into each site; this injection was followed by an injection of 0.05 ml of P-K serum diluted to contain 0.2 ng of IgE. Thus 10^{-9} mole of peptide was competing with 10^{-15} mole of IgE for the binding sites on the mast cells, a ratio of one IgE molecule to 10^6 peptide molecules. In titration experiments in different individuals measurable inhibition (30 to 97 percent) was observed with a 2000- to 2-millionfold excess of pentapeptide (III) to IgE.

A single "displacement" experiment was performed on the donor (V.B.) of the serum used in the P-K inhibition experiments. Approximately 2 nmole each of three peptides—pentapeptide (III), hexapeptide (IV), TASMe (VI)—and the buffer diluent were each injected intradermally into three marked sites. At 1, 5, and 24 hours one of each peptide and one diluent site were challenged with guinea pig antigen by prick puncture. No inhibition of the wheal or flare reaction was observed at any site at 1 and 5 hours. However, at the 24-hour challenge the wheal at the pentapeptide (III) site was approximately 45 percent smaller and that at the hexapeptide (IV) site was 23 percent smaller than the control wheal. No reduction in the size of the wheal was seen at the TASMe (VI) site. This experiment would tend to confirm the data of Ishizaka and Ishizaka (*10*), showing that IgE is not as irreversibly bound to the mast cell as had been thought. Further, it suggests that pentapeptide (III) not only can inhibit or prevent the binding of IgE to the mast cell but it also can displace already bound IgE from the mast cell. In the P-K experiments and the direct skin test experiment the fact that the pentapeptide (III) seemed to have persisted at the site of injection for 24 hours suggests attachment to a specific binding site, since other peptides as well as intact globulins, other than IgE, rapidly diffuse away from the injection site.

The six experiments summarized above and in Table 2 were part of a series of experiments performed with some of the same peptides (without coded labels); all of them yielded comparable results. In addition, some confirmatory evidence of the effectiveness of pentapeptide (III) was provided by two experiments in which the histamine release in vitro from human peripheral leukocytes was measured (*11*).

These experimental results suggest that we have synthesized a pentapeptide whose configuration in physiologic solution is sufficiently similar to that of the natural cell binding site of IgE to compete successfully for IgE binding sites on the mast cell and basophil as well as to displace IgE from the mast cell, as evidenced by the inhibition of the P-K reaction, inhibition of the immediate skin test reaction, and probable inhibition of histamine release from leukocytes in vitro. Other related peptides tested had a less consistent and smaller inhibitory effect. Only the one pentapeptide (number III in Table 2) inhibited the P-K reaction to an extent greater than 50 percent in all six subjects. We are now exploring ways to increase the efficiency of competition for mast cell binding sites by these and other related peptides. The blocking of the immediate hypersensitivity reaction with a small synthetic peptide provides evidence for the feasibility of a new therapeutic approach to the treatment of allergic disease.

Table 2. Average inhibition (percent) of P-K immediate wheal and flare reactions by 1 nmole of each peptide injected prior to injection of serum B containing 10^{-15} mole of IgE, in six adult subjects. Differences of less than 20 percent between averages are not considered significant.

Peptide		Average inhibition	
No.	Sequence	Percent	Range
I	Asp-Pro-Arg	15	0–38
II	Ser-Asp-Pro-Arg	18	0–50
III	Asp-Ser-Asp-Pro-Arg	72	60–89
IV	Ala-Asp-Ser-Asp-Pro-Arg	46	10–61
V	Asp-Thr-Glu-Ala-Arg	58	38–80
VI	Tos-Arg-Sar-Me	24	0–40

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Glomerular Epithelium: Structural Alterations Induced by Polycations

Abstract. *Perfusion of rat kidneys with the polycation protamine sulfate caused glomerular epithelial alterations resembling those observed in human and experimental nephrotic states. The changes included swelling, blunting, and flattening of epithelial foot processes, were accompanied by decreased stainability of glomerular anionic sites, and were largely reversed by subsequent perfusion with the polyanion heparin.*

The visceral epithelial cells of the renal glomerulus are characterized by a complex ultrastructural configuration (Fig. 1a). From the main cell body, primary cytoplasmic extensions send secondary and tertiary processes (pedicels, foot processes) which anchor into the basement membrane (*1*). Between the foot processes are the fil-

tration slits or slit pores. Filling the slits and covering the epithelial cell surface is a thick anionic cell coat, which is rich in sialic acid residues (*2*) and stainable with colloidal iron (*2, 3*). Along with other elements of the glomerular filter (*4*) the glomerular sialoprotein has been postulated to play a role in the ultrafiltration process by acting as an electrostatic barrier for negatively charged plasma proteins (*2*). In disease states associated with proteinuria, such as human lipoid nephrosis and experimental aminonucleoside nephrosis, the normal epithelial architecture is lost and discrete foot processes are replaced by broad masses of cytoplasm which cover the basement membrane (*5*). The morphogenesis of this lesion, which is commonly referred to as "fusion of foot processes," is poorly understood, but it has been reported that a concomitant decrease in glomerular sialoprotein takes place (*6*). Negatively charged surface coats may govern the stability of cell membranes. Thus, polycations known to interact with surface anionic sites (*8, 9*) have been shown to induce morphological alterations that suggest loss of membrane integrity and cellular rigidity (*7*). These considerations prompted us to study the effect of polycations on the ultrastructural architecture of the glomerular visceral epithelial cells.

Sprague-Dawley female rats weighing 180 to 280 g were anesthetized with Nembutal (5 mg per 100 g of body weight) given intraperitoneally, and both kidneys were perfused in situ, by means of an aortic catheter. Perfusion pressure was maintained at 120 mm-Hg and the temperature was held at 37°C. All kidneys were perfused for 1 minute with oxygenated Krebs Ringer bicarbonate buffer in order to wash out the blood; experimental kidneys were then perfused for 10 minutes with various concentrations of the polycations protamine sulfate or poly-L-lysine, while controls were perfused for 10 minutes with Krebs Ringer bicarbonate buffer only or buffer containing neutral (poly-DL-alanine, myoglobin) or anionic (poly-L-glutamic acid, heparin) macromolecules, in concentrations equivalent to the polycationic peptides (*10*). This was followed by perfusion fixation for 20 minutes with 1.25 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer at room temperature. Random blocks of tissue were postfixed for $1^1/_2$ hours in osmium tetroxide, dehydrated, embedded in Epon, thin-sectioned, stained with uranyl acetate and lead citrate, and examined in a Philips 200 microscope.

The ultrastructure of control glomeruli including those exposed to neutral or anionic macromolecules was similar to that observed after direct perfusion fixation of normal kidneys, showing discrete epithelial foot processes and slit pores of normal width (Fig. 1a). Perfusion with protamine sulfate (10, 20, 50, and 500 μg/ml) caused diffuse epithelial changes consisting of cytoplasmic swelling, a constant narrowing of epithelial filtration slits, blunting and flattening of the foot processes, and a reduction in their number. In the extreme form the foot processes were replaced by continuous masses of epithelial cytoplasm covering the basement membrane (Figs. 1b and 2a). In addition, there was close apposition between epithelial cell bodies and their cytoplasmic extensions (Figs. 1b and 2a). Pentalaminar junctions between adjacent foot processes were frequently observed in sections appropriately stained to accentuate membrane structure. Glomeruli perfused with polylysine (5 and 10 μg/ml) showed similar changes, but, in addition, there was appreciable cell damage, in keeping with the known cytotoxicity of this polyamino acid.

Low-magnification micrographs, which were coded to conceal their origin, of at least three different capillary loops from each experimental group were examined by each of us, and we graded the severity of

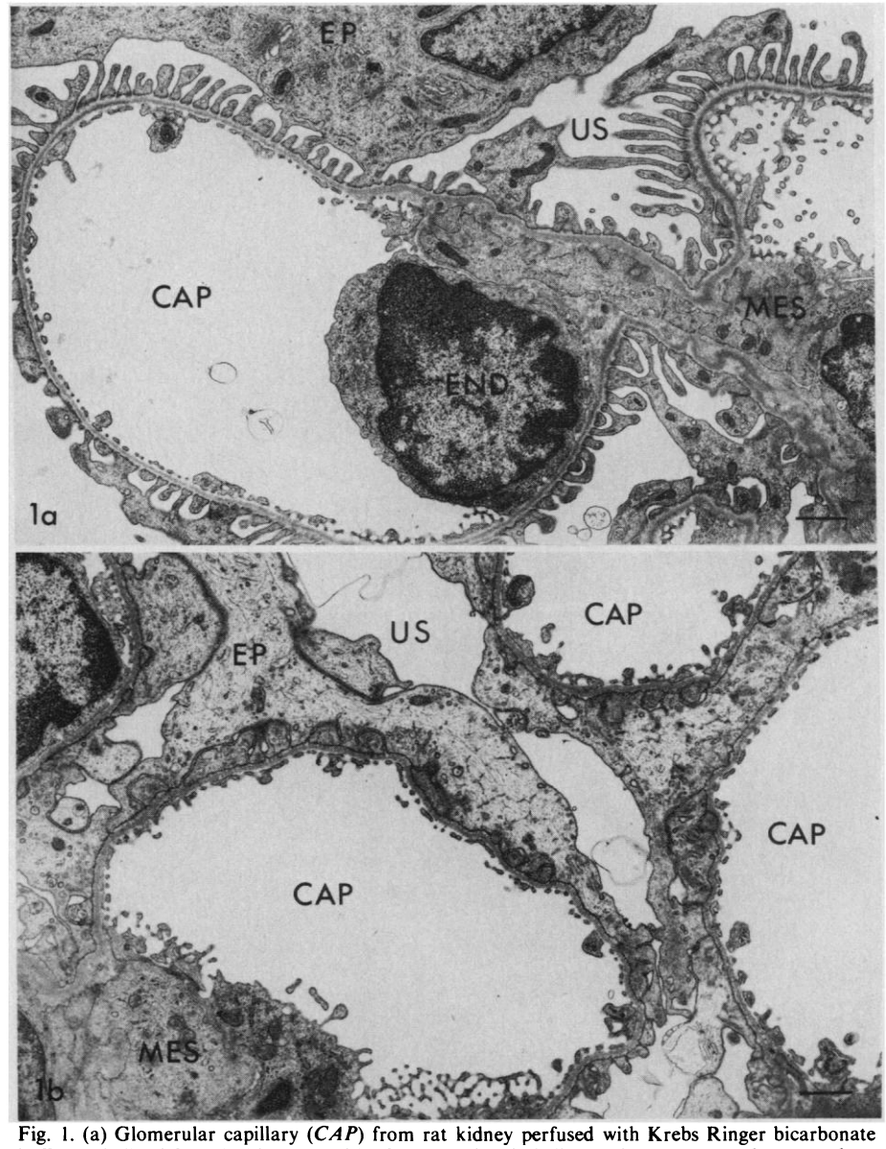

Fig. 1. (a) Glomerular capillary (*CAP*) from rat kidney perfused with Krebs Ringer bicarbonate buffer. It is lined from inside outward by fenestrated endothelium, a basement membrane, and cytoplasmic processes of the visceral epithelial cell (*EP*). These so-called foot processes are discrete and club-shaped and appear to be anchored in the external layer of the basement membrane. Alternating with the foot processes are interstices, the filtration slits or slit pores. Indicators: *END*, endothelial cell; *MES*, mesangium (the centrilobular or stalk area of the glomerulus); *US*, urinary space (scale bar, 1 μm). (b) Glomerular capillaries (*CAP*) from rat kidney perfused with protamine sulfate (50 μg/ml) in Krebs Ringer bicarbonate buffer for 10 minutes. The fenestrated endothelium and basement membrane appear unremarkable. In contrast to (a), however, the number of discrete foot processes of the epithelial cell (*EP*) is greatly diminished. The remaining processes are blunted and closely apposed to each other, giving rise to broad masses of epithelial cytoplasm covering the basement membrane (scale bar, 1 μm).

Table 1. Obliteration of foot processes. Micrographs (three per animal) were reviewed by the three investigators. Their respective gradings were in close agreement. Numbers correspond to number of micrographs: 0, essentially normal morphology; +, focal blunting of foot processes; 2+, diffuse blunting or flattening of foot processes and narrowing of slit pores; 3+, large areas of epithelium devoid of foot processes in addition to above. Controls include ten animals perfused with buffer alone and six animals perfused with buffer containing myoglobin (two), poly-DL-alanine (one), poly-L-glutamic and (two), or heparin (one).

Treatment	Heparin afterward	Animals (No.)	Severity of lesion			
			0	+	++	+++
None (controls)		16	42	6	0	0
Protamine sulfate (50 μg/ml)	No	8	0	3	15	6
	Yes	2	5	1	0	0
Protamine sulfate (500 μg/ml)	No	4	0	0	5	7
	Yes	2	4	2	0	0
Poly-L-lysine	No	3	0	0	0	9

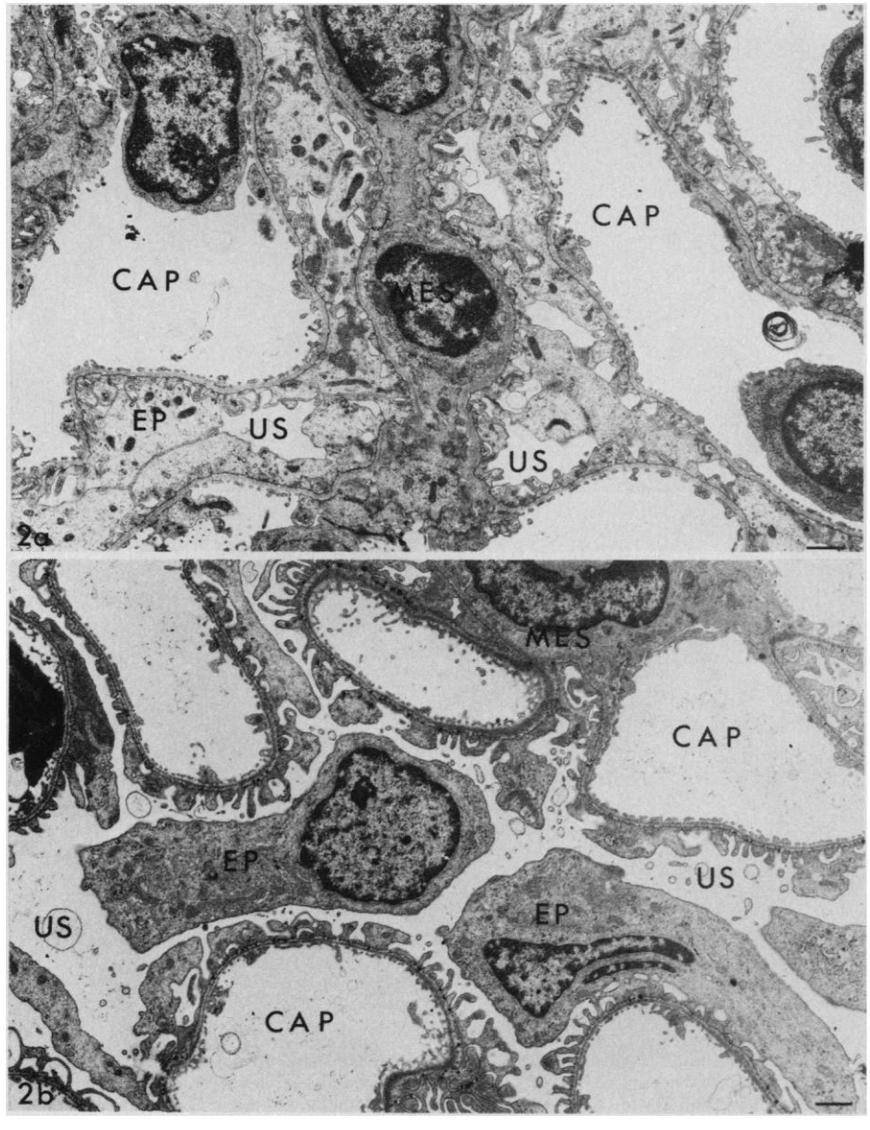

Fig. 2. (a) Glomerular capillaries (*CAP*) from a kidney perfused for 10 minutes with protamine sulfate (500 μg/ml) in Krebs Ringer bicarbonate buffer. There is marked blunting and effacement of foot processes and close apposition of adjacent cell bodies. Immersion fixation (scale bar, 1 μm). (b) Glomerular capillaries (*CAP*) from the same kidney as in (a) after additional perfusion with heparin (100 unit/ml) in Krebs Ringer bicarbonate buffer. Note the reversal toward a normal pattern. Many discrete foot processes are recognized, and the main bodies of the epithelial cells (*EP*) are no longer in contact with one another. There is, however, some blunting and focal fusion of foot processes. The electron-opaque particles observed in the outer layer of the basement membrane may be protamine-heparin complexes. Immersion fixation (scale bar, 1 μm).

the lesion from 0 to 3+ (Table 1). Paraffin sections of kidneys stained for acid mucosubstances by the colloidal iron technique (*11*) indicated marked diminution of staining in glomeruli of animals perfused with 500 μg/ml of protamine, as compared to controls. The histochemical differences were less marked in animals exposed to lower concentrations of the polycations.

Heparin and other polyanions reverse some of the biological effects of polycations (*8, 9, 12*). Experiments performed as follows showed that induced glomerular lesion could be reversed in a similar fashion. Kidneys were perfused for 10 minutes with protamine sulfate (50 or 500 μg/ml) and for an additional 10 minutes with heparin in a neutralizing concentration (10 and 100 unit/ml). A biopsy (Fig. 2a) obtained before exposure to heparin showed epithelial changes including obliteration of foot processes, whereas samples taken after perfusion with heparin showed nearly normal glomerular morphology (Fig. 2b). Glomeruli from such kidneys showed, in addition, the presence of numerous spherical electron-opaque particles, which commonly localized in the basement membrane and in the urinary space (Fig. 2b). In separate experiments heparin and protamine were reacted in vitro, perfused through normal kidneys, and examined by electron microscopy; the resultant precipitates (present in capillary lumina) were similar to those illustrated.

Our experiments show that the complex cytoplasmic contour of the glomerular epithelial cells can be rapidly altered by exposure to polycations which are known to interact with membrane-associated anionic sites (*8, 9, 12*). The mechanisms involved in the morphogenesis of the polycation-induced lesion are unclear. However, it has been suggested that interference with cell surface charge may cause cytoplasmic contraction (*12*), alter cell locomotion (*13*), and increase cell deformability (*14*). Since partial neutralization of glomerular epithelial surface charge occurred in polycation-perfused kidneys (as evidenced by decreased colloidal iron staining), any of the above-mentioned phenomena may have taken place. Since cationic polypeptides cause alterations of cell membrane resistance and permeability (*12, 13, 15*), this may account for the cytoplasmic swelling observed in our experiments. An important feature of the epithelial lesion was the constantly observed close proximity and apposition of adjacent foot processes and cell bodies (see Figs. 1b and 2a). This may have been induced by the loss of mutual repulsive forces (due to decreased surface charge) and the formation of macromolecular "bridges," as has been suggested by Katchalsky for polycation-agglutinated

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.

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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

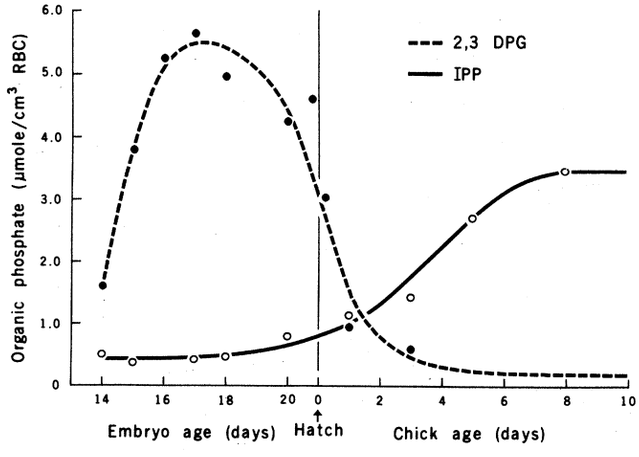

Fig. 1. The 2,3-DPG and IPP contents of erythrocytes from chick embryos and young chicks. The data collected by three methods and shown in Table 1 for the analyses of 2,3-DPG in micromoles per cubic centimeter of red cells were averaged for each age and plotted as a function of time in days of incubation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