less than 0.25% as well as it recognizes rATP III. In the original description of our antiserum (*10*), we erroneously reported, because of a bad lot of synthetic α-rANP(1–28) obtained from Peninsula Labs, that our antiserum recognized α-rANP(1–28) only 10% as well as it recognizes α-rANP(5–28).
13. Disposable syringes were placed on C_{18} Sep-Pak cartridges that were activated by passing 25 ml of HPLC-grade methanol and washed by passing 20 ml of HPLC-grade distilled water. Frozen plasma samples were thawed and rapidly portioned (1 part) into the syringes, and 4 parts of ice-cold 4% acetic acid were added immediately afterward. The acidified plasma samples were pushed through the Sep-Paks at a flow rate of approximately 2 ml/min. Each sample-loaded Sep-Pak was washed with 10 ml of 4% acetic acid solution and samples were eluted within 30 seconds with 4 ml of a solution consisting of 3 parts of acetonitrile and 1 part of 4% acetic acid. The effluent was collected and evaporated to dryness in a Speed Vac concentrator. Portions of the samples were either suspended in buffer for RIA or in 10% acetonitrile containing 0.1% trifluoroacetic acid for HPLC. The recovery of synthetic ATP III, ANF(8–33), or ANF, when added to rat plasma containing EDTA and aprotinin, and extracted as outlined, ranged from 89% to 94%. There were no statistically significant differences in the recovery of the individual peptides.
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18. In conscious animals volume loading did not alter basal values of mean arterial blood pressure (105 ± 1.1 mmHg, mean ± SEM) or heart rate (346 ± 7.7 beat/min) throughout the experiment. In contrast, hyperosmotic challenge in conscious animals resulted in a substantial increase of mean arterial pressure at 2 minutes (128 ± 3.6 mmHg) and 10 minutes (139 ± 3.1) after the onset of the infusion, whereas an increase in heart rate observed at 2 minutes (455 ± 8) had subsided to basal levels by 10 minutes. The basal value of mean arterial pressure was slightly lower in halothane-anesthetized animals (96 ± 2.0 mmHg) than in conscious animals and declined to 73 ± 2.9 mmHg 2 minutes after volume loading, whereas the heart rate response was similar to that of conscious rats. Cardiovascular parameters in the pithed rat were quite different from those of other groups; mean arterial pressure and heart rate, which were 64 ± 5.0 mmHg and 386 ± 19 beat/min before volume loading, were 96 ± 7.5 and 449 ± 18, respectively, 2 minutes after volume loading.
19. A plasma extract obtained from conscious rats was subjected to HPLC with a Gilson dual-pump system (pump A, 0.1% trifluoroacetic acid in water; pump B, 0.1% trifluoroacetic acid in acetonitrile) and an Altex ultrasphere-ODS column (4.6 × 250 mm; 5 μm particle size). Acetonitrile (25%) was maintained for 5 minutes after injection of the plasma extract; a linear gradient from 25% to 55% acetonitrile was then maintained for 60 minutes. Fractions (1 ml) were collected, dried, and subjected to RIA (Fig. 2). Synthetic ANP's were chromatographed under identical conditions and their elution profile is as indicated in Fig. 2.
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Protection of Cattle Against Foot-and-Mouth Disease by a Synthetic Peptide

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A chemically synthesized peptide consisting essentially of two separate regions (residues 141 to 158 and 200 to 213) of a virus coat protein (VP1) from the 0_1 Kaufbeuren strain of foot-and-mouth disease virus was prepared free of any carrier protein. It elicited high levels of neutralizing antibody and protected cattle against intradermolingual challenge by inoculation with infectious virus. Comparative evaluation of this peptide with a single-site peptide (residues 141 to 158) in guinea pigs suggests the importance of the VP1 carboxyl terminal residues in enhancing the protective response.

FOOT-AND-MOUTH DISEASE (FMD) IS a major, although rarely fatal, disease of domesticated animals; an outbreak of the disease in cattle can be financially devastating. In countries where the disease is endemic, the commonly used method of control is vaccination with an inactivated virus preparation. Approximately 10^9 doses of vaccine are administered annually in South America alone (*1*). The use of a synthetic protein–peptide vaccine for FMD has attracted much attention (*2, 3*) since it offers the advantage of a highly pure, defined, and safe alternative to the conventional vaccine.

In early work, isolated VP1 coat protein from the FMD virus (FMDV) was able to induce neutralizing antibody (*4, 5*) and protective immune (*5*) responses in swine and cattle (*6*). These results were later confirmed when cattle and swine were protected against FMDV serotype A challenge after vaccination with a biosynthetic VP1 fusion protein (*2, 7*). Strohmaier *et al.* (*8*), on the basis of a series of chemical and enzymatic degradations of VP1 from the 0_1 Kaufbeuren (0_1K) strain of FMDV, suggested that the important epitopes were centered in two small regions. These two sites are represented by residues 146 to 154 and 201 to 213. The studies of Bittle *et al.* (*9*) revealed a particularly active VP1 peptide of residues 141 to 160 which, when coupled to keyhole limpet hemocyanin (KLH), provided guinea pigs a neutralizing antibody response superior to that provided by VP1 protein. However, the successful extension of these guinea pig results to cattle, the primary target animal, by this conjugate or an analogous substance has yet to be reported (*10*). Furthermore, the presence of KLH or any other carrier as a required ingredient detracts from the advantages of peptide vaccines. The contribution of carrier selection, purity, and conjugate molecular heterogeneity (as introduced by chemical construction) to the final results can make identification of the active entity difficult.

We considered the possibility of a single-peptide vaccine consisting essentially of the two previously identified immunogenic regions connected by a diproline spacer and terminating on each end with cysteine residues. Both peptidyl regions would be included to increase the molecular size while providing a second site of some importance relative to residues 141 to 160 (*11*). The diproline spacer was inserted as a means of increasing the likelihood of interaction between the two sites through a presumed induction of a secondary structural turn. The final aspect of the synthesis was the placement of cysteine residues at or near each terminus for the purpose of polymerization as a possible means of eliminating the necessity of a carrier molecule. The following peptides, corresponding to the sequence in VP1 of the 0_1K serotype, were prepared by solid-phase synthetic methodology (*12*): 141–158-ProCysGly (21-residue peptide), 200–213-ProProSer-141–158-ProCysGly (38-residue peptide), and CysCys-200–213-ProProSer-141–158-ProCysGly (40-residue peptide). The first of

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Table 1. Evaluation of guinea pig immunization against FMDV with synthetic peptides. Dunkin Hartley guinea pigs that had been randomly bred in a closed colony and that weighed 450 to 500 g were immunized by subcutaneous injection. The peptide dose was delivered in 150 μl of a 1:1 mixture of 0.04*M* phosphate buffer and Freund's complete adjuvant. All test animals were challenged by intradermal inoculation of the left hind footpad with a minimum of 3000 guinea pig units of virulent O_1K FMD virus 28 days after vaccination. The criterion of protection was the absence of any secondary lesions for 7 days.

Peptide dose (nmol)	O_1K serum neutralizing titer* (number protected:number challenged)			
	21-residue peptide	21-residue KLH conjugate	38-residue peptide	40-residue peptide
150	2.3 (5:5)	0.8 (0:5)	2.9 (5:5)	3.6 (5:5)
50	0.8 (1:5)	0.8 (0:5)	3.1 (5:5)	3.1 (5:5)
17	0.8 (2:5)	0.8 (0:5)	3.0 (5:5)	3.1 (5:5)
6	0.8 (0:5)	0.8 (0:5)	2.9 (4:4)	2.1 (5:5)
2	0.8 (0:5)	0.8 (0:5)	2.1 (3:4)	2.3 (4:5)
0.6	0.8 (0:5)	0.8 (0:5)	1.0 (5:5)	2.1 (3:5)
0.2	0.8 (0:5)	0.8 (0:5)	1.2 (1:5)	1.8 (1:5)

*Titer is the log of the serum dilution required to neutralize 50 percent of the virus in monolayers of IB-RS2 cells (*14*).

these peptides was also chemically conjugated to KLH through *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) to a level of 18 μmol per gram of KLH (4 percent by weight) (*13*).

The most appropriate method for establishing the relative potency of a series of synthetic peptide vaccines is the determination of their minimum protective molar dose (Table 1). Treatment with the 38- and 40-residue peptides resulted in marked improvement in virus-neutralized titers and protection as compared with that of the 21-residue peptide. These results indicate the importance of antibody production against residues 200 to 213 as an important component of optimal protection against viral challenge. The apparent equivalence, within experimental error, of the 38- and 40-residue peptides seems to minimize any contribution of the amino terminal cysteine residues in the 40-residue peptide. These two double-antigen peptides seem to induce complete protection at less than one-tenth to one-hundredth the dose of the 21-residue peptide. Although this potentiation may be solely a result of increased molecular size, the magnitude of the response suggests a more specific role for residues 200 to 213 and possibly for the diproline spacer. The 21-residue peptide provided more protection than its conjugate with KLH, at an equivalent molar dose. This may be a function of KLH purity, antigen load, or the different methods used in conjugation, because protection with a similar peptide has been reported (*9*). However, the ability to protect guinea pigs in the absence of a specific carrier substantially simplifies the approach to vaccination and eliminates a potential source of variability. The necessity of antigen optimization is of utmost importance because Freund's complete adjuvant is not currently acceptable to the livestock industry. At a molar dose comparable to that which provided complete protection with the 21-residue peptide in Freund's complete adjuvant (150 nmol, 0.3 mg), the 40-residue peptide induced full protection with the commercially accepted aluminum hydroxide–saponin adjuvant.

Although guinea pig vaccination has proven to be a useful model for evaluation of FMD vaccines, the final test of success must be in cattle. The results of vaccinating cattle with the 40-residue peptide, followed by intradermolingual challenge with virulent cattle-adapted virus, are shown in Table 2. After being infected, animals were examined daily for evidence of lesions on the tongue and feet. The serum neutralizing titers (SNT's) (*14*) rose rapidly and, by day 14, reached a plateau that was maintained at least until challenge on day 32. The SNT data were confirmed by enzyme-linked immunosorbent assay (ELISA) of antibody directed against whole virus. The control cattle displayed SNT's, with only two exceptions, that were lower by approximately two orders of magnitude than the peak titers of the vaccinates. An occasional high titer was attributed to analytical variance because no correlation has been established with protection or ELISA determination. Two of the nine animals that were challenged without revaccination were protected, and two others (animals 4 and 5) showed no evidence of generalized infection until day 5 after challenge.

To our knowledge, this experiment represents the first example of protection of cattle against FMD after a single immunization with a synthetic peptide under conditions typical of those used to test the potency of conventional vaccines. The protection was obtained with an O_1-strain FMDV peptide preparation, which has been more difficult in protection studies than peptide-protein of the A serotype (*10*). The magnitude of antibody response was unchanged over a 6-month interval as measured by ELISA. The levels of the neutralizing antibody, however, do not allow the degree of protection of individual cattle to be predicted. This inabil-

Table 2. Neutralizing antibody and immune responses of cattle vaccinated with the 40-residue peptide. Heifers 6 to 8 months old and weighing approximately 150 to 180 kg were immunized by subcutaneous injection. The peptide dose was delivered in 3 ml of a mixture of equal volumes of 0.04*M* phosphate buffer and Freund's complete adjuvant.

Animal	Peptide dose (mg)	Serum neutralizing titer on day after vaccination:						Challenge response*
		0	7	14	21	26	32	
1	1/0.2†	0.8	1.2	2.9	3.0	3.0	3.1	Protected
2	1/0.2†	1.3	1.3	3.1	3.1	3.1	3.1	Protected
3	1/0.2†	1.2	1.7	3.0	3.0	3.0	3.0	Protected
4	0.2	1.0	1.3	3.1	3.1	3.0	3.1	Not protected
5	0.2	0.8	1.3	3.1	3.1	3.1	3.1	Not protected
6	0.2	1.5	1.5	3.1	3.1	3.1	3.0	Not protected
7	1.0	0.9	1.0	2.6	2.4	2.6	2.7	Not protected
8	1.0	1.5	1.3	3.1	3.1	3.0	3.0	Not protected
9	1.0	1.0	1.3	3.1	2.9	3.0	3.0	Not protected
10	5.0	0.8	1.7	3.1	3.1	3.1	3.1	Protected
11	5.0	0.8	1.2	3.1	3.1	3.1	3.1	Protected
12	5.0	1.0	1.3	2.6	2.7	3.1	3.0	Not protected
13	None	0.8	0.8	0.8	0.8	2.0	1.5	Not protected
14	None	1.0	1.0	0.8	1.0	1.0	1.3	Not protected
15	None	1.0	0.8	1.0	0.8	1.0	1.2	Not protected
16	None	1.0	0.8	1.0	0.8	0.8	1.0	Not protected

*Animals 4 to 16 were challenged on day 32 with 10×10^4 cattle units of virulent O_1 FMD virus. Animals 1 to 3 were similarly challenged on day 53. The criterion of protection was the absence of any secondary lesions over 7 days. Serum neutralization titers were determined as for Table 1. †On day 32 the first three subjects only were reimmunized subcutaneously with 0.2 mg of peptide in 3 ml of a mixture of equal volumes of 0.04*M* phosphate buffer and Freund's incomplete adjuvant.

ity is in contrast to the situation with conventional virus vaccines (*15*). Furthermore, the levels of neutralizing antibody normally sufficient for cattle protection with a conventional vaccine are approximately one-tenth those shown in Table 2. Consequently, it is not yet possible to establish the efficacy of an FMD peptide vaccine on the comparative basis of its SNT value with that of a conventional vaccine. The first three animals that were challenged 21 days after reimmunization were completely protected. Antibody titers in these animals as measured by ELISA techniques increased after booster immunization. Currently, this synthetic peptide is the smallest agent in molecular size capable of inducing protection in cattle. Its synthetic nature will allow evaluation of its inherent activity in combination with various adjuvants, free of any bacterially derived contaminants.

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A Mutation in the R Body–Coding Sequence Destroys Expression of the Killer Trait in *P. tetraurelia*

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This report describes a mutant strain of *Caedibacter taeniospiralis* 169 that does not produce refractile (R) bodies or kill sensitive paramecia, but still renders its host resistant to killing by wild-type strains of *Caedibacter taeniospiralis*. The mutation is due to insertion of a 7.5-kilobase, transposon-like element into the R body–coding region of the plasmid pKAP169. The results provide strong evidence that R body synthesis is required for expression of the killer trait.

FORMERLY THE KILLER TRAITS OF *Paramecium* were considered classic examples of cytoplasmic inheritance. It is now known that these traits are due to the presence of bacterial cytoplasmic endosymbionts rather than naked genes (*1*). The most notable group of bacterial endosymbionts that occur in paramecia is the genus *Caedibacter* [commonly known as kappa particles (*2*)]. All caedibacteria are obligate endosymbionts of paramecia and typically exhibit two morphological forms. Most members of any population of caedibacteria do not have any distinguishable internal structures and are referred to as nonbright particles. The remaining individuals, called bright particles, possess a large, hollow, cylindrical inclusion body (approximately 0.5 μm long by 0.5 μm in diameter) known as a refractile body or R body. Only caedibacteria carrying R bodies are toxic to sensitive strains of paramecia (*3*). However, it is unlikely that R bodies themselves constitute the toxin, since purified R bodies from most strains of *Caedibacter* do not elicit toxic effects when ingested by sensitive paramecia (*1*) and since strains of *Escherichia coli* carrying cloned R body–coding sequences from *Caedibacter taeniospiralis* produce R bodies but do not elicit toxic effects when ingested by sensitive paramecia (*4*).

The question that arises is whether R body synthesis, which is a plasmid-determined trait in *C. taeniospiralis*, is required for expression of a killer trait, or whether the observed association between these traits in *Caedibacter* is merely coincidental. The available evidence suggests that R body synthesis and expression of toxigenicity are associated, but does not indicate whether one is required for the other. Widmayer (*5*) reported a mutant of *C. taeniospiralis* 51 (51m43) that had greatly reduced abilities to synthesize R bodies and kill sensitive paramecia but still retained the ability to confer resistance upon its host. Dilts (*6*) reported that plasmids carried by *C. taeniospiralis* 51m43 appear to exist as concatemers, whereas they occur as monomers in the wild type. In addition, we have observed that the amount of plasmid DNA present in a population of *C. taeniospiralis* 51m43 is much less than one would expect from a population of *C. taeniospiralis* of a similar size. Thus we believe that the mutation that occurred in *C. taeniospiralis* 51m43 probably affected plasmid replication, which in turn may have affected R body and toxin synthesis. Unfortunately, *C. taeniospiralis* 51m43 apparently no longer exists. Thus the genetic basis for the mutant phenotypes in *C. taeniospiralis* 51m43 cannot be determined. In this report we describe a mutant of *C. taeniospiralis* 169 that has spontaneously lost the abilities to synthesize R bodies and mediate expression of the killer trait due to insertion of a transposon-like element into the DNA sequence required for R body synthesis.

The mutant strain of *C. taeniospiralis* 169 was discovered during routine examination of subcultures of *Paramecium tetraurelia*

Table 1. Results of tests for killing activity and resistance to killing. Cultures of paramecia were mixed together and observed 6 and 24 hours later to determine whether any characteristic prelethal effects were exhibited by individuals in the mixed cultures (*5*). Abbreviations: −, no killing activity observed; +, killing activity observed; K, killer; R, resistant to killing; NK, nonkiller; and S, sensitive to killing.

Paramecium strain	*Paramecium* strain					Trait
	51K	169	169-1	51S	152	
51K	−	−	−	+	+	K, R
169		−	−	+	+	K, R
169-1			−	−	−	NK, R
51S				−	−	NK, S
152					−	NK, S

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