The results obtained in this study indicate that Salmonella infection in laboratory colonies of mice may be effectively reduced if carriers are isolated and treated with chemotherapy. In our experiments, treatment of infected animals with sulfanilylguanidine resulted in a decrease of Salmonella organisms in stool cultures. In those few instances where chemotherapy is not effective the mice may be sacrificed in order to decrease the sources of infection.

13584

Pigment Observed in Cultures of Hemolytic Streptococci Belonging to Lancefield Group A.*

S. M. WHEELER AND G. E. FOLEY. (Introduced by J. F. Enders.)

From the Department of Preventive Medicine, Harvard Medical School, and House of the Good Samaritan, Boston, Mass.

Pigment production is not often observed in the growth of the hemolytic streptococcus. Durand and Giraud,[1] in a study of 124 cases of human streptococcus infection, reported the isolation of pigmented strains in 11 instances. Olivieri[2] reported the isolation of pigmented hemolytic streptococci from chronic pyelonephritis. Since these studies were made before the development of serological grouping, it is impossible to interpret the observations in terms of the newer classification. More recently, pigment has been observed in strains belonging to Lancefield's Group B, and occasionally to Lancefield's Group D.[3] As far as could be determined from the literature, the phenomenon of pigment production has not been described in strains belonging to Group A.

During an epidemiological study of hemolytic streptococcus infections involving several thousand cultures, pigmented colonies were observed on several occasions in the cultures of 3 patients. A fourth pigmented strain was observed from a case of scarlet fever in an Army camp. The data relating to these strains are summarized in Table I. All strains were classified as to group by the precipitation method of Lancefield,[4] and serological type was determined by

* This work was supported in part by a grant to the House of the Good Samaritan from the Commonwealth Fund.

1 Durand, T., and Giraud, P., *Compt. rendu. Acad. d. sc.*, 1923, **177**, 1333.

2 Olivieri, J., *J. d'urol.*, 1929, **27**, 484.

3 Lancefield, R. C., *J. Exp. Med.*, 1934, **59**, 459.

4 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

TABLE I.
Classification, Frequency and Source of Pigmented Hemolytic Streptococci.

Strain	Source	No. times isolated	Serological group (Lancefield)	Serological type (Griffith)	Non-pigmented strains carried by patients: Previous type	Non-pigmented strains carried by patients: Subsequent type
(1)	throat, scarlet fever	2	A	1-15*	not known	not known
(2)	throat, carrier	2	A	17-23*	28	28
(3)	" "	5	A	3	0	28
(4)	" "	3	A	28	28	0

*Cross-agglutination with type-specific sera.

Note: Recently, 2 more pigmented Group A strains have been recovered from carriers. These were classified respectively as Type 17-23 and Type 28. They were isolated during a streptococcus carrier survey of 700 naval recruits; of whom 176 were positive for Group A hemolytic streptococci.

the Griffith method of slide-agglutination.[5] Cross reactions occurred in these strains only as indicated in Table I.

The results of cultural studies on these strains are summarized in Table II. It seems that the environmental requirements for pigment production on artificial media closely parallel those of the pigmented Lancefield Group B strain included as a control. All media were observed at intervals of 24 hours through 14 days' incubation. Maximum pigment production was observed after 48 hours' incubation on 5.0% horse blood agar, or in buffered dextrose beef infusion broth. Incubation under partial carbon dioxide tension or anaerobic conditions enhanced pigment production in freshly isolated cultures. Partial inhibition was noted in 1.0% horse serum broth. The ability to produce pigment was apparently lost on storage and regained only by prolonged serial culture. Little difference was observed on the various media incubated at 25°C and 37°C.

TABLE II.
Summary of Cultural Results with Various Media.

Strain	Buffered broth	Blood agar	1% serum broth	Loeffler's serum agar	Potato slant	Dextrose serum agar	Sabouraud's agar
(1)	4+	4+	+	—	—	—	—
(2)	4+	4+	+	—	—	—	—
(3)	4+	4+	+	—	—	—	—
(4)	4+	4+	+	—	—	—	—
Control Group A (non pigmented)	—	—	—	—	—	—	—
Control Group B (pigmented)	4+	4+	+	—	—	—	—

4+ = yellowish-brown pigment.
+ = pale yellow pigment.
— = no pigmentation after 14 days incubation.

[5] Griffith, F., *J. Hyg.*, 1934, **34**, 542.

TABLE III.
Results of Solubility Tests on Ether Extracted Pigment.

Strain	Ether	Chloroform	Carbon disulfide	Alcohol (95%)	Water (dist.)	Normal saline	Sulphuric acid (conc.)
(1)	+	+	+	±	—	—	blue-green color
(2)	+	+	+	±	—	—	"
(3)	+	+	+	±	—	—	"
(4)	+	+	+	±	—	—	"
Control Group A (non-pigmented)	—	—	—	—	—	—	no reaction
Control Group B (pigmented)	+	+	+	±	—	—	blue-green color

+ = soluble.
± = very slightly soluble.
— = insoluble, no color in solvent.

After 48 hours' incubation on 5.0% horse blood agar, the colonies were permeated with a yellowish-brown pigment not unlike that observed in old, dried-out cultures of *Staphylococcus aureus*. The pigment, insoluble in the media used, did not enter the zone of hemolysis on the plates, but seemed to be confined to the colony. The sediment in broth cultures gave much the same appearance. The pigment did not diffuse into the broth, but was confined to the deposit of cells in the butt of the tube.

The results of solubility tests are summarized in Table III. Spectrophotometric analysis indicates that the pigment obtained from the Group A strains is similar to that obtained from the Group B control. (Table IV and Fig. 1.) The pigment could not be extracted from whole cells with the usual organic solvents, but was readily extracted in ether from cultures dried over phosphorus pentoxide and ground in a mortar. The ether extracted pigment was insoluble in normal saline. These results, although preliminary, suggest that the pigmented substance is one of the carotinoids, similar to pigments which are widely distributed in nature.[6] It is not known at this time

TABLE IV.
Summary of Spectrophotometric Analyses.*

Pigment	Solvent	Position of bands (mu.)
Group A Strains	Ether	470-492
	Carbon disulfide	490-508
Group B Strains	Ether	470-492
	Carbon disulfide	490-508

Palmer (1922) has shown by spectroscopic analysis that carotine in ether has a wave-length of 475-490 mu. and 485-519 mu. in carbon disulfide.

*These analyses were made under the supervision of Dr. S. Q. Duntley, Color Measurement Laboratory, Massachusetts Institute of Technology.

[6] Palmer, L. S., *Carotinoids and Related Pigments*, ed. 1, New York, Chem. Cat. Co., 1922, p. 218.

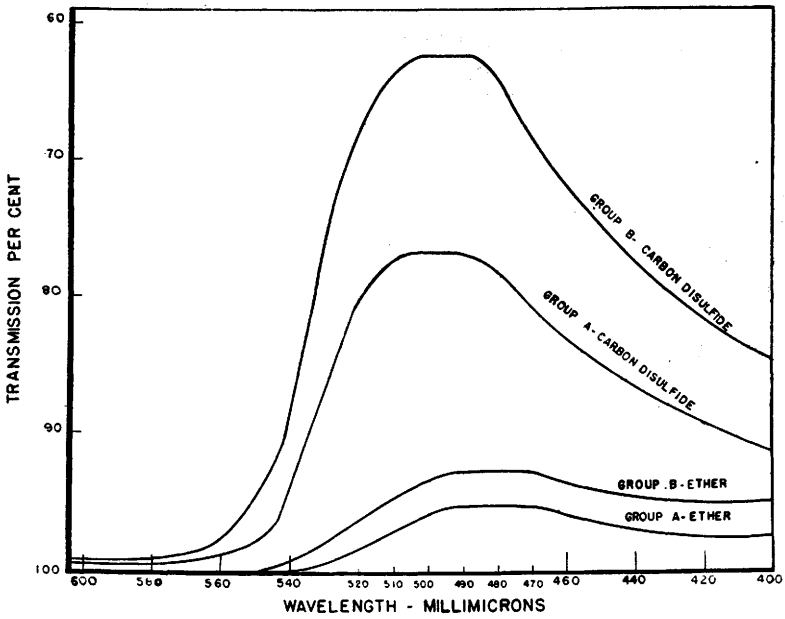

FIG. 1.

Spectrophotometric analyses of pigment extracted from hemolytic streptococci belonging to Group A and Group B.

whether this substance is the same as that recently described by Sevag[7] in a chemical analysis of *"Streptococcus pyogenes,"* in which it was found that the pigmented substance contained a group-specific carbohydrate.

As can be seen in Table I, these pigmented strains were found in throat cultures of only 4 patients over a relatively short period of time. No correlation was observed between pigment and a particular serological type or clinical diagnosis. This does not suggest that pigment production is a characteristic of certain rare strains of Group A hemolytic streptococci. It seems more likely that it is a relatively transient phenomenon occurring only under special environmental conditions.

Failure to obtain maximum pigment production in broth containing 1.0% horse serum, together with the enhancing effect of partial carbon dioxide tension or complete anaerobiasis, suggests a possible relationship of pigment production and the oxidation-reduction potential of the environmental medium. Further experiments are planned using synthetic media of known Eh values to determine the effect of varying oxidation-reduction potentials on pigment production.

[7] Sevag, M. G., *et al.*, *Am. J. M. Sc.*, 1941, **201**, 627.