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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.

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 acid					
0	1/10,000	1/25,000	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

Effect of ascorbic acid on the growth of S. cerevisiæ in different concentrations.

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

Concentration of ascorbic acid					
0	1/10,000	1/25,000	1/50,000	1/75,000	1/100,000
600	250	450	4250	2400	1200
100	—	60	2150	800	275
225	75	130	3000	1700	500

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.

TABLE III

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

	72 hours old culture			120 hours old culture		
	Without ascorbic acid	With ascorbic acid 0.4 mg.	With ascorbic acid (oxidised) 0.4 mg.	Without ascorbic acid	With ascorbic acid 0.4 mg.	With ascorbic acid (oxidised) 0.4 mg.
Asp. niger.	0.0128	0.0222	—	0.0320	0.0304	—
	0.0168	0.0261	—	0.0356	0.0369	—
	0.0098	0.0179	—	0.0250	0.0284	—
	0.0072	0.0139	—	0.0235	0.0229	—
	0.0130	0.0302	—	0.0342	0.0359	—
	0.0087	0.0127	—	0.0223	0.0235	—
	0.0090	0.0158	—	0.0240	0.0252	—
	0.0153	0.0324	0.0129	0.0346	0.0360	0.0353
	0.0122	0.0335	0.0132	0.0348	0.0382	0.0329
	0.0104	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
Asp. oryzæ.	0.0085	0.0234	0.0079	—	—	—
	0.0125	0.0402	0.0140	—	—	—
	0.0042	0.0180	0.0030	—	—	—
	0.0027	0.0098	0.0037	—	—	—
	0.0011	0.0063	0.0020	—	—	—
	0.0032	0.0084	0.0027	0.0285	0.0301	0.0278
	0.0015	0.0039	0.0020	0.0164	0.0153	0.0149
	0.0044	0.0140	0.0035	0.0364	0.0410	0.0338
Asp. flavus.	0.0014	0.0031	—	—	—	—
	0.0017	0.0049	—	—	—	—
	0.0012	0.0042	—	—	—	—
	0.0009	0.0027	—	—	—	—
	0.0015	0.0039	—	—	—	—
	0.0023	0.0052	—	—	—	—
	0.0010	0.0034	0.0017	0.0072	0.0085	0.0063
	0.0020	0.0057	0.0024	0.0123	0.0109	0.0132
	0.0008	0.0030	0.0015	0.0098	0.0104	0.0083
	0.0018	0.0043	0.0012	0.0102	0.0115	0.0121

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

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 IV.

Figures indicate wt. of Aspergillus in g. in 20 c.c. culture.

	72 hours old culture				120 hours old culture.			
	Control	Glutathione 0.4 mg.	Cysteine 0.4 mg.	Ascorbic acid 0.4 mg.	Control	Glutathione 0.4 mg.	Cysteine 0.4 mg.	Ascorbic acid 0.4 mg.
	0.0098	0.0153	0.0130	0.0250	0.0255	0.0263	0.0272	0.0285
	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
	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
	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
	0.0044	0.0096	0.0084	0.0134	0.0186	0.0170	0.0153	0.0206
	0.0035	0.0045	0.0039	0.0118	0.0179	0.0163	0.0140	0.0198
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-dichlorcphenol indophenol in the usual way.

TABLE V.

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

	72 hours old culture.				120 hours old culture.			
	Control	Glutathione 4.0 mg.	Cysteine 4.0 mg.	Ascorbic acid 4.0 mg.	Control	Glutathione 4.0 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
	0.160	0.198	0.179	0.287	0.432	0.349	0.324	0.418
	0.153	0.176	0.164	0.210	0.353	0.276	0.246	0.326
	0.124	0.146	0.130	0.186	0.298	0.240	0.221	0.304
	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

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 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.	600	4240	—
2.	700	3800	—
3.	660	4600	—
4.	600	4000	—
5.	600	4500	—
6.	460	2900	—
7.	700	4500	—
8.	100	840	—
9.	800	3600	—
10.	600	2720	—
11.	900	2126	—
12.	500	1600	560
13.	650	6050	440
14.	780	4650	840
15.	225	1204	350
16.	600	2060	450
17.	400	2600	530
18.	700	3200	590
19.	100	1700	230

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

	Without 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.	700	3200	—
3.	400	6600	—
4.	1380	3600	—
5.	900	2700	—
6.	800	3600	—
7.	1000	4600	—
8.	1200	4000	—
9.	600	4240	—
10.	700	3800	—
11.	660	4000	450
12.	1400	4500	1250
13.	600	2940	840
14.	400	1050	450
15.	700	3200	610

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

TABLE VII.

Effect of ascorbic acid, glutathione, cysteine on the growth of Saccharomyces

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

	Control	Glutathione 0.4 mg. in 20 c.c. culture.	Cysteine 0.4 mg. in 20 c.c. culture.	Ascorbic acid 0.4 mg. in 20 c.c. culture.
	600	3210	1570	4250
	225	760	500	1250
Saccharomyces cerevisiæ.	400	1850	900	2900
	100	1250	450	2000
	500	3200	1250	3600
	400	800	580	1050
	660	2500	1070	4000
Saccharomyces ellipsoideus.	800	2800	1200	3600
	700	2500	1500	3800
	600	2100	1350	3000

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.) formed in 200 c.c. Saccharomyces culture

	72 hours old culture.				120 hours old culture.			
	Control	Glutathione 4.0 mg.	Cysteine 4.0 mg.	Ascorbic acid 4.0 mg.	Control	Glutathione 4.0 mg.	Cysteine 4.0 mg.	Ascorbic 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.

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

	Concentration of ascorbic acid					
	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	—	—	—	229	686
	1543	—	—	—	229	1373
	379	—	—	—	—	190
Staphylococcus aureus.	379	—	—	—	slight turbidity	190
	758	—	—	—	190	569

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

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.
B. coli.	757	—	190	—
	379	—	—	—
	379	—	—	—
	757	—	379	—
	379	—	—	—
	1136	379	379	—
	757	190	379	—
	379	—	—	—
	757	—	—	—
	1136	379	379	—
	379	—	—	—
	379	—	—	—
	757	—	—	—
Staphylococcus aureus.	1137	190	379	—
	758	—	190	—
	570	—	—	—
	758	—	190	—
	379	—	—	—
	1327	570	570	—
B. typhosus.	458	—	—	—
	915	229	229	—
	915	458	458	—
	686	458	458	—
	915	229 (below)	229	—
	458	—	—	—
	1373	458	458	—
B. subtilis.	379	—	—	—
	379	—	—	—
	757	—	379	—
	1136	379	379	—
	757	190	379	—
	379	—	—	—
	1136	379	759	—
	379	—	—	—
	757	—	379	—
	757	—	—	—
	1136	379	759	—
	757	—	—	—
	379	—	—	—
	379	—	—	—
	759	—	—	—
	1136	379	379	—
	379	—	—	—
	1136	759	759	—

TABLE X (*contd.*)

	757	—	—	—
	379	—	—	—
	379	—	—	—
	1136	—	190	—
	379	—	—	—
	379	—	—	—
	379	—	—	—
	379	—	—	—
Aerobacter	757	—	379	—
aerogenes.	1136	190	379	—
	379	—	—	—
	379	—	—	—
	757	—	—	—
	1136	379	379	—
	379	—	—	—
	757	—	—	—
	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%, K_2HPO_4 0.2%, water 100 c.c., *p*H 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
379	—	1516	1516	678
285	—	1895	1516	758
190	—	758	678	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%
Peptone	... 1%
Sodium chloride	... 0.5%
Glucose	... 0.5%
Glycerine	... 2.0%

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

	Concentration of ascorbic acid		
	0	1/50,000	1/20,000
B. diphtheria.	403	202	—
	605	403	—
	1210	403	—
	202	—	—
	806	202	—
	1613	1210	202
Streptococcus hæmolyticus.	994	331	—
	663	331	—
	165	—	—
	331	—	—
	994	165	—
	1157	663	—
	331	165	—

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

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.

TABLE XIII.

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

	Control.	Ascorbic Acid.	Antuitrin S.	Antuitrin G.	Adrenalin chloride	Nicotinic acid.	Insulin.	Pitressin.
	379	—	379	757	569	757	1136	1136
	190	—	379	379	379	757	757	1136
	379	—	757	757	379	569	757	757
B. subtilis.	379	—	379	757	757	757	569	757
	379	—	757	757	757	1136	1136	1136
	379	—	757	569	757	757	569	569
	190	—	379	379	757	379	757	757
	190	—	379	379	379	757	757	757
	202	—	403	403	403	806	1210	1210
	403	—	605	806	806	1613	1210	1613
	403	202	806	1210	806	1210	1613	2016
B. diphtheria.	202	—	403	605	605	806	403	806
	403	—	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
Figures indicate no. of cells per c.c. in thousands								
	320	2900	656	1224	800	1150	1500	1800
	280	1250	608	832	732	800	900	1100
	560	3000	672	664	664	925	1100	1560
Sac. cerevisiæ.	600	4250	696	774	724	1025	1500	2250
	400	2900	900	1150	925	1225	1500	2000
	225	1250	560	700	600	725	780	950
	500	3600	760	1050	825	1300	1625	2500

TABLE XIV (a)

	Number of cells per 0.1 c.c. inoculum.	*Number of cells per c.c. of culture in thousands.*			
		Control.	Glutathione. 1/50,000	Cysteine. 1/50,000	Ascorbic acid. 1/50,000
Sacch. cerevisiæ.	50	600	3240	1445	4050
	25	400	925	580	1625
	23	225	625	500	1050
Sacch. ellipsoideus.	20	400	850	575	1050
	32	660	2500	1070	4000
	15	275	600	400	800

TABLE XIV (b)

	No. of cells per 0.1 c.c. inoculum.	*No. of cells per c.c. in millions after 72 hours' incubation.*	
		Control (without ascorbic acid).	With ascorbic acid.
Staph. aureus.	180	450	—
	240	796	—
	115	360	—

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

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

(1) 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 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 1/100,000 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.

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