NUTRITIONAL FACTORS INFLUENCING THE POLYSACCHARIDE PRODUCTION OF *BRUCELLA SUIS*

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The formation of cellular polysaccharide by cells of the genus *Brucella* has been reported by several investigators: Favilli and Biancalani (1934), Higginbotham and Heathman (1936), and Libby and Joyner (1941). These workers, however, dealt mainly with the immunological characteristics of the polysaccharide, and little emphasis was placed upon the nutritional conditions affecting its production. A comparison of the differences in the media with the chemical characteristics of the polysaccharide material isolated from *Brucella abortus*, *Brucella suis*, and *Brucella melitensis* by Huston *et al.* (1934) and Higginbotham and Heathman (1936) suggests that nutrition may play an important role in the synthesis of polysaccharide by this group of organisms.

Investigations in this laboratory with *B. suis* revealed certain nutritional conditions which significantly altered the polysaccharide content of this organism. The data suggest that this polysaccharide was intracellular and therefore probably unrelated to the immunologically active fractions studied by others. The following presentation is concerned primarily with identification of the nutritional conditions affecting polysaccharide formation. The isolation and chemical characterization of the polysaccharide will be reported later.

METHODS

Brucella suis strain PSIIIK was maintained in stock cultures on agar slants composed of tryptose (2 per cent), glucose (1 per cent), NaCl (0.5 per cent), thiamin·HCl (0.1 μg per ml), and $FeSO_4$ (12.2 μg per ml) at 4 C. Actively growing cultures, to serve as inocula, were prepared by transfer of cells from stock slants to 10-ml amounts of standard (S-) medium (composition in table 1) and incubation with shaking at 37 C for 24 hr. From these cultures, 0.5 per cent inocula (by vol) were transferred to the final growth medium. All cultures were aerated by shaking on a reciprocating shaker operating through a 3-in stroke at 100 cycles per min in a 37 C incubator adjusted to approximately 50 per cent relative humidity.

The media used in this investigation were prepared from deacidified (deionized) casein partial hydrolyzate (Higuchi and Carlin, 1957), yeast autolysate (yeast-75, Vico Products Co.) and a carbon source. Growth and cellular carbohydrate production were determined in media containing a range of concentrations of these components as a basis for the selection of media yielding cells with varying levels of cellular carbohydrate (table 1). In addition, all media contained sodium thioglycolate, 0.0005 M. Media were adjusted to pH 7.5 and sterilized by autoclaving at 123 C for 15 to 20 min. Carbon sources (sterilized by autoclaving) were added aseptically, prior to inoculation. The volumes of medium employed were 10 ml in 140-ml square pyrex bottles (milk testing bottles) and 50 ml in 250-ml Erlenmeyer flasks.

Growth was determined by diluting cultures 10-fold with distilled water in 18- by 150-mm colorimeter tubes and measuring turbidity in a Coleman photonephelometer by comparing with a turbidity standard. Fifty turbidity units are approximately equivalent to an optical density (at 660 mμ wave length) value of 0.5.

The determination of cellular polysaccharide was based on the procedure of Seifter *et al.* (1950). Samples of washed cells were diluted to 3 ml with distilled water in colorimeter tubes which were placed in an ice bath, and 7 ml of anthrone reagent (0.2 per cent anthrone in 95 per cent H_2SO_4, freshly prepared before each series of determinations) were added slowly with shaking. The tubes were covered with marbles and placed in a boiling water bath for 10 min to develop color. After

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TABLE 1

Composition of media yielding cells containing high (H-), standard (S-), and low (L-) levels of carbohydrate (CH_2O)

Medium	Composition			Turbidity*		Cell CH_2O†	
	Casein hydrolyzate	Glucose	Yeast autolysate	45 hr	70 hr	45 hr	70 hr
	%	%	%				
L-	4.0	1.0	1.0	191	186	5.2	3.9
S-	2.0	2.0	0.25	164	206	12.1	13.8
H-	0.5	5.0	1.0	125	165	21.3	24.3

* Turbidity, 1:10 dilution of culture, mean values of 5 to 11 independent cultures.

† CH_2O expressed as mg glucose per 100 mg cell protein. Mean values, 5 to 11 independent cultures.

cooling, color intensities were measured at 620 mμ wave length and readings were converted to "glucose" from a standard curve. Cell polysaccharide was expressed as optical density (of anthrone reaction) per unit standard turbidity or as mg glucose per 100 mg of cell protein. Reducing sugar was measured according to the procedure of Horvath and Knehr (1941) after precipitation of protein with trichloracetic acid.

Protein content of cells was determined according to the biuret procedure reported by Stickland (1951). A 5-ml aliquot of washed cells was transferred to a 12-ml conical centrifuge tube, 1 ml of 20 per cent NaOH was added, and the tubes were covered with marbles and heated for 5 min in a boiling water bath to digest the cells. The alkaline digests were cooled, 0.2 ml of 25 per cent $CuSO_4 \cdot 5H_2O$ was added to each tube and mixed with the digest by shaking each tube immediately; the tubes were left at room temperature for 60 min and then centrifuged at 3,000 rpm for 15 min. The supernatant liquids were transferred to colorimeter tubes and color intensity was measured at 565 mμ wave length. Readings were converted to protein from a standard curve prepared from casein.

All pH values were measured with a Beckman pH meter, using standard or 1-drop glass electrodes.

Polypeptide fractions were prepared by partially hydrolyzing casein in the presence of a cation exchange resin (Underwood and Deatherage, 1952); casein 10 g, and "dowex-50," 25 g, were suspended in 0.1 N HCl, 100 ml, and autoclaved at 123 C. A 30-min and a 60-min hydrolyzate was prepared; both hydrolyzates were filtered, neutralized with alkali, refiltered to remove the small amount of insoluble material, and then dialyzed against cold, running tap water for 2 hr. The 30-min hydrolyzate contained approximately 76 mg per ml biuret positive material before and after dialysis, whereas the 60-min hydrolyzate contained approximately 63 and 54 mg per ml before and after dialysis, respectively.

RESULTS

Preliminary investigation revealed that washed cells of *B. suis* reacted with the anthrone reagent to yield the typical absorption spectrum and color noted for glucose. Chemical analyses of cells for total carbohydrate and free reducing sugar content (described below) indicated that a major portion of the cellular carbohydrate as measured by the anthrone reaction was polysaccharide in nature. Assay of cell suspensions with varying turbidities (25 to 165) demonstrated a linear relationship between cell concentration (turbidity) and optical density of the anthrone reaction, suggesting the applicability of the method for the quantitative determination of cellular polysaccharide (CH_2O).

Examination of the effect of sterilization time and temperature revealed that heating cultures at 70 C for 10 to 60 min did not alter CH_2O content of cells, whereas heating at 100 C for 10 min resulted in approximately a 73 per cent reduction in cell-CH_2O. From these findings, a procedure for the routine assay of cells grown under different nutritional conditions was adopted: cultures were sterilized at 70 C for 15 to 20 min and the cells removed by centrifugation followed by washing (2 ×) with 0.85 per cent NaCl or distilled water. The packed cells were finally adjusted with distilled water to a selected standard turbidity in the range of 75 to 100, and aliquots were assayed for cell-CH_2O and protein as described.

Factors affecting polysaccharide production by growing cells. Cell age:—In order to study the effects of various nutritional conditions, it was necessary first to correlate CH_2O production with the age of the organism. A 250-ml Erlenmeyer flask containing 50 ml of S-medium was inoculated and incubated as described. At varying time intervals, 2-ml aliquots were removed and assayed. The washed cells collected at each time

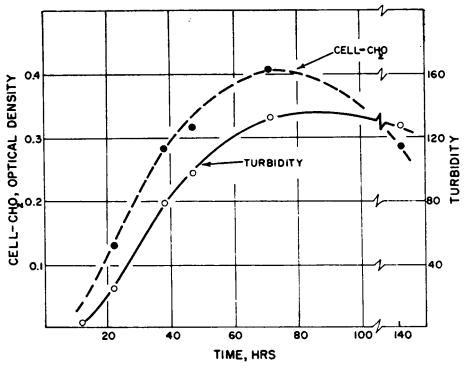

Figure 1. Growth and production of cellular carbohydrate (CH_2O). Closed circles ● represent cell-CH_2O per unit of turbidity (see text).

were adjusted to the same turbidity (T = 75) and equal volumes (3 ml) were used for the anthrone reaction. Therefore, the cell-CH_2O values (optical density) presented in figure 1 represent an increase in carbohydrate per cell with respect to time. It was noted that maximum production of CH_2O coincided essentially with maximum growth. Continued incubation resulted in a decrease of cell-CH_2O. In the subsequent experiments, cells were collected and assayed at or near maximum growth.

Hydrogen ion concentration. The effect of pH on CH_2O production was determined by growing the organism in S-medium (10 ml in square pyrex bottles) adjusted to pH levels of 5.0 to 8.5. The findings revealed that initial adjustment of the medium to pH 7.5 (7.3 after autoclaving) was optimal for growth and synthesis of cell-CH_2O.

Carbon source. The effect of various carbon sources was similarly tested in S-medium. In order to reduce the number of assays, it was assumed that maximum CH_2O production would coincide with maximum growth for each compound tested. The values in table 2 were compiled from three separate experiments; control media, with and without glucose, were included with each experiment. With the exception of glucose, the best CH_2O yields were obtained with D-lyxose, D-ribose, and D-glucosamine·HCl. In most instances increased CH_2O synthesis was accompanied by heavy growth.

Glucose, casein hydrolyzate, and yeast autolysate concentration. In testing the concentration effect of the various components in S-medium, each substance was varied individually while maintaining the other two at a constant level. Two independent experiments were employed for each substance varied and the results were averaged. The organism was grown and assayed as previously described. The CH_2O values recorded in figure 2 represent values obtained from equal aliquots of washed cells adjusted to a standard turbidity (T = 75). The results indicate that maximum synthesis of CH_2O occurs when glucose

TABLE 2

Effect of carbon source on growth and production of cellular carbohydrate (CH_2O)

Carbon Source*	Maximum Turbidity	Cell-CH_2O†
Pentoses		
D-Lyxose	160	0.390
D-Ribose	100	0.314
D-Arabinose	24	0.157
L-Rhamnose	29	0.090
Hexoses		
D-Glucose	199‡	0.490‡
D-Glucosamine·HCl	150	0.377
D-Galactose	188	0.238
D-Fructose	152	0.209
Disaccharides		
D-Trehalose	95	0.218
D-Lactose	40	0.213
Sucrose	81	0.166
D-Maltose (hydrate)	68	0.164
D-Cellobiose	38	0.119
Melibiose	39	0.107
Trisaccharide		
Raffinose (hydrate)	50	0.168
Alcohols		
Adonitol	190	0.229
L-Arabitol	30	0.180
D-Sorbitol	41	0.090
D-Mannitol	33	0.077
Dulcitol	28	0.072
Miscellaneous		
Salicin	10	0.161
Calcium-glucoheptonate	59	0.135
None	35‡	0.097‡

* Final concentration, 2% in 10 ml of media containing deionized casein partial hydrolyzate, 2 per cent; yeast autolysate, 0.25%.

† Optical density of anthrone reaction of 3-ml washed cells adjusted to turbidity = 75 units.

‡ Mean from three independent controls.

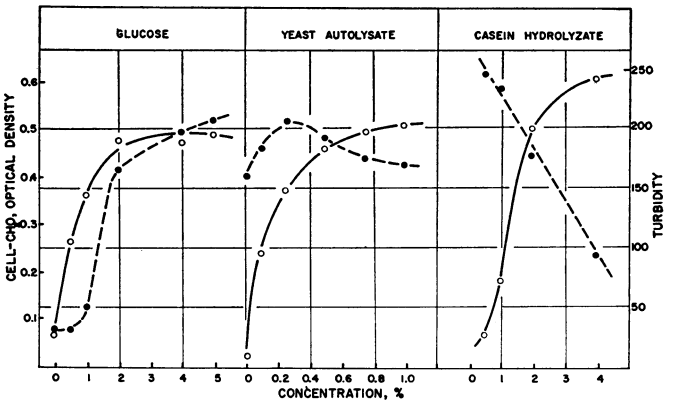

Figure 2. The effect of glucose, yeast autolysate, and casein hydrolyzate concentration on growth and production of cellular carbohydrate. Each curve represents the mean of two experiments. Open circles ○ represent turbidity; closed circles ● represent cellular carbohydrate.

is present in excess of the amount required for maximum growth, whereas the concentration of yeast autolysate had little effect on the CH_2O content of cells. The effect of varying the deionized casein hydrolyzate was most marked. An increase in casein hydrolyzate concentration was accompanied by a decrease in CH_2O synthesis. The reduced growth noted at the 0.5 per cent level of casein hydrolyzate was corrected in subsequent experiments by supplementing the medium with larger amounts of yeast autolysate. The apparent inhibitory effect of the casein hydrolyzate on cell-CH_2O synthesis was further verified by statistical analyses of data from a factorial experiment employing 2 and 4 per cent glucose, 0.25, 0.5, and 1.0 per cent yeast autolysate and 1, 2, and 4 per cent casein hydrolyzate. The analyses of cultures incubated for growth periods of 52 and 72 hr revealed that only casein hydrolyzate was significant in its effect. No interactions were of importance.

From the preceding results, several media were selected which resulted in equivalent growth of cells containing different amounts of CH_2O. The composition of the media, and the growth and relative cell-CH_2O yields are given in table 1. The designations given in table 1 will be employed in the remainder of this report: L-medium (yielding L-cells with low CH_2O), S-medium (yielding S-cells with standard or normal CH_2O), and H-medium (yielding H-cells with high CH_2O). The composition of H-medium was shown later to be optimum for CH_2O formation. In experiments where yeast autolysate and glucose concentrations were varied while maintaining casein hydrolyzate at the 0.5 per cent level, the highest polysaccharide values were obtained at the concentrations reported (table 1) for H-media.

Duplicate analyses of alkaline digests (15 min in 1 N NaOH at 20 to 25 C) of dried cells obtained from the three media revealed that whereas the free reducing sugar contents were essentially the same for L-, S-, and H-cells (1.5, 1.1, and 2.7 per cent, respectively), the total carbohydrate contents varied significantly (1.7, 11.3, and 25.6 per cent, respectively). Analyses of reducing sugar and total CH_2O in supernatant liquids from cultures after 48 and 72 hr growth revealed essentially equal levels of residual carbohydrate in the media which indicated that polysaccharide material was not released from the cell during growth. The marked increase in the extent of endogenous respiration (total μL O_2 uptake) of H-cells, compared to L-cells, together with the decrease of cell-CH_2O values of respiring H-cells during prolonged incubation, indicated that the polysaccharide synthesized by *B. suis* was biologically active and constituted an intracellular reserve material.

Inhibition of polysaccharide CH_2O synthesis by amino acids. The inhibitory action of increased concentrations of casein hydrolyzate noted in the preceding section was attributed to the presence of certain amino acids or peptides present in the hydrolyzate. This was emphasized further by the findings that pretreatment of the casein hydrolyzate with varying concentrations of activated charcoal, calcium phosphate·gel (Keilin and Hartree, 1938) and ethyl ether at different pH levels failed to reduce the inhibitory action of

TABLE 3

Concentration and composition of amino acid groups in "synthetic casein hydrolyzate"

Group and Amino Acid	Concentration*
	g/100 ml
I. Cyclic	
L-Tryptophan	0.060
L-Tyrosine	0.335
L-Proline	0.375
L-Histidine·HCl	0.142
DL-Phenylalanine	0.500
II. Sulfur-hydroxy	
DL-Methionine	0.340
DL-Threonine	0.380
DL-Serine	0.770
L-Cystine	0.018
III. Branched chain	
L-Leucine	0.485
DL-Isoleucine	0.630
DL-Valine	0.650
IV. Dicarboxylic	
L-Glutamic	1.165
DL-Aspartic	0.610
V. Straight chain monobasic and dibasic	
Glycine	0.025
DL-Alanine	0.550
L-Arginine	0.260
L-Lysine·HCl	0.475

* Final concentration = approximately 5.5 g L-amino acids per 100 ml of mixture.

4 per cent casein hydrolyzate employed in L-medium. Moreover, neither the deionization procedure nor the incorporation of "humin free" casein hydrolyzate (obtained by complete decolorization of the hydrolyzate with excess activated charcoal) in the medium altered the results noted for cells grown in L-medium.

To test the effect of amino acids, varying concentrations of a "synthetic casein hydrolyzate," prepared by mixing amino acids to duplicate the amino acid content of casein (Hawk *et al.*, 1948), were added to H-medium, table 3. The results in figure 3 show that the addition of 1.0 ml of the amino acid supplement (7.77 mg per ml medium) resulted in approximately 71 per cent reduction of cell-CH_2O synthesis without inhibition of growth. The amino acid supplement was then subdivided into groups of amino acids according

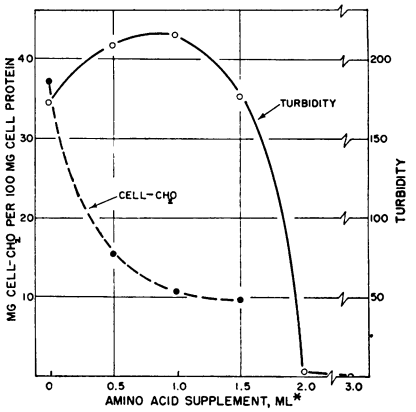

Figure 3. The effect of amino acid supplement in H-medium (medium yielding cells containing high levels of carbohydrate) on growth and production of cellular carbohydrate (CH_2O). (* Volume of amino acid solution added per 10 ml of medium; one ml contains 77.7 mg amino acids.)

to structure (table 3) which were added to H-medium, and the subsequent effect upon CH_2O formation was determined. The results demonstrated that only the addition of group III or V amino acids was significant (table 4), with the most marked effect occurring upon the incorporation of both groups into the media.

An attempt was made to determine which amino acid(s) was responsible. Although the data (table 5) indicate that the inhibition does not appear to be highly specific, it should be noted that the combination of L-lysine (group V) and DL-valine (group III) alone resulted in essentially the same inhibition of growth and of CH_2O production observed for groups III and V (1.5 ml level, table 4) amino acids added *in toto*.

Effect of polypeptides. The two polypeptide fractions described above and a polypeptide fraction supplied by Dr. E. N. Fox (Fox and Krampitz, 1956) were added individually to H-medium at final concentrations of 10, 20 (except pancreatic fraction), or 40 mg per ml. Growth (turbidity) and cellular carbohydrate were assayed after 48 and 72 hr of incubation. The peptide fractions, at each level, stimulated the rate and extent of growth without inhibiting carbohydrate synthesis. In most instances the increase in growth was accompanied by an increase in cellular carbohydrate formation.

Factors affecting polysaccharide production by resting cells. Since cells grown in L-medium do not contain significant levels of cell-CH_2O, the ques-

TABLE 4

Inhibition of cellular carbohydrate (CH_2O) production of growing cells by branched chain (group III), and by straight chain monobasic and dibasic amino acids (group V)

Amino Acids Added to H-Medium*		Turbidity	Cell-CH_2O	
Group III†	Group V†		Found‡	Inhibition
ml	*ml*	*72 hr*	*72 hr*	*%*
None (Control)	—	160	21.3	0.0
1.0	—	133	12.1	43.2
1.5	—	108	11.6	45.6
2.0	—	92	11.0	48.4
3.0	—	35	5.8	72.8
—	1.0	228	19.1	10.4
—	1.5	228	16.6	22.1
—	2.0	222	15.9	25.4
—	3.0	228	13.4	37.1
1.0	1.0	180	13.0	39.0
1.5	1.5	144	7.2	66.2
2.0	2.0	101	8.9	58.3
1.0	2.0	167	9.8	54.0
2.0	1.0	64	11.5	46.1

* H-medium = medium containing high levels of carbohydrate.

† Group III contains 17.65 mg amino acids per ml; group V, 13.1 mg per ml. See table 3 for concentrations of individual amino acids.

‡ CH_2O expressed as mg glucose per 100 mg cell protein.

tion arose as to whether these cells were capable of polysaccharide synthesis in the absence of amino acids. L-cells were grown in 19 square pyrex bottles containing 10 ml of medium per bottle, and incubated for 46 hr as described. The cultures were pooled and the cells were removed, washed, and diluted to 13 ml with saline. In accordance with figure 4, aliquots of the cell suspensions were incubated with and without glucose at pH 6.0 and 7.0 and samples were removed at intervals and assayed for cell-CH_2O. The data show that in the absence of amino acids L-cells can synthesize carbohydrate from glucose, indicating, thereby, that L-, S-, and H-cells did not arise by virtue of population selection by the different media. D-ribose or D-glucosamine·HCl (at 2 per cent final concentration) did not serve as substrates for polysaccharide synthesis, in con-

TABLE 5

Inhibition of cellular carbohydrate (CH_2O) production of growing cells by amino acids

Amino Acids Added to H-Medium*	Turbidity	Cell-CH_2O	
		Found†	Inhibition
	72 hr	*72 hr*	*%*
None (control)	144	28.2	0.0
Glycine	101	25.0	11.4
DL-Alanine	151	16.4	41.9
L-Arginine	154	24.0	14.9
L-Lysine·HCl	162	20.7	28.8
L-Leucine	128	23.2	17.8
DL-Isoleucine	134	18.1	35.9
DL-Valine	108	16.1	43.0
Glycine:			
+ DL-Alanine	144	22.1	21.7
+ L-Arginine	210	31.8	0.0
+ L-Leucine	130	22.5	20.3
+ DL-Isoleucine	144	18.0	36.2
+ DL-Valine	120	12.1	57.1
DL-Alanine:			
+ L-Arginine	170	20.1	25.6
+ L-Lysine·HCl	168	21.3	24.5
+ L-Leucine	166	17.1	39.4
+ DL-Isoleucine	156	21.6	23.1
+ DL-Valine	128	14.2	49.7
L-Arginine:			
+ L-Lysine·HCl	192	29.0	0.0
+ L-Leucine	168	17.4	38.3
+ DL-Isoleucine	162	14.0	50.4
+ DL-Valine	84	5.8	79.5
L-Lysine·HCl:			
+ L-Leucine	146	20.5	27.4
+ DL-Isoleucine	152	16.8	40.5
+ DL-Valine	142	7.4	73.8
L-Leucine:			
+ DL-Isoleucine	110	12.6	55.4
+ DL-Valine	98	10.9	61.4
DL-Isoleucine + DL-valine	102	9.2	67.4

* H-medium = medium containing high level of carbohydrate. Amino acid added per 10 ml medium: glycine, 0.38 mg; DL-alanine, 8.3 mg; L-arginine, 3.9 mg; L-lysine·HCl, 7.1 mg; L-leucine, 7.3 mg; DL-isoleucine, 9.5 mg; DL-valine, 9.8 mg.

† CH_2O expressed as mg glucose per 100 mg cell protein.

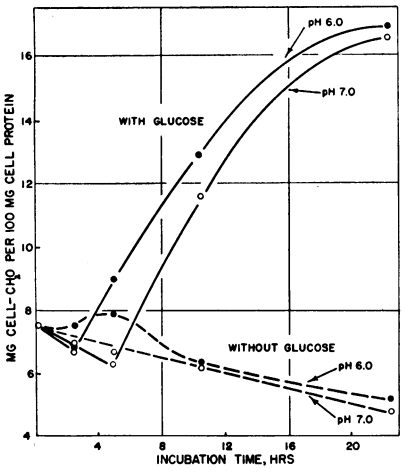

Figure 4. Synthesis of cellular carbohydrate (CH_2O) by resting cells with low levels of carbohydrate (L-cells) at pH 6.0 and 7.0.

Protocol per bottle: 0.2 M phosphate buffer, pH 6.0 or 7.0, 5.0 ml; 10 per cent glucose or water, 2.0 ml; washed cells, 3.0 ml; incubation at 37 C with shaking.

TABLE 6

Total carbohydrate and reducing sugar content of resting L-cells (low CH_2O content) after incubation with and without glucose

Incubation* Conditions	Cell-Carbohydrate†	
	Reducing sugar	Total
	%	%
0 time	2.4	8.1
22.5 hr without glucose	1.7	6.1
22.5 hr with glucose	1.8	21.3

* See figure 4.

† Expressed as "glucose," as per cent by weight of dried cells.

trast to the results noted for these substrates with growing cells (table 2).

At the end of the incubation period (22.5 hr), the cells were washed twice with water, frozen at −20 C, and dried *in vacuo* over $CaSO_4$ overnight. Analyses of the dried cells for reducing sugar and total carbohydrate content (table 6) verified the increase in cell-CH_2O noted in figure 4 as cell polysaccharide. The level noted is in good agreement with the data reported for H-cells in table 1.

Inhibition by amino acids. In a similar experiment, L-cells were incubated with glucose and

TABLE 7

Inhibition of cellular carbohydrate production of resting L-cells (low CH_2O content) by amino acids

Additions*	Inhibition†	
	16 hr	25.5 hr
	%	%
Glucose	0.0	0.0
Glucose + group III amino acids	27.0	20.6
Glucose + group V amino acids	5.4	23.3
Glucose + group III + V amino acids	60.2	49.2
Glucose + DL-valine	8.0	33.4
Glucose + L-lysine·HCl	0.0	0.0
Glucose + DL-valine + L-lysine·HCl	33.2	41.4

Protocol. Per bottle: 0.2 M phosphate buffer, pH 6.0, 5.0 ml; 20 per cent glucose, 1.0 ml; washed cells (45.1 mg protein per ml), 2.0 ml. Amino acids: group III—L-leucine, 7.3 mg; DL-isoleucine, 9.5 mg; DL-valine, 9 8 mg; group V—glycine, 0.38 mg; DL-alanine, 8.3 mg; L-arginine, 3.9 mg; L-lysine·HCl, 7.1 mg. Water to 11.0 ml. Incubation at 37 C with shaking.

* Amino acid solutions neutralized before addition.

† Calculated from anthrone reaction of washed cells at time intervals given.

groups III and V amino acids in phosphate buffer at pH 6.0. To reduce the possibility of cell multiplication, a high concentration of cells, approximately 8.2 mg protein per ml final concentration, was employed. The results, table 7, agree favorably with those obtained with growing cells; i. e., the greatest inhibition of polysaccharide formation (without reduction in growth) was noted when both groups III and V amino acids were present. On the basis of previous findings, only L-lysine and DL-valine were examined individually. These two amino acids resulted in greater inhibition than group III or V added separately. The addition of L-lysine alone gave approximately a 10 per cent increase in carbohydrate production. These data suggest that DL-valine is the most significant factor in inhibition of cellular polysaccharide.

DISCUSSION

The data presented above indicate clearly the significant role played by nutrition in the alteration of the polysaccharide content of *B. suis.*

The identity of this carbohydrate as an intracellular component rather than extracellular (capsular) material is suggested by the data which showed that it was not released into the medium during growth but disappeared (utilized as energy source?) during incubation beyond the stationary phase of growth.

Although only presumptive evidence is presented, it appears that the concentration of certain amino acids present in the casein hydrolyzate were responsible for the decrease in polysaccharide formation in L-media. A similar relationship between amino acids and polysaccharide synthesis was reported by Dawson and Happold (1943). DL-Phenylalanine and DL-tyrosine stimulated the accumulation of a metabolizable polysaccharide in *Escherichia coli*. However, Dagley and Dawes (1949) were unable to confirm this finding but demonstrated an increase in polysaccharide formation up to approximately 18 per cent of the dry cell weight. Glucose, lactose, and galactose served as substrates, with maximum synthesis occurring at pH 7.5 at the onset of the maximum stationary phase. Carruthers and Cooper (1936) obtained no effect upon dextran production from *Leuconostoc mesenteroides* when the peptone medium was supplemented or replaced by the amino acids glycine, D-alanine, asparagine, or glutamic acid. L-leucine occasionally stimulated dextran synthesis.

The inhibition of polysaccharide synthesis in *B. suis* by amino acids, though not specific, was most significant in combinations of amino acids containing DL-valine. It is possible that DL-valine (in combination with other amino acids) blocks the formation of an enzyme(s) necessary for the synthesis of the polysaccharide. This possibility is strengthened further by the observation that DL-valine plus L-lysine inhibited polysaccharide synthesis of resting L-cells and that even in the absence of these amino acids several hours of incubation were required (adaptive enzyme formation) before polysaccharide formation was noted.

Quantitative determination of amino acids by paper chromatography (Levy and Chung, 1953) revealed that L-medium contained 2.11 and 0.45 mg per ml bound and free DL-valine, respectively, whereas H-medium contained 0.26 and 0.08 mg per ml, respectively. Moreover, the addition of 0.98 mg per ml DL-valine to H-medium (table 5) resulted in 43 per cent inhibition of carbohydrate formation. These results, in addition to the observation that various polypeptide fractions added to the medium failed to inhibit polysaccharide formation, strongly suggest that the individual amino acids (or low molecular weight peptides) were responsible for the inhibitory action.

It is also possible that certain amino acids may have stimulated the oxidation of glucose and thereby limited assimilation, or stimulated the utilization of the polysaccharide as it was formed. Both possibilities would result in an apparent "inhibition." The likelihood that the amino acids were preferentially utilized as carbon sources is ruled out by the disappearance of glucose from the medium during growth and the lack of significant growth in the absence of glucose. In order to understand more completely the relationship between the action of amino acids and polysaccharide synthesis in *B. suis*, it would be necessary to initiate studies employing synthetic media.

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SUMMARY

Cultivation of *Brucella suis* in a complex medium containing varying concentrations of deacidified casein partial hydrolyzate, yeast autolysate, and glucose resulted in cells with altered cellular polysaccharide content, ranging from 1.5 to 20 per cent of dry weight. Maximum synthesis of carbohydrate was attained at the termination of the log phase of growth at pH 7.5 with glucose as the carbon source. Production of the polysaccharide was adversely affected by addition to the medium of increasing concentrations of casein hydrolyzate or amino acid supplements containing DL-valine, whereas the addition of polypeptide to the medium failed to alter polysaccharide formation. The combination of DL-valine and L-lysine likewise inhibited polysaccharide synthesis by resting cell suspensions in phosphate buffer and glucose.

The data do not permit specific conclusions as to the mode of action of the amino acids during inhibition of polysaccharide synthesis.

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