eggs metabolize independently of the female worms after being laid.

The determination of biological half-life of ^{65}Zn reveals no significant difference between infected and noninfected animals. Differences may have existed in the distribution of ^{65}Zn among the various body pools which were not detectable in the biological half-life.

Although these studies do not demonstrate that schistosome infection reduces the body zinc stores, they do demonstrate relatively high zinc uptake by both worms and eggs. Schistosome infection may play a role in pathogenesis of zinc deficiency, not by reducing total body zinc stores, but by redistribution of body zinc.

Summary. When $^{65}ZnCl_2$ was injected intraperitoneally into hamsters infected with *Schistosoma mansoni* the incorporation of ^{65}Zn into the parasite, including worms of both sexes and eggs, was much greater than the incorporation of ^{65}Zn into host tissue. Furthermore, the worm/blood ^{65}Zn concentration ratio increased throughout 21 days, being 10 and 20 after 1 day for female and male worms respectively, and 36 and 44 after 21 days. The determination of biological half-life of tracer doses of ^{65}Zn in hamsters heavily infected with *S. mansoni* and in hamsters not infected showed no significant differences in half-life between the two groups up to 50 days postinjection.

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1. Prasad, A. S., Miale, A., Jr., Farid, Z., Sandstead, H. H., Schulert, A. R., and Darby, W. J., Arch Internal Med. **111**, 407 (1963).

2. Prasad, A. S., Miale, A., Jr., Farid, Z., Sandstead, H. H., and Schulert, A. R., J. Lab. Clin. Med. **61**, 537 (1963).

3. Prasad, A. S., Sandstead, H. H., Schulert, A. R., and El Rooby, A. S., J. Lab. Clin. Med. **62**, 591 (1963).

4. Browne, H. G. and Schulert, A. R., Am. J. Trop. Med. Hyg. **13**, 558 (1964).

5. Browne, H. G. and Thomas, J. I., J. Parasitol. **49**, 371 (1963).

6. Ross, J. F., Ebaugh, F. G., Jr., and Talbot, T. R., Jr., Trans. Assoc. Am. Physicians **71**, 322 (1958).

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Effect of Nephrectomy and a Low Sodium Diet on the Increase in Plasma Renin Levels Produced by Hemorrhage* (32778)

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The present experiments were carried out to determine if the kidneys are the source of the increased amounts of renin in the circulation following hemorrhage, and if the amount of renin secreted in response to hemorrhage is

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TABLE I. Effects of Hemorrhage on Plasma Renin Levels.[a]

	Plasma renin (mμg angiotensin II generated per ml per 3 hours)		
	Normal dogs	Nephrectomized dogs	Dogs fed a low sodium diet for 14 days
Control	6.4 ± 2.8	0.9 ± 0.1	31.7 ± 8.3
After hemorrhage (min) 4	10.8 ± 2.3	0.9 ± 0.3	42.8 ± 9.2
30	14.8 ± 5.0	1.2 ± 0.5	102.2 ± 28.8
60	23.8 ± 9.5	1.6 ± 0.4	111.1 ± 28.9

[a] Values are means ± SE.

TABLE II. Effect of Hemorrhage on Systolic Blood Pressure.

	Systolic blood pressure (mm Hg)		
	Normal dogs	Nephrectomized dogs	Dogs fed a low sodium diet for 14 days
Control	161 ± 12	155 ± 19	180 ± 10
After hemorrhage (min) 4	76 ± 6	49 ± 2	81 ± 13
30	75 ± 9	63 ± 11	118 ± 15
60	102 ± 12	86 ± 19	141 ± 8
Amount bled (ml/kg)	26.8 ± 3.6	23.9 ± 2.7	28.1 ± 3.9

increased in animals fed a low sodium diet.

The possibility that extrarenal tissues secrete renin is raised by the demonstration of renin-like activity in the salivary glands of mice (1), the uterus and placenta of several species (2), and the arterial walls of pigs (3). The magnitude of the increase in circulating renin in response to hemorrhage in animals fed a low sodium diet merits consideration because such a diet causes juxtaglomerular cell hypergranularity and hypertrophy (4), and other endocrine glands that are hypertrophied are hypersensitive to stimuli that activate them (5).

Methods. Twelve male mongrel dogs weighing 7.6–16.6 kg were anesthetized with pentobarbital sodium (30 mg/kg) and subjected to hemorrhage of sufficient volume to lower the systolic arterial pressure to 50 mm Hg. Eight of the dogs had been fed a stock diet which provided 40 mEq of sodium per day. Four of these dogs were bilaterally nephrectomized 4 hours before being bled. Four dogs which had been fed a low sodium diet (less than 1 mEq of sodium per day) for 14 days were similarly anesthetized and subjected to hemorrhage.

In each dog, one carotid and one femoral artery were cannulated with polyethylene catheters. The femoral arterial pressure was monitored continuously with Grass model 5 polygraph and a Statham strain gauge. After collecting a control sample of arterial blood from the carotid artery, the dogs were bled over a period of approximately 3–5 min. The amount of blood removed during hemorrhage was recorded. Additional blood samples were obtained 4, 30 and 60 min after the end of hemorrhage. Clotting was prevented by addition of ethylenediamine tetraacetic acid, 3 ml of a 3.8% solution to each 30-ml sample. All samples were centrifuged promptly and the plasma stored in the frozen state until plasma renin activity was assayed by a slight modification of the method of Boucher *et al.* (6).

Results. The effect of hemorrhage on renin levels and blood pressure for the 3 groups of dogs studied are shown on Tables I and II. Plasma renin levels were increased 4 min after the end of hemorrhage in all 4 of the normal dogs with intact kidneys that had been fed approximately 40 mEq of sodium/day. In 3 of the dogs, the levels continued to rise throughout the 60-min period the dogs were studied. However, the mean rises were not

statistically significant ($0.05<p<0.1$) due to the considerable variation from dog to dog. Both systolic and diastolic pressure began to rise promptly after the blood was removed, and by 60 min, the systolic pressure had reached 63% of the control level (Table II).

Hemorrhage in the 4 nephrectomized dogs had essentially no effect on plasma renin levels. In these animals the systolic blood pressure returned toward normal less rapidly than in the normal dogs; 60 min after hemorrhage, it had reached only 55% of the control level. However, there was considerable variation in blood pressure from dog to dog, and the difference is not statistically significant.

The mean plasma renin level prior to hemorrhage in dogs maintained on a low sodium diet for 14 days was significantly greater ($p<0.05$) than comparable values in normal dogs fed 40 mEq of sodium/day. Following hemorrhage, plasma renin levels increased markedly. As in the normal dogs, mean values continued to rise and were highest at 60 min. The mean renin level 60 min after hemorrhage in the 4 dogs fed the low sodium diet was significantly greater than the prehemorrhage control value ($p<0.05$). The mean renin levels at 4, 30, and 60 min after hemorrhage were also significantly higher than the comparable values in dogs fed 40 mEq of sodium/day despite the large standard errors. The blood pressure rose more rapidly in the dogs fed a low sodium diet than it did in the normal dogs; 1 hour after hemorrhage, it had reached 78% of the prehemorrhage value. However, the difference between this value and that seen in the normal dogs is not statistically significant.

Discussion. Hemorrhage has long been known to increase renin secretion (7). In the present experiments, renin levels continued to rise 30 and 60 min after hemorrhage. At this time, the blood pressure and presumably the blood volume were rising toward normal. This finding suggests that something other than simple stretch of the juxtaglomerular cells controls renin secretion; if renin secretion were inversely proportional to the degree of stretch (8), one would expect the maximum renin output to occur when the blood pressure was lowest.

Renin-like activity has been found in the salivary glands and the walls of blood vessels in various animal species (1,3), but the physiological role of this extrarenal renin is unknown. Apparently the material is not mobilized into the circulation by hemorrhage, since nephrectomy blocked the increase in plasma renin levels normally produced by hemorrhage. Of course, no conclusion can be drawn about uterine or placental renin (2), since only male dogs were used in the present experiments. Incidentally, the failure of the renin levels to rise is additional proof of the specificity of the Boucher method used in these studies; hemorrhage is known to increase the plasma levels of vasopressin and catecholamines (9,10), but the high titers of these substances did not interfere with the method. Others have also reported that nephrectomy prevents the increase in plasma renin or angiotensin produced by hemorrhage (7,11,12), although repeated measurements over a period of time after hemorrhage in nephrectomized animals do not appear to have been reported previously. In addition, renin stimulates aldosterone secretion and nephrectomy prevents the increase in aldosterone secretion produced by hemorrhage (see 5).

On the other hand, renin-like activity does not disappear from the plasma after bilateral nephrectomy. The substance in plasma 4 hours after bilateral nephrectomy in the present experiments is probably renin; the half-life of renin is approximately 80 min in dogs (13), and therefore values of approximately 1 mμg/ml would be expected 4 hours after removal of the source of renin. However, measurable amounts of renin have been reported in plasma 24 and more hours after nephrectomy (14,15). Perhaps the material being measured in these instances is renin of extrarenal origin.

The large increments in plasma renin produced by hemorrhage in the dogs fed a low sodium diet could be due to an increased sensitivity of the renin-secreting mechanism to hemorrhage. Others have postulated the existence of such increased responses (16), but they did not present quantitative data. The juxtaglomerular cells hypertrophy and become hypergranulated in animals fed a low sodium diet (4), and other hypertrophied endocrine glands are known to be more sen-

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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1. Bing, J. and Farup, P., Acta Pathol. Microbiol. Scand. **170**, 212 (1965).
2. Ferris, T. F., Gordon, P., and Mulrow, P. J., Am. J. Physiol. **212**, 698 (1967).
3. Gould, A. B., Skeggs, L. T. and Kahn, J. R., J. Exptl. Med. **119**, 389 (1964).
4. Hartroft, W. S. and Hartroft, P. M., Federation Proc. **20**, 845 (1961).
5. Ganong, W. F., Biglieri, E. G. and Mulrow, P. J., Recent Progr. Hormone Res. **22**, 381 (1966).
6. Boucher, R., Veyrat, R., de Champlain, J. and Genest, J., Can. Med. Assoc. J. **90**, 194 (1964).
7. Huidobro, F. and Braun-Menendez, E., Am. J. Physiol. **137**, 47 (1942).
8. Tobian, L., Can. Med. Assoc. J. **90**, 160 (1964).
9. Weinstein, H., Berne, R. M. and Sachs, H., Endocrinology **66**, 712 (1960).
10. Walker, W. F., Zileli, M. S., Reutter, F. W., Shoemaker, A. C., Friend, D. and Moon, F. D., Am. J. Physiol. **197**, 773 (1959).
11. McKenzie, J. K., Lee, M. R. and Cook, W. F., Circulation Res. **19**, 269 (1966).
12. Scornik, O. A. and Paladini, A. C., Can. Med. Assoc. **90**, 269 (1964).
13. Assaykeen, T. A., Otsuka, K. and Ganong, W. F., Federation Proc. **26**, 615 (1967).
14. Toussaint, C. and Verniory, A., New Engl. J. Med. **276**, 350 (1967).
15. Blaufox, M. D., Birbor, A. E., Hickler, R. B. and Merill, J. P., New Engl. J. Med. **275**, 1165 (1966).
16. Bunag, R. D., Page, I. H. and McCubbin, J. W., Circulation Res. **19**, 851 (1966).

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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