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The vitamine requirements of certain yeasts and bacteria.

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In discussing the nutritional requirements of the microörganisms, we cannot overlook the important rôle that the vitamines play. The identity of the vitamine that influences the growth of the lower organisms is still an open question. The bulk of evidence, however, points strongly to the conclusion that this substance is distinct from vitamine B, although it is closely related to it; and our results lead us to draw the same conclusion.

Preliminary experiments with beef-heart infusions, peptone, and autolyzed yeast solutions, have shown us that these media contain substances which have a comparable growth-stimulating action on hemolytic streptococci and yeast cells. Thus a beef-heart infusion gives a profuse growth when inoculated with streptococci, whereas this medium, when decolorized by boiling with 2 per cent. of its weight of norit charcoal, loses its growth-stimulating activity, even on addition of a glucose-salt solution. This confirms some of the results obtained by Mueller. When 1 per cent. peptone or autolyzed yeast solutions are added to the decolorized infusion, the medium again becomes favorable for the growth of streptococci. Analogous results were obtained with these substances on the growth of yeast cells.

As it was more desirable to separate these activating substances from the bulk of impurities with which they are associated in their natural media, we subjected beef-heart infusions and autolyzed yeast solutions to fractional adsorption by means of fuller's earth and norit according to the method of Funk and Dubin. These authors have shown that at least two different substances can be separated from autolyzed yeast by means of fractional adsorption

[1] The data in this paper was taken from a dissertation to be presented by Louis Freedman in partial fulfillment for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

with fuller's earth. By this method it is now possible to separate the vitamine active for yeast growth, which has been provisionally called "vitamine D," from that of the anti-beriberi or B vitamine.

TABLE I.

EFFECT ON GROWTH OF YEAST CELLS AND STREPTOCOCCI OF SHAKING OF AUTOLYZED YEAST WITH FULLER'S EARTH AND NORIT.

No.		Yeast Growth.	Bacterial Growth.
	Fuller's Earth:		
1.	Autolyzed yeast (5% solution)	12.5	++
2.	" " shaken with 50 grams per liter	9.5	++
2(a).	Baryta extract of fuller's earth (from 2)	5.5	++
3.	Autolyzed yeast (filtrate from 2) shaken with 100 grams per liter	5.0	−
3(a).	Baryta extract of fuller's earth (from 3)	4.0	++
4.	Autolyzed yeast (filtrate from 3) shaken with 100 grams per liter	0.5	−
4(a).	Baryta extract of fuller's earth (from 4)	0.0	−
	Norit:		
5.	Autolyzed yeast shaken with 50 grams per liter	10.5	+
5(a).	Acetic-acid extract of norit (from 5)	4.0	++
6.	Autolyzed yeast (filtrate from 5) shaken with 100 grams per liter	3.0	−
6(a).	Acetic-acid extract of norit (from 6)	3.0	+
7.	Autolyzed yeast (filtrate from 6) shaken with 100 grams per liter	0.0	−
7(a).	Acetic-acid extract of norit (from 7)	0.0	−

TABLE II.

SHOWING EFFECT ON GROWTH OF YEAST CELLS AND STREPTOCOCCI OF FRACTIONAL SHAKING OF BEEF-HEART INFUSIONS WITH FULLER'S EARTH AND NORIT.

No.		Yeast Growth.	Bacterial Growth.
	Fuller's Earth:		
1.	Beef-heart infusion 1 c.c. [equiv. to ($\frac{2}{3}$ gm.) beef-heart]	12.0	++
2.	Beef-heart infusion shaken with 50 grams per liter.	3.0[1]	++
2(a).	Baryta extract of fuller's earth (from 2)	1.0	+
3.	Beef-heart infusion (filtrate from 2) shaken with 100 grams per liter	0.0	−
3(a).	Baryta extract of fuller's earth (from 3)	1.0	+
	Norit:		
4.	Beef-heart infusion shaken with 20 grams (2%) per liter	0.0	+
4(a).	Acetic-acid extract of norit (from 4)	7.5[1]	++
5.	Beef-heart infusion (filtrate from 4) shaken with 50 grams per liter	0.0	−
5(a).	Acetic-acid extract of norit (from 5)	0.0	−

[1] Average result of several extractions.

The activated adsorbents were extracted with baryta and glacial acetic acid respectively, and the influence of these extracts were tested on the growth of yeast cells and streptococci. The results which we obtained and which are embodied in Tables I and II, show that the substances which stimulate the growth of streptococci and yeast cells, as extracted from beef-heart and autolyzed yeast solutions, apparently belong to the class of vitamines of the water-soluble B type, but are not identical with B vitamine. They are comparable in activity and show similar properties in that they are easily extracted from their natural sources by the same adsorbents, and are again recovered from the adsorbents without appreciable loss in activity.

ACTION OF PROTEIN HYDROLYSATES ON BACTERIA AND YEAST CELLS.

It is very well known that protein hydrolysates stimulate the growth of certain bacteria, and this stimulating action has been attributed at various times to the presence of unknown substances in the protein molecule. To test out this theory, we subjected to acid hydrolysis twelve animal and ten vegetable proteins, which were prepared and purified by the usual methods, particular care

TABLE III.

QUANTITATIVE ACTION OF PROTEIN HYDROLYSATES ON STREPTOCOCCI.
p_H. of Standard Culture Medium = 7.3.

No.	Hydrolysates of the Proteins. (1 c.c. used in each test.)	Growth.	Change in p_H.
1.	Casein (purified) HCl hydrolysate	+	5.8
1(*a*).	" " (sterile control)	−	7.3
2.	Casein (purified) H_2SO_4 hydrolysate	+	5.3
2(*a*).	" " (sterile control)	−	7.0
3.	Casein (technical) HCl hydrolysate	+	5.8
3(*a*).	" " (sterile control)	−	7.3
4.	Gelatin (commercial)	+	6.0
4(*a*).	" (sterile control)	−	7.2
5.	Gelatin (prepared and purified in laboratory)	−	7.2
5(*a*).	" (sterile control)	−	7.3
6.	Edestin	+	6.5
6(*a*).	" (sterile control)	−	7.3
7.	Yeast protein	+	4.9
7(*a*).	" " (sterile control)	−	7.3

NOTE.—Hydrolysates of 18 other proteins found to be inactive.

being taken to have them free of vitamines. These hydrolysates were tested on the growth of streptococci and yeast cells. The bacterial growth was measured by the increase in the acidity of the medium, by means of the Sörensen Indicator method. The action on yeast was not constant, and in most cases showed a growth inhibition due to the known inhibiting action of certain amino acids.

The results on streptococci, which are summarized in Table III, strongly suggest that the growth-stimulating action of protein hydrolysates is not due to a constituent part of the protein molecule, but to a vitamine-like substance, probably similar to vitamine D, which is present as an impurity, and which cannot be removed by the known methods of protein purification.

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The existence of different immunological types of B. pertussis.

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Twenty-two strains having the typical morphological and cultural characteristics of *B. pertussis* have been studied by means of the agglutination and agglutinin absorption reactions. These tests have demonstrated that the cultures studied fall into two serological groups. If the two groups are tentatively designated as "a" and "b" the results may be briefly described as follows: Anti-serums for group "b" agglutinate the strains of group "b" but agglutinate the strains of group "a" very slightly or not at all. The absorption of group "b" serum by group "a" strains does not appreciably reduce the agglutinins for group "b." Group "a" serum, however, agglutinates group "b" strains to a considerable extent. The absorption of group "a" serum by group "b" strains results in a reduction of the agglutinins for strain "a." The serological differences, therefore, are sharply defined in one direction, but group relationship is shown in the reverse direction. These findings are of immediate interest because of their possible bearing on the use of pertussis vaccines.