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## Studies in the Agglutination Prozone.

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In the course of studies of the mechanism of agglutination, we have sought to explain the pro-agglutinoid zone. Agglutinative sera showing a natural prozone have been so rare in our experience that we have resorted to the study of the prozone in serum produced artificially by heating.

The following is a summary of results observed with such "heat" prozones. Horse and rabbit sera were used. The temperature used for securing this type of prozone is usually stated to be 70° to 75° C. Our observations have shown that with certain sera, temperatures ranging from 56°, or possibly lower, to 76° C. are effective (at pH's 5.0 to 7.0), longer heating being required at the lower temperatures, *e. g.*, 3 minutes at 68° produces a prozone, while 2 to 4 hours is necessary at 60°. Heating to 76-78° destroys all agglutinin. In the case of some of the typhoid sera, the production of a prozone was accompanied by a faint opalescence. Centrifugalization failed to clear the serum but passage through Berkfeld V candles was successful. Accompanying this removal of turbidity, the prozone disappears and coincidentally the titre of the the serum falls (Table 1).

TABLE I.
Effect of Berkfeld filtration upon the prozone serum (R 44).

| *Sera* | *Agglutination* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 | 1/1280 | 1/2560 |
| Untreated serum | c | c | c | c | c | c | c | c |
| Heated (prozone) serum | — | — | — | — | — | c | c | c |
| Filtered prozone serum | c | c | c | c | c | c | — | — |

c = complete agglutination; — = no agglutination.

In the case of a dysentery prozone serum not showing this turbidity a similar eradication of prozone and coincident reduction of titre was obtained by adding kaolin.

When prozone serum (typhoid) was added in proportions of 1:1, 2:1 and 4:1 to an untreated *B. melitensis* serum no inhibition of agglutination of the melitensis organism occurred, *i. e.*, typhoid prozone serum does not produce a prozone in melitensis serum. Also, in the case of closely related bacteria (dysentery strains), the

addition of Shiga prozone serum to Flexner, Mt. Desert, "Y" and Sonne B sera in similar fashion failed to produce prozones. In other words, the prozone effect seems to be highly specific. Prozone serum, 1/20, showing no agglutination, when treated with excess bacteria, shows typical absorption of agglutinin. When this absorption process is carried out with carefully graded doses of bacteria, the prozone goes first, *i. e.*, there would seem to be absorption first of the inhibitory factor and then of normal agglutinin.

As serum is exposed to increasing heat (*B. dysentery*, Shiga), first the prozone appears, then it is removed and finally all agglutinin is destroyed. Table II illustrates this effect.

TABLE II.

Effect of varying heat upon B. Dysentery, Shiga, serum.

| Temperature of heating 6 min. | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 | 1/1280 | 1/2560 | 1/5120 |
|---|---|---|---|---|---|---|---|---|---|
| Unheated | c | c | c | c | c | c | c | c | 3 |
| 60° | c | c | c | c | c | c | c | c | 3 |
| 62° | c | c | c | c | c | c | c | c | 3 |
| 64° | ± | ± | c | c | c | c | c | c | ± |
| 66° | — | — | — | c | c | c | c | c | ± |
| 68° | — | — | — | c | c | c | c | c | — |
| 70° | ± | ± | ± | c | c | c | c | 3 | |
| 72° | c | c | c | c | c | c | 2 | — | |
| 74° | c | c | c | c | c | c | 2 | — | — |
| 76° | ± | ± | ± | — | — | — | — | — | — |
| 78° | — | — | — | — | — | — | — | — | — |

c = complete agglutination, clear supernatant fluid.
3 = heavy flocculation, supernatant fluid not clear.
2 = visible granular appearance.
1 = visible granular appearance with hand lens.
— = no agglutination.

The zone begins at 64°, reaches a peak at 66-68° and fades at 72° C.; coincident with the disappearance of the zone there is a corresponding drop in titre. This finding taken in conjunction with the reduction of agglutinin titre accompanying prozone removal by filtration or by kaolin treatment supports the assumption that the prozone factor is modified agglutinin; in favor of this also is the evidence of absorption of agglutinin in the prozone.

The cataphoretic behavior of bacteria treated with prozone sera in the inhibition dilutions very closely resembles that of bacteria treated with unheated serum. This suggests that there is union in this zone between organisms and modified agglutinin; the absorption experiments above are further proof of this.

We have shown that sensitization of bacteria is selective coating by the globulin of the antibody and that flocculation follows because the coated bacteria now take on the character of particles of denatured globulin. It is probable that the union between organism and modified agglutinin, *i. e.*, film formation, in the prozone, is incomplete because of the modification of agglutinin noted above, and by the same token that flocculation cannot follow. If we accept this explanation it is necessary to assume that in the lower dilutions, where modified and unmodified agglutinin are present together, the former (agglutinoid of Ehrlich) has a greater affinity for the bacteria; this is the original hypotheses set forth by Ehrlich. In support of this there is the selective absorption referred to above.

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## Critical Concentrations of Bioses.

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The observations on yeast growth which have been recorded by numerous workers, together with the results reported here, seem to require some extension of the theory of "bios" originally stated by Wildiers,[1] *viz.*, "That a substance of unknown composition, or bios, is indispensable for the development of yeast." For the development of some species of yeast at least, a certain minimal concentration of a substance or substances is indispensable. Moreover, the following results show that this minimal concentration may be very critical, *i. e.*, a given yeast species may be very sensitive to a small diminution in the concentration of bios below the minimum value.

*Critical Concentration of Bios.* The following type of experimental result has led to the postulation of a theory of "critical" concentrations. A series of 7 different concentrations of "bios" (in the form of a 70% alcoholic extract of autolyzed yeast, from which the alcohol had been evaporated) in a basal salt-sugar medium, was inoculated with varying quantities of yeast cells. After 5 days' incubation at 25° C. a concentration of cells (constant within 22%) was observed corresponding to each "bios" concentration—except the smallest. In the latter case (0.025 mg. of bios per cc.) this constancy was absent, and the smaller seedings attained a final concen-