ON THE MECHANISM OF OPSONIN AND BACTERIOTROPIN ACTION.

I. Correlation between Changes in Bacterial Surface Properties and in Phagocytosis Caused by Sera of Animals under Immunization.

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(Received for publication, February 8, 1929.)

The opposing views of the early cellular and humoral schools of immunologists were to some degree brought into harmony by the demonstration by Denys and Leclef (1), Wright and Douglas (2) and others that phagocytosis could be greatly increased by the presence of serum. Components of normal sera promoting phagocytosis only in relatively high concentration were called by Wright and Douglas *opsonins*. The specific components of immune serum, active either in high or low concentration, have been termed *bacteriotropins* (3). It was shown that the action of opsonins and bacteriotropins was chiefly upon the bacteria or other cells phagocytized, not upon the leucocytes. Treatment by appropriate sera prepared bacteria or other cells for phagocytosis even after the removal of the serum.

Study of the phagocytosis of particulate matter brought out the fact that a number of factors in the phagocytic system could influence the degree of phagocytosis. Thus phagocytosis of carbon particles suspended in sodium chloride solution could be increased somewhat by addition of small amounts of calcium chloride, ethyl alcohol or iodoform, and decreased by departure of the suspending sodium chloride solution from isotonicity (4). Manganese dioxide particles showed an attraction for leucocytes and were very rapidly taken up by them; no such attraction could be demonstrated for carbon or quartz particles (5). Effects on the activities of delicate living cells like leucocytes by factors in their environment are not surprising. However, such effects are not comparable to the enormous increases in phagocytosis produced by the action of bacteriotropins on the cells to be phagocytized.

Important progress was made by formulation of the interfacial tension relations in phagocytosis through the work of Rhumbler (6), Tait (7), and especially of Fenn (8). Fenn showed that in a given phagocytic system the amount of phagocytosis was proportional to the number of collisions between leucocytes and

particles (9). The chance that any given collision may result in phagocytosis would seem to depend to an important degree upon the *interfacial tension relations* at the colliding surfaces; these must be such that the leucocyte and particle will adhere and that the former will spread around and engulf the latter (8). It is understood, however, that the consistency of the protoplasm (10, 11, 12) of the leucocyte and possibly other factors may also enter into the phenomenon.

If then the surface properties of cells are of great importance in determining phagocytosis, have sera any such effects on cell surfaces as might account for their opsonic and bacteriotropic efficiency? Much evidence has been accumulating of late years to indicate that sera do profoundly change the surfaces of cells with which they interact. Studies on bacteria (13), erythrocytes (14), and spermatozoa (15), using the method of cataphoresis, and on erythrocytes (16) and acid-fast bacteria (17), using a method dependent on interfacial tension, have shown that all of these cells do, indeed, undergo certain characteristic changes in surface properties with serum sensitization. The present experiments indicate that these changes are intimately related to phagocytosis.

The experiments described in this paper and the one immediately following represent the first stage of a study directed toward analysis of the mechanism of opsonin and bacteriotropin action in physical-chemical terms. They are intended to answer the question, "What changes do sera effect in acid-fast bacteria in preparing them for phagocytosis?"

Methods.

Various strains of acid-fast bacteria, treated with serial dilutions of normal and immune sera, have been studied as follows:—(1) The bacteria remained in serum dilutions overnight and the *agglutination* readings were then made for each tube. (2) The serum-bacterial mixtures were strongly centrifugated and the sediments were *resuspended* by shaking until the untreated control tubes showed even suspension. (3) The sensitized bacteria were washed and their wetting or interfacial tension properties were then estimated in the *interface reaction.* The bacteria were observed microscopically in an oil-water boundary surface. (4) The cataphoretic velocity of the washed, sensitized bacteria was measured in a microcataphoresis cell. These 4 reactions together gave a picture of the cohesiveness, of the wetting properties, and of the surface potential difference of the bacteria tested for phagocytosis. (5) While such data were being obtained mixtures of rabbit leucocytes with, (a) bacteria and serum dilutions, or, (b)

sensitized, washed bacteria were rotated in stoppered vials on a Robertson agitator (18). Smears were made from each mixture, fixed and stained, and 100 (or 200) leucocytes in each smear were observed microscopically. The percentage of leucocytes which had taken up bacteria was recorded.

Such details of the several reactions as seem necessary are given below:

Bacteria were removed with a platinum loop from the surface of a glycerol-agar slant. They were rubbed against the bottom of a Pyrex test tube by a glass rod with rounded end. A few drops of 0.85 per cent sodium chloride solution were added after the first grinding, and the bacterial mass was rubbed up into a paste; this was diluted with 0.85 per cent sodium chloride with further grinding and shaking. This suspension was largely freed from clumps by centrifugation and brought to the desired turbidity with saline.

1. Agglutination.—1 cc. of the bacterial suspension was mixed with 1 cc. of each serum dilution. The tubes were left in the ice-box over night and in the morning were usually removed to the 37° room for 30 to 60 minutes before reading. Agglutination or sedimentation was read without shaking up the sediment. ++++ indicates complete agglutination with clearing, +++, ++, + and tr. indicate decreasing degrees of agglutination or sedimentation. The lesser degrees of agglutination recorded in many experiments with mammalian tubercle bacilli may well have indicated only sedimentation and not macroscopic agglutination, strictly speaking. Such sedimentation was never considered positive however, unless distinctly in excess of the sedimentation in the saline control tube.

2. Resuspension.—Under various special conditions, for instance with mammalian tubercle bacilli, agglutination as ordinarily carried out is unsatisfactory. To meet these cases a modified agglutination or "resuspension" reaction has been developed (19). After making the agglutination readings, all tubes were centrifugated at high speed until the bacteria were completely sedimented or practically so. The supernatant fluid was decanted and two drops of 0.85 per cent sodium chloride solution were added to the bacterial sediment in each test tube. The tubes were arranged in a rack with the control tubes in the middle, *i.e.*, tubes in which the bacteria had been mixed with 0.85 per cent sodium chloride solution without serum. The rack was now shaken uniformly until the sediment in the control tubes was first brought into even suspension. The bacteria which had been sensitized resuspended in flocculi whose coarseness increased with the concentration of serum and with the affinity of the serum components for the particular bacteria used.

This reaction has the advantage of eliminating certain imperfectly controlled variables in the second or flocculation stage of the agglutination reaction. The bacteria after sensitization are forcibly pressed together by centrifugal force.

Their subsequent resuspension depends primarily upon and gives a roughly quantitative estimate of their cohesion.

3. Interface Reaction.—After resuspension, 0.85 per cent sodium chloride solution was poured into each tube, all tubes were again centrifugated, the supernatant liquid was decanted, and the sediments were again resuspended. A portion of this sensitized washed suspension in each tube was used for the interface reaction (17) and a portion for cataphoresis. In the present experiments, the bacteria were observed microscopically in the interface between tricaprylin and 0.85 per cent sodium chloride solution. The degrees of alteration of the wetting properties of the bacteria after sensitization are charted as plus signs.

4. Cataphoresis.—A slightly modified form of the Northrop-Kunitz (20) microcataphoresis cell was used. For details see (21). The cataphoresis cell was mounted over a dark-field condensor. Three readings were made at each of 3 levels, namely, at $\frac{1}{12}$, $\frac{3}{12}$, and $\frac{5}{12}$ of the distance from the bottom to the top of the inside of the cell. A better technique used in the later experiments was to make 3 readings at each of the 2 "stationary levels," namely, at $\frac{21}{100}$ and at $\frac{79}{100}$ of the inside depth of the cell (22). The algebraic sum of the rates of migration at the several levels was used for calculation. The cataphoretic velocity is given in μ per second per volt per centimeter fall in potential along the cataphoresis cell. If we make certain assumptions (23), which are not rigidly accurate, however, velocities may be converted into millivolts potential difference at the bacterial surface by multiplication by 12.6.

Phagocytosis.—The importance of using serial dilutions of sera in careful phagocytosis work has long since been demonstrated (3, 24), but has been neglected. Moreover much work in phagocytosis has been vitiated and discredited by the difficulties and uncertainties of the Leishman-Wright opsonic index method. Introduction of the beautiful technique of Robertson and his co-workers (18) has therefore marked an important advance. The Robertson apparatus has been adapted to the needs of the present experiments, and a technique for staining and handling several titration series at the same time has been developed.

Leucocytes.—Exudative leucocytes from rabbits were used as the phagocytic cells according to a method elaborated in Hamburger's laboratory (25). They were obtained by injecting about 200 cc. of sterile 0.9 per cent sodium chloride intraperitoneally (on the right side about the mid-clavicular line and midway between costal margin and pelvis) into a rabbit by means of a hollow needle attached to a rubber tube and funnel; after 3 to 4 hours about 80 to 100 cc. of the fluid was recovered by puncturing the peritoneal cavity with a stout (No. 15 bore) needle near the mouth of which a few side openings had been drilled. The fluid was received in a flask containing a solution of 0.7 per cent sodium chloride + 1.1 per cent sodium citrate in proportion of 3 parts of peritoneal fluid to 1 part of saline-citrate solution. (In order to obtain fluid very rich in leucocytes an injection of 200 cc. of sterile 0.9 per cent sodium chloride solution was given on the evening before the experiment, to be followed in the morning by a second injection as described above.) The suspension was centrifuged for 2 minutes at a speed

of 1100 revolutions per minute; the centrifuge was started and stopped gradually; the supernatant fluid was poured off and the sediment resuspended in 0.9 per cent sodium chloride and centrifuged again for 2 minutes at the same speed. About 0.1 cc. of leucocytes were obtained from 15 cc. of peritoneal fluid. Usually the leucocytes from 3 rabbits were used, furnishing about 10 cc. of a very dense leucocytic suspension. Approximately 95 per cent of the cells were polymorphonuclear leucocytes; disintegrated cells and large monocytes constituted the remaining 5 per cent. The rabbits showed no ill-effects from the injection and withdrawal of fluid. The same rabbits were used weekly for 8 months. When leucocytes were obtained oftener a different series of rabbits was employed.

Phagocytic Mixture.—Into a series of small hard-glass vials of uniform size (50 mm. in length, 9 mm. inside diameter), 0.1 cc. of serum dilutions was placed (a fresh pipette was used for each dilution to insure greater accuracy). Immediately before adding 0.2 cc. of the leucocytic suspension (which was never more than ½ hour old), 0.1 cc. of bacterial suspension was introduced. Each of the vials contained, therefore, 0.4 cc. of fluid. They were stoppered with freshly paraffined well-fitting corks, and immediately placed on a Robertson rotating machine. The speed of rotation was 4 times per minute; the rotation was continued for 15 minutes. Temperature was that of the room, 25°±1.5°C. At the end of this period the tubes were quickly placed in racks and plunged in ice water for several minutes. A minute drop of human serum (heated for 30 minutes to 56°) was added to each vial immediately before making the spreads; this caused the leucocytes to adhere to the slide. The first vial of each series did not require the addition of serum.

Making of Spreads.—Spreads were made on thoroughly cleaned and polished numbered glass-slides. A small drop of the mixture was placed near one end of the slide with a clean pipette, using a fresh pipette for each vial, and drawn across the slide by a spreader (a slide with the corners broken off); the edge of the spreader was then dipped into a beaker of water and wiped clean and dry. The spread slide was rapidly dried by holding it before an electric fan which was standing in front of a radiator or electric heater. Rapid drying is essential for obtaining well-spread non-contracted leucocytes. The dried slides were exposed for 2 to 10 minutes to formalin vapors (by placing them, spread side downwards) over flat dishes containing undiluted formalin. After removal from formalin vapors they were placed in a row and the flame of a Bunsen burner passed rapidly over them.

Staining.—The fixed spreads were stood in staining racks and placed for 10 minutes in a covered jar containing Ziehl-Neelsen carbol-fuchsin previously heated to 70°C. They were then thoroughly rinsed in water. Each slide was decolorized separately by shaking it, with forceps, for 5 to 10 seconds in 10 per cent sulfuric acid. (This was found to decolorize the cells satisfactorily; the bacilli were brilliantly stained.) The slides were counterstained, usually for 20 seconds, in a 1:10,000 Azure II solution; (the dilution was made from a more concentrated solution; the diluting distilled water was rendered slightly alkaline by adding 0.3

cc. of a 15 per cent sodium carbonate solution to each 200 cc. of water). The staining solution was always freshly prepared. The slides were then rapidly washed, and dried, first between blotting papers and then by holding them in front of an electric fan (or placing them in the incubator).

Estimation of Degree of Phagocytosis.[1]—The numbers of leucocytes that had ingested bacilli were determined by counting 100 cells (sometimes 200 cells). Each of two observers counted 50 (or 100) cells; the results agreed very closely. Care was taken not to regard mere adherence to the leucocyte as evidence of phagocytosis. In the vast majority of instances there was no doubt as to whether a cell had ingested bacteria or not.

The agglutination, resuspension, interface and cataphoresis reactions were done at the Phipps Institute by one group of workers; portions of the sera and bacterial suspensions used in these tests were meanwhile sent to the other collaborators at the Medical School, by whom the phagocytosis tests were made. The results were not combined until both sets of observations had been completed.

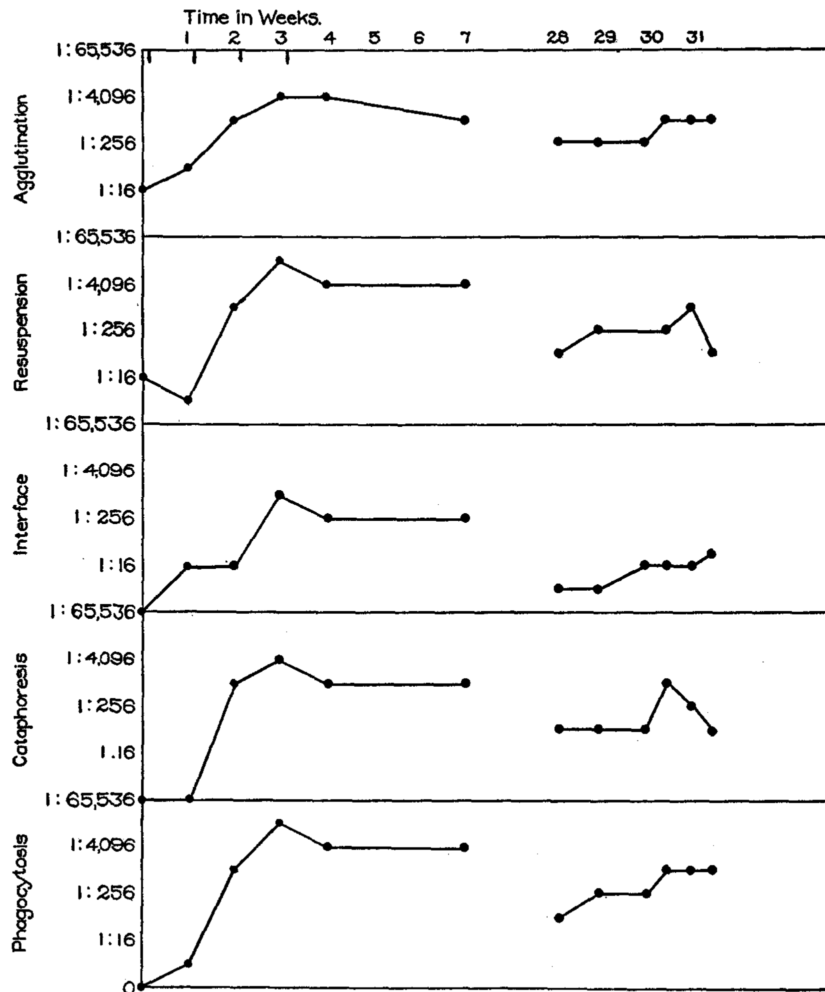
By use of the reactions and procedure outlined above we are able to follow changes in the cohesiveness, the surface potential difference and the wetting properties of the bacterial surfaces, and to compare these changes with the increase in phagocytosis brought about by the same sera on other portions of the same bacterial suspensions. Positive resuspension and interface reactions each indicate increased cohesiveness between the bacteria of the suspension tested. A positive interface reaction indicates also bacterial surfaces less readily wet by oil than are those of the controls. The change (decrease) in surface potential difference is directly proportional to the decrease in cataphoretic velocity brought about by serum sensitization. Each of these changes in bacterial surface properties is closely correlated with the increase in phagocytosis brought about by serum, as will be shown in this and the two succeeding papers.

In Text-figs. 1 to 4 are plotted the titers for the several reactions of the sera of 4 rabbits, as withdrawn during the course of active immunization. The times in weeks between the beginning of the experiment and the bleedings of the rabbits are plotted on the abscissa axis.[2]

[1] In the present study phagocytosis is defined as ingestion of bacilli by polymorphonuclear leucocytes; the effect on the bacilli ingested is not here under consideration, nor is the question of phagocytosis by other types of cells than polymorphonuclear leucocytes.

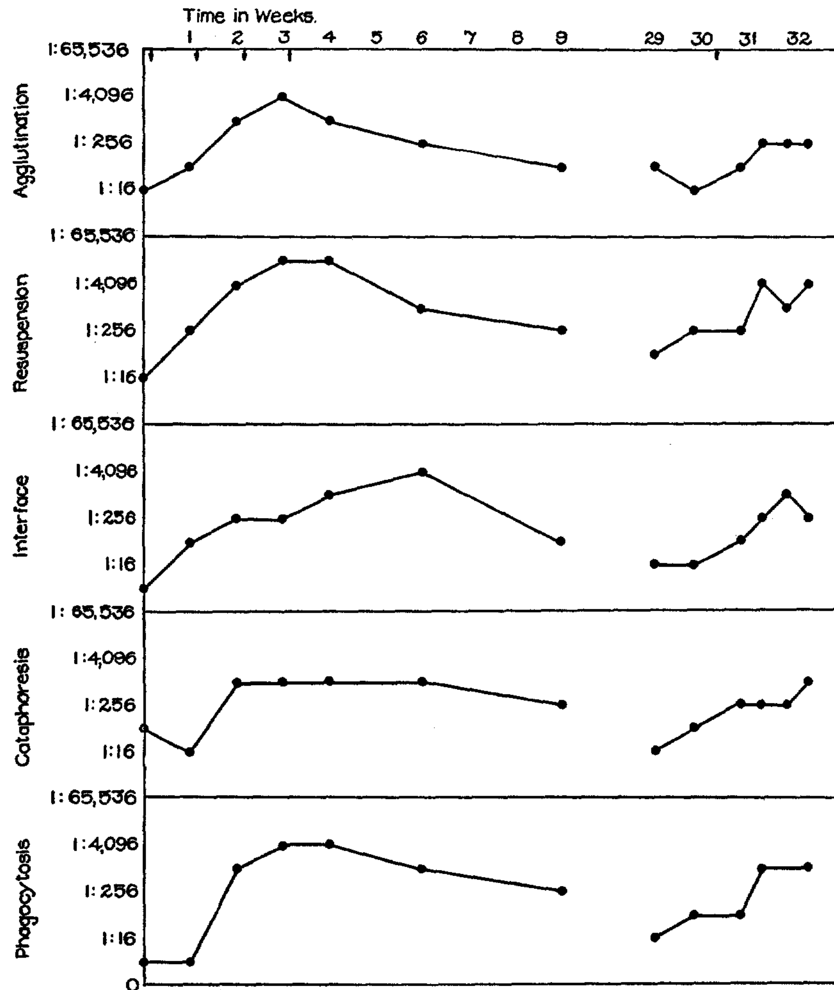
[2] The sera were separated from the freshly clotted blood, and were inactivated for 30 minutes at 56°C. before use. The saline bacterial suspensions were ad-

Rabbit 19-72, injected with *M. chelonei.*



TEXT-FIG. 1. The course of active immunization of a rabbit with turtle bacillus. Titers of the several reactions are plotted against time in weeks. Time of intravenous injections of living antigen is indicated by arrows. Note the parallelism between phagocytosis and bacterial surface changes in this and succeeding text-figures.

Rabbit 20-02 injected with *M. avium* (Arloing strain).



TEXT-FIG. 2. Active immunization of a rabbit with an avian tubercle bacillus. The open circle (first cataphoresis titer) indicates titer not reached but estimated.

Intravenous injections of saline suspensions of the living bacilli used for immunization are indicated by small arrows on this axis. The

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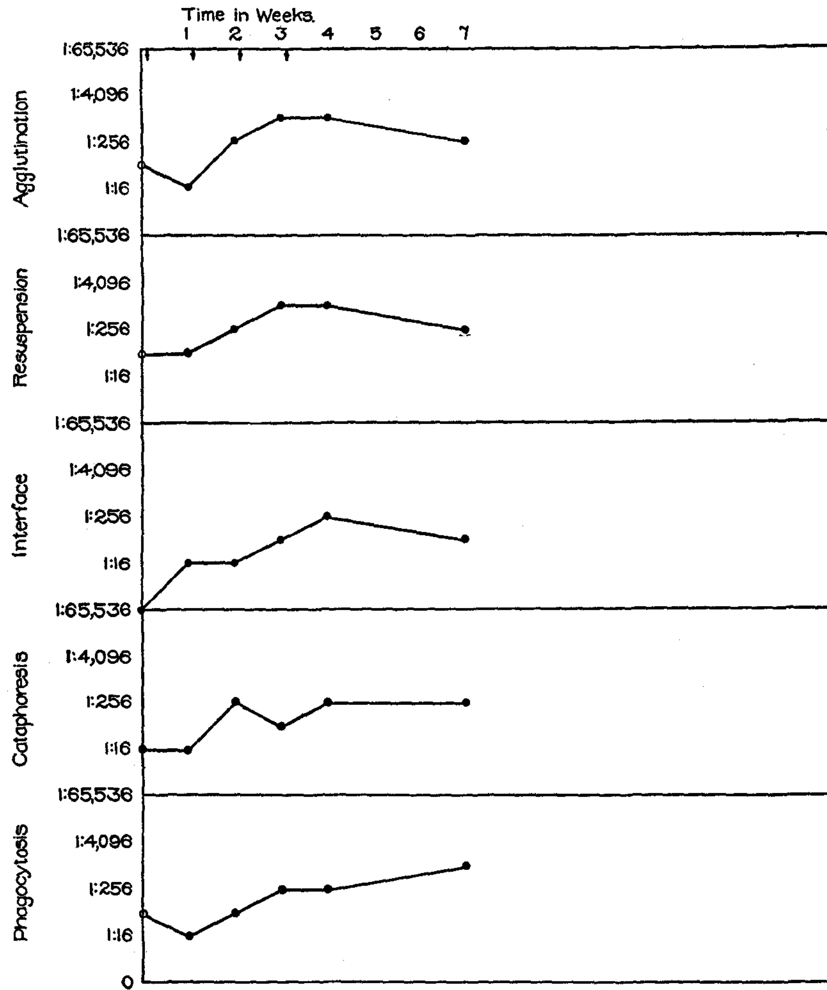
justed as nearly as possible by comparison with a turbidity standard to the same turbidity for each experiment; the living microorganism homologous with the antiserum was used for all tests.

Rabbit 20–03, injected with *M. avium* (Prague strain).

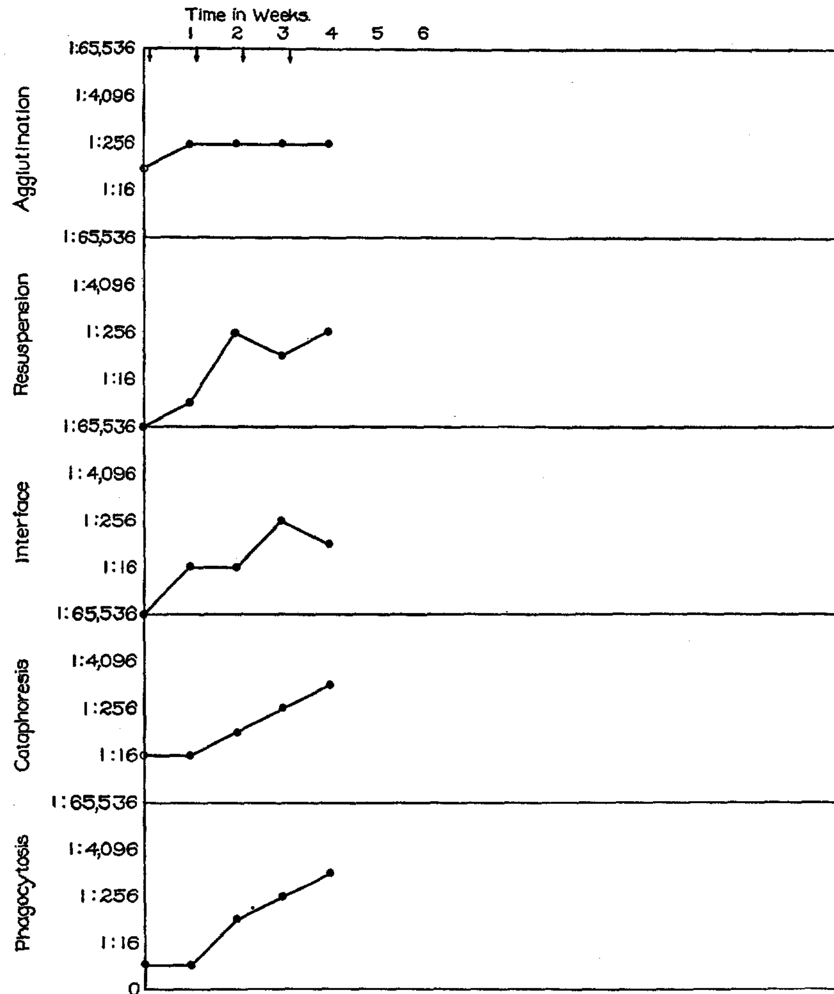


TEXT-FIG. 3. Active immunization of a rabbit with an avian tubercle bacillus. Symbols as in Text-figs. 1 and 2.

titers of the sera are indicated on the ordinate axis; the highest dilutions of the sera giving clearly positive results are taken as titers; traces or doubtful reactions are not included.

Text-figs. 1 and 2 show the course of active immunization due to

Rabbit 20-37, injected with *M. tuberculosis* (Bovine III strain).



TEXT-FIG. 4. Active immunization of a rabbit with an avirulent bovine tubercle bacillus. Symbols as in Text-figs. 1 and 2.

4 weekly injections, the subsequent slight falling of titer, the titers after a 5 months interval and the rise following a final injection of the antigens. The rabbits yielding the data shown in Text-figs. 3 and 4 died of intercurrent disease after the first immunization.

The general correspondence is very striking between the bacterial surface changes and the increase in phagocytosis brought about by the sera at all the stages of immunization. The absolute titers for the several reactions do not necessarily correspond; this was to have been anticipated, however, since it had already been recorded, for instance, that the interface reaction was relatively insensitive (17), and that agglutination with mammalian tubercle bacilli (see Text-fig. 4) was unsatisfactory (19). An occasional point also is obviously off the correct curve (*e.g.*, Text-fig. 2, interface value at 6 weeks). However the significant fact is the striking general parallelism of the titers indicating bacterial surface change and phagocytic increase; this parallelism is, we believe, complete, within the limits of experimental error.

Influence of the Intrinsic Properties of the Bacterial Surface.—Serum which prepares bacteria for phagocytosis, then, has in approximately corresponding degree altered the bacterial surface properties, *i.e.*, increased the cohesiveness, decreased the surface potential difference and altered the wetting properties. The correspondence between these bacteriotropic and surface effects strongly suggests that they are due to a common cause, namely, to deposition of a sensitizing substance or substances from the serum on the bacterial surface. It has already been shown that serum agglutination involves a surface deposition of serum components (26).

It is of interest further to inquire: (a) whether phagocytosis and agglutination occur at a constant critical surface potential difference or whether a factor or factors other than potential difference enter into the determination of phagocytosis and agglutination, and (b) whether phagocytosis and agglutination can be formulated in terms of the properties of the sensitizing substance alone or are dependent both on the properties of the sensitizing substance and on the intrinsic properties of the bacteria undergoing sensitization. These questions may be answered from the data in Table I.

Table I gives the cataphoretic velocities at which phagocytosis and agglutination began, at which phagocytosis reached 50 per cent and at which agglutination approached completeness. Phagocytosis and agglutination are seen to begin and to reach the levels indicated within

TABLE I.

The Relation of Cataphoretic Velocity to Agglutination and Phagocytosis.

1. Antigen	2. Time of immunization	3. Unsensitized antigen	4. Phagocytosis begins	5. Agglutination begins	6. Phagocytosis 50 per cent or more	7. Agglutination +++ or ++++	8. Minimum potential difference
	weeks	*c.v. in μ/sec.*	*c.v. in μ/sec.*	*c.v. in μ/sec.*	*c.v. in μ/sec.*	*c.v. in μ/sec.*	*c.v. in μ/sec.*
M. avium (Arloing strain)	1	1.92	1.18	1.73	1.50	1.18	1.18
	2	1.67	1.50	1.50	1.34 1.34	1.30	0.92
	3	1.96 1.83	1.82 1.55	1.82 1.49	1.55 (17 mv.)	0.89 0.88	0.49 0.69
	4	1.83 (23 mv.)	1.71 (20 mv.)	1.55 (19 mv.)	1.00	0.61 (11 mv.)	0.43 (9 mv.)
	6	1.67	1.45	1.00	1.32	0.57	0.49
	9	1.93	1.64	1.32		0.75	0.63
M. tuberculosis (Bovine III strain)	1	1.37	0.92	1.34	Not reached	Not reached	0.92
	2	1.37 1.57	1.13 1.22	1.34 1.36	0.83 0.80	Not reached	0.83 0.72
	3	1.98 (20 mv.)	1.55 (15 mv.)	1.55 (17 mv.)	0.58 (10 mv.)	Not reached	0.58 (9 mv.)
	4	1.56	1.29	1.23	0.98	Not reached	0.54
M. chelonei	1	1.54	1.43	1.45	Not reached	1.43	1.43
	2	1.36 1.44	1.09 1.29	1.09 1.21	1.04	1.04 1.04	0.54 0.59
	3	1.24 (18 mv.)	1.17 (16 mv.)	0.95 (15 mv.)	0.95 1.02	0.65 (13 mv.)	0.26 (7 mv.)
	4	1.29	1.15	1.15	0.89 (13 mv.)	0.89	0.41
	7	1.78	1.59	1.41	1.20	1.20	0.32
M. avium (Prague strain)	1	1.01	0.83	0.83	Not reached	Not reached	0.56
	2	1.05 1.09	0.75 0.89	0.82 0.99	0.60	0.60	0.60 0.63
	3	1.18 (14 mv.)	1.04 (11 mv.)	1.17 (12 mv.)	0.71 0.69	0.66 0.64	0.66 (8 mv.)
	4	1.10	0.78	1.22	0.57 (9 mv.)	0.52 (8 mv.)	0.52
	7	1.13	1.07	0.93	0.89	0.80	0.80

In columns 1 and 2 are given the antigen and the duration of immunization at the time the sensitizing serum was drawn; in column 3 the cataphoretic velocity (c.v.) of the control unsensitized antigen in salt solution is given.

In columns 4 and 5 are given the cataphoretic velocities corresponding to the beginning of phagocytosis and agglutination.

In columns 6 and 7 the cataphoretic velocities are given when phagocytosis has reached 50 per cent or over, and agglutination +++ to ++++.

The last column shows the lowest cataphoretic velocity reached by the antigen in each experiment.

The values in each category are averaged and the mean value is calculated as potential difference in millivolts.

The table shows that phagocytosis and agglutination occur within certain zones of cataphoretic velocity; but these zones are different for the different organisms.

fairly definite zones of surface potential difference,[3] but the potential zones are different for the different organisms. Consider for instance the Arloing and Prague strains of avian tubercle bacilli of similar growth habit; the Arloing strain has a relatively high surface potential difference (average, 23 millivolts), and the Prague a low potential difference (average, 14 millivolts). The phagocytosis and agglutination titers for the Arloing strain fall at 20 and 19 millivolts and for the Prague strain at 11 and 12 millivolts; phagocytosis of Arloing reached 50 per cent at about 17 millivolts, of Prague at about 9 millivolts; agglutination approached completeness with Arloing at about 11 millivolts, with Prague at about 8 millivolts. Yet in the highest concentrations of immune sera both strains were reduced to about the same potential difference.

We conclude, therefore, (a) that phagocytosis and agglutination do tend to occur within certain zones of surface potential difference; since, however, these zones are different for the different organisms it is obvious that another factor or factors than potential difference must enter into these phenomena.

Further consideration of Table I and of other data leads to the conclusion (b) that phagocytosis and agglutination cannot be formulated in terms of the properties of the sensitizing substance alone but depend both on the properties of the sensitizing substance or substances and on the intrinsic properties of the bacteria undergoing sensitization.

Evidence pointing to this conclusion may be summarized as follows: Agglutination of Bovine III never approached completeness although the surface potential difference of this strain was reduced well below the level required for +++ or ++++ agglutination of Arloing or the turtle bacillus (*chelonei*). This difficult agglutinability is characteristic for mammalian tubercle bacilli in general, and has given rise to a number of artifices, *e.g.*, agglutination at high temperature, agglutination of a substitute strain (Arloing strain), and the resuspension reaction (19). Phagocytosis of certain difficultly agglutinable strains of mammalian tubercle bacilli, on the other hand, may be high even without serum sensitization. This point is illustrated in Table

[3] Surface potential difference is measured by and is proportional to cataphoretic velocity.

TABLE II.

Atypical Phagocytosis of Human Tubercle Bacillus Sensitized with Aged Serum.

M. tuberculosis, strain H 37, in anti H 37 serum	Serum dilutions							
	1:4	$1:4^2$	$1:4^3$	$1:4^4$	$1:4^5$	$1:4^6$	$1:4^7$	Sodium chloride control
Agglutination	++	+ to tr.	+	tr.	+ to tr.	tr.	tr.	tr.
*Resuspension	+++	+++	+++	++ to +	+	++ to +	0	tr.
*Interface	More positive than control							
*Cataphoresis, μ/*sec*	0.70	1.16	1.87	2.12	2.14	2.14	2.28	2.31, 2.12
*Phagocytosis, *per cent*	5	17	88	81	85	89	88	71

* Bacteria sensitized and washed before testing.

II in which it is shown that a human tubercle bacillus of low virulence was phagocytized by 71 per cent of cells even without sensitization. Agglutination in the instance shown in Table II was slight although resuspension and cataphoresis showed the binding of agglutinin; the phagocytic prezone is characteristic of the aged serum used for sensitization; (see paper following). Tests with several other cultures of human tubercle bacilli gave 50 per cent or higher phagocytosis in the unsensitized 0.85 per cent sodium chloride suspension controls. Phagocytosis of this high order in the unsensitized controls has not been found with any type of acid-fast bacteria other than the human tubercle bacillus, and has not been found with all cultures of the human bacillus. It has already been recorded that the human tubercle bacilli we have studied in the interface differ from other types of acid-fast bacteria in their wetting properties (27).

Phagocytosis and agglutination are thus dependent both upon the properties of the sensitizing serum substance or substances and upon the intrinsic properties of the bacteria themselves.[4] Serum sensitization tends to promote both phagocytosis and agglutination. The intrinsic properties of the bacterium may be either favorable or unfavorable to phagocytosis or agglutination and may even influence the 2 phenomena in opposite directions. Since agglutination is a matter of the cohesion of the bacteria themselves, and phagocytosis necessitates adhesion of the bacteria to, and engulfing by, leucocytes, the relative independence of the influence of the original intrinsic bacterial properties on the 2 phenomena is not surprising.

SUMMARY.

Methods are described for investigating the relation between phagocytosis of bacteria by polymorphonuclear leucocytes, and certain physical-chemical properties of the bacterial surface.

Serum sensitization causes the following changes in the properties of acid-fast bacteria: (a) increased cohesiveness, (b) decrease in

[4] However it is possible that with maximum sensitization by a strong homologous serum the deposited sensitizing substance may form a complete film enveloping the sensitized bacterium. We are not sure whether the sensitizing film ever becomes complete or not. If it does, the original properties of the bacterial surface would doubtless no longer be factors in agglutination and phagocytosis.

surface electric potential difference, (c) decrease in wettability of the bacteria by oil, and (d) increased phagocytosis.

Tests have been conducted periodically with the sera of 4 rabbits under active immunization with as many strains of acid-fast bacteria; the parallelism between the alteration in bacterial surface properties and the promotion of phagocytosis by these sera has been, within the experimental error, complete.

The percentage of phagocytosis of a given bacterial suspension has been found to depend both upon the sensitizing serum component or components deposited upon the bacterium and upon the intrinsic properties of the unsensitized bacterial surface.

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