THE EFFECT OF VITAMIN C AND CERTAIN OTHER SUBSTANCES ON THE GROWTH OF MICRO-ORGANISMS

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The relation of vitamin C to the growth of plant organisms has attracted attention for some time. Havas (1) observed that vitamin C in concentrations of 1/10,000 and 5/10,000 had an accelerating effect on the growth of wheat seedlings but it had an inhibitory effect in a concentration of 2.5/1,000, and in larger concentrations it produced practically a lethal effect. Hausen (2) observed that 40 mg. of ascorbic acid increased the dry weight of treated pea plants to an extent of 35-75% in sterile culture experiments. Similar observations were made by Davis and his co-workers on the willow, tomato and castor oil plants (3). Discussing Hausen's results, Virtanen (4) remarks that "it is reasonable to regard vitamin C as a phytohormone which is indispensable to plants."

Very little information, however, has been available regarding the effect of vitamin C on micro-organisms, and we undertook this work in 1936, as it seemed to us that the study of the effect of vitamin C on such simple organisms might throw some light on the mechanism of the action of vitamin C. A note on preliminary results has been published (5). A few papers have, however, appeared subsequently bearing on the relation between vitamin C and certain bacteria (6-8). Some of this work is discussed later.

In the present work we have carried out some experiments on the effect of vitamin C on the growth of different fungi and bacteria in a synthetic medium. The cultures used were those of Aspergillus niger, Asp. oryzæ, Asp. flavus, Saccharomyces cerevisiæ, S. ellipsoideus, B. subtilis, B. typhosus, B. coli, Ærobacter ærogenes, Staphylococcus aureus, Streptococcus hæmolyticus and B. diphtheria.

METHOD

The synthetic medium of Reader (9) with slight modification was used in all the experiments. The inoculum throughout the experiments consisted of 0.1 c.c. of a suspension of a loopful of the culture (24 hours old) in 5 c.c. of sterile water. The estimation of growth was carried out by the determination of the dry weight in the case of *Aspergillus*, by counting with the hæmocytometer in the case of *Saccharomyces* and nephelometrically in the case of bacteria.

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(1/50,000) to 1 the first 72 approximately acid, which ha

THE EFFECT OF VITAMIN C ON THE GROWTH OF FUNGI

At the beginning, in a few experiments different concentrations of ascorbic acid were used and 1/50,000 was found to have the maximum stimulating effect. A stronger concentration like 1/10,000 was, however, inhibitory to the growth of Aspergillus and Saccharomyces.

TABLE I

Effect of ascorbic acid on the growth of Asp. niger in different concentrations.

Figures give the wt. of Asp. niger in g. in 20 c.c. culture (72 hours old).

	•	Concentration	of ascorbic ac	id	
0	1/10,000	I/25,000	1/50,000	1/75,000	1/100,000
0.0113	0.0059	0.0087	0.0274	0.0162	0.0145
0.0153	0.0087	0.0130	0.0324	0.0246	0.0187
0.0072	0.0020	0.0053	0.0140	0.0108	0.0065

TABLE II

7è 1.

Effect of ascorbic acid on the growth of S. cerevisiæ

in different concentrations.

Figures indicate no. of cells per c.c. in thousands.

		(K) 2 2	Concentr	ation o	of ascorbic aci	d	
	o	1/10,000	1/25,0	00	τ/50,000	1/75,000	1/100,000
-					5, 75. 5	- (. ¹	
	600	250	450		4250	2400	1200
ale	100		60		2150		
					2150	800	275
	225 · · E ¹ 00.0	75 5355.5	130	 71/22	3000-	1700	500
	1. J. T.	to the state		1		A	

With all the varieties of Aspergillus, the addition of vitamin C (1/50,000) to the medium resulted in very markedly increased growth during the first 72 hours; after five days, however, the total growth was approximately equal to that in the flask in which no ascorbic acid or ascorbic acid, which had been previously oxidised irreversibly, had been added.

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on the effect a synthetic Asp. oryzæ, B. typhosus, cus hæmoly-

ion was used ents consisted hours old) in d out by the by counting phelometrically

TABLE III

	72 hou	urs old cult	ure :	120 hours old culture				
	Without ascorbic acid	With ascorbic acid o.4 mg.	With ascorbic acid (oxidised) 0.4 mg.	Without ascorbic acid	With ascorbic acid 0.4 mg.	With ascorbic acid (oxidised) 0.4 mg.		
	0.0128	0.0222		0.0320	0.0304	·		
	0.0168	0.0261		0.0356	0.0369	<u> </u>		
140	0.0098	0.0179		0.0250	0.0284			
Asp. niger.	0.0072	0.0139		0.0235	0.0229	÷		
Tab	0.0130	0.0302		0.0342	0.0359			
	0.0087	0.0127		0.0223	0.0235			
	0.0090	0.0158		0.0240	0.0252			
		0.0324	0.0129	0.0346	0.0360	0.0353		
	0.0153	0.0324	0.0132	0.0348	0.0382	0.0329		
	0.0122	0.0170	0.0118	0.0256	0.0242	0.0249		
	0.0059	0.0202	0.0043	0.0219	0.0232	0.0231		
	0.0113	0.0274	0.0103	0.0325	0.0342	0.0318		
			\$					
	0.0085	0.0234	0.0079	_	_			
	0.0125	0.0402	0.0140		—			
	0.0042	0.0180	0.0030			_		
Asp. oryzæ.	0.0027	0.0098	0.0037	—				
	0.0011	0.0063	0.0020					
	0.0032	0.0084	0.0027	0.0285	0.0301	0.0278		
in the second	0.0015	0.0039	0.0020	0.0164	0.0153	0.0149		
	0.0044	0.0140	0.0035	0.0364	0.0410	0.0338		
						÷		
	0.0014	0.0031			-			
· .	0.0017	0.0049		_	-			
	0.0012	0.0042		_	-			
Asp. flavus.	0.0009	0.0027		_				
	0.0015	0.0039						
	0.0023	0.0052				-		
<i>c</i> .	0.0010	0.0034	0.0017	0.0072	0.0085			
	0.0020	0.0057	0.0024	0.0123	0.0109			
	. 0.0008	0.0030	0.0015	0.0098	0.0104			
	0.0018	0.0043	0.0012	0.0102	0.0115	0.012		

Wt. of Aspergillus in 'g. in 20 c.c. culture.

From Table III it will be noticed that the growth was simply stimulated by the addition of ascorbic acid and maximum growth was attained

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Asp. niger.

Asp. flavus.

Asp. oryzæ.

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earlier than in the controls; controls reached the maximum in five days, the final growth being approximately the same in both sets of experiments. Experiments were carried out with other reducing agents such as glutathione and cysteine to find how far the action of ascorbic acid is specific. From Table IV it will be seen that, though ascorbic acid has a greater stimulating action than the other two reducing substances, the latter stimulate growth quite markedly.

TABLE	LV.

Figures indicate	wt.	of	Aspergillus	ın	g.	in	20	<i>c.c.</i>	culture.	
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2X7 19	72 ho	urs old	culture		120	hours	old cult	ure.
22	Control	Glutathione o.4 mg.	Cysteine o.4 mg.	Ascorbic acid o.4 mg.	Control	Glutathione 0.4 mg.	Cysteine 6.4 mg.	Ascorbic acid 0.4 mg.
2	0.0098	0.0153	0.0130	0.0250	0.0255	0.0263	0.0272	0.0285
211	0.0128	0.0250	0.0186	0.0287	0.0348	0.0327	0.0310	0.0382
Asp. niger.	0.0072	0.0163	0.0116	0.0210	0.0160	0.0126	0.0147	0.0180
	0.0059	0.0130	0.0096	0.0178	- 0.0384	0.0326	0.0284	0.0344
1 1	0.0024	0.0067	0.0053	0.0114	0.0224	0.0240	0.0186	0.0238
Asp. flavus.	0.0031	0.0083	0.0064	0.0130	0.0253	0.0223	0.0198	0.0244
2 Million Contraction	0.0012	0.0058	0.0030	0.0082	0.0146	0.0106	0.0090	0.0132
	0.0015	0.0072	0.0043	0.0092	0.0160	0.0176	0.0138	0.0153
hantani () 	0.0044	0.0096	0.0084	0.0134	o.0186	0.0170	0.0153	0.0206
	0.0035	0.0045	0.0039	0.0118	0.0179	0.0163	0.0140	•
Asp. oryzæ.	0.0027	0.0053	0.0047	0.0110	0.0163	0.0168	0.0119	0.0184
	0.0042	0.0076	0.0058	0.0140	0.0210	0.0190	0.0176	0.0225

SYNTHESIS OF ASCORBIC ACID BY FUNGI

Several experiments were conducted to see whether the fungi are themselves capable of synthesising ascorbic acid. From Table V it will be observed that in all the cases the organisms were capable of synthesising vitamin C. In the experiments where glutathione and cysteine were used, an appreciably larger quantity of ascorbic acid was formed. The estimation of ascorbic acid was carried out with the trichloroacetc acid extracts of the micro-organisms by titration with 2:6-dichlorophenol indophenol in the usual way.

Vith scorbic acid cidised) mg.

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	.72 ho	urs old	culture.	• • •	120	hours o	old cultu	ire:
	Control	Glutathione 4.0 mg.	Cysteine 4.0 mg.	Ascorbic acid 4.0 mg.	Control	Glutathione 4.o mg.	Cysteine 4.0 mg.	Ascorbic acid 4.0 mg.
Aspergillus niger.	0.146	0.208	0.183	0.246	0.376	0.253	0.208	0.353
det i st	0.160	0.198	0.179	0.287	0:432	0.349	0.324	0.418
a (1447)	0.153	.0.176	0.164	0.210	Q.353	0.276	0.246	0.326
1999 - 19	· 0.124	0.146	0.130	0.186	0.298	0.240	0.221	0.304
and the second sec	0.127	0.168	0.156	0.186	0.208	0.186	0.173	0.230
Aspergillus oryzæ.	0.116	0.130	0.117	0.167	0.178	0.159	0.140	0.286
	0.104	0.148	0.130	0.159	0.174	0.168	0.152	0.280
	0.122	0.153	0.143	. 0.193	0.221	0.208	0.189	0.243
······	0.091	0.146	0.140	0.173	0.198	0.189	0.160	0.208
	0.104	0.160	0.144	0.208	0.208	0.190	0.183	0.221
Aspergillus flavus.	0.078	0.124	0.104	. 0.140	0.176	0.151	0.140	0.188
	0.083	0.153	0.130	0.168	0.182	0.194	0.156	0.198

TABLE V. Figures indicate mg. of ascorbic acid formed in 200 c.c. culture

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Saccharomyce ellipsoideus.

With Saccharomyces also the addition of ascorbic acid markedly stimulated the growth, with the only difference that the growth was always greater than that in the negative controls even after five days (Table VI).

TABLE VI.

Effect of ascorbic acid on the growth of Saccharomyces cerevisiæ (after 72 hours) in 20 c.c. culture

	- -	Without a	scorbic acid.	With ascor 0.4		With ascorbi (oxidised) o.	c acid 4 mg.
	Expt. no	Numb b. per th	er of cells c.c. in ousands.	Number of per c.c. thousand		Number of per c.c. thousand	cells in s.
-	1.		600	4240			
	2.	8	700	3800			
	3.	en pinera v	660	4600			
£	4.	1.6.4	600	4000) · · ·		2
1	5.		600	4500		_	
	6.	·······	400	2900		· · · · ·	a.
	7.		700	4500			
	8.	· · · · · · · · · · · · · · · · · · ·	100	840	.1 11		
		AL . A.	800	3600			
1	10.		600	2720			
Č.,	II.	3	900	2126	1.54 3.5	11	
11	12.		500	1600		560	
	13.	1. A.	· 650	6050	*	440	
÷.,	. 14.		780	4650	the second se		20020
	15.		225	1204	19 C C C C C C C C C C C C C C C C C C C	350	
	16.		600	2060		450	9
	17.	instrain.	400	2600		-530	£.*1.
	18.		700	3200		590	• • • •
	19.		100	1700		230	-

VITAMIN C ON THE GROWTH OF MICRO-ORGANISMS

	Wit	hout ascorbic acid.	With ascorbic acid 0.4 mg.	With ascorbic acid (oxidised) 0.4 mg.
	Expt. no.	Number of cells per c.c. in thousands.	Number of cells per c.c. in thousands.	Number of cells per c.c. in thousands.
	1.	660	4000	
2	2.	700	3200	
	3.	400	6600	
	4.	1380	3600	
	5-	900	2700	-
	6.	800	3600	
ę.,	7.	1000	4600	_
	8.	1200	4000	4 <u>21.313</u>
2.	9.	600	4240	
à.	10.	700	3800	-
-	II.	660	4000	450
	12.	1400	4500	1250
100	13.	600	2940	840
5.	14.	400	1050	450
80 J	· 15.	700	3200	610

Effect of ascorbic acid on the growth of Saccharomyces ellipsoideus in 20 c.c. culture (after 72 hours)

bi Similar experiments with glutathione and cysteine were carried out and gave similar results as with ascorbic acid (Table VII).

TABLE VII. To be Effect of ascorbic acid, glutathione, cysteine on the

growth of Saccharomyces

Figures indicate no. of cells per c.c. in thousands.

no.		Control	0.4 20 C	athione mg. in .c. cul- ure.	Cysteine 0.4 mg. in 20 c.c. cul- ture.	Ascorbic acid 0.4 mg. in 20 c.c. culture.
A REAL	t) -	-600		3210	1570	4250
100 A.S.	3140. 0	225	•	760	500	1250
Saccharomyces	-	400		1850	900	2900
cerevisiæ.		. 100		1250	450	2000
		500		3200	1250	3600
		400	· · · ·	800	580	1050
		660		2500	1070	4000
Saccharomyces		800		2800	1200	3600
ellipsoideus.		700		2500	1500	3800
	lan a	600		2100	1350	3000

Ascorbic acid 4:0 mg. 0.353 0.418 0.326 0.304 0.230 0.286 0.280 0.243 9 0.208 0 0.221 3 0.188 0 0.198 56 markedly is always

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scorbic acid d) 0.4 mg. of cells in c.c. usands. _ ---_ -.... 560 440 840 350 450 530 590

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Parallel estimations of the vitamin C content of the cells have shown that it increases with the increasing growth which will be seen from Table VIII.

TABLE	VIII.

Wt. of ascorbic acid (mg.) forme	d in 200 c.c. Saccharom	yces culture
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•	72 hoi	urs old	culture.		120	hours	old cultur	æ.
8 3)	Control	Glutathione 4.0 mg.	Cysteine 4.0 mg.	Ascorbic acid 4.0 · mg.	Control	Glutathione 4.0 mg.	Cysteine 4.0 mg.	acid 4.0 mg.
	0.025	0.059	0.046	0.186	0.062	0.188	0.139	0.386
S. cervisiæ.	0.038	0.078	0.062	0.223	0.091	0.268	0.198	0.448
	0.032	0.081	0.049	0.195	0.087	0.244	0.178	0.462
	0.072	0.146	0.120	0.296	0.188	0.264	0.203	0.524
S. ellipsoideus.	0.048	0.078	0.096	0.136	0.125	0.185	0.164	0.329
	0.104	0.160	0.129	0.208	0.230	0.319	0.288	0.582

THE EFFECT OF VITAMIN C ON THE GROWTH OF BACTERIA

The proliferation of bacteria like B. subtilis, B. typhosus, B. coli, Aerobacter aerogenes, Staphylococcus aureus, Streptococcus hæmolyticus and B. diphtheria, appeared to be inhibited instead of stimulated by ascorbic acid.

In these cases also (except with the last two bacteria mentioned above) ascorbic acid of strength 1/50,000 was found to give the best inhibitory result. Ascorbic acid of higher dilution also had inhibitory action (Table IX).

TABLE IX.

Effect of ascorbic acid on the growth of different bacteria in different concentrations.

	Concentration of ascorbic acid									
6 ¹⁷	0	1/10,000	1/25,000	1/50,000	1/75,000	1/100,000				
	757				190	569				
B. subtilis.	1136				190	757				
	379					190				
	1373				229	915				
	686				170	458				
B. typhosus.	915	<u> </u>			229	686				
	1543				229	1373				
	379	-				190				
Staphylococcus	379	. —	-		slight	190				
aureus.					turbidity					
	758		-		190	569				

Figures indicate no. of cells per c.c. in millions.

*In this and subsequent Tables (---) indicates no recognisable growth.

B. typhosus.

Staphylococc

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B. coli.

B. subtilis.

VITAMIN C ON THE GROWTH OF MICRO-ORGANISMS

Glutathione and cysteine in concentrations of 1/50,000 also have some inhibitory effect on the growth of the bacteria studied (Table X).

TABLE X. Figures indicate no. of cells per c.c. in millions.

	Control.	Glutathione.	Cysteine.	Ascorbic Acid.
	757	23 7/	190	
	379			
	379			
	757	10 <u></u> 0	379	
	379	()	_	
	1136	379	379	
B. coli.	757	190 ·	379	
	379	1975 - 19		
	757			
	1136	379	379	
	379			_
	379	· · · · · · · · · · · · · · · · · · ·		
	757	-		<u> </u>
	1127			
	1137 758	190	379	
Staphylococcus aureus.		-	190	
suphylococcus auteus.	570			
	758		190	·
	379		<u> </u>	
	1327	570	570	
	458		, <u>11</u> ,	
	915	229	229	
(1)1	915	458	458	· · · · · · · · · · · · · · · · · · ·
B. typhosus.	686	458	458	_
	915	229 (below)	229	
	458			
	1373	458	458	
8.24	379		<u> </u>	
	379			
	757		379	
	1136	379	379	
	757	190	379	
	379	_		
	1136	379	759	
8	379	575	139	_
	, 757		379	_
			5/9	
	757			N
B. subtilis.	757	270	750	
B. subtilis.	1136	379	759	—
B. subtilis.	1136 757	379	759	. —
B. subtilis.	1136 757 379	379	759	_
B. subtilis.	1136 757 379 379	379 	759	
B. subtilis.	1136 757 379 379 759			
B. subtilis.	1136 757 379 379 759 1136	379 — — — 379	759 	
B. subtilis.	1136 757 379 379 759			

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ture	
Ascorbic	acid 4.0 mg.
9	0.386
8	0.448
78	0.462
03	0.524
б4	0.329
88	0.582

B. coli, yticus and orbic acid. ned above) inhibitory Table IX).

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	23
1	
	1/100,000
	569
	757
	190
1	915
	458
	686
	1373
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	569

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· · · · · · · · · · · · · · · · · · ·	757	<u>, iz</u> 11, i	1			
A 60 (911) 111 141	379	_				
	379	· -		100		
17 - 78 h =	1136	_	190		—	
	379				—	
122	379	<u></u>			-	
(i) - 43	379	n				
1. A A A A A A A A A A A A A A A A A A A	379					
		_	379			
Aerobacter	757	190	379			
aerogenes.	1136	190				
	379		· · ·			
	379	_			<u> </u>	10.0
(4) (4)	757 1136	379	379			
(* *)	379					
a.); a	757					
44 K)	1136	379	379		-	
	757		379			

In much lower concentrations of vitamin C viz. in 1/100,000, there is no inhibitory effect, but there is no stimulating effect either. In this respect the fungi therefore behaved very differently from the bacteria.

Kodama and Kojima (10) have also observed an inhibitory effect of vitamin C on the growth of *Staphylococcus* cultures but Farber states that he found a stimulating action of vitamin C on the growth of *Staph. aureus*. We, therefore, reinvestigated the question by using the casein digest medium of Farber and confirmed our finding obtained with Reader's medium regarding the inhibitory effect of vitamin C on *Staph. aureus* (Table XI). The casein digest medium used by us was as follows:

Tryptic digested casein 1.0%, NaCl 1.0%, K2HPO, 0.2%, water 100 c.c., pH adjusted to 7.2-7.4.

TABLE XI.

Effect of ascorbic acid on the growth of Staph. aureus

Figures indiciate no. of cells per c.c. in millions

Reader's medium.		•	Tryptic digested casein.		
Control.	1/50,000	Control	1/50,000	1/20,000	
758		758	678	379	
		1516	1516	678	
379 285		-1895	1516	758	
10.000		758	678	190	
190 379	. —	1137	758	379	

THE EFFECT OF VITAMIN C ON THE GROWTH OF B. DIPHTHERIA AND STREPTOCOCCUS HÆMOLYTICUS

As the two organisms *B. diphtheria* and *Streptococcus hæmolyticus* did not grow well in Reader's medium, the following medium was used?

Beef extract	 0.3%	11	_1 <u>r</u>	
Peptone	 1%		14.1	. R.
Sodium chloride	 0.5%	÷	7.2	2.1
Glucose	 0.5%			
Glycerine	 2.0%			1912.1.4
and the constraint and the second second	 		2.22	000 100

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With these two organisms also vitamin C had an inhibitory effect in a concentration of 1/20,000. With 1/50,000 concentration the inhibitory effect is somewhat less (Table XII).

TABLE XII.

The effect of ascorbic acid on the growth of B. diphtheria and Streptococcus hæmolyticus

Figures indicate no. of cells per c.c. in millions

			0		1/50,00	00	1/20,000	>
			403	1. 1.	202			
÷.,		340 B	605	s	403			
	B. diphtheria.	242 - 12	1210	i	403			
1.		a	, 202		· · · ·	304		
	194 g	S. 8 -	806		202		_	
i detta 1. genere			1613	<i>.</i>	1210		202	
2.2		1	994		331			
1	Streptococcus		663	8 ¹⁹ 10	-331	· • •		hi al
	hæmolyticus.	-	165		. —			
have the			331	12	· ·		—	
		8	994		165		1	
3.	2		1157		663	14		
Sec. 1			331		165		· · · <u>· · ·</u> · · ·	1.12

EFFECT OF NICOTINIC ACID, ADRENALIN AND INSULIN ON THE GROWTH OF B. SUBTILIS, B. DIPHTHERIA AND SACCH. CEREVISIÆ

It was considered of interest to investigate what effect another vitamin like nicotinic acid and hormones available in the pure form like adrenalin and insulin would have on the growth of typical bacteria and fungi. For bacteria, *B. subtilis* and *B. diphtheria* were used, the former grown in Reader's medium and the latter in the medium indicated above. Sacch. cerevisiæ was used as a typical fungus and it was grown in Reader's medium. The addenda were all used in 1/50,000 concentration, except in the case of *B.* diphtheria, where it was used in 1/20,000 concentration. The results (Table XIII) shows that in all cases there was a stimulation of the growth of the organisms, both bacteria and fungi. In this respect these addenda differ from ascorbic acid which in the same concentration

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bitory effect of arber states that f Staph. aureus. n digest medium nedium regarding XI). The casein

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ted	casein.
	1/20,000
	379
	678

758

190

stimulates the growth of fungi only. Hormone preparations like Antuitrin S, Antuitrin G and Pitressin (Parke Davis & Co.) had also a stimulating effect on the above micro-organisms in a dilution of 1/50,000, but no significance can be attached to these results as the hormones concerned have not yet been obtained in a chemically pure state. The effect of adrenalin, insulin and nicotinic acid deserves further investigation in order to find whether it is merely due to provision of extra nitrogenous material as a nutriment or due to some specific stimulation.

EFFECT OF ASCORBIC ACID ON THE GROWTH OF A SMALL INOCULUM OF SACCH. CEREVISIÆ AND STAPH. AUREUS

It was considered desirable to carry out two typical experiments, one with a fungus and another with a bacterium, with a very small and definite number of cells in the inoculum to start with and counting the cells at the end of a stated period of incubation. These results obtained with Sacch. cerevisiæ, Sacch. ellipsoideus and Staph. aureus are given in Tables XIV (a) and (b); they confirm the previous results regarding the action of ascorbic acid on bacteria and fungi.

	Figures	indicat	e no.	of	cells per	C.C. 1	n millioi	ns	
	Control.	Ascorbic	Acid. Antuitrin	s.	Antuitrin G.	Adrenalin chloride	Nicotinic acid.	Insulin.	Pitressin.
	3	79 -		379	757	569	757	1136	1136
		90 -		379	379	379	757	757	1136
		79 -		757	757	379	569	757	757
B. subtilis.		79 [.] -		379	757	757	757	569	757
J. Subtins.		79 -		757	757	757	1136	1136	1136
		79 -		757	569	757	757	569	569
		90 -		379	379	757	379	757	757
		90 -		379	379	379	757	757	757
		02 -	_	403	403	403	806	1210	1210
		.03 -		605	806	806	1613	1210	1613
			202	806	1210	806	1210	1613	2016
B. diphtheria.		02 -		403	605	605	806	403	806
D. dipititeria.		.03 -	_	806	1210	806	806	1210	1613
		202 -		403	806	605	403	403	806
		202 -		403	806	403	806	806	806
		202 -	_	605	806	403	806	403	1210
- 77	Figures	indicat	e no.	of	cells per	c.c. in	thousa:	nds	
	1000		900	656	1224	800	1150	-1500	1800
			250	608		732	800	900	1100
			000	672	664	664	925	1100	1560
Sac. cerevisiæ			250	696	774	724	1025	1500	2250
Sac. Cerevisia		1999 - 19 1 9	900	900		925	1225	1500	2000
			250	560		600	725	780	959
¥			600	760		825	1300	1625	2500

TABLE XIII. indicate no. of cells per c.c. in millions

> enjuvice. Although to explore th important fac aspects of its investigation of might throw s Some curious 1/50,000, vita studied but an of vitamin C is At the same vitamin C, so growth, given it is added in growth of the c of these and c vitamin C by t of vitamin C, and cysteine w to a less marke

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REPORT.

Sacch. cerevis

Sacch. ellipso

Staph. au The cocci

VITAMIN C ON THE GROWTH OF MICRO-ORGANISMS

olo	1.435	TABLE XI	V (a)	1. S. S. S. S. S. S.	
bot Sin Equ	Number of cells per 0.1 c.c. inoculum.	Number of	cells per c.c.	of culture in	thousands.
$z_{i} = \frac{1}{2} \frac{1}$		Control.	Glutathione. 1/50,000	Cysteine.	Ascorbic acid. 1/50,000
65					
n17.	50	600	3240	1445	4050
Sacch. cerevisiæ.	25	400	925	580	1625
Acres 1	23	225	625	500	1050
Elin	20	400	850	575	1050
Sacch. ellipsoideus.	32	660	2500	1070	4000
hedt -	15	275	600	400	800
D-52		TABLE XI	V (b)	×** •	
Nux No.	o. of cells 1 c.c. inoculu		of cells per 72 hours	c.c. in million incubation.	s after
			rol (without orbic acid).	Wit	h ascorbic acid.
Staph. aureus.	180 240 115		450 796 360		<u> </u>

TABLE XIV (a)

The cocci were counted by proportionate counting method.

DISCUSSION

Although considerable work has been carried out in various laboratories to explore the mechanism of action of vitamin C in the body and some important facts have been discovered, a connected picture of the various aspects of its rôle in life is not yet possible. It was considered that an investigation on the effect of vitamin C on primitive unicellular organisms might throw some light on the fundamental mode of action of vitamin C. Some curious results have been obtained. Thus in concentrations of 1/50,000, vitamin C has a stimulating action on the growth of the fungi studied but an inhibitory action on that of bacteria. If the concentration of vitamin C is increased to 1/10,000, the growth of the fungi is also inhibited. At the same time the fungi themselves have the power to synthesise vitamin C, so that it would seem that although vitamin C is needed for their growth, given the time, they can themselves synthesise it. If, however, it is added in a preformed condition at the start of the incubation, the growth of the cells is hastened, though in course of 5 days or so the growth of these and of the controls is largely equalised owing to the synthesis of vitamin C by the latter. In trying to investigate the specificity of this action of vitamin C, other naturally occurring reducing substances like glutathione and cysteine were also found to stimulate the action of vitamin C though to a less marked degree. Similarly these reducing agents also inhibited the

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(a) bic

1210

1800

1100

2250

2000

950

2500

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G. C. DAS GUPTA AND B. C. GUHA

growth of certain bacteria like vitamin C. It would thus seem probable that the action of vitamin C with reference to the growth of fungi is associated with some sort of reducing action and that the agent concerned in the natural process of proliferation is vitamin C, whose redox potential and other characteristics are perhaps more suitable for the complicated biochemical mechanism underlying the cell-division of the fungi than glutathione or cysteine, which simulates the action of the vitamin to some extent. It is possible that the same properties of vitamin C adversely affect the growth of bacteria in 1/50,000 concentration and that of fungi in 1/10,000 concentration. Beyond this, it is not possible to speculate at the present stage.

SUMMARY

(I) Ascorbic acid has got a stimulating effect on the growth of the following fungi-Aspergillus niger, Asp. oryzæ, Asp. flavus, Sach. cerevisiæ, S. ellipsoideus, in a synthetic medium in a concentration of 1/50,000 and an Se . inhibitory effect in a concentration of 1/10,000:

(2) Ascorbic acid has got an inhibitory effect on the growth of the following bacteria-B. subtilis, B. typhosus, B. coli, Aerobacter aerogenes, Staphylococcus aureus, Streptococcus hæmolyticus and B. diphtheria, in a synthetic medium in a concentration of 1/50,000 except in the last two cases, which require a concentration of 1/20,000. In concentration of I/100,000 vitamin C has neither any stimulating nor any inhibitory action on these bacteria.

(3) The above effect of vitamin C is simulated by other reducing substances like glutathione and cysteine though to a less marked degree. (4) In the case of certain fungi, it has been found that they can synthesise their own ascorbic acid. The addition of ascorbic acid at the start of the incubation apparently produces stimulation of growth during the first two or three days, which later becomes roughly equal to that of the controls owing to the latter synthesising their own vitamin C. (5) Nicotinic acid, adrenalin and insulin have a stimulating action on the growth of bacteria and fungi in 1/50,000 concentration but significance regarding specificity may not be attached to these results at present.

Our thanks are due to the Indian Research Fund Association for a research grant."

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