started, under these conditions. In other experiments it has been observed to continue into the 5th day.

In some experiments we have observed somewhat greater inhibition of lysis than that seen in the series summarized in Table I. We have never seen anything approaching complete inhibition of lysis, however.

The technic used measures the inhibition of hemolytic damage in relation to control preparations. It does not distinguish between reduction in the amount of hemolysin formed and subsequent neutralization.

The results are in harmony with those obtained by others using neutralization-tests on active culture fluids. If Meyer's conclusions were intended to apply to Prontosil-soluble, then our results are not in accord with his finding that hemolysin-formation is prevented when β streptococci are grown in presence of the drug.

It seems to us that tissue-culture clots holding erythrocytes in fixed position in relation to the developing colonies provide a means of registering quantitatively the activity of hemolysin as formed and under conditions as nearly comparable to those existing in the body as can be arranged *in vitro* at present.

Conclusion. *Beta* hemolytic streptococci growing in tissue-culture media containing Prontosil-soluble, 1:1000, produce hemolysin. The amount available for erythrocytic destruction is moderately reduced as compared with controls. This effect is not secondary to bacteriostasis.

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Response of Leukocytes to Colonies of Streptococci Growing in Tissue-culture Media.*

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In discussing the mechanism of action of sulfanilamide, Mellon and Bambas[1] state, " . . . the growth inhibition brought about by the indirect action of sulfanilamide predicates a suspension in the pro-

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1 Mellon, R. R., and Bambas, L. L., *Med. Rec.*, 1937, **146**, 247.

duction of necrobiotic substances (leucocidins, etc.) by the streptococci. As a result, the phagocytes are probably mobilized in proportion to the suppression of the negative chemotactic substances."

Recently McCutcheon, Coman, and Dixon[2] studied the response of leukocytes to particles of kaolin and Lloyd's reagent and to a strain of β streptococci, with a view to determining whether or not a true negative chemotropism could be demonstrated. The leukocytes were suspended in plasma which was flooded over the particles or organisms to be studied. They observed a true negative chemotropic response to particles of kaolin and Lloyd's reagent, *i. e.*, the cells were repelled. The response to the strain of streptococci used was sometimes positive and sometimes negative.

In experimental animals infected with β streptococci it has been noted by Domagk,[3] Long and Bliss,[4] and others that phagocytosis is apt to be less marked in the controls than in the animals treated with sulfanilamide or derivatives. However, it has not been possible to demonstrate that these substances directly influence phagocytosis *in vitro* (Finklestone-Sayliss, *et al.*;[5] Osgood and Brownlee;[6] Coman[7]).

The theory advocated by Bliss and Long[8] and by Gay and Clark[9] postulates a reduction in toxic substances due to the bacteriostatic action of sulfanilamide but it is not assumed that negative chemotropism is the explanation for the reduced phagocytic activity shown by cells in untreated animals.

In the paper referred to above, McCutcheon, Coman, and Dixon point out that the terms "negative chemotropism" and "negative chemotaxis" have been very loosely used in the past and that either a true negative chemotropism (actual repulsion) or merely the absence of positive chemotropism would result in an essential failure of phagocytosis. It is evident that proper distinction has not always been made between that reduction in, or failure of, phagocytosis which is due to true negative chemotropism and that due to other causes.

In the case of the β streptococcus, it is well known that phagocyte-

2 McCutcheon, M., Coman, D. R., and Dixon, H. M., *Arch. Path.*, in press.

3 Domagk, G., *Z. f. Klin Med.*, 1937, **132**, 775.

4 Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

5 Finklestone-Sayliss, H., Paine, C. G., and Patrick, L. B., *Lancet*, 1937, **233**, 792.

6 Osgood, E. E., and Brownlee, I. E., *J. A. M. A.*, 1938, **110**, 349.

7 Coman, D. R., *Arch. Path.*, in press.

8 Bliss, E. A., and Long, P. H., *J. A. M. A.*, 1937, **109**, 1524.

9 Gay, F. P., and Clark, A. R., *J. Exp. Med.*, 1937, **66**, 535.

killing substance or substances are produced. Infected, untreated controls show not only fewer phagocytic cells in exudates but also a leukopenia. The cells, especially the granulocytes, show degenerative changes. It becomes important, therefore, to determine whether, in addition to this general toxic depression of phagocytes, true negative chemotropism is a factor in producing the observed diminution of phagocytosis.

Tissue-cultures have been utilized in studying the response of rabbit's leukocytes to 2 strains of streptococci. Fragments of buffy coat from centrifuged blood were planted in clots composed of one part autogenous plasma (heparinized) and 2 parts chick-embryo extract made with autogenous serum. Methods have been described by one of us[10, 11, 12] previously. The tissue-extract was inoculated before mixing with the plasma with suitable dilutions (usually between 10^{-5} and 10^{-8}) of 18- to 24-hour broth cultures of the streptococcus. The dilutions were made in Tyrode's solution. The cultures were planted on 22 mm round cover-slips (volume approximately 0.15 cc). After clotting, the cultures were incubated (Maximow moist chambers) at 37.5°C.

Bacterial colonies usually become microscopically visible by the 6th hour and they grow rapidly in this medium. If the medium contains approximately 150,000 colonies per cc, growth is essentially complete at 12 hours. With 5 colonies or less per cc, growth may continue into the 5th day.

Cell-migration is prompt and rapid in this medium. As the edge of the dense field of migrating cells approaches and passes the growing colonies one has an excellent opportunity to observe the response of the leukocytes to the streptococci. After making observations on the living state, cultures may be fixed in Carnoy and stained in dilute Delafield's hematoxylin and mounted as whole preparations, or fixed in Zenker-formol for serial sections. Good differential staining of sections is obtained with Dominici.

Two strains of streptococci have been used in these studies: (a) a β strain recovered from a case of meningitis which recovered on treatment with sulfanilamide. This strain was very sensitive to sulfanilamide in tissue-culture media; (b) a β strain recovered from a fatal septicemia in man. This strain was also sensitive to sulfanilamide *in vitro*.

Observations on the living cultures during the first 24 hours

10 King, J. T., *Arch. f. Exp. Zellforsch*, 1930, **9**, 341.
11 King, J. T., *Arch. f. Exp. Zellforsch*, 1931, **10**, 467.
12 King, J. T., *Arch. f. Exp. Zellforsch*, 1937, **20**, 208.

showed that the advancing edge of the leukocytic migration-zone presented a finely or coarsely granular appearance according to whether the bacterial colonies were small and numerous or larger and fewer in number. This finding is in marked contrast to the edge of the migration-zone in sterile cultures where the edge is smooth and the distribution of cells quite uniform.

Examination of fixed and stained *in toto* mounts showed that the bacterial colonies were densely infiltrated with leukocytes. Colonies that are just being approached by the leukocytes show infiltration predominantly on the side from which the cells are approaching while colonies that have been passed by the edge of the migration zone are more uniformly infiltrated.

Study of stained serial sections showed that phagocytosis was taking place actively in each instance.

Using the first strain described, special attention was given to the effect of varying the number of colonies. It was noted in cultures containing a larger number of streptococci that activity of granulocytes was depressed earlier. In one series stained as *in toto* mounts at 24 hours, 2 dilutions of the same bacterial culture were used for inoculating the tissue-culture medium. The dilutions in the final medium were 1:1200 and 1:120,000. In the higher concentration there were practically no living cells in the periphery of the migration-zone at 24 hours while in the lower concentration the toxic depression was not so marked. As would be expected, the width of the migration-zone varied directly with the dilution of bacteria. The average maximal width of the zone was 227.5† for the 1:1200 dilution and 360 for the 1:120,000. These values are taken to indicate the activity of the granulocytes since the periphery of the migration-zone contains few non-granular elements.

There is evidence suggesting that the non-granular elements are less sensitive to the toxic substance formed by the streptococci than are the granulocytes. This question is being investigated further.

Conclusions. Observations made on 2 strains of β streptococci show that rabbit's granulocytes exhibit positive chemotropic response to both strains. There is a progressive toxic depression of phagocytosis varying with the number of organisms present. For the strains investigated true negative chemotropism does not play a part in the depression of phagocytosis.

† 114 units = 1 mm.