since serum incubated for several days with [^{131}I]iodide shows only a trace of radioactivity in the albumin and none in any other fraction.

Further work is in progress on the nature of the α_2-activity and of the processes causing it, including the possibility of bacterial action.

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The influence of sulfur compounds on molybdenum toxicity in *Aspergillus niger*

From investigations carried out with several organisms, it has become evident that the toxic effects of molybdenum can be counteracted, in many cases, by sulfur-containing compounds, such as inorganic sulfate, or the amino acids cysteine and methionine. GRAY AND DANIEL[1] showed that methionine, at very high dietary levels, suppressed the growth-inhibitory effect of molybdenum in rats. Later, VAN REEN AND WILLIAMS[2] demonstrated that inorganic sulfate and cysteine had similar beneficial effects. In *Neurospora crassa*, SIVARAMA SASTRY *et al.*[3] found that molybdenum toxicity depressed the biosynthesis of methionine and that, at levels of molybdenum causing around 50% inhibition of growth, methionine completely prevented the growth suppression when supplemented at the low concentration of 5 μg/ml in the basal medium. Since this was the first demonstration of a direct interference with sulfur amino acid metabolism by molybdenum in microorganisms, it was thought of interest to explore whether this was a general phenomenon. The present communication deals with the influence of several sulfur compounds on molybdenum toxicity in the mold *Aspergillus niger*.

The strain of *A. niger* used herein was the same as that used in this laboratory by ADIGA *et al.*[4] in earlier studies and was grown on a basal medium with the following percentage composition: glucose, 15; KH_2PO_4, 0.25; NH_4NO_3, 0.25; $MnSO_4 \cdot 4H_2O$, 0.01: $MgSO_4 \cdot 7H_2O$, 0.025; $ZnSO_4 \cdot 7H_2O$, 0.00065. The mold was grown for 4 days on 10 ml of the above medium adjusted to pH 2.5–3.0, in 100-ml pyrex conical flasks at 30 ± 1°. Molybdenum was added aseptically after being sterilized separately from the rest of the medium. The same procedure was also followed in the case of L-cysteine. Na_2SO_4 and DL-methionine were added directly to the culture medium.

At the end of the experiment, the mycelial felts were removed, washed thoroughly, dried at 60° overnight and weighed. The uptake of molybdenum by the mold was determined by estimating the amount present in the well-washed mycelia after wet digestion by the method of DICK AND BINGLEY[5].

Ammonium molybdate was inhibitory to growth of *A. niger* in the range 2–10 mg Mo/10 ml. The ability of sulfur compounds in counteracting growth depression was studied at two toxic levels of 5 mg Mo and 10 mg Mo per 10 ml, respectively. Typical results are presented in Table I.

TABLE I

THE INFLUENCE OF SULFUR COMPOUNDS ON GROWTH AND MOLYBDENUM UPTAKE BY *A. niger* IN MOLYBDENUM TOXICITY

Supplements to 10 ml culture medium	*Dry wt. of mycelium (mg)*	*Mo uptake (μg/100 mg dry wt. of mycelium)*
None	189.0	—
5 mg Mo	46.5	4119
5 mg Mo + 200 mg methionine	172.4	160
5 mg Mo + 100 mg Na_2SO_4	48.0	2336
10 mg Mo	24.1	4350
10 mg Mo + 200 mg methionine	162.0	807
10 mg Mo + 100 mg Na_2SO_4	28.0	2765

The data indicate that inorganic sulfate cannot prevent molybdenum toxicity in *A. niger*, even though it partially prevents molybdenum uptake in the small amounts of mycelium eventually formed. No appreciable protection was obtained even when Na_2SO_4 was included at levels upto 1.5 g/10 ml. Cysteine in the range of 5–100 mg/10 ml caused only an increased toxicity.

Methionine almost completely prevented the toxic effects of 5 mg Mo/10 ml and had an appreciable effect even at the higher toxic level of 10 mg Mo/10 ml. Moreover, it may be seen that concomitant with enhancement of growth, there is a drastic reduction in the amount of molybdenum accumulated by the mold. The prevention of growth inhibition obtained is, in all probability, a direct consequence of this suppression of molybdenum uptake. A point of interest is the comparatively large amount of methionine needed, in striking contrast to only 50 μg required to restore growth to normal under similar conditions in *N. crassa*[3]. In view of this situation, it is unlikely that molybdenum toxicity in *A. niger* is directly associated with an inhibition of sulfur amino acid biosynthesis. Further work is in progress to elucidate

the mechanism by which molybdenum becomes toxic in this mold, as well as to study in detail the control of molybdenum uptake by methionine.

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Biosynthesis of valine and isoleucine in plants

The bulk of evidence available in recent years points to the following sequence of reactions leading to the biosynthesis of valine: pyruvate → α-acetolactate → α,β-dihydroxyisovalerate → α-ketoisovalerate → valine. In the case of isoleucine biosynthesis an analogous sequence in which α-aceto-α-hydroxybutyrate is an intermediate has also been shown. WAGNER *et al.*[1] demonstrated the conversion of α-acetolactate and α-aceto-α-hydroxybutyrate to α,β-dihydroxyisovalerate and α,β-dihydroxy-β-methylvalerate, respectively, using cell-free extracts of *Neurospora crassa*. On the basis of the existence in these extracts of a reductase which catalysed the conversion of synthetic α-keto-β-hydroxyisovalerate and α-keto-β-hydroxy-β-methylvalerate to the corresponding dihydroxy acid precursors of the two amino acids, these authors considered that these ketohydroxy compounds were intermediates in the conversion of α-hydroxy-β-ketoacids to the dihydroxy acids. However, RADHAKRISHNAN *et al.*[2] clearly demonstrated that in the case of *Escherichia coli* and *Neurospora crassa* this conversion is catalysed by a "reductoisomerase" which is distinct from the reductase. The participation or otherwise of the α-keto-β-hydroxy compounds in this reaction is still unknown. The above sequence of reactions has been amply confirmed by other workers in *E. coli*[3], in yeast[4] and in *Salmonella*[5].

In this report evidence is provided for the existence of enzyme systems involved in the biosynthesis of valine and isoleucine in plants. Seeds of green gram (*Phaseolus radiatus*) germinated for 24 h were ground in a mortar, the slurry centrifuged at 12000 × *g* for 15 min and the supernatant (crude extract) was used as the enzyme source. The enzyme system catalysing the conversion of pyruvate to α-acetolactate has been partially purified and the crude extract used for demonstrating the presence of other systems.

The α-acetolactate-forming system is extremely labile and the presence of a a metal (Mn^{2+}, $5 \cdot 10^{-4}$ *M*) and a thiol compound (cysteine or β-mercaptoethanol, $5 \cdot 10^{-4}$ *M*) is required for stabilisation throughout the procedure of fractionation.