This work was presented in partial fulfillment of the requirements for the degree of Master of Arts at Indiana University. The author is grateful to Dr. S. E. Luria for advice, and to the Indiana Federation of Women's Clubs for a fellowship.

TABLE 1

Determination of the frequency of mutant cells in normal cultures

Seven flasks with 50 ml of nutrient broth were each inoculated with a well-isolated colony of *Eberthella typhosa* O 901, and incubated with shaking for 36 hours. The cells were harvested and washed twice by centrifugation (30 minutes at 2,500 rpm). The cell deposits were resuspended in 10 ml of sterile 0.85 per cent NaCl solution, and used to inoculate plates and tubes of minimal medium. The titer of each suspension was determined by colony count.

	CULTURE NO.						
	1	2	3	4	5	6	7
Inoculum, cells per plate	3.2×10^8	1.8×10^8	2.6×10^8	1.4×10^8	2.6×10^8	1.3×10^8	1.1×10^8
Colonies of the mutant in individual plates	106	2	0	0	0	1	0
	130	0	1	0	0	0	1
	135	0	0	1	0	1	0
	117	1	0	0	0	5	0
		1	0	0	0	0	0
		0	0	0	0	0	0
		0	2	0	2	0	0
		0	2	0	0	1	0
		0	3	0	0	1	0
		0	0	0	0	4	1
Average	122	0.47	0.8	0.1	0.2	1.3	0.2
Ratio: Mutant cells/normal cells	3.8×10^{-7}	2.2×10^{-9}	3×10^{-9}	7×10^{-10}	7.7×10^{-10}	1×10^{-8}	1.8×10^{-9}
Smallest inoculum that gave growth in liquid medium, cells per tube	1.2×10^6	3.6×10^8	2.6×10^8	2.8×10^8	5.2×10^8	2.6×10^8	2.2×10^8
Calculated no. of mutants in the inoculum that gave growth in liquid medium	0.45*	0.79*	0.78*	0.19*	0.4*	2.6*	0.39*

* The average of the figures followed by an asterisk is 0.8.

THE RELATIONSHIP BETWEEN PRODIGIOSIN PRODUCTION AND CATALASE ACTIVITY

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Received for publication June 12, 1948

Prodigiosin, the pigment of *Serratia marcescens*, is agreed to be a tripyrrole methene. Waring and Werkman (Arch. Biochem., **1**, 428) have shown that iron

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deficiency in the medium inhibits pigment formation. They have also shown (Arch. Biochem., **4**, 75) that catalase activity in *Aerobacter indologenes* falls with decreasing iron content. There exist variant strains of *Serratia marcescens* that do not produce pigment, and it appeared possible that a comparison of colored and uncolored variants could be used to demonstrate a direct relationship between pigment production and catalase activity.

Catalase activities of our normal stock pigmented strain and a white variant that had appeared spontaneously and bred true through continuous transfers were compared on pour plates by means of Oxford cups containing hydrogen peroxide. Table 1 shows the differences noted in the diameter of zones of inhibition.

Heavy saline suspensions of the two strains were studied spectrophotometrically in the Coleman photoelectric spectrometer. Two absorption curves of the pigmented strain were prepared. For the first curve, the white strain was placed in the reference cell to obtain a comparative absorption curve for the pigmented strain against the white variant. To obtain the second curve, a

TABLE 1

CONCENTRATION OF HYDROGEN PEROXIDE	DIAMETER OF ZONE OF INHIBITION AFTER 24 HOURS AT ROOM TEMPERATURE	
	White variant	Normal form
per cent	*cm*	*cm*
3	4.5	3.5
0.3	1.9	1.3
0.03	0.75	0.75

suspension of the normal strain that had been kept unpigmented by growth at 37 C was used in the reference cell. It was assumed that this curve would reveal differences of absorption that were due solely to the pigment. Both curves followed the general shape of that for prodigiosin (Ehrismann and Noethling: Biochem. Z., **284**, 381). The normal red against white curve had a number of sharp peaks, which did not in most instances appear on the other curve. In contrast, the normal red against unpigmented red curve showed plateaus at these points, indicating identical absorption characteristics. A series of peaks at 430, 505, 540 to 550, and 630 mμ was interpreted as the catalase absorption spectrum.

The two experiments outlined above show that the normally pigmented strain of *Serratia marcescens* has a considerably higher catalase activity than the unpigmented variant, and that there appears to be a correlation between ability to produce pigment and catalase activity.

Assuming the porphin nucleus to be synthesized *in vivo* by the same steps that have been used in the laboratory synthesis, the following is proposed as a hypothetical explanation of the origin of prodigiosin:

$$\begin{array}{ccccccc} & & \text{Tripyrryl methanes} & \longrightarrow & \text{Prodigiosin} & & \\ & & \downarrow\uparrow & & & & \\ \text{Substrate} & \longrightarrow & \text{Dipyrryl methenes} & \longrightarrow & \text{Porphin} & \longrightarrow & \text{Catalase} \end{array}$$

The production of tripyrryl methanes during the laboratory synthesis of dipyrryl methenes has been demonstrated by Corwin and Andrews (J. Am. Chem. Soc., **58,** 1086). It is suggested that when catalase production exceeds the demand for it, the equilibrium may shift, favoring an increase in concentration of the intermediate di- and tripyrryl stages. This suggestion is supported by the following experimental observations: (1) The pigment is produced at room temperature, and only by old cells. (2) The absorption curve of the normal red against the white variant showed a sharp peak at 450 mμ, corresponding to the absorption maximum given for alkyl dipyrryl methenes (Granick and Glider: Advances in Enzymol., **7,** 360). (3) There is a lack of pigment production when growth of the normal strain occurs at 37 C. The recognized instability of catalase at this temperature could result in reducing the amount of active enzyme to such an extent that the envisaged side reaction production of prodigiosin would not occur.