red blood cells (*8*). Interference with surface coats and close apposition between plasma membranes have been considered prerequisites for actual cell fusion (*16*), a phenomenon that we are unable to rule out as a contributing cause of the observed foot process abnormalities. The rapidity of induction of epithelial configurational changes by polycation perfusion and reversibility following subsequent heparin perfusion indicate that the cytoplasmic processes of glomerular epithelial cells are not rigid structures, but undergo changes in shape in response to altered local environment or cell surface.

Protein leakage through the glomerular filter has been considered to be related to the development of foot process abnormalities (*5, 17*). Conversely, a cellular defect, leading to the development of epithelial pockets and vacuoles or "percellular channels" has been held to be partly responsible for abnormal protein leakage through the glomerular filter in rat aminonucleoside nephrosis (*18*). Decrease in anionic sites was histochemically demonstrated in nephrotic glomeruli, and alterations in sialic acid metabolism have been related to the onset of proteinuria in rat aminonucleoside nephrosis (*6*); but precise data on their temporal relationship to the development of epithelial changes are not available. It seems reasonable to consider that a decrease in anionic sites, in the glomerular basement membrane as well as on the epithelial surface, may modify the permeability characteristics of the glomerular filter; thus proteinuria and morphological foot process abnormalities occur concomitantly in the nephrotic syndrome, perhaps as a result of defective synthesis of negatively charged structural proteins by the epithelial cells. The polycation-induced glomerular alterations bear striking resemblance to the pathological changes seen in the nephrotic syndrome and are presumably caused by artificial reduction of epithelial surface charge. Whether interference with epithelial and basement membrane anionic sites by polycations also results in altered glomerular permeability remains to be established.

M. W. SEILER
M. A. VENKATACHALAM, R. S. COTRAN
Departments of Pathology,
Peter Bent Brigham Hospital,
West Roxbury Veterans Administration Hospital, and *Harvard Medical School,*
Boston, Massachusetts 02115

References and Notes

1. K. W. Zimmerman, *Z. Mikrosk. Anat. Forsch. (Leipz.)* **32**, 176 (1933); B. V. Hall, *Proceedings of the Fifth Annual Conference on Nephrotic Syndrome* (National Nephrosis Foundation, New York, 1954), p. 117; D. C. Pease, *J. Histochem. Cytochem.* **3**, 295 (1955); J. Rhodin, *Exp. Cell Res.* **8**, 572 (1955); E. Yamada, *J. Biophys. Biochem. Cytol.* **1**, 551 (1955); M. Arakawa, *Lab. Invest.* **23**, 489 (1970); P. M. Andrews and K. R. Porter, *Am. J. Anat.* **140**, 81 (1974); H. Latta, in *Handbook of Physiology*, Section 8, *Renal Physiology* (American Physiological Society, Rockville, Md., 1973), pp. 1–29.
2. S. C. Mohos and L. Skoza, *Science* **164**, 1519 (1969); *Exp. Mol. Pathol.* **12**, 316 (1970).
3. D. B. Jones, *Lab. Invest.* **21**, 119 (1969).
4. The renal glomerulus is a system of capillary loops functioning as an ultrafilter for plasma and preventing molecules approximately the size of albumin and larger from entering the urinary space. The capillary wall consists of three consecutive layers: a fenestrated endothelium, a continuous basement membrane, and the glomerular visceral epithelium. Both the basement membrane and the filtration slits in between epithelial foot processes have been ascribed roles in the normal process of exclusion of proteins from the urinary space [M. G. Farquhar, S. L. Wissig, G. E. Palade, *J. Exp. Med.* **113**, 47 (1961); J. P. Caulfield and M. G. Farquhar, *J. Cell Biol.* **63**, 883 (1974); R. C. Graham and M. J. Karnovsky, *J. Exp. Med.* **124**, 1123 (1966); M. A. Venkatachalam, M. J. Karnovsky, H. D. Fahimi, R. S. Cotran, *ibid.* **132**, 1153 (1970); R. Rodewald and M. J. Karnovsky, *J. Cell Biol.* **60**, 423 (1974); K. H. Langer, N. Paul, W. Thoenes, *Cytobiologie* **7**, 268 (1973)].
5. M. G. Farquhar, R. L. Vernier, R. A. Good, *J. Exp. Med.* **106**, 649 (1957); R. L. Vernier, B. W. Papermaster, R. A. Good, *ibid.* **109**, 115 (1959); M. G. Farquhar and G. E. Palade, *ibid.* **114**, 699 (1961).
6. A. F. Michael, E. Blau, R. L. Vernier, *Lab. Invest.* **23**, 649 (1970); E. B. Blau and J. E. Haas, *ibid.* **28**, 477 (1973); E. B. Blau and A. F. Michael, *Proc. Soc. Exp. Biol. Med.* **141**, 164 (1972).
7. P. M. Quinton and C. W. Philpott, *J. Cell Biol.* **56**, 787 (1973).
8. A. Nevo, A. DeVries, A. Katchalsky, *Biochim. Biophys. Acta* **17**, 536 (1955); A. Katchalsky, D. Danon, A. Nevo, A. DeVries, *ibid.* **33**, 120 (1959); A. Katchalsky, *Biophys. J.* **4** (Suppl.), 9 (1964).
9. M. Mamelak, S. L. Wissig, R. Bogoroch, I. S. Edelman, *J. Membr. Biol.* **1**, 144 (1969).
10. The test substances used have the following molecular weights, which are well below that of albumin and hence could diffuse freely through the glomerular filter: protamine sulfate, approximately 7,000; poly-L-lysine, 1,000 to 4,000; poly-κ-glutamic acid, 14,700; myoglobin, 17,000; and heparin, 17,000.
11. J. F. Rinehart and S. K. Abul-Haj, *AMA (Am. Med. Assoc.) Arch. Pathol.* **52**, 189 (1951).
12. D. Gingell and J. F. Palmer, *Nature (Lond.)* **217**, 98 (1968); D. Gingell, *J. Embryol. Exp. Morphol.* **23**, 583 (1970).
13. L. Wolpert and D. Gingell, *Symp. Soc. Exp. Biol.* **22**, 169 (1968).
14. L. Weiss, *J. Cell Biol.* **26**, 735 (1965).
15. P. W. Brandt and A. R. Freeman, *Science* **155**, 582 (1967).
16. G. Poste and A. C. Allison, *J. Theor. Biol.* **32**, 165 (1971).
17. R. L. Vernier, B. W. Papermaster, K. Olness, E. Binet, R. A. Good, *Am. J. Dis. Child.* **100**, 476 (1960); E. R. Fisher and H. R. Hellstrom, *Lab. Invest.* **11**, 617 (1962).
18. M. A. Venkatachalam, M. J. Karnovsky, R. S. Cotran, *J. Exp. Med.* **130**, 381 (1969); M. A. Venkatachalam, R. S. Cotran, M. J. Karnovsky, *ibid.* **132**, 1168 (1970); K. H. Langer, N. Paul, W. Thoenes, *Virchows Arch. Abt. B. Zellpathol.* **17**, 91 (1974).
19. We thank D. Sandstrom and B. A. Gray for their technical assistance and V. Samuels for help in preparing the manuscript. This work was supported by grants AM16749 (M.A.V.) and HL08251 (R.S.C.) from the National Institutes of Health.

12 February 1975

2,3-Diphosphoglycerate in Erythrocytes of Chick Embryos

Abstract. *2,3-Diphosphoglycerate, heretofore considered absent in avian erythrocytes, occurs in the erythrocytes of embryos to the extent of 4 to 5 micromoles per cubic centimeter of erythrocytes before hatching; it disappears from the cells within 8 days after the embryo hatches.*

In 1925 Greenwald (*1*) showed that approximately two-thirds of the organic phosphate of pig erythrocytes is 2,3-diphosphoglyceric acid (2,3-DPG). Subsequently it has been shown that a high concentration of 2,3-DPG is characteristic of erythrocytes of most mammals. In all animal cells barely detectable amounts of 2,3-DPG are present where 2,3-DPG serves as a cofactor for the enzyme phosphoglycerate mutase (*2*). In 1967 it was discovered that 2,3-DPG serves as an important allosteric regulator of hemoglobin function in mammalian erythrocytes (*3, 4*). Until that time the position of the oxygen-dissociation curve in any given species had been considered "fixed," except for the well-known effects of *p*H, temperature, and CO_2. It is now generally accepted that regulation of hemoglobin oxygen affinity by 2,3-DPG serves as an important adaptive mechanism for the organism during periods of hypoxia (*5*).

Equally distinctive is the presence of

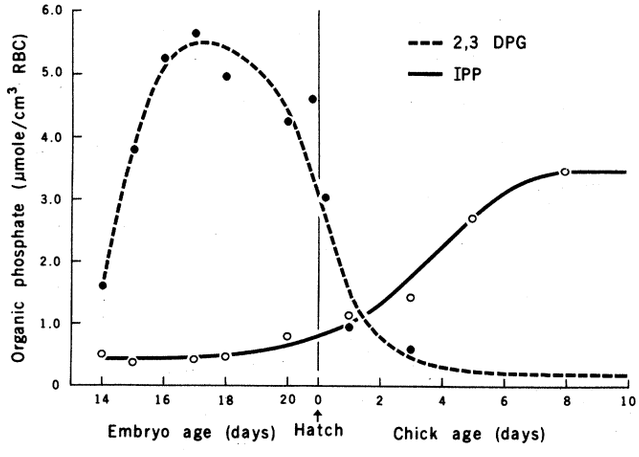

Fig. 1. The 2,3-DPG and IPP contents of erythrocytes from chick embryos and young chicks. The data collected by three methods and shown in Table 1 for the analyses of 2,3-DPG in micromoles per cubic centimeter of red cells were averaged for each age and plotted as a function of time in days of incubation or days after hatch. The levels of IPP in micromoles per cubic centimeter of cells for each respective age are plotted as a function of time.

Table 1. 2,3-Diphosphoglyceric acid and inositol pentaphosphate in erythrocytes from chick embryos and chicks. The embryo age is indicated as the day of embryonic development (DE) from start of incubation. "Pipped" means embryos that have pipped the shell but have not hatched. "Hatched" are chicks that have been out of the shell for 1 to 2 hours. Chick age is shown as day since the chick (DC) hatched. Enzymatic assay of 2,3-DPG was determined by reaction kinetics on perchloric acid extracts of erythrocytes. Trichloroacetic acid extracts of erythrocytes were chromatographed on Dowex 1-X8 formate columns. The 2,3-DPG was determined by reaction of portions of column fractions with chromotropic acid and by wet-ash phosphate analysis. IPP was assayed by wet-ash phosphate analysis of column fractions. Numbers in parentheses indicate the number of determinations and ± values are standard deviation.

Age	2,3-DPG (μmole/cm^3)			IPP (μmole/cm^3)
	Enzymatic assay	Phospho-glyceric acid	P_i	P_i
14-DE	1.62 ± 0.10 (4)	1.63 ± 0.53 (2)	1.57 ± 0.06 (2)	0.51 ± 0.01 (2)
15-DE	3.22 ± 0.20 (4)	4.14 (1)	4.02 (1)	0.38 (1)
16-DE	5.26 ± 0.12 (4)			
17-DE		5.43 (1)	5.88 (1)	0.42 (1)
18-DE	4.67 ± 0.30 (6)	5.09 ± 1.29 (3)	5.10 ± 0.21 (3)	0.48 ± 0.06 (3)
20-DE		3.89 ± 0.27 (2)	4.60 ± 0.51 (2)	0.79 ± 0.28 (3)
Pipped	4.61 ± 0.32 (8)			
Hatched	3.04 ± 0.12 (6)			
1-DC		0.76	1.13	1.15
3-DC		0.57	0.58	1.42
5-DC				2.69
8-DC				3.47

inositol polyphosphates in the erythrocytes of birds (*6*) and some turtles (*7*). This material, making up approximately three-fourths of the cellular organic phosphate, was first thought to be inositol hexaphosphate, phytic acid (IHP), but has recently been identified more accurately (*8*) as the 1,3,4,5,6-pentaphosphate of inositol (IPP). The supposition that the IPP in avian erythrocytes might serve as a regulator of hemoglobin function in a similar manner to that of 2,3-DPG in mammalian red blood cells, although not proved, is supported by studies in vitro (*3, 9*) and in vivo (*10, 11*). In fact, IHP binds more tightly to hemoglobins and is more effective in lowering oxygen affinity than is 2,3-DPG. The increases of IPP in the chick red cell shortly after it hatches (Fig. 1) undoubtedly accounts for the observed decrease in affinity for oxygen in whole blood during this period of development (*10, 11*)

We have been studying the changes in P_{50}, hemoglobin type, and organic phosphate in erythrocytes from newly hatched chicks (*10*). On extending these studies to embryos we were quite surprised to find high concentrations of 2,3-DPG, comparable to those in human red cells (4 to 5 μmole per cubic centimeter of packed cells), in the erythrocytes of the embryo shortly before hatching (Table 1). The amount of 2,3-DPG increases in the erythrocytes from the 14-day embryo to a maximum in the 16- or 17-day embryo and then drops precipitously in erythrocytes from embryos at the time of hatching (Table 1; Fig. 1). On the other hand, IPP is present in only small amounts in the erythrocytes until hatching and then accumulates rapidly.

The material we are measuring is identical with 2,3-DPG in that it possesses the characteristic elution position from Dowex 1-X8 with an ammonium formate buffer system (*12*), gives the appropriate color in assays for glycerate (*13*), the ratios of phosphate (*14*) to glycerate are 2 : 1, and stimulates phosphoglycerate mutase activity in an enzymatic assay for 2,3-DPG (*15*).

The significance of the presence of 2,3-DPG in the red blood cells of the chick embryo is not readily apparent. It accounts for approximately 45 percent of the cell phosphates and is the major organic phosphate in the chick embryo red blood cell during the last week of incubation. The accumulation and rapid elimination of 2,3-DPG from the erythrocyte of the embryo during this period is puzzling. In other studies we have found that 2,3-DPG interacts with chicken hemoglobins, shifting the oxyhemoglobin dissociation curve to the right just as it does with mammalian hemoglobins.

The sudden change from 2,3-DPG as the predominant organic phosphate of erythrocytes of the embryo to IPP shortly after hatching indicates an abrupt activation and inactivation of genes. It seems reasonable to propose that the sudden changes in oxygen tension that result from the changeover from passive diffusion into the egg to active respiration in the newly hatched chick may trigger these events. Whether these events may be related to the switchover from fetal to adult hemoglobin synthesis remains to be determined.

RUSSELL E. ISAACKS*
DONALD R. HARKNESS
Research Laboratories of the Veterans Administration Hospital and *Department of Medicine, University of Miami School of Medicine, Miami, Florida 33125*

References and Notes

1. I. Greenwald, *J. Biol. Chem* **63**, 339 (1925).
2. E. W. Sutherland, T. Posternak, C. F. Cori, *ibid.* **181**, 153 (1949).
3. R. Benesch and R. E. Benesch, *Biochem. Biophys. Res. Commun.* **26**, 162 (1967).
4. A. Chanutin and R. R. Curnish, *Arch. Biochem. Biophys.* **121**, 96 (1967).
5. D. R. Harkness, *Adv. Intern. Med.* **17**, 189 (1971).
6. S. Rapoport, *J. Biol. Chem.* **134**, 403 (1940).
7. _____ and G. M. Guest, *ibid.* **138**, 269 (1941).
8. L. F. Johnson, and M. E. Tate, *Can. J. Chem.* **47**, 63 (1969).
9. I. Tyuma, K. Imai, K. Shimizu, *Biochem. Biophys. Res. Commun.* **44**, 682 (1971); G. R. Janig, K. Ruckpaul, F. Jung, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **17**, 173 (19 C. Vandecasserie, A. G. Schnek, J. Leonis, *Eur. J. Biochem.* **24**, 284 (1971); T. Ochiai, T. Gotoh, K. Shikama, *Arch. Biochem. Biophys.* **149**, 316 (19 C. Vandecasserie, C. Paul, A. G. Schnek, J. Leonis, *Comp. Biochem. Physiol.* **44A**, 711 (1973).
10. R. E. Isaacks *et al.*, *Comp. Biochem. Physiol*, in press.
11. M. Oshima, T. G. Taylor, A. Williams, *Biochem. J.* **92**, 42 (1964).
12. R. E. Isaacks, D. R. Harkness, G. A. Froeman, S. A. Sussman, *Comp. Biochem. Physiol.*, in press.
13. G. R. Bartlett, *J. Biol. Chem.* **234**, 469 (1959).
14. _____ *ibid.* p. 466.
15. J. C. Towne, V. W. Rodwell, S. Grisolia, *ibid.* **226**, 777 (1957).

* Mailing address: Basic Sciences Research, Veterans Administration Hospital, 1201 NW 16 Street, Miami, Florida 33125.

20 February 1975

Meteor-Generated Infrasound

In their report Donn and Balachandran (*1*) have made a complex problem appear overly simple. They suggest that, independent of other present means of meteor detection, they can determine a meteor influx rate with the use of acoustical methods. On the contrary, I suggest that their present speculation linking the pressure waves they record to meteors is not well founded. No cause-and-effect relationship has been established. All the supporting evidence they cite is indirect.

The following points stand out with respect to their hypothesis:

1) Recent theoretical treatments by both Tsikulin (*2*) and ReVelle (*3*) predict that an air wave signal with a period of 3 seconds (at maximum signal amplitude) as shown in figure 1 of Donn and Balachandran (*1*) at a range of about 50 km requires for its production a very large object ($\gtrsim$ 1 m in diameter, depending on the Mach number of the meteor). The theoretical analysis assumes that under certain flow conditions the meteor-atmosphere interaction can be treated with the use of a cylindrical blast-wave, line-source model. Specific details can be found in ReVelle (*3*). Such a large