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.

TABLE I. Species Differences in Amino Acid Composition of Insoluble Collagen of Uterus. (Residues/1000 residues)

Amino acid	Insoluble collagen of uterus[a]						Soluble collagen of uterus	
	Rat	Guinea pig	Rabbit	Pig	Cow	Human	Pig(2)	Human(1)
Cysteic acid	0	0	1	0	1	0	1	1
Hydroxyproline	102	114	115	107	108	104	102	94
Aspartic acid	49	51	51	48	49	48	43	43
Threonine	17	16	17	16	15	16	16	16
Serine	39	38	32	36	33	33	34	31
Glutamic acid	77	74	74	75	73	75	74	74
Proline	113	111	108	117	113	109	121	122
Glycine	340	342	340	341	346	345	334	337
Alanine	101	102	103	101	103	105	113	115
Valine	22	19	22	22	23	25	25	29
Methionine	5	5	4	5	4	5	4	3
Isoleucine	11	10	11	10	13	12	10	11
Leucine	24	23	23	23	24	25	23	25
Tyrosine	4	2	3	5	4	4	3	2
Phenylalanine	12	11	12	12	12	12	12	12
Hydroxylysine	16	10	11	11	9	9	10	7
Lysine	18	25	25	24	25	26	25	26
Histidine	5	6	5	5	5	5	4	5
Arginine	52	48	48	49	49	49	51	52
Lysine/hydroxylysine	1.1	2.5	2.3	2.2	2.8	2.9	2.5	3.7

[a] Mean value from 2 sets of determinations.

from several species of animals, including human beings.

Methods. Uterine samples from rats (9 months old), guinea pigs (1.5 years old), rabbits (1 year old), pigs (6–8 months old), cows (4–5 years old) and human beings (30–50 years old[2]) were used in this study. Tissues were cut into small pieces; frozen with dry ice and pulverized in a stainless steel beaker with a stainless steel weight. One gm of the pulverized tissue was extracted with 10 ml of 1 *M* NaCl.[3] The extract was discarded. This extraction was repeated once. The residue was washed with water. The water wash was discarded. The water washed residue was extracted twice with 0.5 *M* acetic acid and the extracts were discarded. The insoluble collagen was extracted from this final residue by the method of Fitch *et al.* with 0.3 *M* trichloroacetic acid at 90°C (3); evaporated to dryness at 100° with a stream of air and hydrolyzed in 6 *N* HCl at 110° for 16 hours in a sealed tube under nitrogen. The excess HCl was removed by reduced pressure distillation. Aliquots containing approximately 250 μg of amino acid were analyzed in a Technicon amino acid analyzer.[4] The amino acid composition was calculated in numbers of residues per 1000 residues. Values for threonine, serine, valine, methionine, and tyrosine were corrected for destruction and liberation during hydrolysis, according to the tabulation of Piez *et al.* (4).

Results and Discussion. In the present method, the 1 *M* NaCl and 0.5 *M* acetic acid were used to remove soluble noncollagenous proteins and soluble collagens. The method of Fitch *et al.* was used to extract the insoluble

[2] The authors are deeply indebted to Dr. William Newman, Professor of Pathology, George Washington University School of Medicine, for making available the tissues used in this study.

[3] All manipulations were carried out at 5°C; all extracts were stirred with a magnetic stirrer for 48 hrs.; and all centrifugations were at 40,000 rpm in a Spinco Model L. centrifuge for one hr.

[4] Technicon Chromatography Corp., Ardsley, N.Y.

collagen and to leave behind the remaining noncollagenous protein and elastin. The very high level of hydroxyproline and hydroxylysine indicated that these preparations were not contaminated with noncollagenous proteins. The amino acid composition of insoluble collagen in uteri from several species is shown in Table I. For easy comparison, the amino acid composition of soluble collagens in uteri from pigs and human beings are listed at the end. The principal difference in amino acid composition was a higher content of hydroxyproline and hydroxylysine in insoluble as compared to soluble collagen. More hydroxyproline was present in the insoluble collagen of guinea pigs and rabbits than in that of the other species studied. The insoluble collagen of rats showed the highest content of hydroxylysine and the lowest content of lysine. In agreement with the findings of Piez (5), despite the variations which may occur in the amounts of lysine and hydroxylysine present in the collagen of different organs of rats or in the uteri of various species, the summation of these two amino acids always represents a constant. Serine in the insoluble collagen of the rat and the guinea pig was higher than in any other species studied. Uterine insoluble collagen was found to contain more glycine and aspartic acid and less proline, alanine, and valine than the soluble collagen of the same organ. The cysteic acid content, which was observed as 1 residue/1000 residues in soluble collagen of uteri of pig and human beings, was absent from the insoluble collagen of these two species, but 1 residue/1000 residues were found in the insoluble collagen of the rabbit and cow. The present study is in agreement with our early findings (1,2) that there is a higher content of hydroxyproline and hydroxylysine in uterine collagen than in dermal collagen.

Summary. Insoluble collagen was prepared from the uteri of rat, guinea pig, rabbit, pig, cow, and human being, after extracting the salt- and acetic acid-soluble collagens. The insoluble collagen of uterus contained a significantly higher content of hydroxyproline and hydroxylysine than the soluble collagen from uterus. The highest content of hydroxyproline, 115 residue/1000 residues, was found in the insoluble collagen of rabbit uterus. The highest content of hydroxylysine, 16 residues/1000 residues, was found in rat uterine insoluble collagen.

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Production of Copper Deficiency in the Rat by an Egg Albumin Diet* (32781)

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Copper deficiency in experimental animals is characterized by impaired growth, anemia, and lower copper levels in certain tissues. Hair pigmentation is a sensitive indicator of the copper status of the animal, and achromotrichia is a characteristic symptom of copper

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