The only conclusion which seems possible from this result is that broth exerts either a protective effect on the organism or an inhibitory effect on the drug and that it must be removed by the dilution in urine.

Table II is an attempt to evaluate the minimal concentration of sulfanilamide and the maximal initial count compatible with stasis. It is apparent that initial counts as high at least as 1000 per cc. yield good bacteriostasis with a concentration of 1:10,000. There is furthermore a suggestion that the concentration of 1:10,000 is very near the critical value for a fairly wide range of initial counts, which is particularly interesting in view of the frequent appearance of this particular value *in vivo*.

Initial experiments employing a pH of 6.0 rather than 7.2 indicate that the action of sulfanilamide is considerably enhanced in the more alkaline range. A concentration of 1:1000 at pH 6.0 appears to be effective in somewhat the same degree as a concentration of 1:10,000 at pH 7.2. This agrees with the observations of Helmholz.[3]

Practically, these results effect a correlation between *in vitro* tests and *in vivo* clinical results where, according to Kenny, disparities existed with the technic employed by her.

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Sulfanilamide and the Macrophage Response to Hemolytic Streptococcal Peritonitis in Mice.

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Because of an apparent disparity between the results of Long and Bliss[1] and Mellon, Cooper, and Gross[2] concerning the rôle of phagocytosis in experimental hemolytic-streptococcal infections in mice under treatment with sulfanilamide, the following inquiry was undertaken. The disparity later came to center about the relative importance of the neutrophils and the clasmatocytes in phagocytosis. In our study we had suggested the importance of strain-differences to explain the original disparity; although it was realized that our experimental set-up was not designed to answer the questions involved. This paper purports to do that.

[3] Helmholz, H. F., *J. A. M. A.*, 1937, **109**, 1039.

[1] Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

[2] Mellon, R. R., Gross, P., and Cooper, F. B., *J. A. M. A.*, 1937, **108**, 1858.

Technic. A representative technical set-up with our Stoddard mucoid strain follows. Thirty mice were injected intraäbdominally with 0.5 cc. of a 1:50,000 dilution of a 12-hour veal-infusion broth culture of the highly virulent Stoddard mucoid strain at 9:45 A. M. Subcutaneous injections of 0.8 cc. of a one percent aqueous solution of sulfanilamide were given to 15 of the mice at the end of 10 hours when the organisms were present in smears of the exudate.

Withdrawal of the peritoneal exudate was made at regular intervals after inoculation, beginning at 3 hours, and repeated at 2- or 3-hour intervals, except during the overnight period—and continuing in this manner usually until the end of the third day. In both control and test groups, certain of the mice were killed at varying periods and smears were made of their spleens, for subsequent examination. Another series of experiments was run with the C-203 strain, used by Long and Bliss, in order to compare the results with our Stoddard strain.

Results with the Stoddard Strain. The first cells to appear in the inflammatory exudate were the polymorphonuclear leukocytes, at the 4-hour period. In 6 hours lymphocytes and monocytes began to appear and after 8 or 10 hours the ratio of neutrophils to monocytes was roughly 3:2. At this stage there was no phagocytosis to speak of, and the heavily encapsulated organisms were easily seen free in the fluid.

Fourteen hours after inoculation and 4 hours after the first treatment the cell-pictures in the treated and untreated animal began to diverge. In the treated animals the neutrophils became necrotic, the monocytic cells greatly increased, and although the number of free encapsulated organisms was fewer, phagocytosis nevertheless was rare.

After 24 to 36 hours the macrophages and monocytes completely dominated the cell-picture, and there was a moderate amount of phagocytosis by the macrophages. The neutrophils at this period were very necrotic, insignificant in number, and played no part in phagocytosis. At the 52-hour period no organisms were seen free in the fluid in the peritoneal cavity, the cells were very scanty, and they were mostly of the polyblastic and macrophagic type, with an occasional cell actively phagocytic.

Corresponding with the replacement of the neutrophils by the macrophages at the 24-hour period, there was a rapid disappearance of the organisms from the peritoneum. Cultures at 24 and 48 hours were negative. The negligible degree of phagocytosis seen in the splenic smears, and the moderate amount in the peritoneal cavity suggested yet an additional unknown factor in the disappearance of the microörganisms.

In the untreated mice there was little phagocytosis by either neutrophils or macrophages of the myriads of streptococci present. The ratio of the former cell to the latter was 3 or 4 to one, at any period observed; and all these mice died in 24 to 72 hours.

Results with the C-203 Mucoid Strain. Without going into unnecessary detail we may say that in general comparable series of experiments were run on this strain. The cellular response and the phagocytosis were notably different from those seen with the Stoddard mucoid strain, there being very much less divergence in the cell-picture at the end of 24 hours following treatment with this C-203 strain. The neutrophilic component of the exudate was decidedly more in evidence and the phagocytosis of the organisms at all stages was more conspicuous in this cell. The phagocytic picture at different stages of the disease was in general a confirmation of the observations of Long and Bliss with this strain.

A third strain isolated from a serious case of Ludwig's angina was avirulent for mice and was rapidly phagocyted by the neutrophils in large numbers in from 3 to 6 hours after inoculation.

It is thus clear that strain-differences, even apart from the virulence-factor *per se* are of much importance in advancing the time of the appearance of the macrophages at the site of inoculation; and when the strain is lacking in virulence they may appear scantily and tardily, due presumably to the lack of necessity for them.

The remarkable fact that within 8 to 12 hours after the administration of sulfanilamide these macrophages have practically replaced the neutrophils first suggested to us a specific mobilizing influence of the drug on this highly important defensive cell. And yet the undoubted bacteriostatic effect of the drug *in vivo* may apparently accomplish the same purpose indirectly, by suspending the microörganisms' elaboration of necrobiotic substances which presumably are negatively chemotactic for the cells.

This consideration together with the difference in response with the C-203 strain make the latter explanation decidedly more probable. The Stoddard Strain may have possessed special resistance to phagocytosis by the neutrophils, thus requiring the presence of the macrophages to dispose of these organisms. All of our work to date is opposed to the conception of a direct and exclusive action of the drug on the organisms, as stressed originally by Long and Bliss,[1] but recently regarded by Long as untenable.[3] On the other hand, it appears as one mediated (and potentiated) by the host's tissues.[4]

[3] Long, P. H., personal communication.

[4] Mellon, R. R., and Bambas, L. L., *M. Rec.*, 1937, **146**, 247.

Summary. Strain-differences in Group A hemolytic streptococci evoke marked differences in the inflammatory cell-response in mice undergoing treatment of experimental peritonitis with sulfanilamide. Some mucoid strains are readily phagocyted by the neutrophils; others seem to require the presence of macrophagic cells almost to exclusion in order to dispose of them. Some non-virulent strains, even in the absence of treatment, are phagocyted and destroyed by the neutrophils alone. Phagocytosis of virulent strains is conditioned by the previous bacteriostatic action of the drug, which appears as an indirect one.

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A Comparison of the Effectiveness of Alpha and Beta Lactose in the Control of Intestinal Reaction.*

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The reaction of the intestinal contents and its control has assumed increasing importance during recent years with the growing realization that acidity and alkalinity are important factors in the digestion and absorption of food and in the progress of various pathological conditions of the intestinal tract. With this knowledge has come an increased interest in the factors influencing the reaction and the methods for their control. One of us[1] has published data indicating that there is a certain constancy about the reactions of the various sections of the intestine which have the appearance of physiological constants and which the body strives to maintain. Unpublished results have confirmed this and demonstrate a rather efficient mechanism for the accomplishment of this end. Apparently changes of long duration are difficult to maintain. The only method whose effectiveness has been adequately demonstrated is that of increasing the acidity by the administration of large amounts of lactose. Several studies have been made of this process.[2, 3]

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† Fellow of the Henry Strong Denison Foundation.

1 Robinson, C. S., *J. Biol. Chem.*, 1935, **108**, 403.

2 Robinson, C. S., Huffman, C. F., and Mason, M. F., *J. Biol. Chem.*, 1929, **84**, 257.

3 Robinson, C. S., and Duncan, C. W., *J. Biol. Chem.*, 1931, **92**, 435.