possibly others in this group may have been due to the early escape of lens tissue before the corneal wound had finally closed.

In the other 9 eyes in which no lenses were found the specimens were fixed later than one week after operation. Therefore, how long the implanted lens tissue remained in these eyes it is impossible to state exactly. However, the appearance of the dorsal iris may give some clue. For example, in a 15-day case the reaction of the dorsal iris was comparable to that of a 7-day lensectomized eye, indicating that the onset of lens regeneration had been retarded. This delay might have been due to the temporary presence of reimplanted lens tissue. More detailed studies are planned to test further this possibility.

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Studies of the Action of Sulfonamides upon the Bacterial Cell.

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The mode of action of sulfonamides upon susceptible bacterial species has been the subject of much study. It appeared to us that one of the most fundamental aspects of this problem was to determine whether active compounds differ from closely related inactive substances in their respective abilities to reach or penetrate the bacterial cell. It was conceivable that p-aminobenzene sulfonamide (sulfanilamide) diffuses into the streptococcal cell (and is concerned in its metabolism) whereas its chemotherapeutically inert isomers, the ortho and meta compounds, do not diffuse into the organism. Since this does not appear to be true, the inactivity of certain compounds cannot be explained by this one fundamental property. In the light of our results diffusion into the organism may or may not be a requisite for a chemotherapeutic agent.

Twenty-liter batches of broth cultures of *Streptococcus hemolyticus*, strain C-203, were grown for 48 hours. The organisms were then collected by means of a continuous flow Sharples centrifuge and washed with 250 cc normal saline 6 to 8 times. The cells were collected after each washing by means of horizontal centrifugation. After final washing the bacteria were dried over P_2O_5 in a vacuum desiccator. The same procedure was repeated with cultures in broth

containing 20 mg % sulfanilamide, orthanilamide and metanilamide, respectively. The drug broth cultured bacteria were washed 6 to 8 times until the wash fluid contained no demonstrable sulfonamide compound as determined by the Marshall test. Accurately weighed samples of dried organisms from each of these test cultures were quantitatively extracted in 3% trichloracetic acid for 2 hours at 37°C and the amount of each substance so eluted was also determined by the Marshall method. These experiments were performed twice with each of the 3 compounds and several samples from each experiment were subjected to analysis.

Table I shows that 4 to 12 mg of drug per 100 g dry weight of bacteria were extracted from each of the samples regardless of which of the isomers was used except that there was a rough correlation with solubilities of the respective compounds. This may be taken to indicate that the drugs either penetrate the cell or are strongly

TABLE I.
Concentrations of Sulfanilamide and Its Isomers in Washed Streptococci Grown in Broth Containing These Drugs.
Conc. in mg per 100 g Dry Wt of Streptococci.

Control	Sulfanilamide	Orthanilamide	Metanilamide
0 (blank 1.0)	5.0-9.0	4.0-7.0	7.0-9.0
0 "	5.0-9.0	4.0-7.0	7.0-12.0

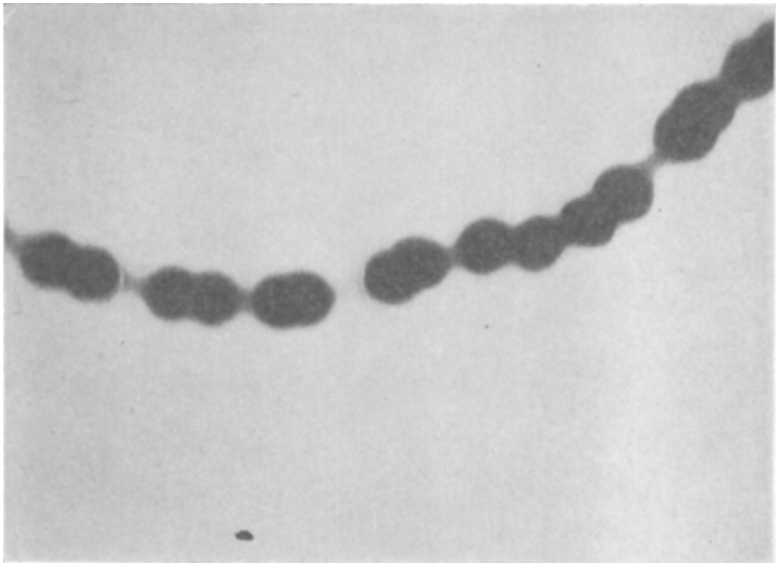

PLATE 1.
Streptococcus hemolyticus, Strain C-203, 24-hour culture grown in plain beef infusion broth. Cells diluted with saline.

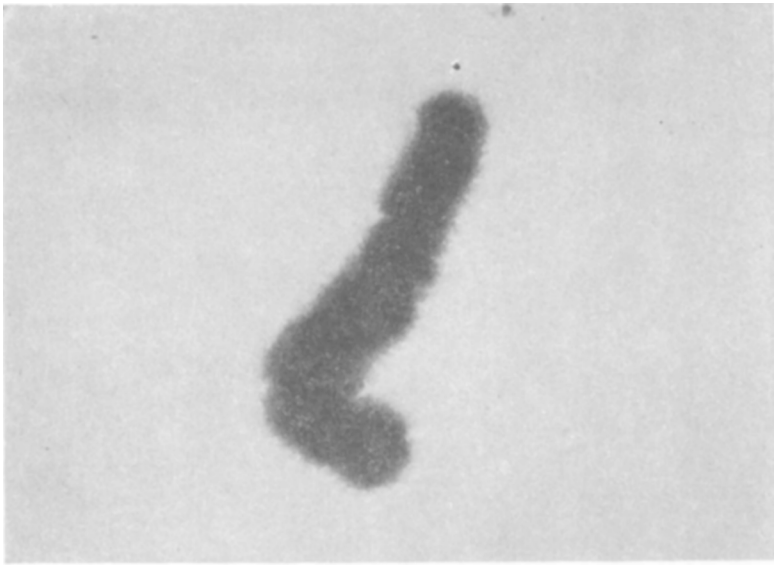

PLATE 2.
Streptococcus hemolyticus, Strain C-203, 24-hour culture grown in broth containing 10 mg % sulfanilamide. Cells diluted with saline.

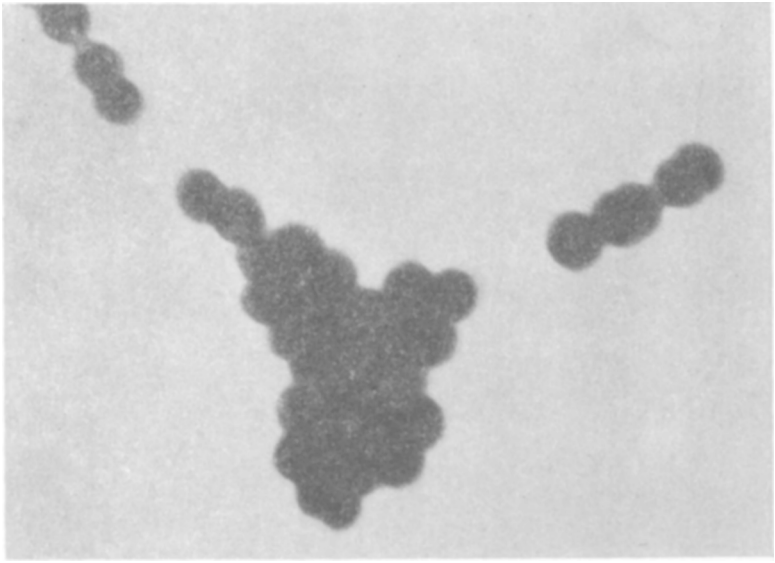

PLATE 3.
Streptococcus hemolyticus, Strain C-203, 24-hour culture grown in broth containing 10 mg % sulfanilamide. Cells washed in saline 3 times.

adsorbed upon the surface so that 6 to 8 washings do not remove them; also that there is no difference in this between the active sulfanilamide and its inactive ortho and meta isomers. However,

this result gives no indication whether this localization is due to adsorption or diffusion of all the compounds or perhaps diffusion in the case of sulfanilamide and adsorption in the case of the inactive ortho and meta isomers.

It was felt that the electron microscope* might offer valuable aid

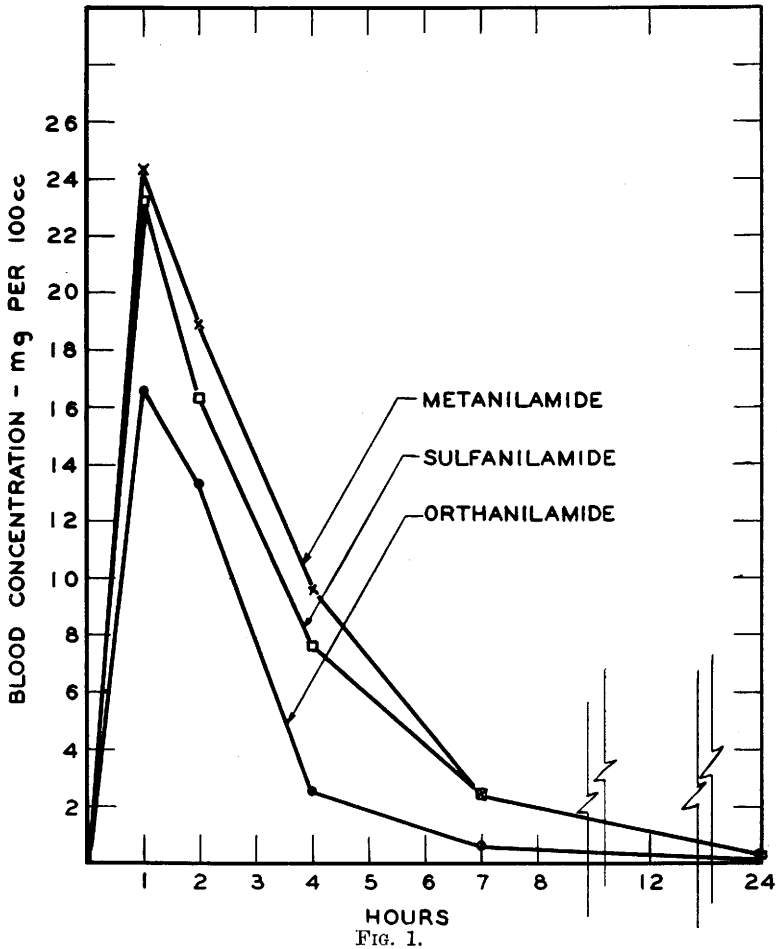

FIG. 1.

Blood concentration-time curves in mice following 10 mg oral doses of sulfanilamide, orthanilamide and metanilamide.†

* We are grateful to Mr. Charles Burton of these laboratories for the electron photomicrographs.

† Quantitatively pooled blood from 10 mice for each curve. Color readings made on Hardy spectrophotometer. Orthanilamide and metanilamide presented difficulty in obtaining standard curves. Maximum color formation for diazotized and coupled orthanilamide required 30 minutes. Metanilamide in about 12 experiments never yielded a constant standard curve. The results shown here for metanilamide are therefore only approximate but not appreciably in error.

in answering this question. Numerous photographs of streptococci growing in plain broth showed the normal appearance of the organism by this method to be quite constant (Plate 1). The examination of cells grown in broth containing 10 mg % of either sulfanilamide or its isomers and washed only once or not at all showed characteristic crystals surrounding the organisms (Plate 2). However, when the organisms were washed 2 or 3 times, the appearance of the streptococci was similar to normal organisms, the crystals having been washed away (Plate 3). Referring to the first experiments, this evidence insofar as it may apply suggests that the drugs diffuse into the organism rather than that they are adsorbed onto the surface of the bacterial cell.

Figure 1 shows the blood concentration time curves of sulfanilamide and its ortho and meta isomers as obtained in mice following oral administration of 10 mg of the respective compounds. All of these substances are well absorbed from the gastrointestinal tract and are therefore available for action *in vivo*.

In vivo and *in vitro* studies on the ortho and meta isomers prove them to be completely inert as chemotherapeutic agents in streptococcal and pneumococcal infections whereas the activity of the para isomer, sulfanilamide, is well known. It must be concluded, therefore, that availability to the host by absorption and distribution, and to the bacteria by strong adsorption or diffusion through the cell, is not a criterion of chemotherapeutic activity of any given compound. Inactive substances may manifest the properties of availability to the host and diffusion into or adsorption onto the bacterial cell but do not inhibit reproduction or otherwise destroy the organism by participating in the metabolism, in the manner that has been suggested for active substances such as sulfanilamide.

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Preservation of *Bartonella muris* in the Frozen State.

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Bartonella muris has been cultivated only with difficulty and but few workers have reported success.[1, 2, 3] All used Noguchi's lepto-