not being perfused, any pulmonary contribution must be made by the respiratory areas of the lung. But in view of the fact that flow was not augmented in spite of the development of massive pulmonary edema, it seems unlikely that alveolar tissue is provided with a mechanism for lymph drainage capable of conducting an appreciable flow.

11134

In vitro Conversion of Prontosil-Soluble to Sulfanilamide by Various Types of Microörganisms.*

WESLEY W. SPINK, FRANK W. HURD AND JEAN JERMSTA.
(Introduced by C. J. Watson.)

From the Departments of Internal Medicine and Bio-Physics, The University of Minnesota, Minneapolis.

Fuller[1] has shown that both prontosil and prontosil-soluble are partly converted to sulfanilamide in the body. He also demonstrated *in vitro* that stannous chloride and sodium hydrosulphite could reduce prontosil-soluble to form sulfanilamide and amino-acetylamino-naphthol-disulphonic acid. When he gave prontosil-soluble to normal mice, only one-fourth appeared in the urine as sulfanilamide, whereas in mice infected with hemolytic streptococci and given prontosil-soluble, nearly one-half was excreted as sulfanilamide. Bliss and Long[2] succeeded in completely reducing prontosil-soluble *in vitro* with cysteine hydrochloride, and the resultant product proved to be actively bacteriostatic for hemolytic streptococci. They also observed that hemolytic streptococci and a strain of anhemolytic streptococcus were capable of decolorizing prontosil-soluble.[3]

Our purpose is to show that various types of bacteria are capable of converting prontosil-soluble to sulfanilamide. Under standard conditions, the facility with which this change takes place varies with different microörganisms. While such compounds as stannous chloride actually reduce prontosil-soluble to form free sulfanilamide, the mechanism is not as clearly defined whereby the conversion is

* Aided by a grant from the Graduate School of the University of Minnesota.

1 Fuller, A. T., *Lancet*, 1937, **1**, 194.

2 Bliss, E. A., and Long, P. H., *Johns Hopkins Hosp. Bull.*, 1937, **60**, 149.

3 Long, P. H., and Bliss, E. A., *Clinical Use of Sulfanilamide and Sulfapyridine and Allied Compounds*, 1939, 89.

accomplished by a biological system. It would appear that microorganisms achieve the same end result by reduction.

In seeking a standard method for quantitating this conversion power of bacteria, different procedures were tried. At first, a method described by Quastel and Whetham was used.[4, 5] "Resting cultures" of organisms were prepared by seeding a culture in broth, and allowing growth to proceed for 2 days. The broth culture was then centrifuged, the sediment washed 5 times in sterile distilled water, and aerated overnight to remove any oxidizable substance. Nitrogen was then bubbled through the concentrated culture to remove the oxygen. The sample was then diluted to a volume of 10 cc with distilled water. A one percent aqueous solution of glucose was used as the hydrogen donator. That concentration of an aqueous prontosil-soluble solution was used which yielded a 1 to 10,000 concentration of sulfanilamide when completely decolorized. Into a sterile pyrex test tube was placed 1 cc of culture, 1 cc of prontosil-soluble solution, 1 cc of glucose solution, 1 cc of 7.2 phosphate buffer, and 3 cc of distilled water. A control tube contained all the foregoing materials except that a molecular equivalent of an aqueous methylene blue solution was substituted for prontosil-soluble. The tubes were evacuated with a motor-driven vacuum pump, and then sealed in a gas-oxygen flame. The tubes were incubated in a water bath at 37°C. Under these conditions, several strains of staphylococci decolorized the prontosil-soluble and methylene blue solutions. The methylene blue solution was reduced in all instances within a very few minutes, whereas decolorization of the prontosil-soluble took several hours, and in some observations, days.

A more simple and effective method was finally adopted. Organisms were grown in peptone-broth media, with the exception of hemolytic streptococci, and *Streptococcus viridans,* in which cases brain-broth was used. The broth cultures were centrifuged, and the organisms resuspended in 5 cc of peptone-broth or brain-broth. The following were added to a sterile test tube: 0.1 cc of resuspended culture, 0.1 cc of aqueous prontosil-soluble solution (diluted to give a 1 to 10,000 concentration of sulfanilamide when completely decolorized) and 3.4 cc of broth. Control tubes were set up containing the same materials, except that methylene blue solution was substituted for prontosil-soluble in one tube, and in another tube organisms were omitted. The tubes were stoppered with cotton plugs, and incubated in a water bath at 37°C. The tubes

4 Quastel, J. H., and Whetham, M. D., *Biochem. J.*, 1925, **19**, 520.

5 Quastel, J. H., and Whetham, M. D., *Biochem. J.*, 1925, **19**, 645.

were examined at frequent time intervals for evidence of decolorization of the prontosil-soluble and methylene blue.

It was assumed that when complete decolorization of the prontosil-soluble took place, free sulfanilamide was one of the products formed. In a series of 7 observations, the contents of tubes showing complete decolorization were centrifuged, and the amounts of free sulfanilamide in the supernatant fluid were determined by the colorimetric method of Marshall and Litchfield.[6] This method depends upon diazotizing the sulfanilamide, and then coupling it with dimethyl-alpha-naphthylamine to form a purplish red dye. The spectral distribution courve of this dye solution was obtained with the use of the spectrophotometer, and was compared with the absorption curve for pure sulfanilamide, after it was diazotized and coupled in the same manner. The spectral distribution curves were the same for both solutions.

Twenty-two cultures of microörganisms were used in this study. The 6 strains of hemolytic streptococci belonged to Lancefield's Group A, and were supplied to us by Dr. F. Heilman of the Mayo Clinic. Several of the other cultures that were used were obtained from Dr. W. P. Larson of the Department of Bacteriology at the University of Minnesota.

The results are tabulated in Table I. It is noted that the organisms varied in their ability to decolorize prontosil-soluble. While

TABLE I.
Decolorization of Prontosil-soluble Solution by Various Microörganisms.

Organism	Total Hours of Incubation and Degree of Decolorization
Staph. aureus No. 24	93—complete
" " " 104	53—50%
" " " 114	18½—complete
" " " 25	90— "
" " " 107	21— "
" " " 16	22— "
Hem. strept. " 39	72—less than 10%
" " " 43	72— " " 10%
" " " 57	72— " " 10%
" " " 33	72— " " 10%
" " " 38	118—40%
" " " 56	20—complete
Strept. viridans	20— "
B. subtilis No. 1	24— "
" " " 2	92½—none
B. proteus " 1	18—complete
" " " 2	18— "
B. pyocyaneus No. 1	20— "
" " " 2	22— "
B. coli communis	27— "
" " *communior*	96—60%
D_4 (Unidentified Gram neg. Bac.)	16—complete

[6] Marshall, E. K., Jr., and Litchfield, J. T., Jr., *Science*, 1938, **88**, 85.

many strains produced the same change, the elapsed time necessary for complete decolorization varied considerably. When organisms, with the exception of the streptococcus, were suspended in a 2% aqueous solution of peptone instead of peptone-broth, there was a diminution in the speed and degree of decolorization.† A concentrated suspension of organisms yielded more complete decolorization than an unconcentrated suspension. Conversion was more rapid and complete in the presence of oxygen than under anaerobic conditions. Presumably, oxygen favored the growth of the organisms, which in turn produced a greater supply or greater activity of enzyme. Therefore, the optimum conditions for the *in vitro* conversion of prontosil-soluble depended upon a large inoculum of bacteria, the most favorable media for growth, and an available source of oxygen.

It is of interest that anaerobic conditions favored a more effective reduction of methylene blue. This difference in behavior between the 2 dyes may be partly explained on the basis that the decolorization of methylene blue is a reversible phenomenon, whereas it is irreversible for prontosil-soluble.

Completely decolorized prontosil-soluble solution yielded 10 mg per 100 cc of free sulfanilamide when determined by the method of Marshall and Litchfield.[6]

Summary. Different strains of bacteria vary in their ability to decolorize prontosil-soluble. It was shown that it was more difficult for microörganisms to decolorize prontosil-soluble than methylene blue.

11135

Functional Spinal Cord Regeneration in Adult Rainbow-Fish.

JOHN H. KEIL. (Introduced by Davenport Hooker.)

From the Department of Anatomy, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Studies on the regenerative capacity of teleosts have given conflicting results. Koppanyi and Weiss[1] reported functional recovery and morphological reconstruction in the severed spinal cord of the adult goldfish. The return of function after spinal section in adults

† The difference in conversion-power of hemolytic streptococci, when compared to the other strains of microörganisms studied, may be due in part to the media used for the streptococcus, although brain-broth did not appear to affect the action of a strain of *Streptococcus viridans.*

1 Koppanyi, T., and Weiss, Paul, *Anz. d. Akad. d. Wissen, Wien.*, 1922, **7**, 206.