sitive to stimuli that normally increase their secretion (5). However, the greater increment in circulating renin could also be due to a slower rate of removal of renin from the circulation. It is also impossible on the basis of the data presently available to rule out increased angiotensin formation per unit of renin in the animals fed the low sodium diet.

Summary. In pentobarbital-anesthetized normal dogs previously fed a diet providing approximately 40 mEq of sodium per day, hemorrhage caused an increase in circulating renin levels. The values continued to rise 30 and 60 min after hemorrhage when blood pressure was returning toward normal. Plasma renin levels were very low 4 hours after bilateral nephrectomy and they failed to rise following hemorrhage in the nephrectomized dogs. Dogs fed a low sodium diet for 14 days had higher prehemorrhage renin values and a much greater increase in plasma renin following hemorrhage than the animals fed 40 mEq of sodium per day.

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Antibacterial Action of Melittin, a Polypeptide from Bee Venom (32779)

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The ability of bee venom to increase the radiation resistance of mice was recently reported from this Laboratory (1). Studies are now underway to determine the fraction(s) of bee venom responsible for this effect. Pursuant to this study, it was noted that one of the fractions, melittin, possesses potent antibacterial properties. Melittin is the largest single component (by weight) of bee venom; it is a polypeptide of mol. wt. 2850, and evidence suggests that in bee venom it exists mostly as a tetramer (2).

In 1941 Schmidt-Lange discovered that bee venom was antibacterial (3), and this observation was extended by Ortel and Markwardt in 1955 (4,5). They investigated the effect of bee venom against 13 gram-positive and nine gram-negative bacteria and showed that the gram-positive bacteria were the most sensitive. In the present work, we studied the antibacterial effect of melittin and the range of its activity. In the course of this

TABLE I. Antibacterial Effect of Bee Venom, Melittin, and Penicillin on Gram-Positive Organisms.

Organisms	Zone of inhibition (diam., mm)					
	Bee venom (300 μg)	Melittin (300 μg)	Penicillin (units) 500	50	5	0.5
Strep. fecalis	8.5	9.5				
Strep. liquefaciens	8.0	7.8				
Staph. aureus, strain 3A	9.0	9.3	35	32	27	22
53	8.1	8.3	18	13	0	0
80	8.0	8.3	0	0	0	0
Corynebacterium sp.	10.5	12.0				
gram-pos. cocci no. 1	8.8	9.3				
2	8.5	8.5				
3	8.8	9.0				
4	8.8	9.8				
5	8.3	9.3				
gram-pos. rods no. 1	8.8	9.8				
2	11.8	11.8				
3	0	0				
4	0	0				

work it was found that the penicillin-resistant *Staphylococcus aureus* strain 80, was sensitive to the antibacterial action of melittin.

Materials and Methods. Venom was collected by the method of Benton *et al.* (6). The crystalline venom was pooled and separated into components on a Sephadex G50 column (2). To establish the purity of the melittin fraction the ultraviolet absorption spectrum of melittin was obtained prior to and after the experiments. A comparison of the two spectra showed the melittin had not been degraded by air oxidation.

Most of the organisms tested were isolated from various animal sources. The three strains of *Staph. aureus* were obtained from the collection of Drs. V. Hurst and V. Sutter of the University of California Medical Center. The 9 unidentified organisms were isolated from the gastrointestinal tract of a strain of mice routinely used in the laboratory. A total of 30 different organisms were tested—equal numbers of which were gram-positive and gram-negative. The organisms to be tested were inoculated from stock cultures into 10 ml of brain heart infusion (BHI) broth, and incubated for 18 hours at 37°C. An exception to this was *Pseudomonas fluorescens* which was incubated at 25°C.

The method used was similar to that employed in standard antibiotic testing. Fresh BHI agar plates, dried in an incubator at 37°C for 0.5 hour, were flooded with a suspension of the organism. The excess fluid was removed with a Pasteur disposable pipette, and the plates allowed to air dry. Three plates per organism were used in each experiment.

Sensitivity discs were made from no. 7 filter paper. These discs measured approximately 7 mm in diameter, and held 10 μl of fluid. Sterile discs were immersed in the bee venom or melittin test solution and placed on the dry plates with sterile forceps. The plates were then incubated overnight. The diameter of the zone of inhibition was measured and the mean values computed.

In order to evaluate the antibacterial effect of venom and of melittin, their inhibitory effects at the level of 300 μg were compared to that of a standard antibiotic, penicillin. Tenfold dilutions in sterile water were made of buffered potassium penicillin G 200,000 units (Squibb), which were assayed in the same manner as the bee venom and melittin solutions.

Results. Each of the thirty organisms was evaluated several times for its sensitivity to bee venom and to melittin. The results are

summarized in Tables I and II. In this survey, the gram-positive organisms (Table I) proved to be more sensitive to the test substances than the gram-negative ones (Table II)—86% compared to 46%. In most cases a slightly higher inhibitory effect was achieved with the melittin fraction, a difference not large enough to be considered significant. It is therefore concluded that the antibacterial activity of the melittin fraction is of the same magnitude as that of whole bee venom.

A comparison of the antibiotic activity of melittin and penicillin against three strains of *Staph. aureus* is shown in Table I. All three strains were equally sensitive to melittin, while the magnitude of the response to penicillin varied with the strain and the concentration. The experimental technique previously described was varied slightly for one experiment. The test cultures were incubated for 5 hours in a water bath rather than 18 hours in an incubator prior to use. It was thought that with a lighter lawn the zones of inhibition would be larger and more easily measured. However, there was no significant difference between the zones created on the 5-hour culture and those on the 18-hour culture.

The antibacterial data expressing the equivalency of the number of units of penicillin to 1 mg of melittin are given in Table III, for those organisms sensitive to both melittin and penicillin. The large equivalence ratios exhibited by the gram-negative bacteria reflect the relative impotency of penicillin towards these microorganisms. Among the gram-positive organisms tested, the antibacterial effect of 1 mg of melittin was equivalent to that of 0.1–93 units of penicillin.

TABLE II. Antibacterial Effect of Bee Venom and of Melittin on Gram-Negative Organisms.

Organism	Zone of inhibition (diam., mm)	
	Bee venom (300 μg)	Melittin (300 μg)
Aerobacter aerogenes	8.0	7.5
Aerobacter cloacae	8.0	8.0
Bethesda, Ballerup	7.6	8.0
Citrobacter freundii	0	0
Citrobacter freundii (aberrant)	0	0
Escherichia coli	0	0
Mima polymorpha	10.5	12.0
Proteus mirabilis	0	0
Proteus morganii	0	0
Pseudomonas aeruginosa	7.8	9.0
Pseudomonas fluorescens	0	0
Pseudomonas maltophilia	8.0	7.9
Salmonella derby	0	0
Salmonella newport	7.8	8.5
Serratia marcescens	0	0

TABLE III. Antibacterial Activity of Melittin Compared with Penicillin.

Organism	Penicillin units equivalent to 1 mg of melittin
Gram-negative	
Aerobacter cloacae	1700
Bethesda, Ballerup	93
Mima polymorpha	930
Salmonella newport	93
Gram-positive	
Staph. aureus, strain 3A	0.093
53	93.0
Strep. fecalis	1.70
Strep. liquefaciens	17.0
Gram-pos. cocci no. 1	0.093
2	0.093
3	0.093
4	0.093
5	0.093
Gram-pos. rods no. 1	0.093
2	0.16

Discussion. These experimental data show that a specific chromatographic fraction of bee venom, identified as melittin, is the component responsible for its antibacterial activity on the microorganisms tested. More gram-positive organisms are sensitive to melittin than are gram-negative organisms. Presumably, this antibiotic action of melittin is associated with its polypeptide structure.

A penicillin-resistant strain of *Staph. aureus*, strain 80, was found to be sensitive to the action of melittin. It is possible that other drug-resistant microorganisms may exhibit a similar sensitivity to this polypeptide.

In order to interpret the present results in terms of the antibacterial activity of a single bee sting an attempt was made to estimate the venom content of a single sting. Other in-

vestigators (8–10) have estimated numerically the venom content of one bee sting, and have arrived at varying results. For our experimental purposes, one bee sting was defined as 100 μg of solid venom in a solution volume of 0.3 μl. From the experimental results it can be calculated that a single bee sting has the antibiotic potency of up to 9 units of penicillin for a variety of gram-positive bacteria, and a range of 9–170 units of penicillin when measured against a selected group of gram-negative organisms.

It would be of considerable interest to determine whether the antibacterial property of melittin, particularly that against penicillin resistant Staphylococci, is associated with the whole polypeptide macromolecule, or whether smaller molecular fragments would also exhibit this antibacterial activity.

Summary. Bee venom and a derived polypeptide fraction (melittin) were shown to have antibacterial activity against a penicillin-resistant strain of *Staphylococcus aureus* (strain 80). This activity was demonstrated by a method similar to that used for plate sensitivity tests. Both whole bee venom and melittin were also able to inhibit the growth of 20 of the 30 different bacterial organisms tested. More gram-positive organisms (86%) were sensitive to bee venom and to melittin than were gram-negative organisms (46%). Among the gram-positive organisms tested the antibacterial effect of 1 mg of melittin was equal to that 0.1–93 units of penicillin; for a group of gram-negative organisms the equivalent penicillin level 93–1700 units.

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Connective Tissue. XVI. Species Difference in Amino Acid Composition of Insoluble Collagen of Uterus (32780)

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Recently, we reported the preparation and amino acid composition of soluble collagen of human and porcine uteri (1,2). In those studies we found differences between the amino acid composition of soluble collagen of uterus and that of skin in each species, and between the uteri of the two species. The content of hydroxyproline and hydroxylysine in soluble collagen was higher in uterus than in skin and higher in pig than in human uterus. The soluble collagen of pig uterus contained more proline and less valine than that of human uterus. During the period of our previous work on uterine collagen, we had constantly observed the unusually high ratio of hydroxylysine/lysine in rat uterus.[1] This led to the present study on the amino acid composition of uterine collagen in various species. Since the yield of soluble collagen was very low, it was not possible to prepare it from uteri of small animals. The present study deals only with insoluble collagen of uteri

[1] Unpublished data.