widely in effectiveness (Fig. 3). In these experiments the uptake of the basic compounds was more than 100 times greater than the uptake of albumin.

The striking parallelism between the uptake of fluorescent compounds and radioactive albumin represented in Fig. 3 could be most simply explained if both molecules were taken up in association. However, no trace of complex formation between albumin and poly-L-ornithine could be detected in vitro (*3*).

The stimulatory effect of histones and polyamino acids could be reduced by increasing the serum concentration in the incubation medium. Several metabolic inhibitors were tested, including sodium fluoride, dinitrophenol, potassium cyanide, and iodoacetate, but only the last had some effect. At a concentration of $10^{-4}M$, iodoacetate decreased by half the stimulation produced by 30 μg/ml of histones, but in the absence of histone the albumin uptake was entirely insensitive to iodoacetate. Also, the temperature dependence of albumin uptake was different when measured in the presence or in the absence of histones, with a Q_{10} above 2 in the presence of histones, and below 2 in their absence. These distinct metabolic characteristics reflect differences in the mechanism of these two forms of uptake and suggest that histones do not in fact increase the basic albumin uptake per se. Rather they initiate a penetration process of a different character, in the course of which albumin appears to be driven into the cell in a more passive way. This hypothesis is in agreement with the fact that albumin lags behind histones in terms of absolute uptake, even when the ratio of albumin to polymer in the medium is greater than 1.

Protamine and basic polyamino acids are known to increase phagocytosis in leukocytes in concentrations comparable to those we used (*4*). It is tempting therefore to postulate that the effects we have measured result from a comparable stimulation of membrane movements. However, we have no morphological evidence as yet pointing to an increase in pinocytosis or endocytosis; until such data are available we prefer not to exclude other possible modes of action, such as group translocation or molecular rearrangements in the membrane itself. Reversible changes in fine structure have been demonstrated in the membranes of red blood cell ghosts treated with polylysine (*5*). Moreover, histone concentrations as low as 1 μg/ml have been shown to act on a different type of membrane and to induce marked changes in mitochondrial metabolism and in mitochondrial adenosine triphosphatase activity (*6*). In view of this plural action and of the low threshold of activity, histones and basic polyamino acids must be considered to be highly potent membrane-active agents.

The clumping, detachment, and cell damage we observed at higher protamine and histone concentrations (100 μg/ml and more) are consistent with the data of Becker and Greene (*7*). It is relevant to point out in this respect that the majority of investigators studying the interaction of histones and protamine with living cells have used concentrations of 100 μg/ml and more (*7–11*). The interpretation of results obtained under such conditions must take into account the possibility of cytotoxic reactions, which may obscure the biological significance of the experimental observation; this note of caution may also apply to the current studies of the role of histones in genetic regulation (*9*).

The effect of histones and related substances on macromolecular penetration does not seem to be restricted to proteins. Protamine has been shown—albeit in different concentration ranges—to increase the amount of foreign RNA capable of functioning in fibroblasts (*11, 12*). Preliminary data from our laboratory indicate that polysaccharides such as inulin are influenced in a comparable fashion. It appears therefore that histones and polyamino acids might constitute useful tools for introducing into mammalian cells a variety of macromolecules of biological or therapeutic interest.

Finally, our data provide good evidence for the selective uptake of various proteins. Arginine-rich histones are taken up 10 times more readily than lysine-rich histones and some 1000 times more so than human serum albumin. This selectivity adds to the biological interest of the uptake process and raises the possibility that cellular histones might exert regulatory functions at extranuclear sites.

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Nucleoside Phosphatases of Fetal and Maternal Blood Cells: Electron Microscope Study

Abstract. *Surface membranes of the blood cells located within the maternal blood sinuses of the rat placenta contain reaction products of nucleoside phosphatases. Fetal blood cells separated from maternal blood by the placental labyrinth show no activity during normal or prolonged gestation. Neonatal blood cells examined 34 hours after parturition show these enzyme activities.*

Recent studies of the fine structural localization of enzymes capable of hydrolyzing nucleoside phosphate esters have shown that there is enzyme activity on the surface membranes of a variety of adult blood cells (*1*). In our study, erythrocytes, leukocytes, and platelets localized within maternal blood sinuses of the chorioallantoic placenta of the rat also showed discrete nucleoside phosphatase reaction products on their surface membranes.

However, all types of fetal blood cells separated from maternal blood by trabecular cords of fetal tissue showed no such enzyme activity on the 14th through 22nd days of gestation. When parturition was delayed 3 days past term (to 25 days) fetal blood cells remained unreactive. On the other hand, plasma membranes of neonatal blood cells examined 34 hours after parturition displayed activity to nucleoside phosphate esters. It would thus appear that birth is a physiological stimulus for the increase in these enzyme activities.

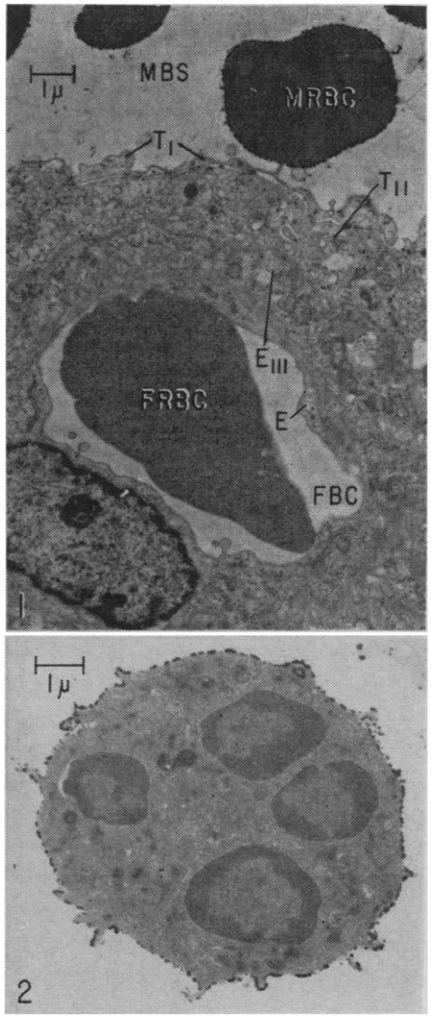

Fig. 1 (top). Placental labyrinth at 18 days, incubated with ATP as substrate. Dense reaction product coats surface membranes of maternal erythrocytes (*MRBC*) within a maternal blood sinus (*MBS*). Precipitates also appear on the surface membranes of trophoblast I (T_I) and in the intercellular space between trophoblasts I and II (T_{II}). Element III (E_{III}) as well as the endothelium (*E*) lining the fetal capillary (*FBC*) lack activity. Reaction product is not present on the surface membrane of the fetal erythrocyte (*FRBC*). Fig. 2 (bottom). Neonatal neutrophil at 34 hours. Washed prior to fixation and incubated with ATP as substrate. Activity coats surface membranes as in Fig. 1.

Freshly excised placentas from rats in the 14th, 18th, 22nd, and 25th days of gestation were cut into small pieces and fixed in 3 percent glutaraldehyde (*2*) buffered to *p*H 7.4 with 0.1*M* sodium cacodylate for 1 hour at 3°C. Thirty-four hours postnatally 1 ml of blood from each of five rats was taken by cardiac puncture and treated with heparin (100 U.S.P. units per milliliter). After the plasma had been removed by centrifugation, the cells were washed in three changes of 0.15*M* KCL and resuspended in cold fixative. Further procedures for the neonatal blood were the same as for the placental tissues, except for centrifugation and resuspension with each change of solution. After fixation tissues were washed overnight in cold 0.1*M* cacodylate buffer containing 0.22*M* sucrose. Frozen sections (50μ) as well as small blocks (1 mm^3) of tissue were incubated for 30 minutes in a standard Wachstein-Meisel (*3*) medium, *p*H 7.2, containing lead nitrate as the capture reagent and one of the following substrates: adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP). For control experiments, tissues were incubated either in media in which adenosine phosphate esters were replaced by equimolar concentrations of β-glycerophosphate or in substrate-free media. No appreciable changes in *p*H occurred as a result of incubation, which, in all cases, was carried out at room temperature.

After incubation the tissues were washed in cold buffer, fixed 2 hours in 2 percent buffered osmic acid (*p*H 7.4) containing 0.22*M* sucrose, dehydrated in a graded series of ethanol, and embedded in Epon 812. Thin sections were cut with glass knives on a microtome (Porter-Blum I) and examined without additional staining in an electron microscope (RCA EMU 3F).

The final product (lead phosphate) resulting from the hydrolysis of ATP, ADP, and AMP substrates was deposited, in each case, as a fine, granular precipitate on the external surface of blood cells (Figs. 1 and 2). Of these substrates, no one showed more activity than another. The other dense granules appearing in the matrix of both fetal and maternal erythrocytes represent inactive single ribosomes (*4*). In the neonatal blood there appeared to be more final reaction product on the limiting membranes of leukocytes and platelets than there was on erythrocytes. Maternal blood showed a more even distribution of activity between cell types. In both cases the reaction was especially prominent where these cells were in contact with one another. When β-glycerophosphate was used as substrate no reaction occurred; hence alkaline phosphatase was not responsible for the activity demonstrated. Nonspecific staining by lead ions was completely absent after incubation of the tissues in substrate-free media.

Both Post and Tosteson *et al.* found an ATP-cleaving enzyme system in broken erythrocyte membranes (*5*). Their results suggest that this nucleoside phosphatase is involved in the active transport of Na^+ and K^+ across intact erythrocytes. With neonatal blood cells which were washed prior to fixation, it is possible to say that the activity found represents a system of membrane-bound enzymes rather than soluble plasma enzymes which had adsorbed to the cell surface during fixation. The absence of reaction product on fetal blood cells probably reflects low levels of enzyme activity, undetectable by electron cytochemical procedures until after birth. It is unlikely that complete inhibition by glutaraldehyde occurs, since a similar inhibition would also be expected to occur in maternal blood cells. Dawkins found such a pattern of enzyme appearance in fetal and neonatal liver (*6*). Furthermore, many other tissues show increasing activity of enzymes after birth as an adaptation to neonatal life (*7*).

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