonatal tissues is essential for the survival of mammalian offspring (*1*). In this way, developing mammals passively acquire immunity until their own immune systems mature. In the neonatal rat, maternal immunoglobulins that occur in the milk are transported across the proximal small intestine during the first 3 weeks after birth. The transport process is highly selective for immunoglobulin G (IgG). Other immunoglobulin classes, as well as other serum and milk proteins, are transferred in much smaller amounts or not at all and instead are digested intracellularly by absorptive cells in the distal jejunum and ileum (*2*). The transport of IgG is a saturable process, can be competitively inhibited, and requires the presence of the Fc (crystallizable fragment) region of the IgG molecule (*2, 3*). This evidence is consistent with the hypothesis that transport of maternal IgG is mediated by cell membrane Fc receptors (*1–4*).

Potential antigens within the mother are in some instances also transferred to the young and can lead to the suppression or enhancement of the immune response (*5–7*) or, if the antigen is a pathogen, active infection of the young (*8*). However, the mechanisms whereby antigens are passed to the young are poorly understood. By using the electron microscope we have documented the transfer of antigens in the form of antigen-antibody complexes across the proximal small intestine of the suckling rat.

Horseradish peroxidase (HRP), a globular protein (40,000 daltons) that can be readily visualized in the electron microscope, was used as the antigen for our transport experiments. We obtained an antiserum to HRP by immunizing adult rats and rabbits with HRP in complete Freund's adjuvant. Control antiserums were obtained from animals immunized with dinitrophenylated bovine-γ-globulin (DNP-BGG). The IgG fractions of the antiserums were purified by precipitation with ammonium sulfate and then by chromatography with diethylaminoethyl (DEAE) cellulose. The antigenic specificity of each IgG fraction was assessed by immunodiffusion, and the concentration of specific antibody to HRP present in each of the antiserums was estimated by quantitative precipitation. The concentration of total IgG in each preparation was then adjusted to 10 mg/ml in 0.1*M* phosphate buffer, *p*H 6.0. Soluble immune complexes of HRP and antibody to HRP (PAP) were prepared by adding a twofold molar excess of HRP to the antiserum to HRP. An equal amount of HRP was added to the antiserum to DNP-BGG to obtain a control mixture of HRP and IgG (PIg). Neonatal rats (10 days old) were anesthetized with ether, and a 2- to 3-cm segment of proximal jejunum was ligated in situ. A 0.1-ml sample of either PAP or PIg was injected into the lumen of the segment. After 15, 30, 60, or 120 minutes, pieces of ligated jejunal tissue were excised and fixed in 2 percent buffered glutaraldehyde, and then incubated in a mixture of H_2O_2 and diaminobenzidine (DAB) to reveal the location of HRP (*9*). After postfixation in osmium tetroxide, the tissue was dehydrated, embedded, and sectioned for examination in the electron microscope. We followed a similar procedure in assessing HRP transport in the jejunum of 22-day old rats.

Our results reveal strikingly different patterns for the cellular distribution of HRP between tissue exposed to PAP and control tissue incubated with PIg. Within 30 minutes of exposure to PAP, HRP was identified throughout the columnar epithelial cells of the jejunum (Fig. 1a). Reaction product was found within endocytotic pits at the microvillar surface and within small underlying tubules and vesicles. HRP was also apparent near the Golgi region of the cell in small vesicles that have previously been identified as coated vesicles (*2, 3*). Most important, after incubation for 30 minutes or longer, HRP reaction product was seen with progressively increasing concentration in the extracellular spaces between epithelial cells. Coated vesicles containing antigen were frequently observed in close proximity to the lateral border of epithelial cells and at times appeared to be discharging HRP into the extracellular space. Identical results were obtained when immune complexes were prepared with rabbit IgG, a heterologous Ig that is transported across the intestine of the suckling rat (*1*). Thus, the cellular distribution of HRP that results from the exposure of jejunal cells to PAP is essentially the same as the distribution of maternal IgG in the absence of antigen within these same cells (*2–4*).

In the neonatal tissue exposed to PIg, HRP was found only in apical regions of the cell regardless of incubation time (Fig. 1b). The HRP occurred predominantly in large apical vacuoles and multivesicular bodies and, when compared to PAP-exposed cells, was qualitatively much less abundant overall within the cells. After incubation of the cells with PIg HRP was never observed in the more basal regions of the cells, within coated vesicles, or within the extracellular spaces. When jejunal segments of 22-day-old rats were exposed to either PAP or PIg, HRP was not detected within or on the surface of any of the absorptive cells examined.

To investigate whether immune complex transport in newborn rats is mediated by the Fc region of the IgG molecule as has been shown for the transport of uncomplexed IgG (*3*), we prepared immune complexes from $F(ab')_2$ fragments derived from rabbit antibody to HRP di-

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gested with pepsin (*10*). Although the $F(ab')_2$ fragments were able to bind and precipitate HRP as demonstrated by immunodiffusion, no transport of HRP by neonates was observed when intestinal segments were exposed to these immune complexes. These results suggest that the membrane receptors for uncomplexed IgG are probably also responsible for the transport of immune complexes. Accordingly, specific antibody can link the bound antigen to the receptor and thus can cause the antigen to be carried piggyback fashion through the cell.

The results from our experiments with PIg demonstrate that some free antigen can enter immunoglobulin-transporting cells. We believe that pinocytosis stimulated by the presence of nonspecific IgG could account for the nonselective uptake of HRP in the fluid volume of pinocytotic vesicles. However, free HRP that fortuitously enters the cell is not linked to the IgG receptor and therefore is not transported. Instead, this HRP is confined to apical vacuoles and multivesicular bodies. The segregation of free antigen from the IgG transport pathway must serve to protect, in part, the vulnerable neonate from antigenic contamination. In contrast, in the adult rat, small amounts of free antigen are apparently transferred across the intestine to the circulation, but immune complex formation appears to decrease significantly such antigen transfer (*11*).

It is not clear what influences the absorption of immune complexes might have on the neonate. The antigen in the complex may (i) dissociate and possibly infect the newborn, (ii) suppress or enhance an active immune response, or (iii) induce tolerance. There is evidence that any one of these three alternatives might occur. Uhr and Baumann (*12*) have demonstrated the possibility of immune complex dissociation. Complexes of diphtheria toxin and antitoxin were injected into adult rabbits or guinea pigs and, in some cases, resulted in death or diphtheric paralysis. Gill and co-workers (*5*) discovered that radioactive antigens injected into female rats before mating are transferred to the litter. In these experiments, the offspring of genetically high responding strains demonstrated a suppressed antigen-specific response when challenged by antigen. Litters from low responders expressed an enhanced response.

Developing mammals are particularly prone to the induction of tolerance (*8*). Benjamin and co-workers (*7*) have shown in neonatal mice that human IgG, which is selectively absorbed by the intestine nearly as readily as homologous mouse IgG (*1, 3*), can induce specific, long-lasting tolerance in the neonate. Their experiments support the theory that during early development the failure of antigenic exposure to stimulate an immune response may represent a mechanism for the generation of self-tolerance (*13*). In addition, Auerbach and Clark (*6*) have demonstrated with mice that agents that induce tolerance can be passed from tolerant mothers to their young during nursing. However, when offspring from tolerant mothers nursed from nontolerant dams, tolerance was not induced. Finally, antigen-antibody complexes have been recognized as potent inducers of tolerance in several humoral and cell-mediated immune responses (*14*). Thus, in addition to passive immunity, tolerance can also be induced in the neonate, possibly by the transfer of immune complexes from the mother to young.

It appears certain that the transfer of antigen can indeed affect the well-being of the newborn and the developing immune system. Our findings demonstrate that antigen-antibody complexes tra-

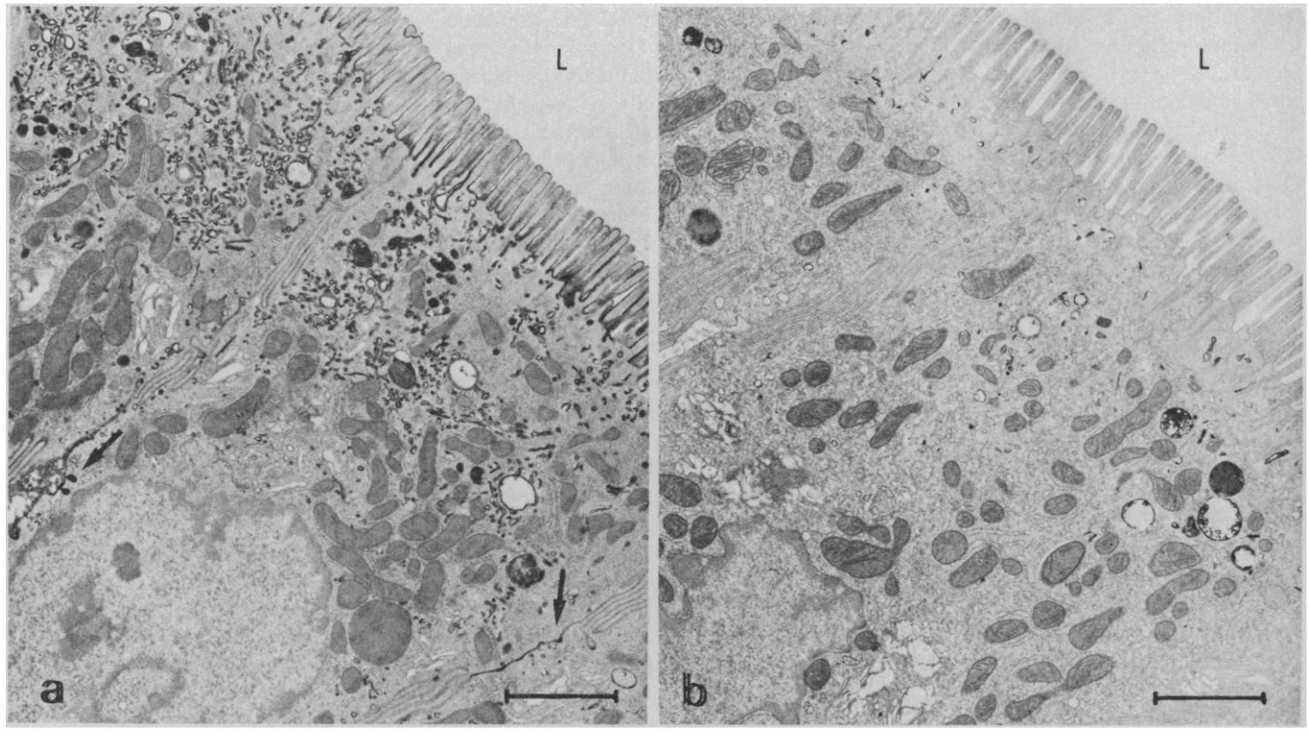

Fig. 1. (a) Electron micrograph of jejunal epithelial cells from a 10-day-old rat exposed to immune complexes of HRP and antibody to HRP (PAP) for 60 minutes. Initially, PAP, as visualized as an electron dense reaction product of HRP, binds to the luminal plasmalemma at the bases of the microvilli and then enters the absorptive cells by endocytosis. The HRP is subsequently transferred to coated vesicles that fuse with the basolateral membranes of the cells (arrows) and release antigen into the intercellular spaces. (b) Cells incubated for 60 minutes with HRP and IgG directed against a different antigen (PIg). In the absence of specific antibody, HRP does not bind to the luminal brush border membrane and, in general, enters the cells in much smaller amounts than after incubation with PAP. At all time points, antigen is confined largely to vesicles in the apical cytoplasm immediately beneath the terminal web. Transfer of antigen to the extracellular spaces is not detected (*L*, intestinal lumen; scale bars, 2 μm).

verse a neonatal tissue formerly believed to transport only maternal antibodies. Thus, selective transport of IgG-antigen complexes represents a possible mechanism for entry into the neonate of a wide variety of antigens. We must therefore consider the transmission of immune complexes as an extremely important, possibly frequent event that may influence the development of immune capability.

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References and Notes

1. F. W. R. Brambell, *The Transmission of Passive Immunity from Mother to Young* (North-Holland, Amsterdam, 1970).
2. B. Morris, *J. Physiol (London)* **245**, 249 (1975); R. Rodewald, *J. Cell Biol.* **45**, 635 (1970).
3. A. E. Beer and R. E. Billingham, *The Immunobiology of Mammalian Reproduction* (Prentice-Hall, Englewood Cliffs, N.J., 1976); R. Rodewald, in *Maternofoetal Transmission of Immunoglobulins*, W. A. Hemmings, Ed. (Cambridge Univ. Press, Cambridge, 1976), p. 137; T. A. Waldmann and E. A. Jones, in *ibid.*, p. 123.
4. B. Morris and R. Morris, *J. Physiol. (London)* **265**, 429 (1977); R. Rodewald, *J. Cell Biol.* **58**, 189 (1973).
5. D. V. Cramer, T. J. Gill III, G. Knauer, *Am. J. Pathol.* **90**, 317 (1978); B. K. Davis and T. J. Gill III., *J. Immunol.* **115**, 1166 (1975).
6. R. Auerbach and S. Clark, *Science* **189**, 811 (1975).
7. D. C. Benjamin, *J. Immunol.* **119**, 311 (1977); J. F. Halsey and D. C. Benjamin, *ibid.* **116**, 1204 (1976).
8. D. V. Cramer, H. W. Kunz, T. J. Gill III, *Am. J. Obstet. Gynecol.* **120**, 431 (1974); J. P. Soloman, *Foetal and Neonatal Immunology* (North-Holland, Amsterdam, 1971).
9. R. C. Graham and M. J. Karnovsky, *J. Histochem. Cytochem.* **14**, 291 (1966).
10. A. Nisonoff *et al.*, *Arch. Biochem. Biophys.* **89**, 230 (1960).
11. W. A. Walker, S. N. Abel, M. Wu, K. J. Bloch, *J. Immunol.* **117**, 1028 (1976).
12. J. W. Uhr and J. B. Baumann, *J. Exp. Med.* **113**, 935 (1961).
13. F. M. Burnet, *The Clonal Selection Theory of Immunity* (Cambridge Univ. Press, Cambridge, 1959).
14. K. E. Hellström and I. Hellström, *Adv. Immunol.* **18**, 209 (1974); N. R. St. C. Sinclair, R. K. Lees, S. Abrahams, P. L. Chan, G. Fagan, C. R. Stiller, *J. Immunol.* **113**, 1493 (1974); P. W. Wright, R. E. Hargreaves, S. C. Bansal, I. D. Bernstein, K. E. Hellström, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2539 (1973).
15. Supported by NIH grant AI 11937. We thank T. Zakin for technical assistance, D. A. Begg, D. C. Benjamin, and D. E. Normansell for criticisms of the manuscript, and G. Johnson and O. Wells for secretarial help.

4 June 1979

Strong Electrical Currents Leave the Primitive Streak of Chick Embryos

Abstract. *The electrical fields above chick embryos were explored with a vibrating probe. These fields indicate that steady currents with exit densities of the order of 100 microamperes per square centimeter leave the whole streak and return elsewhere through the epiblast. The epicenter of these strong exit currents lies near Hensen's node. They are probably pumped into the intraembryonic space by the epiblast and then leak out of the streak because it is a zone of junctional disruption.*

Recent explorations with a vibrating probe show that a wide variety of developing systems drive strong steady electrical currents through themselves (*1*). Currents of 1 to 100 μA/cm^2 traverse developing systems from a level as simple as an algal egg (*2*) through one as complex as the regenerating stump of a newt limb (*3*). Moreover, substantial evidence indicates that these currents—or at least some of them—act back to affect development (*4*). However, an important intermediate stage of developing system—which may be roughly called the epithelial one—has not been explored. We now report a first exploration of developmental currents at this stage—specifically, through the early chick embryo. At the primitive streak stages, the embryo consists largely of two flat epithelia separated by a narrow intraembryonic space. The upper sheet, or epiblast, contains a 1- to 2-mm long groove (the primitive streak) through which epiblast cells enter en route to forming all, or almost all, of the internal tissues. We have found that strong steady currents pour out of the whole streak and return elsewhere through the epiblast. In the measurement plane, 0.2 mm above the epiblast, current densities of up to 10 to 20 μA/cm^2 were found; ones on the order of 100 μA/cm^2 are estimated to leave the streak.

Large pieces of vitelline membrane with an adherent embryo were peeled off the yolk and explanted, right side up, into a measurement chamber according to a modification of Nicolet and Gallera's method (*5*) together with Jaffe and Nuccitelli's vibrating probe technique (*6*). The explant was supported on a dense, inert oil (Mediflor, Minnesota Mining and Manufacturing) and covered with a layer of thin albumen (of 92 ohm-cm resistivity) in turn covered with a light nontoxic oil (Klearol, Witco). The probe's meniscus setter was placed at the upper water-oil interface. The preparation was maintained at 38°C. Typical signals were 0.3 to 3 μV; root-mean-square noise levels (with a 10-second time constant), 0.02 μV. We visualized the top surface of the 20- to 40-μm thick vitelline membrane by depositing upon it some 10 μm of crystals of the rare earth oxide, Barnesite (which is sold for lens grinding). We studied a dozen explants and succeeded in exploring the patterns of tangential currents above three of them during streak stages 3 to 5. These all seemed relatively healthy since they later formed several pairs of somites, a well-formed head fold, and a neural tube.

Figure 1 shows a scan of the current component flowing across the streak. The direction of the current reversed sharply above the streak, with current flowing away from both sides of it. This outward current reached a peak density of 8 to 10 μA/cm^2 at 0.1 to 0.2 mm from the streak and then continued, with diminishing intensity, out to 1 mm from the streak. Farther out, toward the edges of the explant, the current reversed direction again, so that it returned from these edges. We obtained similar results from all of the seven similar scans made across various levels of the streak (from front to rear) of the three explants successfully explored. They indicate that current leaves the whole streak (as well as the periphery) and returns elsewhere through the epiblast (Fig. 1C).

Scans of the cross component were also made in several vertical lines extending through various points, particularly the circled point of peak density, shown in Fig. 1A. This curve (not illustrated) fell steadily from 16 μA/cm^2 at 0.15 mm above the embryo's top surface to 11 μA/cm^2 at 0.2 mm, 6 μA/cm^2 at 0.3 mm, and 3 μA/cm^2 at 0.5 mm; this is about the shape predicted by potential theory. Integration of this curve indicates that about 1 μA leaves each centimeter length of the streak. If we assume that current leaves a strip about 0.1 mm wide, it leaves with a density of the order of 100 μA/cm^2.

Figure 2 shows a scan of the current component flowing parallel to the streak and within a line just above the streak. Current direction reversed sharply about 0.2 mm behind the front end of the streak, that is, behind Hensen's node. Current flowed away from all sides of this central reversal point or epicenter—forward to the most anterior point explored (0.2 mm in front of the node) and backward to the most posterior point explored (0.6 mm behind the rear end of the streak) as well as laterally. The densest forward current (25 μA/cm^2) lay just posterior to Hensen's node, and two similar peaks of backward current lay just posterior to the epicenter and just posterior to the caudal end of the streak. Figure 2A also shows the parallel component with-

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