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-

tration which was 72% less than that with the largest seeding. The figures are given in Table I.

TABLE I.

Bios concentration (mg. per cc.)	Cell concentration found after 120 hrs. (millions per cc.).			
	Seedings 2.0 millions	0.4 millions	0.2 millions	0.04 millions
1.45	87.6	84.8	82.0	88.0
0.73	70.5	67.0	58.4	54.8
0.36	44.5	43.8	43.8	43.1
0.18	42.4	42.4	42.0	38.1
0.09	40.0	38.0	37.0	36.0
0.045	25.6	21.2	20.8	20.1
0.025	20.1	10.1	5.6	5.6

If the concentration of bios falls below a certain minimum (in the above experiment .045) then there is a marked difference between the behavior of large and small seedings. Further investigation is necessary before any explanation of this effect can be given. However, the determination of the minimum or "critical" concentration for any given preparation of bios may be made a simple means of gauging its potency. Such a determination measures the ability of a preparation to enable *small* inoculations to grow—a criterion of true bios activity as implied by Wildiers.

Obviously many factors may influence the determination of crit-

TABLE II.
Sensitivity of yeast to changes in concentration of bios.

Nos.	Bios concentration* (mg. per cc.)	Concentration of cells after 6 days at 25° C. (millions per cc.)			
		Seedings	840,000 cells	3,000 cells	Difference per cent
1	0.041	(Volumes constant at 5cc.)	10.1	11.0	8.9
2	0.038		12.4	11.7	5.7
3	0.036		11.7	9.9	15.4
4	0.033		11.0	6.0	45.5
5	0.030		10.6	9.2	13.2
6	0.027		9.6	7.8	18.8
7	0.027		9.2	2.6	71.7
8	0.025		9.9	2.6	73.5
9	0.022		8.9	2.7	69.7
10	0.019		11.0	1.2	89.1
11	0.016		12.8	5.6	56.3

*The different concentrations of "Bios" were obtained by adding to the basal medium a constant volume of "Bios" solution. The latter was diluted successively 5 times to prepare Nos. 1 to 6 inclusive. A fresh solution was used and successive dilutions of it made in preparing 7 to 11. This possibly explains the discrepancies between Nos. 6 and 7.

ical concentration. Activity cannot as yet be measured in terms of absolute units. If, however, the principle of "critical" concentrations is sound—and the experiments reported seem to show that it is—then it is possible, by keeping the conditions of test uniform, to determine the relative potency of a series of preparations. The method described above, furnishes a means of testing the activity of isolated fractions.

Preliminary studies of some factors which may influence critical concentration values have yielded the following results. (See Table II and seq.)

The experiment reported in Table II was continued to the 19th day. The percentage differences between the concentration of cells attained by the 2 seedings after 19 days were as follows:

Medium No.	1	2	3	4	5	6	7	8	9	10	11
% Diff.	10.7	14.5	10.7	20.3	12.9	3.4	33.2	24.9	35.2	56.2	30.4

Reference to Table II shows clearly that if the concentration of Bios is above .027 the final cell production is practically independent of the seeding. Below .027 marked differences appear. .027-.030 is therefore the approximate critical concentration for this bios preparation.

Is Volume of Medium a factor in critical concentration determination? Two tubes of 5 cc. and a flask of 1500 cc. of basal medium containing 0.045 mg. "bios" per cc. were each seeded with 3,000 cells. After 10 days, but not earlier, the concentration was 16 million cells per cc. in each of the 3 cultures. In spite of the difference in volumes and hence in cells per cc. (2 cells per cc. in one case and 600 per cc. in the other 2) no difference in final cell concentration is detectible provided critical concentration of bios is present.

TABLE III.

	Bios concentration (mg. per cc.)	Time days	Cell concentrations attained (millions per cc.) Seeding 500,000	
A. Alpha-Bios*	0.025	9	6	
"	0.1, 0.05	9	3-4	
"	0.0125 & 0.006	9	3-4	
But at a seeding of 5,000 cells and .025 bios the crop was only 1.				
			Seeding 500,000	5,000
B. Beta-Bios†	0.1	7	16	—
"	0.075	7	14	15
"	0.05	7	12	6
"	0.025	7	4	—
This result indicates .05-.075 as the critical concentration of Beta-Bios.				

*Prepared by R. W. Kerr after method Eddy, Kerr and Williams.[2]
†Reported by R. W. Kerr in this Journal.[3]

Following the above methodology various bios preparations have been tested to establish critical concentrations. (See Table III.)

In the case of Alpha-bios no critical concentration was established, but with 0.025 mg. per cc. the highest growth occurred. In higher concentrations there was some inhibition of growth. The critical concentration of Beta-bios was 0.075 to 0.05 mg. per cc., so that its activity is only one-half that of a crude alcoholic extract of autolyzed yeast, (see above, critical concentration of 0.03 to 0.025 mg. per cc.). This confirms the results obtained in Dr. Eddy's laboratory and elsewhere and supports the view that several factors of the bios type are involved. Tests are in progress on Kerr's synthetic bios. Indications to date are that a critical concentration is demonstrable, but the limits are not yet determined.

Discussion. The results reported here find confirmation in the work of Clark,[4] who found that a maximum constant crop is obtainable that is independent of the size of seeding; presumably the concentrations of wort he employed were all larger than the critical concentration value. The type of curve obtained by Eddy, Heft, Stevenson and Johnson[5] for the yeast stimulatory power of alfalfa extract indicated that a certain maximum effect was obtained with a certain concentration of extract, and that higher concentrations have no greater effect. It seems probable that here the optimum effect was with a "critical" concentration of alfalfa extract bios. The principal advantage of the "critical" concentration method of study is that it takes account of effect on small seedings compared with effect on large seedings, and therefore measures the "true bios activity" in the sense of Wildiers, *i. e.*, the power of enabling small inoculations to grow.

Summary. A study of the effect of varying simultaneously the size of seeding and the concentration of bios, shows that a "critical" concentration of bios can be defined, in the presence of which a small seeding will attain the same cell concentration as a large seeding; while a diminution of the bios concentration below the critical value (by as little as 0.005 mg. per cc.) has been recognized by the large divergence in the cell concentrations attained. The "critical" concentrations of some crude yeast extract, purified Alpha and Beta bioses have been studied.

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[1] Wildiers, *La Cellule*, 1901, xviii, 313.

[2] Eddy, Kerr and Williams, *J. Am. Chem. Soc.*, 1924, xlvi, 2846.

[3] Kerr, R. W., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 344.

[4] Clark, N. A., *J. Phys. Chem.*, 1922, xxvi, 42.

[5] Eddy, Heft, Stevenson and Johnson, *J. Biol. Chem.*, 1921, xlvii, 249.