mediated by the sympathetic nervous system, an action independent of its direct vasoconstrictor action. The parallelism of the increase in response to tyramine and the rise in arterial pressure suggests that a common mechanism is concerned.

These observations bear on the nature of renal and essential hypertension in man. The dogs behaved very much like labile essential hypertensives. The amount of angiotensin need not be large to elicit relatively severe hypertension by its action on the sympathetic nervous system.

Dickinson and Lawrence (*2*) found a delayed rise of pressure in rabbits infused with small amounts of angiotensin. They suggested that the hypertension was due to cerebral vasoconstriction; it seems more likely to us that the peripheral sympathetic nervous system is affected.

JAMES W. MCCUBBIN
R. SOARES DEMOURA
IRVINE H. PAGE, FREDERICK OLMSTED
Research Division, Cleveland Clinic Foundation, Cleveland, Ohio

References and Notes

1. J. W. McCubbin and I. H. Page, *Science* **139**, 210 (1963).
2. C. J. Dickinson and J. R. Lawrence, *Lancet* **1963-I**, 1354 (1963).
3. Supported in part by grant H-6835 from the National Heart Institute.

19 July 1965

Hemolysin Production in the Development of Staphylococcal Lesions

Abstract. *The presence of hemolysin in staphylococcal lesions of the rabbit kidney was detected by overlaying sections of kidney with blood agar. Hemolytic activity against rabbit, human, sheep, calf, and guinea pig erythrocytes was present and was not abolished by heating the sections to 60°C for 30 minutes. In developing lesions, hemolytic activity appeared before necrosis or exudation.*

Although culture filtrates of pathogenic staphylococci contain a large number of substances capable of damaging cells and tissues, none of these toxins have been shown directly to play a role in the pathogenesis of staphylococcal infections. Neither has it been clearly demonstrated that staphylococci produce toxins while growing in a host's tissue, although production of hemolysin in peritoneal exudates has been observed (*1*). Since the production in vitro of many of the toxins is influenced by the conditions of culture, staphylococci could fail to elaborate any or all toxins under the conditions of growth in a particular tissue. If this were true the toxin could have no role as a pathogenic agent in that tissue. On the other hand, if a toxin is produced in a tissue, careful attention must be paid to the temporal relation between the appearance of the toxin and the development of tissue injury in assessing the pathogenic role of the toxin.

Table 1. Hemolytic activity and histologic changes in rabbit kidneys infected with staphylococci. Each animal received 10^8 cells of *Staphylococcus aureus* (Wood 46 strain) per kilogram of body weight by intravenous injection at 0 hour. RBC, red blood cells.

Time after inoculation (hr)	Staphylococci (avg. No. per gram of kidney)	Hemolysis of:*		Histologic findings*			
		Rabbit RBC	Human RBC†	Degeneration	Bacteria	Necrosis	Leukocytes
6 to 8	3.9×10^2	3/6	2/6	4/6	0/6	0/6	0/6
9 to 12	4.3×10^5	7/9	2/9	5/9	2/9	1/9	0/9
18	6.8×10^6	5/5	5/5	5/5	5/5	5/5	2/5

* Number of animals with positive findings in kidney tissue over number infected animals examined. † In each instance, kidney sections showing hemolysis of human erythrocytes also showed hemolysis of rabbit erythrocytes.

Two probably pathogenic staphylococcal products are α-hemolysin and δ-hemolysin, both of which are produced in vitro by most pathogenic strains (*2*, pp. 239, 343, 247). Alpha-hemolysin causes local necrosis when injected directly into the skin and is lethal to rabbits when injected intravenously (*2*, p. 260); δ-hemolysin has much less pronounced toxic properties (*3*). Alpha hemolysin lyses rabbit erythrocytes readily. Sheep, cow, and goat cells are much less sensitive to this hemolysin; human, guinea pig, and horse cells are completely resistant (*2*, p. 231; *3*). Delta hemolysin is active against rabbit, human, guinea pig, monkey, horse, rat, and sheep erythrocytes (*2*, p. 242; *3*). The α- and δ-hemolysins can be further distinguished from each other by tests of their heat stability. In crude preparations, the activity of α-hemolysin is completely abolished by heating at 60°C for 30 minutes (*2*, p. 231). According to some reports, δ-hemolysin is partially inactivated by heating at 65°C for 30 minutes (*3*). Others state that purified δ-hemolysin is stable at 65°C or 100°C for 2 hours (*2*, p. 243).

The production of hemolysin in staphylococcal lesions of the rabbit kidney is reported here. Renal infections were induced by injecting approximately 10^8 staphylococci per kilogram of body weight into the ear veins of white New Zealand rabbits (2 kg). The animals were killed at intervals after inoculation by intravenous injection of sodium pentobarbital. Blocks of renal tissue were frozen with dry ice within 10 minutes of the animal's death. To detect the presence of hemolysin, frozen sections of kidney (prepared in a cryostat), approximately 8μ thick, were covered with a thin layer of agar containing 5 percent of triply washed erythrocytes as well as 5 μg/ml of sodium methicillin to prevent bacterial growth. The diluent was either 0.85 percent NaCl or 5.5 percent glucose in 0.01*M* phosphate buffer, *p*H 6.8 to 7.0. The necessary thinness of the blood-agar layer was achieved by placing a drop of the liquid material on the tissue and rapidly covering it with a glass cover slip (22 × 22 mm). Preparations were incubated for 1 to 3 hours at 37°C in a moist chamber. Hemolysis could be recognized in transmitted light with the naked eye.

In the initial studies, animals were killed 2 to 4 days after intravenous inoculation of a locally isolated, human-pathogenic strain of *Staphylococcus* (Kyser). Grossly visible, small abscesses were present in the kidneys. Hemolytic activities against rabbit, human, sheep, calf, and guinea pig erythrocytes were demonstrated in the lesions. Hemolysis was evident after 30 minutes of incubation in some instances, and, usually, after 2 hours it was fully developed. The hemolytic activity could be reduced, but not abolished, by maintaining the sections at 60°C for 30 minutes before applying the blood agar. Heating the sections for 10 minutes at 100°C abolished all hemolytic activity.

The possibility that the hemolysis was due to the presence of products of damaged tissue in the lesions was considered. Necrotic, 2-day-old lesions of similar histologic appearance to the

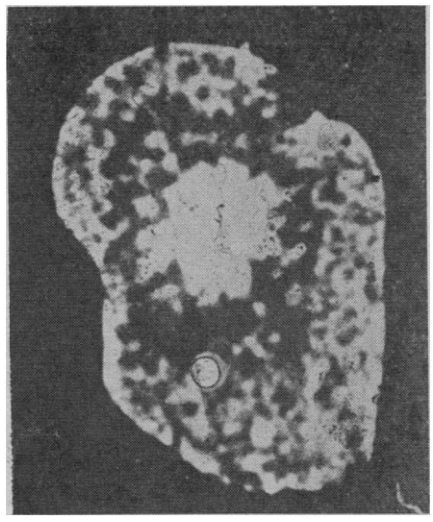

Fig. 1. Hemolysis in human blood agar covering a section of rabbit kidney, 6 hours after inoculation (rabbit No. 117) (× 6). Clear zones of hemolysis are seen against a dark background of unhemolyzed erythrocytes. The peripheral band of spotty hemolysis is over the renal cortex; the central solid zone of hemolysis is over the outer medulla.

staphylococcal lesions were produced either by infection with *Escherichia coli* or by making multiple punctures of the kidney with a red-hot needle. No hemolytic activity could be detected in any of these lesions.

Although it appeared that staphylococci could produce hemolysin when growing within the renal parenchyma of a living animal, it was necessary to exclude the possibility that the conditions for producing hemolysin were present only in the necrotic tissue and exudate of the lesions. If the hemolysin is considered to have an important pathogenic role it should be produced in the tissue before necrosis or exudation. To investigate the temporal relations among hemolysin production, bacterial growth, and tissue injury, 20 rabbits were inoculated intravenously with 10^8 cells of *Staphylococcus aureus* (Wood 46 strain) per kilogram of body weight. Two or three animals were killed at each hour from 6 to 12 hours after inoculation; five were killed 18 hours after inoculation. Half of one kidney was homogenized for quantitative culture. At least five tissue blocks from each animal were studied for the presence of hemolysin by the method already described, rabbit and human erythrocytes being used in each test. Histologic sections prepared from the same tissue blocks used for the hemolysin studies were stained conventionally and studied microscopically (Table 1).

Hemolytic activity was detected in three of the six rabbits killed 6 to 8 hours after inoculation (Fig. 1). At this time the kidneys contained an average of 3.9×10^2 bacteria per gram of tissue, but no bacteria could be found in the microscopic sections. There were no distinct histologic changes, but very subtle degeneration was present in some groups of renal tubules. By 9 to 12 hours after inoculation, hemolytic activity was present in the kidneys of seven of nine rabbits studied. The mean bacterial count was 4.3×10^5 per gram of tissue, and small colonies of bacteria were seen in the interstitial tissue of some of the specimens. Large zones of slight tubular degeneration were seen in several specimens. In one animal with microscopically visible bacterial colonies, small foci of early necrosis were seen adjacent to some of the colonies. No leukocytic infiltrates were present. Eighteen hours after inoculation, hemolytic activity was present in the kidneys of all five animals. The mean number of bacteria per gram of tissue had risen to 6.8×10^6; bacterial colonies were seen frequently in the sections and were larger than at 10 to 12 hours. Foci of renal necrosis were found in all animals. Occasionally it was possible to see necrosis in a concentric zone around a bacterial colony. Slight polymorphonuclear leukocytic infiltration was present at the periphery of some of the foci of necrosis. Six of the 15 rabbits in whose kidneys hemolysin was detected had activity against rabbit cells only; all of these were in the 6-to-12-hour group. There were no kidneys that showed hemolysis of human erythrocytes but no hemolysis of rabbit cells. In the kidneys in which both rabbit and human hemolysis was detected, the activity against rabbit cells was always somewhat greater than against human cells.

Evidence of hemolysin production during the genesis of staphylococcal lesions has been obtained in these experiments. The characterization of the hemolysin, however, was not certain. Its activity against various species of erythrocytes indicated that it could not consist entirely of α-hemolysin and suggested the presence of δ-hemolysin. Because of conflicting opinions about the heat stability of δ-hemolysin it is difficult to fully interpret the results. The appearance of detectable hemolysin before the occurrence of necrosis was consistent with the hypothesis that the hemolysin contributed to the subsequent tissue damage. The evidence at hand, however, did not exclude the possibility that other substances were responsible for at least part of the injury.

EUGENE A. FOSTER
Department of Pathology, University of Virginia School of Medicine, Charlottesville

References and Notes

1. Z. A. Cohn, *Yale J. Biol. Med.* **35**, 12 (1962).
2. S. D. Elek, *Staphylococcus Pyogenes and Its Relation to Disease* (Livingstone, London, 1959).
3. J. E. Blair, in *Bacterial and Mycotic Infections of Man*, R. J. Dubos, Ed. (Lippincott, Philadelphia, ed. 3, 1958), p. 314.
4. Supported by research grant HD-857 from the National Institutes of Health. I thank Eleanore Sturgill for technical assistance.

25 June 1965

Melphalan Therapy and Exercise

In his refutation of the report of Bergsagel *et al.* that myeloma patients excreting type II (λ) Bence Jones protein fail to respond to melphalan therapy, Osserman [*Science* **149**, 564 (1965)] cites two such patients who did very well on this drug—a golfer ("in the 90's") and a pool swimmer ("100 to 150 yards daily"). As the pool swimmer cited, I find the underestimation of my athletic prowess annoying. The actual distance covered by my daily swim is 500 to 550 yards. This performance has been maintained regularly over almost 4 years of melphalan treatment. . . .

NAME WITHHELD

5 August 1965

Assessment of Drugs

Schneiderman, Myers, Sathe, and Koffsky [*Science* **144**, 1212 (1964)] have introduced "a substitute ranking measure for the therapeutic index . . . that would be based on minimizing the losses from the failure to cure plus the losses due to toxicity." The authors say that this new measure would allow better ranking of the net effectiveness of drugs than the therapeutic index. We do not agree. We object particularly on the following grounds:

1) Any drug-ranking measure should yield better results for drugs that provide good therapeutic properties over a wide range of dosage than for those