However, the released parasites again dispersed throughout the larval breeding area, and 2 weeks later all housefly pupae that were collected were parasitized.

The grid counts made at the test area (Fig. 2) showed that the population of houseflies increased from the prerelease count of 2.3 flies per grid to about 6.2 flies per grid, dropped because of the releases, and then on 14 July, perhaps because of immigration, increased to 7.2 flies per grid. However, by 21 July—that is, within 35 days after the first release—the count had decreased to the lowest level before the treatment and continued to decline throughout the remainder of the study. Indeed the counts never exceeded 1 fly per grid during the remainder of the test, although adequate larval breeding medium was always present except during a short period in August just after the manure was removed.

At the check farm, grid counts were begun 24 June. The weekly larvicide treatments reduced the fly population to less than 1 fly per grid by 7 July, but subsequent intermittent larvicide treatments failed to check the increase in the housefly population. On 18 August, weekly insecticidal treatments were started again and the housefly population dropped.

Therefore, in this study parasites were as effective as the recommended insecticidal treatments in the control of houseflies (*6*). The costs of producing and releasing the wasps were relatively low and slightly less than those for pesticides. This approach is highly pest-specific, does not adversely affect the quality of the environment, and eliminates many of the problems that are normally associated with pesticides.

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Peptide Inhibition of the Prausnitz-Küstner Reaction

Abstract. *A pentapeptide was synthesized with the same amino acid sequence that occurs in a unique region of the ε chain of immunoglobulin E near the cysteine residue participating in the linkage between the two heavy chains. This pentapeptide has the capacity to block a standard Prausnitz-Küstner reaction as well as to inhibit a known positive skin test reaction. Other similar synthetic polypeptides had less or no inhibitory activity. The likelihood that this pentapeptide amino acid sequence does in fact represent the structure of the specific immunoglobulin E binding site for the mast cell and basophil is considered.*

Immunoglobulin E (IgE) binds to mast cells and basophils (*1*). The cell binding site has been localized in the Fc portion of the molecule, and as such is thought to reside in the second, third, or the fourth domain (or all of these) of the constant region of the ε heavy chain (*2*). Efforts to isolate a smaller fragment containing the binding site by enzymatic digestion of the Fc portion of IgE have not been successful. Thus, although the IgE Fc inhibits the Prausnitz-Küstner (P-K) reaction in man, no other smaller fragments of Fc examined retain activity (*3*). This observation has given rise to the notion that intrachain disulfide bonds play a crucial role in the conformation essential for skin binding, and thus inhibitory polypeptides might not be readily synthesized (*3*). We have reasoned that a relatively short polypeptide, representing a small region of the Fc fragment of the ε chain, is responsible for fixation of IgE molecules on the sterically complementary mast-cell receptor (*4*).

By comparing the amino acid sequence of a fragment of ε chain of IgE (from PS myeloma) believed to include the hinge region, and that of fragment III of IgE (from ND myeloma) with the homologous regions of the γ, α, and μ chains, five small regions (one decapeptide, one hexapeptide, and three pentapeptides) unique to the second and third constant domains of the ε chain were located (*5*) (Table 1). Of these the pentapeptide Asp-Ser-Asp-Pro-Arg was synthesized (*6*). This peptide was found to have the capacity to inhibit the binding of IgE to the mast cells of the skin as measured by the inhibition of the P-K reaction (*7*). With the publication (*5*) of the complete sequence of the ε chain of human IgE (ND), this pentapeptide was located as amino acids 320 through 324. The hexapeptide Ala-Asp-Ser-Asp-Pro-Arg was also prepared, as well as a tripeptide and a tetrapeptide contained within this sequence: Asp-Pro-Arg and Ser-Asp-Pro-Arg. In addition another synthetic peptide, the methyl ester of tosyl-L-argininylsarcosine (TASMe) and another pentapeptide Asp-Thr-Glu-Ala-Arg were synthesized for comparison testing (*8*).

Table 1. Formulas of five amino acid sequences unique to the ε chain near the hinge region.

Residue numbers (*5*)	Sequence
266–275	Asp-Val-Asp-Leu-Ser-Thr-Ala-Ser-Thr-Glu
289–293	Leu-Ser-Gln-Lys-His
320–324	Asp-Ser-Asp-Pro-Arg
354–359	Ala-Pro-Ser-Lys-Gly-Thr
367–371	Ala-Ser-Gly-Lys-Pro

The P-K reaction was utilized to measure the capacity of each peptide to inhibit the wheal and flare (immediate hypersensitivity) response. This classic method involves the intradermal injection of allergic serum (containing IgE specific for a known antigen or allergen), waiting 20 or more hours, and then challenging at the same sites with a prick or intradermal injection of a solution of the specific antigen. The extent of the positive reaction can be ascertained by measurement (in millimeters) of the diameter of the wheal (and flare) that develops over the following 10 to 30 minutes. All these studies were performed with a single, proven, P-K donor serum (B) with which we have had considerable experience (*9*). The typical sequence of events was intradermal injection of 0.1 ml of the peptide solution or control (buffered saline diluent) solution, followed in 1 to 24 hours by intradermal injection of 0.05 ml of the P-K serum into each of the previously injected sites. After 20 to 24 hours, each site was prick-punctured with the antigen solution, blotted dry in 5 minutes, and the wheal and flare were measured in both their narrowest and widest diameters at 15, 20, and 25 minutes. (Flares were measured only for confirmation of the occasionally difficult to measure wheal).

The average percentage inhibition of the standard P-K reaction with the use of six different peptides in six individuals is shown in Table 2. Results are the average of duplicate measurements on each individual at three different times, subtracted from the average control wheal measurements, and divided by the average measurement of each individual's control wheal. Control wheals in different individuals varied from 8 to 40 mm^2, with a mean of 17 mm^2. In this set of experiments, each peptide was used at a dilution of approximately 6 μg/ml and 0.1 ml was injected

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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8. Equivalent amounts of each peptide were provided to the experimenter with labels coded so that the contents were unknown to him during the P-K tests. Concentrated solutions were prepared in sterile H_2O; dilutions for use between 0.01 and 1.0 μmole/ml were made in phosphate buffered saline, *p*H 7.4, and the solutions were passed through a 0.45-μm Millipore filter. Microbiologic safety standards were observed with cultures (aerobic and anaerobic) and intraperitioneal injection into mice prior to use. Peptide solutions were either injected intradermally 1 to 24 hours prior to the P-K serum or mixed with dilutions of the P-K serum for simultaneous injection. In the "displacement" or direct skin test inhibition experiment, peptide solutions alone were injected ½, 5, and 24 hours prior to antigen challenge.

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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-