# BLOCKING ANTIGENS OF SHIGELLA ALKALESCENS AND ITS VARIANTS

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# BLOCKING ANTIGENS OF SHIGELLA ALKALESCENS AND ITS VARIANTS

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# INTRODUCTION

The recent trend in the laboratory diagnosis of bacillary enteric infections is toward the use of the slide agglutination test. This method makes possible identification of intestinal pathogens within forty-eight hours of receipt of specimens, an interval which can be further reduced when epidemic conditions prevail.

The reliability of the slide agglutination test depends primarily on the production of highly specific, high-titer antisera and the existence of the organisms in an antigenically "smooth" state at the time of test. In most instances, cultures of Shigella of recent isolation are antigenically smooth and readily agglutinated by specific antisera. There are, however, certain freshly isolated strains of Shigella which can be agglutinated in specific antiserum only after heat treatment. These occur with sufficient frequency to be annoying to the diagnostic bacteriologist and a puzzle to the immunologist.

It is the purpose of this work to investigate the inagglutinable state of one species of Shigella.

# I. LITERATURE

The inagglutinability of certain members of the genus Shigella in homologous antisera when tested in the live state has long been known. It is also known that such strains can be rendered agglutinable by boiling. This characteristic was apparently first noticed in Shigella dysenteriae (Shiga), (2, 14). Braun and Unat (3, 4) reported a heat-labile antigen in strains of Shigella paradysenteriae, Flexner, which inhibited O-agglutination. They also reported a method of treating serum by "immunizing" it against heated organisms (4). Similar reports of destruction of insensitivity to agglutination have been made by Schutze (13), Weil, Black and Farsetta (20) and Olitzki et al (12).

That Shigella alkalescens possesses a heat-labile antigen of a similar type has been repeatedly mentioned. In discussing the antigenic relationships of Shigella alkalescens, Stuart et al (16) state that heat treatment of certain strains was necessary to obtain agglutination. Wheeler (18) mentions the necessity of treating Shigella alkalescens antigens for the slide test to temperatures of 60 to 70 degrees Centigrade for one hour. Fulton (8) also found "some strains (of Sh. alkalescens) require heating to render them agglutinable."

The explanation of the phenomenon of inagglutinability or "blocking" was first sought in the cellular structure of the

bacilli exhibiting this characteristic rather than in the properties of their specific antisera. Stuart and Rustigian (16) felt the behavior of the blocking antigen was suggestive of the presence of capsular substance in certain strains of Shigella alkalescens. Weil (19) believed the phenomenon to be connected with some structural element of the cell, although examination under the light microscope and also by electron microscope revealed no difference in the morphology of agglutinable and inagglutinable cells.

More recently a number of studies upon the properties of normal and hyperimmune human sera have disclosed (12) the presence of non-agglutinating antibody in human sera to Shigella Shiga and Salmonella typhi. The development of prozones occurring with certain human sera and somatic suspensions of Salmonella typhi were found (10) to be determined solely by the treatment of the bacterial suspension with heat.

Some investigators have attempted other methods of solving the problem presented by the presence of "blocking" factors in agglutination tests. The sensitization of antigens by treatment with dye particles (11), the development of precipitin tests (5) and the separation and concentration of thermolabile precipitinogens for use as antigen in the production of antisera against heat-labile substances (15) are some of the methods undertaken. Thomen and Frobisher (17) and

Archer (1) have shown that it is possible to produce specific bacteriophage against the dysentery organisms. These investigators mentioned that bacteriophages were effective with cultures rendered inagglutinable by other procedures due to roughness as well as with smooth cultures. The use of 'phage typing as a diagnostic tool awaits refinement of the procedures and greater knowledge of the interrelationships existing between the various dysentery 'phages.

It has been the experience in the Bureau of Laboratories, Michigan Department of Health (6) that the majority of strains of Shigella alkalescens are inagglutinable by slide test upon primary isolation. The isolation of other species of Shigella with "blocking" substance has not been noted, perhaps because of the limited species and types encountered. So unique is this characteristic of Shigella alkalescens that it has proved to be a presumptive identity test for the species. Ferguson et al (7) found that strains which appear to be Shigella from their reactions on triple sugar agar, and which are stable in physiological saline and unstable in acriflavine hydrochloride solution*, are subsequently confirmed in identity as Shigella alkalescens.

No explanation has been made for the instability of these strains in acriflavine solution . It is noteworthy that

*acriflavine monohydrochloride, 1:500, in physiological saline

strains of Salmonella typhi containing Vi antigen are also unstable in acriflavine solution, leading one to conjecture that the "blocking" substance of Shigella alkalescens, like the Vi of Salmonella typhi, may be a surface antigen.

The present investigation is an attempt to determine the possible antigenicity of "blocking" substance in Shigella alkalescens and to learn if practical application can be made of the information.

## II. MATERIALS USED

### A. Registry Antiserum.

The antiserum referred to herein as Registry antiserum was prepared by the immunization of rabbits with a strain of Shigella alkalescens obtained from Dr. Joseph Felsen, Dysentery Registry, The Bronx Hospital, New York City. This organism possessed the biochemical characteristics of a classical strain of Shigella alkalescens, was citrate negative and inagglutinable in acriflavine solution. There was no evidence that this strain contained blocking substance.

### B. Acriflavine

The acriflavine solution was a 1:500 concentration of acriflavine (trypaflavine) monohydrochloride in 0.85 percent sodium chloride.

C. Sterilizing Filter Pads

Type ST (Bacterial Tests) filter pads manufactured by the Hercules Filter Corporation, Patterson, New Jersey, were used in Seitz filters for sterilization of serum.

D. Agglutination Slides

Agglutination slides, Type A-1474X, obtainable from Clay-Adams Company, Incorporated, New York, were used. A slide of this type permits the simultaneous mixing of a number of different antigens with portions of antiserum and holds at a minimum the amount of evaporation which occurs during the mixing and incubation period.

## III. EXPERIMENTAL METHODS

A. Production of Antisera

Two cultures, Nos. 2269 and 5628, were selected at random from a group of thirty-seven isolations of Shigella alkalescens which failed to agglutinate in the Registry antiserum and which were agglutinated in acriflavine hydrochloride solution. These isolations were made in the Diagnostic Division of the Michigan Department of Health, Lansing.

Antisera against these strains were produced in rabbits by the following immunization procedure: Two intracutaneous injections of 0.5 ml. each were made on alternate days with antigen

killed by the addition of 0.4% formalin to saline suspensions of the organisms. After a two-day period of rest, intravenous injections of live antigens were begun. These were given on alternate days beginning with a 0.25 ml. volume and increasing the dosage 0.25 ml. at each injection until a dosage of 1.0 ml. was reached. Inoculations were continued at this volume until a total of 5.0 ml. of live antigen had been given. All antigens, both killed and live, were adjusted to a turbidity equalling that of a No. 2 McFarland nephthelometer (approximately 600 million organisms per cc.).

Twenty-four hours after the final injection, the animals were bled out and the serum immediately separated from the cells. Sterilization was accomplished by passage through a Seitz filter, using a Hercules Type ST pad. No preservative was used.

Strain 2269 produced an antiserum with such a low titer for its homologous organism that it was considered to be useless as a basic serum from which to prepare an absorbed serum for the slide agglutination test. The titer of the antiserum produced by Strain 5628, as determined by tube agglutination tests, was 1:20,000 with the homologous antigen and 1:80,000 with the Registry strain.

B. Preparation of Absorbed Antiserum

The steps in the preparation of an absorbed antiserum may be followed by reference to Table I. The raw serum, diluted

TABLE I. SHIGELLA ALKALESCENS ANTISERUM, #5628

| Living Antigens | Slide Agglutination Reactions | | | |
|---|---|---|---|---|
| | Unabsorbed Serum (Dil.1:10) | Following First* Absorption (Dil.1:10) | Following Second** Absorption (Dil.1:10) | Following Final Absorption (Dil.1:10) |
| Flexner V, 3090 | ++++ moderate | + slow | ± slow | - |
| Flexner W, IIb C | +++ moderate | + slow | ± slow | - |
| Flexner W, IIa R | ++ slow | + slow | ± slow | - |
| Flexner X, 694 | ++++ rapid | + slow | ± slow | - |
| Flexner X, 1533 | - | - | - | - |
| Flexner X, 1806 | ++++ instant | + mod. | - | - |
| Flexner X, 1807 | ++++ instant | + mod. | - | - |
| Flexner Y, R | ++++ instant | ++ mod. | ± slow | - |
| Flexner Y, 6094 | ++++ rapid | ++ mod. | + slow | - |
| Flexner Y, 412 | ++++ instant | ++++ rapid | + slow | - |
| Flexner Z, R | - | - | - | - |
| Flexner Z, 2450 | - | - | - | - |
| Boyd 103, 1049 | - | - | - | - |
| Boyd 170, C | - | - | - | - |
| Boyd P-143 | - | - | - | - |
| Boyd P-119, M | + moderate | - | - | - |
| Boyd 88 | + slow | - | - | - |
| Boyd P-288, N.M. | - | - | - | - |
| Boyd P-274, M | ++ moderate | - | - | - |
| Boyd D-1, M | - | - | - | - |
| Boyd D-19, M | ++++ rapid | + slow | - | - |
| S. typhi, 703 | ++++ moderate | + slow | - | - |
| Sh. ambigua, N.Y. | + slow | - | - | - |
| Sh. ambigua, H | ++++ slow | ++ slow | + slow | - |
| Sh. alcaligenes | + slow | - | - | - |
| Sh. alkalescens,R | ++++ rapid | ++++ rapid | ++++ rapid | ++++ rapid |
| Sh. alkalescens #5628 | ++++ rapid | ++++ rapid | ++++ rapid | ++++ rapid |
| Sh. sonnei, 1055 | - | - | - | - |

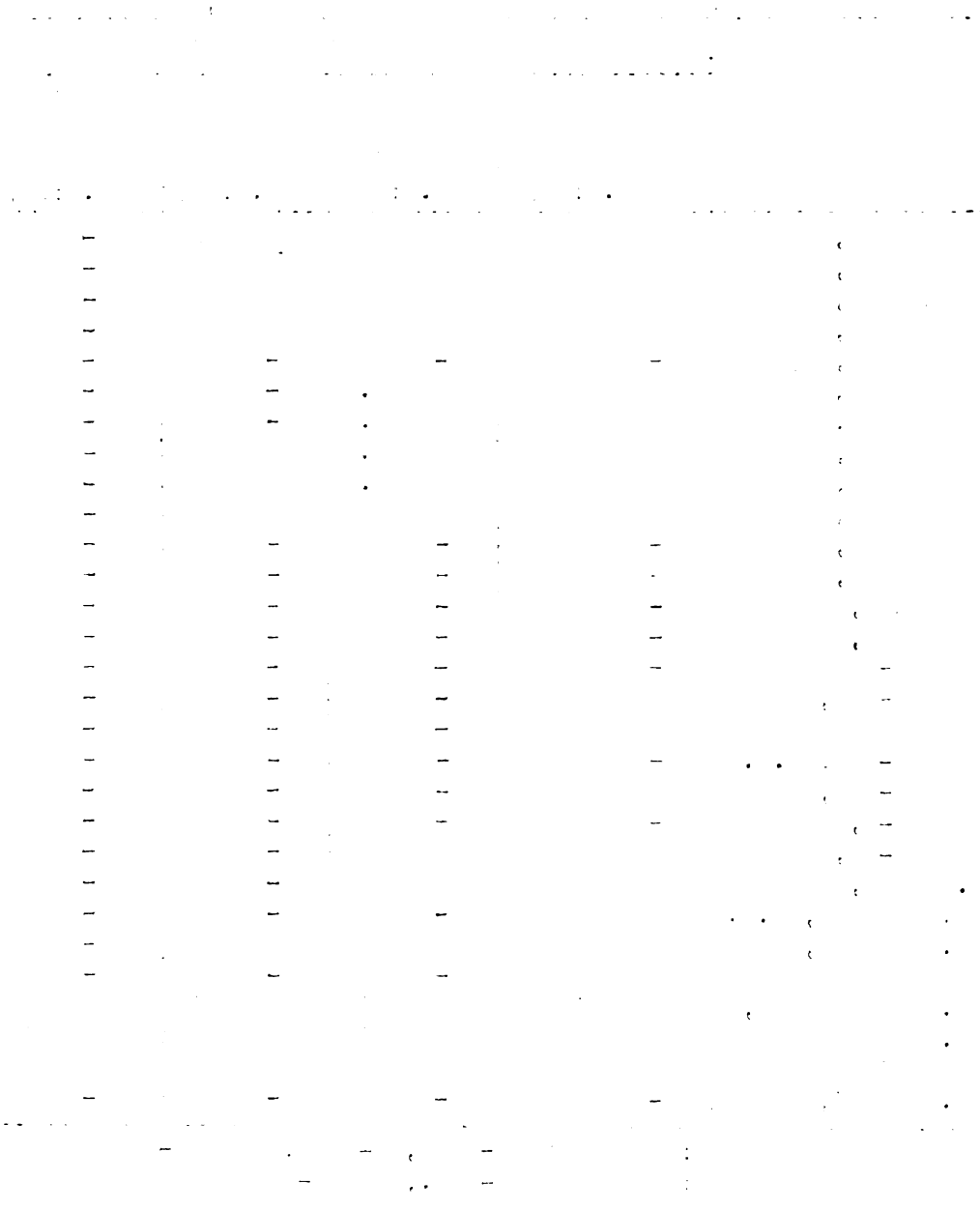
*Absorbing strains: Flexners X-1806, X-1807, and X-694

**Absorbing strains: Flexners Y-Reg., and Y-412

++++ Complete agglutination

++ Agglutination, 50%

± Doubtful agglutination



with physiological saline 1:10, was tested by the slide agglutination technique with live, eighteen-hour, smooth cultures of representative strains of Shigella which had been grown upon nutrient blood agar. The cross reactions are shown in column 1. The technique of the slide test, the method of absorbing the serum, and the interpretation of the results are the same as previously described (6, 7). In this case, the strains selected for removal of antibodies causing cross reactions in the unabsorbed serum were:

Shigella paradysenteriae, Flexner X, Strain 1806
Shigella paradysenteriae, Flexner X, Strain 1807
Shigella paradysenteriae, Flexner X, Strain 694
Shigella paradysenteriae, Flexner Y, Registry
Shigella paradysenteriae, Flexner Y, Strain 412

The result of the absorption of the serum with these organisms is shown in columns 2, 3 and 4 of Table I. Following removal of the factors responsible for cross reactions, the titer of the absorbed serum, as determined by tube agglutination tests, was 1:10,000 with the homologous antigen and 1:40,000 with the Registry strain; the slide test was shown to yield a ++++ rapid reaction with both the 5628 and Registry antigens.

## IV. EXPERIMENTAL RESULTS

### A. Reactions of Absorbed Antiserum with Stock Cultures

The absorbed antiserum was first tested in parallel with the antiserum produced with the Registry strain. The initial comparison was performed with a group of thirty-seven strains of

Shigella alkalescens which had been collected from the Diagnostic Division over a period of eight months and stored in the lyophilized state until the time of test. Members of this group of cultures were tested in the unheated state with the 5628 absorbed antiserum and the absorbed Registry antiserum by means of the slide agglutination technique. The results of this experiment appear in Table II.

All but three strains, Nos. 4746, 6590 and 7144, exhibited an agglutination reaction of ++ or stronger with the absorbed 5628 antiserum. With the exception of Strain 5708, which also failed to react with acriflavine, all strains gave a negative or doubtful agglutination reaction with the absorbed Registry antiserum.

It will be noted that the three strains, Nos. 4746, 6590 and 7144, reacted in acriflavine, providing presumptive evidence that blocking substance was present. This was substantiated by their failure to react in the absorbed Registry antiserum before heat-treatment and their agglutination in the same antiserum after heat-treatment. One possible explanation for this behavior may be the fact that Nos. 4746 and 7144 were shown to be citrate positive, motile variants of Shigella alkalescens. These two characteristics were often found in strains inagglutinable with absorbed 5628 antiserum.

TABLE II. COMPARATIVE REACTIONS OF STOCK CULTURES

| Culture Number | Live Antigens | | | Heat-treated Antigen and Reg. Serum (Dil.1:20) |
|---|---|---|---|---|
| | In Acriflavine | With #5628 Antiserum (Dil.1:10) | With Registry Antiserum (Dil.1:20) | |
| 726 | ++++ | ++++ rapid | - | ++++ moderate |
| 2123 | ++++ | +++ rapid | ± | ++++ moderate |
| 2145 | ++++ | ++++ rapid | + | ++++ moderate |
| 2184 | ++++ | ++++ rapid | ± | ++++ moderate |
| 2263 | ++++ | ++++ rapid | - | ++++ rapid |
| 2269 | ++++ | ++++ rapid | - | ++++ moderate |
| 3178 | ++++ | ++++ rapid | + slow | ++++ moderate |
| 3787 | ++++ | ++++ rapid | - | ++++ moderate |
| 2455 | ++++ | ++++ rapid | ± | ++++ rapid |
| 3855 | ++++ | ++++ rapid | - | ++++ rapid |
| 4215 | ++++ | ++ mod. | - | ++++ moderate |
| 4420 | ++++ | ++++ rapid | - | ++++ moderate |
| 4432 | ++++ | ++++ mod. | - | ++++ rapid |
| 4727 | ++++ | ++++ rapid | - | ++++ rapid |
| 4746 | ++++ | - | - | ++++ rapid |
| 5464 | ++++ | ++++ mod. | - | ++++ moderate |
| 5628 | ++++ | ++++ rapid | - | ++++ rapid |
| 5692 | ++++ | ++++ rapid | - | ++++ moderate |
| 5708 | - | ++++ instant | ++++ instant | |
| 5889 | ++++ | ++++ mod. | + slow | ++++ moderate |
| 6590 | ++++ | - | - | ++++ moderate |
| 6734 | ++++ | ++++ rapid | - | ++++ moderate |
| 6743 | ++++ | ++++ rapid | + | ++++ moderate |
| 7144 | ++++ | - | - | ++++ moderate |
| 7015 | ++++ | +++ rapid | - | ++++ moderate |
| 7050 | ++++ | ++ mod. | - | ++++ moderate |
| 7060 | ++++ | ++++ rapid | - | ++++ rapid |
| 7078 | ++++ | ++++ rapid | - | ++++ rapid |
| 7081 | ++++ | ++++ instant | - | ++++ moderate |
| 7146 | ++++ | +++ rapid | - | ++++ moderate |
| 7149 | ++++ | ++ slow | ± | ++++ moderate |
| 7156 | ++++ | +++ slow | - | ++++ rapid |
| 7218 | ++++ | ++++ rapid | - | ++++ rapid |
| 7276 | ++++ | ++++ rapid | ± | ++++ rapid |
| 7286 | ++++ | ++++ rapid | ± | ++++ rapid |
| 7544 | ++++ | ++ mod. | ± | ++++ rapid |
| 7589 | ++++ | ++++ rapid | ± | ++++ rapid |
| 7577 | ++++ | ++++ rapid | ± | ++++ moderate |
| Registry | | ++++ rapid | ++++ rapid | |

++++, Complete agglutination

++, Agglutination, 50%

After heating in a boiling water bath for thirty minutes, saline suspensions of all strains gave a ++++ agglutination reaction with the absorbed Registry antiserum by the slide technique.

B. Reactions of Absorbed Antiserum with Freshly Isolated Cultures

The same general procedure was followed for the parallel testing of the absorbed 5628 antiserum and the absorbed Registry antiserum with freshly isolated cultures. These cultures were characterized by (1) agglutinability in acriflavine hydrochloride solution, (2) inagglutinability in physiological saline and (3) failure to react with the absorbed Registry antiserum in the slide test.

A total of fifty-two fresh isolations were examined. Fourteen of these failed to agglutinate with the absorbed 5628 antiserum. The balance were agglutinated to some degree in the absorbed 5628 antiserum. The results of these typings are shown in Table III. Heat treatment of antigen, followed by test of agglutinability in absorbed Registry antiserum, was discontinued after the first few strains were examined. This test was performed in the Diagnostic Laboratory as a basis for identification of the cultures as strains of Shigella alkalescens and repetition of the test appeared superfluous. The inagglutinable strains were subjected to further test as will be reported later in this paper.

TABLE III. COMPARATIVE REACTIONS OF FRESH CULTURES

| Culture Numbers | Live Antigens | | | Heat-treated Antigen and Reg. Serum (Dil. 1:20) |
|---|---|---|---|---|
| | In Acriflavine | With #5628 Antiserum (Dil.1:10) | With Registry Antiserum (Dil. 1:20) | |
| **886 | ++++ | ++++ | - | ++++ |
| *3454 | ++++ | - | - | ++++ |
| 222 | ++++ | +++ | - | ++++ |
| 223 | ++++ | +++ | - | ++++ |
| 259 | ++++ | +++ slow | - | ++++*** |
| 449 | ++++ | +++ slow | - | |
| 842 | ++++ | +++ | - | |
| 954 | ++++ | ++++ moderate | - | |
| 1040 | ++++ | ++++ moderate | - | |
| 1095 | ++++ | +++ moderate | - | |
| 1099 | ++++ | +++ | - | |
| 3704 | ++++ | - | - | |
| 3717 | ++++ | ++++ moderate | - | |
| 3917 | ++++ | ++++ moderate | ++ slow | |
| 4340 | ++++ | - | ++ slow | |
| 4817 | ++++ | +++ rapid | - | |
| 6757 | ++++ | +++ moderate | - | |
| 6881 | ++++ | ++ moderate | - | |
| 7139 | ++++ | ++ moderate | - | |
| 7745 | ++++ | +++ moderate | - | |
| 4201 | ++++ | +++ moderate | - | |
| 5186 | ++++ | +++ moderate | - | |
| 5535 | ++++ | ++++ rapid | - | |
| 2649 | ++++ | ++ moderate | - | |
| 2875 | ++++ | ++ moderate | - | |
| 3105 | ++++ | +++ moderate | - | |
| 3143 | ++++ | +++ moderate | - | |
| 3278 | ++++ | ++ slow | - | |
| 3437 | ++++ | - | - | |
| 3643 | ++++ | ++ | - | |
| 3694 | ++++ | ++ | - | |

*Similar reactions obtained with cultures 3487, 755, 1718, 1277, 4192, 4792, 5146, 7015, 4527 and 4135.

**Similar reactions obtained with cultures 2682, 728, 3490, 3520, 96, SB435, SB208, 771, 774, 868, 1346, 6325 and 6757.

***Balance of cultures gave ++++ reactions; tested in Diagnostic Laboratory prior to inclusion in study.

C. Inactivation of Heat-labile Substance

Experiments were conducted to determine the time and temperature required to destroy or inactivate the heat-labile substance in the Shigella alkalescens strains under study. Suspensions of Strain 5628 in physiological saline were subjected to temperatures of 56 degrees Centigrade and 100 degrees Centigrade for varying periods of time; the latter temperature was secured by immersing small tubes containing saline suspensions of the organism in a distilled water bath held at a rolling boil. These results are shown in Table IV.

It was found that one hour and twenty-five minutes was required to inactivate the heat-labile substance at the 56-degree temperature. Inactivation was secured in the boiling water bath in 1.5 minutes. Agglutination in the absorbed Registry antiserum appeared within 1.5 to 2.0 minutes after combination with heat-treated antigens and rapidly progressed to the ++++ degree. The prolongation of the period in the boiling water bath failed to speed up the initial appearance of agglutination.

A representative group of Shigella alkalescens containing the heat-labile substance yielded the same results upon being subjected to the boiling water bath. (See Table V.). The one exception was Strain 2269. This was one of the strains which had been selected at random for the production of anti-blocking antiserum. However, it had produced serum with a titer so low as to be considered useless for the experiments under consider-

TABLE IV. INACTIVATION OF STRAIN 5628

| Time | Temperatures, Degrees Centigrade | |
|---|---|---|
| Minutes | 100 | 56 |
| 1 | ++++ | - |
| 2 | ++++ | - |
| 3 | ++++ | - |
| 4 | ++++ | - |
| 5 | ++++ | - |
| 10 | ++++ | - |
| 15 | ++++ | - |
| 30 | | - |
| 45 | | - |
| 60 | | ++ |
| 85 | | +++ |
| Overnight | | ++++ |

Quantity of Test Material: 0.5 ml. saline suspensions

TABLE V. COMPARATIVE AGGLUTINATION REACTIONS BETWEEN LIVING AND INACTIVATED ANTIGENS

| Culture Numbers | Registry Antiserum(Dil. 1:20) | | #5628 Antiserum |
|---|---|---|---|
| | Unheated | Heated 1.5 min. | Unheated |
| 4215 | - | ++++ | - |
| 4746 | - | ++++ slow | - |
| 7015 | - | ++++ | - |
| 7081 | - | ++++ | - |
| 7144 | - | ++++ | - |
| 7146 | - | ++++ | - |
| 7149 | - | ++++ | - |
| 7156 | - | ++++ | - |
| 7544 | - | ++++ | - |
| 4432 | - | ++++ | ± |
| 7050 | - | ++++ | ± |
| 7276 | - | ++++ | + |
| 5708 | ++ slow | ++++ | ++++ |
| 2269 | - | - | ++++ |
| 726 | - | ++++ | ++++ |
| 2123 | - | ++++ | ++++ |
| 2145 | - | ++++ | ++++ |
| 2184 | - | ++++ | ++++ |
| 2263 | - | ++++ | ++++ |
| 3787 | - | ++++ | ++++ |
| 3855 | - | ++++ | ++++ |
| 4420 | - | ++++ | ++++ |
| 4727 | - | ++++ | ++++ |
| 5464 | - | ++++ slow | ++++ |
| 5692 | - | ++++ | ++++ |
| 5889 | - | ++++ | ++++ |
| 6734 | - | ++++ | ++++ |
| 6743 | - | ++++ | ++++ |
| 7060 | - | ++++ | ++++ |
| 7078 | - | ++++ | ++++ |
| 7286 | - | ++++ | ++++ |
| 7589 | - | ++++ | ++++ |
| 7577 | - | ++++ | ++++ |
| 2455 | - | ++++ | ++++ |
| 3178 | - | ++++ | ++++ |
| 5628 | - | ++++ | ++++ |

ation. The behavior of this strain might be interpreted as indication that it contained a greater concentration of blocking substance which caused it to fail to be affected by the 1.5 minute heating period.

D. Biochemical Reactions

A compilation of the data obtained by a study of the biochemical reactions of these organisms disclosed the fact that nine (Nos. 3454, 3487, 7144, 4746, 7149, 7081, 7015, 7146, and 7156) of the eighty-seven cultures studied were capable of utilizing citrate in three to five days and were motile in semi-solid agar. The biochemical reactions of the remaining seventy-eight strains conformed to the classical reactions of Shigella alkalescens in their ability to ferment dextrose, maltose, mannite, xylose, sorbitol, dulcitol and produce indole. By reference to Tables II and III, it will be noted that the organisms which utilized citrate failed to react with the absorbed 5628 antiserum altogether or were agglutinated weakly and slowly by it.

E. Examination of Inagglutinable Strains

As previously mentioned, fourteen of the freshly isolated strains failed to be agglutinated by absorbed 5628 antiserum. Eleven of these were selected for further investigation in comparison with thirteen cultures which had been shown to be

agglutinated in varying degrees by the absorbed 5628 antiserum. Of this group of twenty-four cultures, all but three (Nos. 1277, 4192 and 4340) were agglutinated by the unabsorbed 5628 antiserum as recorded in Table VI. Of the three inagglutinable strains, strain 1277 has been shown to be a paracolon; strains 4192 and 4340 were repeat isolations from the same person and have been shown on further study not to be members of the Shigella alkalescens group.

F. Absorption Experiments

One portion of absorbed 5628 antiserum was further absorbed with Shigella alkalescens organisms of the Registry strain which had been heated for fifteen minutes in a boiling water bath. A second portion of absorbed 5628 antiserum was further absorbed with unheated Registry organisms. After the initial absorptions, both portions of the absorbed 5628 antiserum were capable of completely agglutinating suspensions of the Registry strain but showed only a + or ++ reaction with the suspensions of the 5628 strain. A repetition of the absorption resulted in that portion of the anti-blocking antiserum which had been absorbed with the unheated suspensions of the Registry strain giving a doubtful to negative agglutination with the Registry strain and no reaction with the 5628 strain. The portion absorbed with the heated suspension of the Registry strain continued to be capable of agglutinating the Registry strain with a ++ to +++ reaction and the 5628 strain with

TABLE VI. COMPARATIVE REACTIONS IN UNABSORBED AND ABSORBED 5628 ANTISERUM

| Culture Numbers | Unabsorbed Antiserum | Absorbed Antiserum |
|---|---|---|
| 96 | ++++ instant | ++++ instant |
| 222 | ++++ instant | +++ instant |
| 259 | ++++ rapid | ++ slow |
| 449 | ++++ rapid | +++ slow |
| 755 | +++ moderate | - |
| 842 | ++++ moderate | ++++ rapid |
| 868 | ++++ instant | ++++ rapid |
| 954 | ++++ rapid | ++++ moderate |
| 1040 | ++++ rapid | ++++ moderate |
| 1099 | ++++ rapid | +++ |
| 1277 | ± | - |
| 1718 | ++++ rapid | - |
| 3704 | ++++ rapid | - |
| 3717 | ++++ rapid | ++++ moderate |
| 3917 | ++++ rapid | ++++ moderate |
| 4135 | ++++ rapid | - |
| 4192 | - | - |
| 4340 | - | - |
| 4527 | ++++ instant | - |
| 4793 | +++ moderate | - |
| 5146 | ++++ instant | - |
| 6325 | ++++ moderate | ++++ rapid |
| 6757 | +++ moderate | ++++ rapid |
| 7015 | +++ instant | - |

a ++ to +++ reaction after the repeated absorption. It would appear that the Registry strain possesses the blocking antigen in some degree but in quantities insufficient for manifestation by the slide technique.

Unfortunately complete reciprocal absorptions employing Registry antiserum and 5628 organisms were not possible because (1) heating renders the 5628 organisms agglutinable in Registry serum and (2) a test for completeness of absorption would be pointless due to the normal inagglutinability of 5628 strains im Registry antiserum.

## V. DISCUSSION

The blocking substances appear to be a constant characteristic of the strains of Shigella alkalescens exhibiting them on primary isolation. They are also capable of stimulating antibody production in the form of agglutinins. The agglutinins produced by these so-called "rough" strains include those possessed by the classical strains of Shigella alkalescens plus a slight, but definite, antibody against the "heat-labile" antigen.

An antiserum was built against a strain which had been carried upon artificial media and also lyophiled for some time. However, it agglutinated the majority of stock cultures and reacted equally well with freshly isolated strains.

Five of the nine organisms which failed to be agglutinated with the absorbed 5628 antiserum possessed the common

characteristic of motility and ability to utilize citrate after approximately three to five days. It would appear that these variants differ antigenically as well as biochemically from both the classical strains and those containing the heat-labile blocking antigen.

The results obtained by testing the inagglutinable strains against the unabsorbed 5628 antiserum suggested that there might have been a decrease in the titer of the unabsorbed serum due to storage. It appeared that the quantity of specific antibody had been depleted in the absorbed antiserum. Tube agglutination tests employing unabsorbed 5628 antiserum with both the Registry strain and strain 5628 failed to confirm this assumption. The absorption process apparently completely removed or decreased certain important antibodies present in the unabsorbed antiserum.

It is interesting to note that unheated suspensions of Registry strain organisms were capable of removing all antibodies for both the Registry and 5628 strain from the absorbed 5628 antiserum. The Registry strain, therefore, would appear to possess the "blocking" antigen as a recessive or masked characteristic. The only effect of heating appears to be in rendering the Registry strain a little less agglutinable in the absorbed 5628 antiserum; its reaction is more on a par with that of the 5628 organisms whereas formerly it exceeded them.

The saving in time between the hour previously recommended in literature and the one minute actually required to inactivate the strains included in this study has been applied successfully to cultures in routine diagnostic work. Since this particular part of the work was completed and was in use, Fulton and Curtis (8) have reported results of a similar nature.

## VI. SUMMARY

1. Using as antigen Shigella alkalescens organisms possessing heat-labile "blocking" factor, it is possible to produce an agglutinating antiserum in rabbits. This antiserum possesses a titer, demonstrated by tube agglutination tests, of 1:20,000 for the homologous strain and 1:80,000 for the Registry strain.
2. An absorbed anti-blocking antiserum, suitable for use in testing by means of the slide technique, can be prepared from raw anti-blocking antiserum by absorption with the appropriate organisms.
3. Such an absorbed antiserum agglutinated 73 (84%) of the eighty-seven "rough" strains of Shigella alkalescens under test, regardless of the interval elapsing between isolation and testing.
4. The blocking antigen is heat-labile and can be inactivated by temperatures of approximately 100 degrees Centigrade in 1.5 minutes.
5. Organisms failing to be agglutinated by the absorbed anti-blocking antiserum were readily agglutinated by the unabsorbed anti-blocking antiserum. It appears that removal of factors producing cross reactions with other members of the Shigella group depletes the anti-blocking antibodies sufficiently to prevent agglutination of certain strains.

6. Of nine citrate positive, motile variants possessing the major antigens of Shigella alkalescens, four gave evidence of containing some degree of blocking antigen; the remaining five failed to react with anti-blocking antiserum.

7. Absorption of absorbed anti-blocking antiserum with unheated suspensions of the Registry strain removed all antibodies for both the Registry and 5628 strains.

8. Absorption of absorbed anti-blocking antiserum with heated suspensions of the Registry strain fails to remove either the Registry or 5628 antibodies. The reactivity of Registry-absorbed anti-blocking antiserum with Registry antigen was lessened but no change was noted in the degree of reaction with 5628 antigen.

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