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Zinc Uptake by *Neurospora*

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Among the minor constituents of living tissues zinc is one of great interest (see Lehman, 1939; Broh-Kahn & Mirsky, 1948; Warburg, 1948; Bertrand, 1948). It constitutes 0.33 % of carbonic anhydrase (Keilin & Mann, 1940), and it is a constituent of uricase which contains 1.3 % of zinc (Davidson, 1938; Holmberg, 1939) and possibly of other enzymes. It was found to be essential for growth and citric acid formation of *Aspergillus niger* (Bernhauer, 1928), for growth and fumaric acid production of *Rhizopus nigricans* (Foster & Waksman, 1939) and for growth and subtilin formation by *Bacillus subtilis* (Feeney, Lightbody & Garibaldi, 1947).

The presence of 0.2 mg. of zinc in the food of mice is stated by Bertrand (1948) to prolong the life of the animals from 2–3 weeks to 2–2.5 months.

Wassiljew (1935) demonstrated that the effect of zinc was not the same for biochemically different strains of *A. niger*, and he went so far as to suggest a characterization of *A. niger* strains based on their reactivity to zinc. Foster & Waksman (1939) interpret the function of zinc as catalyzing a more complete destruction of the glucose molecule with a consequent greater efficiency of energy utilization by the fungus.

In connexion with studies on the growth of *Neurospora crassa* we determined the amount of zinc necessary to obtain a maximum growth of that mould.

METHODS

Throughout the experiments mycelium from one spore was used. This was produced by crossing the two wild-type strains, nos. 25a and 1A of Beadle (1945). The culture solution was that described by Ryan, Beadle & Tatum (1943); it was autoclaved, and the cultures were treated under sterile conditions. Inocula were taken from cultures 4–5 days old.

Labelled zinc as chloride and varying amounts of non-radioactive zinc as sulphate were added to culture solutions at the start of the experiments. From the specific activities of the zinc present in the culture solution and in the mould, and the amount of zinc added to the culture solution, the zinc content of *Neurospora crassa* was calculated. We are much indebted to the Atomic Energy Commission of the U.S.A. and the Chief of the Isotope Branch, Dr Poul Aebersold, for supplying the zinc containing the radioactive isotope ^{65}Zn .

The activity of the ^{65}Zn taken up by *Neurospora* was less than 1 μcurie , and thus the radiation emitted could hardly produce any noxious action on the mould.

After centrifuging and thorough washing the *Neurospora* was dried at 105°, brought into solution by wet ashing, 5 mg. zinc as sulphate added as carrier, and the zinc precipitated with 8-hydroxyquinoline. The precipitate was filtered through a perforated aluminium dish covered by a layer of filter paper, and the dish placed under a Geiger-Müller counter of the type described by Madsen (1945). A sample of the active ZnCl_2 solution was treated in the same way. By comparing the activities of this 'standard' precipitate of known zinc content and that of the above-mentioned precipitates, the percentage of the zinc originally present in the culture solution which accumulated in the mould was calculated.

RESULTS

The results of some of our experiments, in which each *Neurospora* was grown in 150 ml. culture solution of varying zinc content for 5 days at 25°, are given in Table 1. This table and Fig. 1 show that the presence of 1 part of zinc in about 30,000 parts of dry mould is sufficient for an almost maximal growth, and the presence of 12.5 $\mu\text{g.}$ zinc in 150 ml. culture solution suffices to give that zinc content. It is advisable to add a somewhat larger amount of zinc to the culture solution, e.g. 200 $\mu\text{g./l.}$ as recommended by Ryan *et al.* (1943) or 450 $\mu\text{g./l.}$ as stated by Mitchell & Houlakan (1946).

Table 1. Amount of zinc taken up by *Neurospora*

Zinc in culture solution ($\mu\text{g.}$)	Wt. of mould (mg.)	Zinc ($\mu\text{g./mg.}$ dry mould)	Percentage of the ^{65}Zn content of culture solution present in 1 mg. dry mould
2.5	106.6	0.021	0.84
	106.9	0.013	0.50
	97.8	0.012	0.48
	90.0	0.014	0.55
Average	100.3	0.015	0.59
12.5	175.2	0.033	0.26
	169.7	0.033	0.26
	151.7	0.035	0.28
	144.3	0.040	0.32
Average	158.9	0.035	0.28
50.0	208.6	0.090	0.18
	178.8	0.11	0.21
	168.1	0.10	0.19
	166.7	0.11	0.22
Average	180.5	0.10	0.20
250.0	153.8	0.13	0.033
	172.3	0.12	0.043
	147.7	0.11	0.044
	161.9	0.14	0.055
Average	158.9	0.13	0.049
2000.0	186.3	0.30	0.015
	184.5	0.34	0.017
	158.3	0.38	0.019
	164.0	0.38	0.019
Average	168.3	0.35	0.018

Fig. 1. Effect of varying zinc content of the culture solution on the dry weight (○) and zinc content (×) of *Neurospora* after the lapse of 5 days.

Fig. 1 shows the very pronounced increase in weight of the mould, from 71 to 100 and 159 mg., after the addition of 2.5 and 12.5 mg. of zinc respectively to the culture solution. In experiments of shorter duration the effect of zinc on the increase in

weight was still more pronounced (Fig. 2). After 48 hr. 28 mg. were obtained in the absence of added zinc, while adding 30 $\mu\text{g.}$ of zinc to a 150 ml. culture produced 83 mg. of mould.

Fig. 2. Dry weight of *Neurospora* without (○) and after (×) addition of 30 $\mu\text{g.}$ of zinc.

After 5 hr., a 150 ml. culture solution containing 2.5 $\mu\text{g.}$ zinc produced 100.3 mg. and one with 12.5 $\mu\text{g.}$ zinc, an almost maximal weight of 158.9 mg. In the former case 1 mg. *Neurospora* contained 0.015 $\mu\text{g.}$ zinc; in the latter roughly twice that amount (0.035 $\mu\text{g.}$). An increase in the zinc content of the culture solution to 2000 $\mu\text{g.}$ did not increase the weight of *Neurospora* observed after the lapse of 5 days (Table 1). The capacity of the fungus to take up zinc was, however, not exhausted, as shown by the fact that an eightfold increase in the zinc concentration of the solution resulted in an almost threefold zinc uptake by the mould.

Feeney *et al.* (1947) determined the amount of zinc to be added to the culture solution in order to obtain maximal growth and maximal antibiotic formation in cultures of *Bacillus subtilis*. This was found to be 0.40 mg./l., while in the case of *Neurospora crassa* an almost maximal growth was observed by us in the presence of as little as 0.08 mg./l. of zinc in the culture solution.

Migration of zinc from the *Neurospora* into the culture solution

By making use of isotopic indicators the migration of ions from the plant into the culture solution has been observed in several cases, and the effect on the

migration process of unlabelled ions in the solution has also been studied. Minute amounts of labelled lead, when taken up by the roots of *Vicia Faba*, could to a large extent be removed by an excess of non-labelled lead added to the nutrient solution, but to a minor extent only if the solution did not contain lead (Hevesy, 1923).

Mullins & Brooks (1939) placed cells of *Nitella coronata* first in a solution containing radioactive potassium (^{42}K) and later in solutions of different chlorides. Sodium and lithium were found to be much less effective in removing labelled potassium than potassium itself. We investigated the exodus of labelled zinc into the culture solution both in the absence and presence of zinc in the solution in which the mould containing labelled zinc was placed.

In these experiments *Neurospora* grown for 5 days at 28°, after repeated washing with distilled water, was divided into five approximately equal parts. One part was kept as a control, two parts immersed separately in 150 ml. culture solution containing no zinc for 24 hr. at 12°, while two parts were kept under similar conditions in a culture solution containing 30 µg. zinc.

Table 2. *Migration of labelled zinc from Neurospora into the culture solution*

Sample	Activity
Control	100
<i>Neurospora</i> after being kept in zinc-free solution for 24 hr. at 12°	88, 84
<i>Neurospora</i> after being kept in zinc-containing solution for 24 hr. at 12°	74, 75
Zinc-free solution after 24 hr.	9, 10
Zinc-containing solution after 24 hr.	14, 19
Penultimate wash water	1

In all our experiments more labelled zinc left the mould when placed in a solution containing zinc. Table 2 shows the results of a typical experiment.

Uptake of cobalt by Neurospora

We compared the uptake of Co^{++} (using ^{58}Co as an indicator) with that of zinc by *Neurospora*. Four moulds were grown for 5 days at 28° in the presence of 25 µg. of cobalt in the culture solution. They were found to take up 0.013, 0.011, 0.011 and 0.011 % of the cobalt content of the solution per mg. of their dry weight (i.e. 0.0033, 0.0028, 0.0028 and 0.0028 µg.). The cobalt uptake corresponds to only about one-twentieth of the uptake of zinc under similar conditions, showing that the moulds do not possess such an effective mechanism for fixing cobalt as they do for zinc.

SUMMARY

1. By making use of labelled zinc (^{65}Zn) the amount of zinc necessary to obtain maximal growth of *Neurospora crassa* was studied.

2. Maximal growth requires that at least 1/30,000 part of the dry weight of the *Neurospora* be composed of zinc, and can be obtained by raising the mould in a culture solution containing 0.08 mg. or more of zinc/l.

3. When placed in a culture solution containing inactive zinc more labelled zinc migrates from *Neurospora* into the solution at 12° than in the absence of zinc.

4. Cobalt uptake, studied by making use of labelled cobalt (^{58}Co), amounts only to one-twentieth of the uptake of zinc under corresponding conditions.

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