Zinc in the Metabolism of a Strain of *Aspergillus niger*

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SUMMARY: Cultures of *Aspergillus niger* partially deficient in zinc produce much larger quantities of acid per unit of glucose used than do normal cultures. This fact has been the basis of the current suggestion that zinc plays a part in the utilization of carbohydrate by fungi. The experiments described here show that partial deficiencies, not only of zinc but also of iron, copper, manganese, phosphorus, magnesium and potassium all had a similar effect on the production of acid by a strain of *A. niger*. It would appear that the effect of zinc on the way in which carbohydrate is utilized is not a specific effect and indicates no more about the role of zinc than about the role of any other inorganic nutrient.

It has been suggested (Foster, 1939; Foster & Waksman, 1939; Foster, 1949) that zinc plays a part in the utilization of carbohydrate by fungi because under conditions of partial zinc deficiency less carbohydrate is oxidized completely and a greater proportion is diverted to form organic acids (Butkewitsch & Orlow, 1922; Bortels, 1927; Waksman & Foster, 1938; Foster, Woodruff & McDaniel, 1943; Foster & Waksman, 1939; Shu & Johnson, 1948). Foster & Waksman (1939) concluded that zinc has a specific effect on carbohydrate metabolism.

Experiments on the metabolism of *A. niger* when partially deficient in several other inorganic nutrients as well as zinc are described in this paper. Certain of these findings have already been reported briefly elsewhere (Chesters & Rolinson, 1950).

METHODS

Surface cultures of a strain of *A. niger* isolated in this laboratory were grown in 100 ml. wide-mouthed conical Hysil or Pyrex flasks. The flasks were cleaned with chromic-sulphuric acid mixture, washed six times with tap water, twice with distilled water and once with double-distilled water prepared in Pyrex glass stills. Each flask was closed with a plug of non-absorbent cotton-wool wrapped in muslin. The flasks were sterilized in the autoclave at 15 lb./sq.in. for 15 min. and 20 ml. of medium used per flask. The basal culture medium contained (w/v): glucose, 5%; NH_4NO_3 , 0.3%; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05%; KH_2PO_4 , 0.05%; Fe, 0.5 p.p.m.; Zn, 0.5 p.p.m.; Cu, 0.05 p.p.m. and Mn, 0.05 p.p.m. This medium was freed from traces of zinc, iron, copper and manganese by shaking 750 ml. with two successive quantities of approximately 0.1 g. of 8-hydroxyquinoline (oxine) and 5 ml. chloroform in a 1 l. Pyrex separating funnel at pH 7.4 and then again at pH 5.2. Nitric acid (Analar) was used to adjust the pH. After each extraction with oxine the solution was washed twice with 5 ml. chloroform. The medium was finally washed three times with 5 ml. chloroform and once with 10 ml. to remove all traces of oxine. B.P. grade

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chloroform was found to be satisfactory. Any chloroform remaining in the medium was removed during autoclaving. After purification, each trace element was then added in the quantity required, using stock solutions prepared from A.R. grade reagents and double-distilled water.

Inoculation was carried out by transferring a mass of spores from a 4-day culture and distributing them on the surface of the medium with a sterile platinum wire. The cultures were incubated at 28°.

Mycelial weights were determined by filtering the pellicles on tared filter-papers in a Büchner funnel and weighing the papers together with the mycelium after drying overnight at 80°.

RESULTS

The effect of zinc deficiency on the production of acid

Cultures containing graded amounts of zinc were harvested at 4 days and analysed. The results are shown in Fig. 1. Maximum acid accumulation occurred when zinc was partially deficient. Growth at this point was approxi-

Fig. 1. The effect of zinc deficiency on the growth (□—□), glucose uptake (△—△) and acid production (○—○) of *A. niger* after 4 days' incubation.

mately 70 % of the maximum. With larger quantities of zinc, less acid accumulated and more growth was made for the amount of glucose used. Fig. 2 shows the result of analyses made at different times during the fermentation by partially deficient and normal cultures. These results show that even the normal cultures do produce a considerable amount of acid but that at 4 days it has been almost entirely metabolized by the fungus. Acids are produced until the glucose becomes exhausted, whereupon the acids themselves are utilized as carbon sources. At 4 days, most of the acid in the normal cultures has been utilized, whereas in the partially deficient cultures the accumulated acid is almost entirely intact. In this respect, analyses at 4 days give an exaggerated account of the effect of zinc on the formation of acid. Nevertheless, more acid is produced by cultures partially deficient in zinc than by normal cultures.

The effect of deficiencies of certain other inorganic nutrients on acid metabolism

Cultures containing graded amounts of iron, copper, manganese, magnesium, potassium, or phosphorus were harvested at 4 days and analysed. The results are shown in Tables 1 and 2. It will be seen that partial deficiencies of iron,

Fig. 2. The effect of zinc deficiency on the course of metabolism of *A. niger*. Full lines, 1.0 p.p.m. of zinc present in the medium. Broken lines, 0.15 p.p.m. of zinc present in the medium. Δ , glucose uptake; $\circ$, acid production.

Table 1. *The effect of zinc, iron, copper and manganese on acid production by a strain of Aspergillus niger*

Metal in medium (p.p.m.)	Zinc		Iron		Copper		Manganese	
	Mycelial wt. (g.)	ml. 0.1 N acid	Mycelial wt. (g.)	ml. 0.1 N acid	Mycelial wt. (g.)	ml. 0.1 N acid	Mycelial wt. (g.)	ml. 0.1 N acid
0	—	—	—	—	0.250	7.5	0.29	33.5
0.005	—	—	—	—	0.310	33.0	0.359	18.3
0.01	—	—	0.135	15	0.322	24.7	0.348	17.0
0.02	—	—	0.197	30	—	—	0.374	15.2
0.05	0.150	10.1	0.264	49.6	0.362	8.2	0.372	13.4
0.1	0.230	29.9	0.328	43.7	—	—	—	—
0.2	0.351	16.0	—	—	—	—	—	—
0.5	0.382	5.0	0.367	13.0	0.339	4.0	—	—

copper, manganese, magnesium, potassium or phosphorus have the same effect on the production of acid as a partial deficiency of zinc. Certain of these cultures were analysed after *c.* 48 hr. when the accumulation of acid in the normal cultures was at a maximum. As shown above, a more accurate account of the formation of acid is given at this time. These results are shown in Table 3. It will again be seen that for each metal, more acid is produced per unit of glucose used when the culture is partially deficient.

Table 2. *The effect of phosphorus, magnesium and potassium on acid production by Aspergillus niger*

(When the concentration of potassium dihydrogen phosphate was varied the concentration of potassium was held constant by the addition of potassium chloride. In the case of potassium chloride the potassium dihydrogen phosphate in the medium was replaced by sodium dihydrogen phosphate.)

Con- centration (g./100 ml.)	Potassium dihydrogen phosphate		Magnesium sulphate		Potassium chloride	
	Mycelial wt. (g.)	ml. 0.1 N acid	Mycelial wt. (g.)	ml. 0.1 N acid	Mycelial wt. (g.)	ml. 0.1 N acid
0.002	—	—	—	—	0.112	6.6
0.005	0.100	—	0.172	7.7	0.219	9.7
0.01	0.167	6.9	0.312	20.6	0.318	25.1
0.02	0.326	33.3	0.336	4.9	0.339	6.4
0.03	0.351	18.0	0.344	3.0	0.337	1.8
0.05	0.390	4.6	—	—	—	—

Table 3. *The effect of deficiencies of iron, copper and manganese on acid production after 50 hr. incubation*

Nutrient	Concentration (p.p.m.)	ml. 0.1 N acid pro- duced/g. glucose used after 50 hr. incubation
Iron	0.05	34
	0.5	17.2
Copper	0.01	37.8
	0.5	25.4
Manganese	0.0	35.0
	0.05	27.8

The results of Tables 1–3 show that although a deficiency of zinc results in the formation of larger quantities of acid, this effect is by no means peculiar to zinc. Partial deficiencies of all the inorganic nutrients examined have the same effect. It is not clear how a deficiency of zinc or any other inorganic nutrient affects the manner in which glucose is metabolized.

The effect of zinc on the amount of glucose used

Cultures were grown in a medium containing 0.15 p.p.m. of zinc; at this concentration, growth was 75 % normal. After 3 days' growth, the medium was removed aseptically and replaced with fresh sterile medium. In one set of flasks this medium contained 10 p.p.m. of zinc, in a second set 0.5 p.p.m. and in a third set, no zinc. The NH_4NO_3 was omitted from the replacement medium so as to diminish subsequent growth of the mycelial felts. The cultures were analysed at intervals and the results are shown in Table 4.

Table 4. *The effect of zinc on the uptake of glucose*

Con- centration of zinc (p.p.m.)	Glucose used (g.)			Mycelial wt. (g.) 72 hr.	Acid produced (ml. 0.1 N)	
	24 hr.	48 hr.	72 hr.		48 hr.	72 hr.
0	0.27	0.41	0.49	0.38	30.3	36.1
0.5	0.27	0.45	0.46	0.38	24.1	28.2
10.0	0.28	0.40	0.47	0.39	13.7	17.6

Although the presence of zinc altered the manner in which glucose was utilized, the metal had no significant effect on the amount of glucose used.

The effect of zinc on the utilization of the acids formed during the fermentation

Cultures containing 0.15, 0.2 and 2.0 p.p.m. of zinc in the medium were incubated, and the utilization of the acids formed during the early part of the fermentation was followed. The results are shown in Fig. 3. Although cultures

Fig. 3. The effect of zinc deficiency on the utilization of acids formed during growth. The concentrations of zinc in the media were 2.0 p.p.m. (●—●), 0.2 p.p.m. (○—○) and 0.14 p.p.m. (○---○).

partially deficient in zinc produce larger quantities of acid, such cultures are capable of utilizing the accumulated acids at the same rate as those cultures supplied with adequate amounts of zinc.

DISCUSSION

A deficiency of zinc undoubtedly affects the way in which glucose is metabolized. When zinc is partially deficient, more glucose is diverted to form organic acids and less is oxidized completely to carbon dioxide and water. From the point of view of liberating energy, this acid fermentation is a wasteful process and less growth is possible per unit of glucose used. Zinc therefore affects the 'economic coefficient' (mycelial weight/glucose used) of the organism. Javillier (1912*a, b*) referred to this as a physiological characteristic of the metal, and Foster (1939) and Foster & Waksman (1939) concluded likewise that zinc has a specific effect on carbohydrate metabolism. However, the results presented here show that iron, copper, manganese, magnesium, potassium and phosphorus as well as zinc, all have the same effect on acid production and on the efficiency of glucose utilization at least by this strain of *A. niger* and that these effects cannot be regarded as characteristic of a deficiency of zinc alone.

The conception that zinc has a specific effect on carbohydrate metabolism may be due to the relative ease with which zinc deficiencies in fungi can be

obtained. Culture media may be partially deficient in this metal without any special attempt to make them so. Iron, and especially copper and manganese on the other hand are usually present as impurities even in A.R. grade reagents in sufficient quantities for maximum growth, and the addition of these nutrients to such a medium would obviously have no effect.

It would appear from the results presented here that rather less is known of the precise role of zinc than the published work would seem to suggest. From all the work with fungi on the effect of zinc on the economic coefficient and on the accumulation of various organic acids, no more can be concluded about the role of zinc than about the role of any other inorganic nutrient. Experiments on the effect of zinc on carbohydrate metabolism show that the metal has no effect on the rate of glucose uptake nor on the rate at which the accumulated acids are subsequently metabolized.

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