Verdoglobinuria: *

An Ominous Sign of Pseudomonas Septicemia in Burns

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During the past year, a child terminal from extensive burns was noted to excrete a bilious green urine. Since the eschar was heavily infected with *Pseudomonas aeruginosa*, it was assumed that a secondary urinary tract infection was responsible for the color. However, the urine was amazingly free of cells and on culture produced *E. coli*. All attempts to separate the responsible pigment were unsuccessful. It was even insoluble in chloroform, which is specifically used in the extraction of pyocyanin, the blue-green antibiotic secreted by *Ps. aeruginosa*.

Routine laboratory tests failed to solve the puzzle. The urine was negative for bile, blood, hemoglobin, melanin, and melanogen, and it contained a normal amount of urobilinogen. Since the urine changed from green to a tea-color on standing at room temperature for 24 hours, tests for porphyria and homogentisic acid were performed, but these were within normal limits.

No description of this phenomenon could be found in the literature. In a report of 19 patients who were noted to develop a dark, smoky-black urine just preceding or accompanying septicemia, Larson attributed the color change to hematuria or hemoglobinuria.[2] However, the urine of our patient was green, and did not contain erythrocytes or hemoglobin.

A more detailed analysis of the specimen disclosed that the urine contained a globin identical (or very similar) to the one in hemoglobin, that iron was present in approximately the correct ratio for hemoglobin, and that the pigment could be broken down to produce biliverdin, by splitting off the iron and globin. On spectrophotometry and electrophoresis, it was determined that the unknown compound was verdoglobin, a product of hemoglobin degradation in the reticulo-endothelial system.[1]

Why or how verdoglobin came to be excreted in the urine was yet to be explained. Survey of the burn cases treated during the preceding six years yielded 11 other patients who had died with the same syndrome. These 12 patients represented 10.6 per cent of the burn deaths. Since then, three additional patients with the syndrome have been encountered. The 15 patients form the basis of this report.

Case Material

The patients ranged in age from two to 85 years. There was the expected distribution for race and sex. With one exception, burns were greater than 30 per cent of body surface area and totally third degree (Table 1). All had gluteal and perineal involvement, permitting early fecal contamination of the wound.

Local and systemic therapy, although

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TABLE 1. *Verdoglobinuria—Experience with 15 Patients*

Burn	Greater than 30%, mainly third degree; all with gluteal and perineal burns
Wound treatment	Soaking led to earlier sepsis
Sepsis	Onset within 3 weeks
Red Cell Loss	All with significant hemolysis
Verdoglobinuria	Onset no earlier than 2 weeks post burn; green—11; tea colored—4
Survival	2/3 died within 3 days of onset of verdoglobinuria
Mortality	100% Death classic for Gram negative septicemia

variable, was in every instance compatible with present day methods. However, soaks seemed to increase the infection. A wound acceptable for grafting was achieved in only three, and in none of these did a graft survive. Administration of antibiotics, either prophylactically or therapeutically, was of no avail. Significant fever appeared early, and definite sepsis was established within ten days, in all but one case. Considerable loss of red cell mass was evidenced by a severe anemia, by necessity for more than the expected number of transfusions, or by both. Only energetic local wound care and intensive supportive measures permitted survival beyond the third week post-burn.

Verdoglobinuria usually appeared during the third or fourth week. It was bilious green in 11 and tea-colored in four. Strangely, the urine pigmentation was intermittent, tending to disappear following a dressing change or after hydration intensive enough to produce a diuresis. Six patients exhibited the same green color in stools or gastric aspirant.

Mortality was 100 per cent. Two-thirds of the patients died within three days and none survived longer than four weeks after the onset of verdoglobinuria. Although death was classic for Gram negative septicemia, five had an additional major complication. Autopsy obtained in only six revealed metastatic abscesses of Gram negative organisms in four of the patients.

Bacteriology

In the terminal stages, *Ps. aeruginosa* could be cultured from the burned surface of 12 patients, although an additional case appeared grossly to have this bacterium as its dominant organism (Table 2). The two remaining patients had some other Gram negative bacillus colonizing the eschar. Of ten wounds which initially cultured organisms other than *Ps. aeruginosa,* an overgrowth of pseudomonads occurred in eight. Blood cultures were positive in eight cases, but *Ps. aeruginosa* was obtained in only four. Cultures of the urine from nine patients yielded *Pseudomonas* in six and some other Gram negative organism in three.

Immunology

It was apparent that some hemolytic process was involved in these patients, because of their significant tendency towards extreme anemia and the presence of increased amounts of uroporphyrin and coproporphyrin in the urine. An attractive hypothesis was the possibility that poly-

TABLE 2. *Verdoglobinuria—Bacteriology of 15 Patients*

Source	Pseudomonas	Gram Negatives	Staphylococcus	Negative	Not Done
Wound	12	5 (3)	5 (5)	—	1 (1)
Blood	4	1	3	6	1
Urine	6	3	—	—	6

( )—later Pseudomonas overgrowth by culture or appearance.

TABLE 3. *Antibody Response to Pseudomonas Infection in Burns*

Antigen	Antibody	Initial Titer							Terminal Titer						
		2	4	8	16	32	64	128	2	4	8	16	32	64	128
Pt. RBC	Ps. his.	3	2	2	2	—	—	—	S	4	S	4	2	—	—
Pt. RBC	Gamma globulin	—	—	—	—	—	—	—	+	—	—	—	—	—	—
	Pt. serum	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Coombs serum	—	—	—	—	—	—	—	±	—	—	—	—	—	—
Ps. RBC	Ps. his.	S	S	4	4	3	3	+							
Ps. RBC	Gamma globulin	4	S	S	4	2	2	—							
Ps. RBC	Pt. serum	—	—	—	—	—	—	—	S	S	S	S	S	S	S
Ps. RBC	Coombs serum	±	—	—	—	—	—	—							

saccharide from the *Pseudomonas* became attached to the patient's erythrocytes, sensitized the red cells to an autoimmune process, and thereby produced the Thomsen-Friedenreich type of hemagglutination and hemolysis.[4]

Sequential sera were obtained from the last three patients and titers were noted to rise from zero to greater than 1:256 on testing with *Pseudomonas* sensitized erythrocytes (Table 3). The maximum titer was always obtained in a terminal stage. Never was a positive reaction noted prior to two weeks postburn.

Attempts to demonstrate coating of the patient's erythrocytes with antibody to *Pseudomonas* were unsuccessful. Coombs testing of both unmodified and trypsinized red cells was consistently negative. It was possible, however, to demonstrate that some *Pseudomonas* polysaccharide was indeed sensitizing the patient's erythrocytes. By testing with absorbed rabbit hyperimmune serum, made to *Pseudomonas* 0 antigen, small but definitely increasing titers of polysaccharide antigen were noted to coat the patient's red cells.

It was therefore concluded that, although an autoimmune process may well have played a minor role, the two powerful hemolysins of *Pseudomonas* were responsible for the major red cell loss.[3]

Fluorescence

Unfortunately, verdoglobinuria was not detectable in ordinary light until the patient had reached the final stage of *Pseudomonas* sepsis. It was noted, however, by chance, that verdoglobin has a peculiar chalky-green fluorescence in ultraviolet light. Urines from burned patients, as well as from cases of various hemolytic anemias, were tested for such fluorescence, and three of the burned individuals had a greenish fluorescent pigment in their urine. A fatal outcome was thus predicted in these three, but only two died of *Pseudomonas* septicemia. The third patient made an unevent-

TABLE 4. *Urinary Fluorescence With Ultraviolet Light*

	Acetic Acid	Neutral	Ammonia
Pyocyanin	—	—	—
Fluorescin	—	Green	Green
Verdoglobin	Chalky green	Chalky green	Chalky blue
Pseudomonas urinary infection	—	Green	Green
Staphylococcus septicemia	—	—	—
Pseudomonas septicemia	Chalky green	Chalky green	Chalky blue

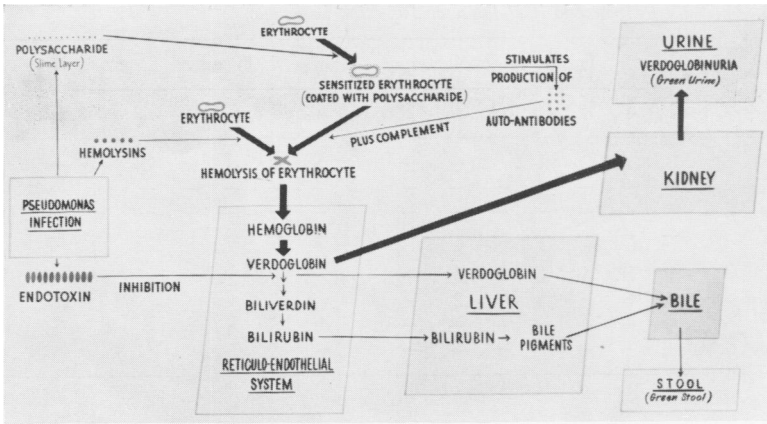

FIG. 1. Mechanism of Verdoglobinuria.

ful recovery. Re-examination of the third patient's urine with culture and fluorescence at a varying pH disclosed that the urine contained *Pseudomonas* and a sufficient amount of fluorescin to produce the false positive reaction. With extreme pH changes obtained by use of glacial acetic acid and ammonia, fluorescin and verdoglobin could be differentiated (Table 4). Solutions of fluorescin have no fluorescence when diluted with concentrated glacial acetic acid, while verdoglobin loses its green fluorescence to become chalky blue on addition of ammonia.

Discussion

The mechanism of verdoglobinuria can only be theorized (Fig. 1). Verdoglobin is produced in the reticulo-endothelial system during hemoglobin catabolism. Since Gram negative endotoxemia is known to paralyze this system, it would appear that catabolic reactions just beyond the verdoglobin stage are inhibited. *Pseudomonas* toxin, whether slime layer polysaccharide or true somatic endotoxin, seems to be particularly incriminated. Verdoglobin accumulates and is excreted primarily in the urine, although liver uptake of this substance with excretion into the bile probably also takes place. At present, attempts are being made to produce verdoglobinuria in the experimental animal, with hopes of testing this hypothesis.

Fluorescence in ultraviolet light appears to be a fairly accurate method for detecting verdoglobinuria some days to weeks prior to the onset of clinically green urine with its fatal picture of Gram negative septicemia. It is hoped that this earlier diagnosis of *Pseudomonas* septicemia may permit specific treatment at a stage when all is not hopeless. With appropriate and intensive therapy, a burned patient with Gram negative septicemia may yet survive.

Summary

A green colored urine was noted in 15 burned patients dying of Gram negative septicemia, particularly that of *Pseudomonas*. The responsible pigment was identified as verdoglobin, an intermediary product of hemoglobin catabolism in the reticulo-endothelial system. It is postulated that absorbed *Pseudomonas* toxin inhibits the reticuloendothelial system, thereby permitting accumulation of verdoglobin and its excretion in the urine.

Immunologic studies suggest that the associated hemolytic anemia is mainly the result of *Pseudomonas* hemolysins and not of an autoimmune process.

Verdoglobinuria was detectable by ultraviolet fluorescence many days prior to its recognition in ordinary light. This should permit more detailed study of *Pseudomonas* septicemia, as well as recognition at a stage when the patient is not terminal.

Acknowledgment

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Discussion

Dr. H. Harlan Stone (closing): When I first saw this urine, I was astounded by its color. Considerable time was required to determine exactly what was producing the color change.

(Slide) This slide shows our antibody studies. As you can see, using the patient's serum obtained when the patient was first admitted, there was a zero titer to red cells which had been coated with Pseudomonas antigen. Terminally this titer was quite high. In fact it was greater than 1:2,048.

On testing the patient's red cells with Coomb's serum, there was no reaction, even terminally. Therefore, we concluded that the hemolytic anemia was not on the basis of an autoimmune process, but rather secondary to Pseudomonas hemolysins. (Slide) Verdoglobin is fluorescent in uultraviolet light, and you will note the chalky green color. (Slide) This slide is merely a summary of the differential fluoresence that one can obtain. The fluorescent pigment can be detected in the urine up to three weeks prior to the time the patient develops clinical Gram negative septicemia.