
Effect of Tin on Biologic Aspects of *Cunninghamella echinulata* and *Fusarium oxysporum*, under Various Cultural Conditions

Mohamed I. Naguib, Aleya A. Hamed and Nahed Z. Haikal

Botany Department, Faculty of Science, Cairo University, Cairo Egypt

ABSTRACT. In absence of external carbon source, the loss in weight from 6-day old mycelial felts was greater with *Fusarium oxysporum* than with *Cunninghamella echinulata* when supplied with nitrate, though carbon dioxide output was almost similar.

Although the three tin compounds seemed to exert no effect on growth of *Cunninghamella* when supplied with nitrate alone, stannous ions stimulated growth on either urea or peptone media. On the other hand, the three compounds were toxic to *Fusarium* when supplied with urea. With an adequate supply of carbon, the inorganic tins enhanced, but Plictran inhibited, growth of *Cunninghamella*. Nitrate favoured greater absorption of sugar than either urea or peptone. Stannic and Plictran were suppressive to glucose uptake by *Cunninghamella* when supplied with either nitrogen sources, whereas stannous exerted almost no effect with nitrate or urea but was suppressive with peptone. The tin compounds also hindered glucose uptake by *Fusarium*, a phenomenon that was alleviated or even reversed with urea feeding.

Inorganic tin stimulated respiration of *Cunninghamella* in the presence of nitrate, while respiration was inhibited in the presence of urea. Plictran inhibited respiration in media containing either nitrate or urea. On the other hand, the three tin containing compounds enhanced respiration of *Fusarium* regardless of the nitrogen source. Further, the presence of glucose in media containing tin attenuated respiration of *Cunninghamella*, regardless of the nitrogen source.

The results show that glucose slightly suppressed nitrogen absorption from urea or peptone, but remarkably increased nitrogen uptake from nitrate media. In most cases, the various tin compounds inhibited nitrogen absorption by *Cunninghamella* when glucose was supplied to the media. In the absence of glucose, the tin compounds stimulated urea and peptone absorption.

The dangers from trace elements (titanium, iron, copper, zinc, lead, cadmium and tin) in the environment were tentatively discussed by Woolrich (1973), who con-

cluded that their concentration in air was not currently threatening to public health. In nature, several plant species accumulate strontium and tin, especially in areas of sulphur deposition (Pyataeva 1966). Such plants may serve as indicators of pollution by such elements (Bardyuk and Ivashov 1969), since the tin content of such plants may reach 100-300 times the original background level.

The role of organotin compounds against pathogenic fungi was substantiated by Picco (1965). They have been reported to be efficient against rice blast disease (Tamura 1965) and potato blight (Jarvis *et al.* 1967, Koula 1971). On the other hand, *Penicillium lilacinum* (Yama *et al.* 1975) and kernel smut of rice (Whitney 1977) were highly resistant to these compounds.

Organotin compounds are also known for their microbiostatic effect against various gram positive bacteria (Noseler 1970, Srivastava and Rupainwar 1971) and algae (Stroganov *et al.* 1966, Deschiens 1968). The same was also recorded for inorganic tin compounds (Walter and Windeler 1976, Lewis and Miller 1977).

The aim of this investigation was to elucidate the difference, if any, in biology of a pathogenic and non-pathogenic fungus towards administration of tin (in both valencies) as well as in its organic form (Tricyclohexyl tin hydroxide), commonly known as Plictran.

Material and Methods

Cunninghamella echinulata and *Fusarium oxysporum* f. sp. *lycopersici* were isolated from Egyptian soil and maintained on modified Dox agar (Naguib 1967). With a sterile graduated pipette, 0.5 ml of spore suspension was aseptically transferred to each 250 ml conical flask containing 50 ml of sterile modified Dox medium in which sucrose was substituted by an equivalent amount of glucose. Nitrate nitrogen was replaced by an equivalent amount of urea or peptone to determine whether the source of nitrogen would affect the response of the fungi under test.

The 6-day old mats were drained, washed several times with sterile distilled water and then supplied with freshly prepared media containing different concentrations of tin in both organic and inorganic forms. Flasks were further incubated at 28°C for 48 hr, during which time the carbon dioxide output (Said and Naguib 1953) as well as analyses of the media for their glucose and nitrate content and mycelial dry weight at 80°C, were recorded at 24-hr intervals. The modified Nelson solution (Naguib 1964) and the procedures of Paech and Tracey (1956) were applied for determination of the above mentioned criteria, respectively.

At least five replicate samples were used for each determination.

Results

Dry Weight Gain

Figure 1 shows that in the absence of glucose, the loss in weight of urea-fed *Cunninghamella* felts was much greater, whereas that of peptone-fed felts seemed similar to that of potassium nitrate-fed samples. On the other hand, urea seemed to induce the same reduction in dry weight of *Fusarium* that nitrate feeding produced, whereas peptone seemed to overcome this phenomenon.

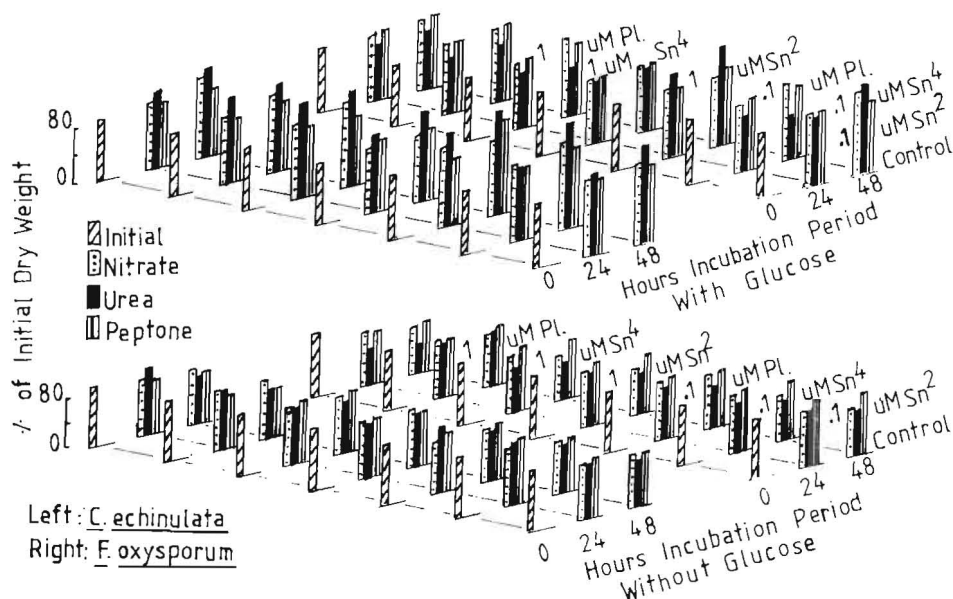


Fig. 1. Effect of various concentrations of tin compounds on the percentage fluctuations in dry weight of 6-day old mycelial felts of *Cunninghamella echinulata* and *Fusarium oxysporum* supplied with different nitrogen sources in presence or absence of glucose.
Pl. = Plictran.

In the presence of nitrate, all tin concentrations appeared to have no effect on the percent dry weight gain of both organisms. Urea and peptone produced a slight, if any at all, reduction in the dry weight of *Cunninghamella*, except for the high rate of Plictran which severely attenuated the gain in dry weight and the 1.0 μM stannous ion rate which slightly counteracted the loss of weight in the presence of peptone. Furthermore, the various tin compounds favoured a severe reduction in dry weight gain by *Fusarium* when urea or peptone was supplied as the nitrogen source; with the response more prominent in the presence of 1.0 μM Plictran.

In the presence of glucose, an equal percent gain in dry weight was recorded for both *Cunninghamella* and *Fusarium* in the presence of nitrate. On the other hand, urea nitrogen seemed to favour the maximum gain for both organisms, particularly with *Cunninghamella*, whereas peptone induced almost the same percent gain for *Cunninghamella* and least for *Fusarium* when compared with potassium nitrate feeding.

With nitrate containing media, in the presence of glucose, neither stannic ion nor Plictran produced a measurable effect, whereas the stannous ion favoured a better gain in dry weight of *Cunninghamella*. Replacement of nitrate with urea attenuated the gain in dry weight by all tin-supplied samples with the 0.1 μM inorganic tin favouring the greatest gain. All tin containing compounds favoured greater loss in weight of *Cunninghamella* when supplied with peptone, except 0.1 μM stannic ion which was stimulatory.

Fusarium differed from *Cunninghamella* in that all tin compounds attenuated the dry weight gain in the presence of nitrate with Plictran providing the greatest gain. With urea, stannous ion caused a very steep drop in dry weight, whereas the other ions, except for 0.1 μM stannic, produced less or no effect. The 0.1 μM stannic was stimulatory in the presence of peptone, whereas the other tin compounds were slightly, if at all, suppressive.

Absorption of Glucose

Figure 2 shows that larger amounts of glucose were absorbed by *Fusarium* than by *Cunninghamella*. Both organisms absorbed greater amounts of glucose in pre-

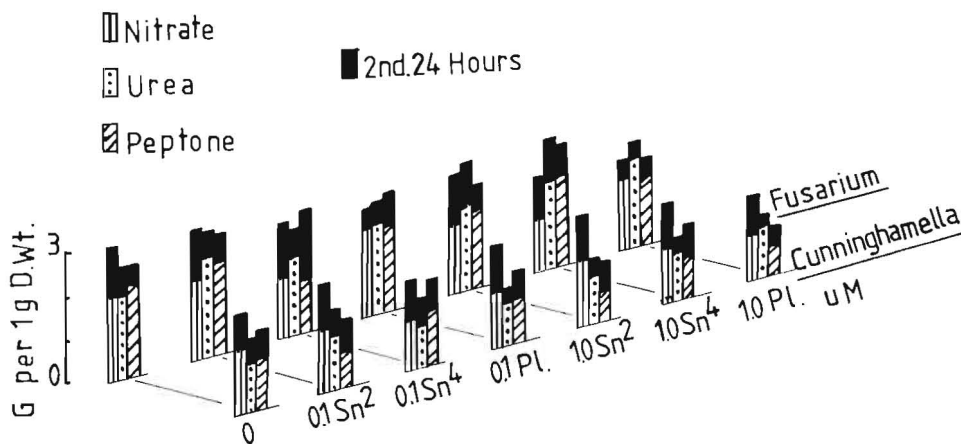


Fig. 2. Effect of various concentrations of tin compounds on glucose uptake by mycelial felts of *Cunninghamella echinulata* and *Fusarium oxysporum* supplied with different nitrogen sources. Pl. = Plictran.

sence of either urea or peptone, which favoured almost the same rate of sugar uptake.

In presence of nitrate, the tin compounds seemed to have little or no effect on glucose uptake by *Cunninghamella*, except at 1.0 μM Plictran, where a noticeable decrease, particularly during the first sample, was detected. On the other hand, although the inorganic tin hindered glucose uptake by *Fusarium* during the first part of the experiment, the organism was able to compensate for this lag in uptake in the presence of the low tin concentration, with the higher dose produced a continued inhibition. Plictran was suppressive to sugar absorption, a phenomenon that appeared late in the experiment, in the presence of the low dose of the substance.

In presence of urea, the various tin compounds had little effect on glucose absorption by *Cunninghamella*, except in presence of 1.0 μM of the inorganic salts and Plictran, which was inhibitory during the experiment. With *Fusarium*, the inorganic tin appeared stimulatory, whereas Plictran seemed to have no effect.

1.0 μM inorganic tin and Plictran were also suppressive to glucose uptake by *Cunninghamella* during the first 24 hr in presence of peptone, a phenomenon that was apparent in *Fusarium* only with the high Plictran level, whereas tin compounds and small Plictran doses were slightly stimulatory.

Carbon Dioxide Evolution

Figure 3 shows that, in absence of glucose, there are minor differences in carbon dioxide output between *Cunninghamella* and *Fusarium*; the highest production accompanied nitrate and the lowest accompanied peptone feedings.

In presence of urea or peptone, the various tin compounds were suppressive to carbon dioxide evolution by *Cunninghamella*, with the effect more prominent with the latter compound. Plictran also inhibited carbon dioxide production in presence of nitrate, whereas inorganic tin compounds, except 0.1 μM stannous, were stimulatory.

All tin compounds markedly enhanced carbon dioxide output by *Fusarium* in the presence of either nitrogen source, except 1.0 μM stannic which retarded this process in the presence of nitrate. Plictran induced the maximum effect with nitrate or urea, whereas 1.0 μM stannous or 0.1 μM Plictran exerted the highest effect in the presence of peptone.

Glucose supplementation favoured higher carbon dioxide output by both organisms. Still, nitrate initiated maximum, while peptone caused the least carbon dioxide production by *Cunninghamella*. *Fusarium* evolved considerably larger amounts of carbon dioxide, in the presence of glucose, than *Cunninghamella* under nitrate or peptone, but lesser with urea feeding.

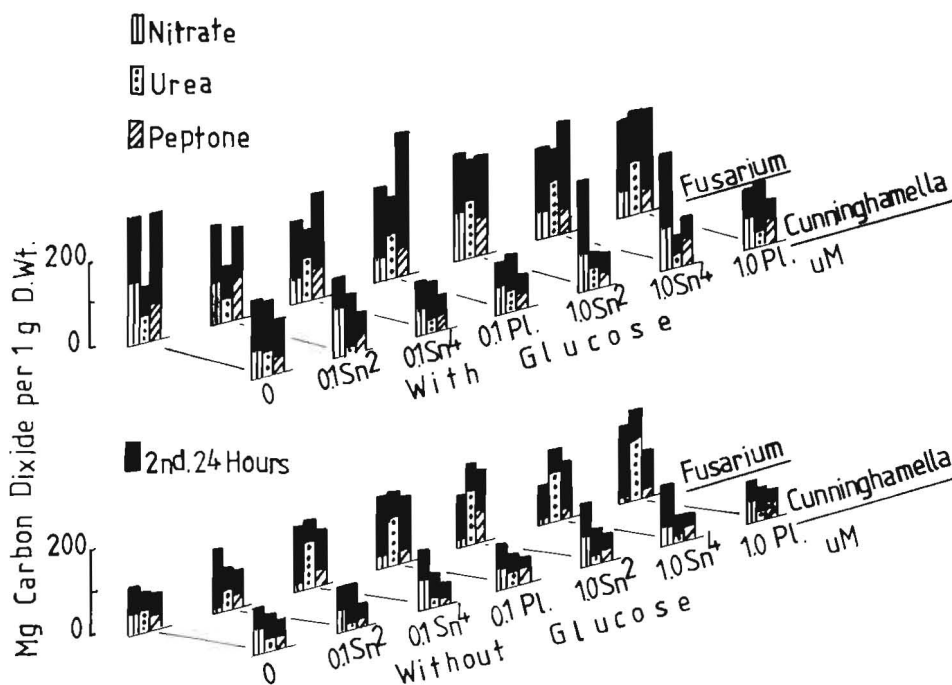


Fig. 3. Effect of various concentrations of tin compounds on carbon dioxide output by mycelial felts of *Cunninghamella echinulata* and *Fusarium oxysporum* supplied with different nitrogen sources in presence or absence of glucose. Pl. = Plictran.

All tin compounds were highly suppressive to carbon dioxide evolution by glucose-fed *Cunninghamella*, particularly with peptone as nitrogen source. 1.0 μM inorganic tin salts in presence of nitrate were the exception, since they enhanced carbon dioxide output. The applied tin compounds were also stimulatory to carbon dioxide production by glucose-fed *Fusarium* in presence of urea but were suppressive in presence of the other nitrogen sources, except peptone which enhanced carbon dioxide output in the presence of 0.1 μM Plictran.

Under all conditions, such fluctuations were highly apparent during the first 24 hr of the experiment.

Nitrogen Absorption

Figure 4 reveals that in absence of glucose both *Cunninghamella* and *Fusarium* absorbed similarly large amounts of nitrogen from urea media and the lowest amounts from nitrate source. With nitrate and peptone, the various tin compounds

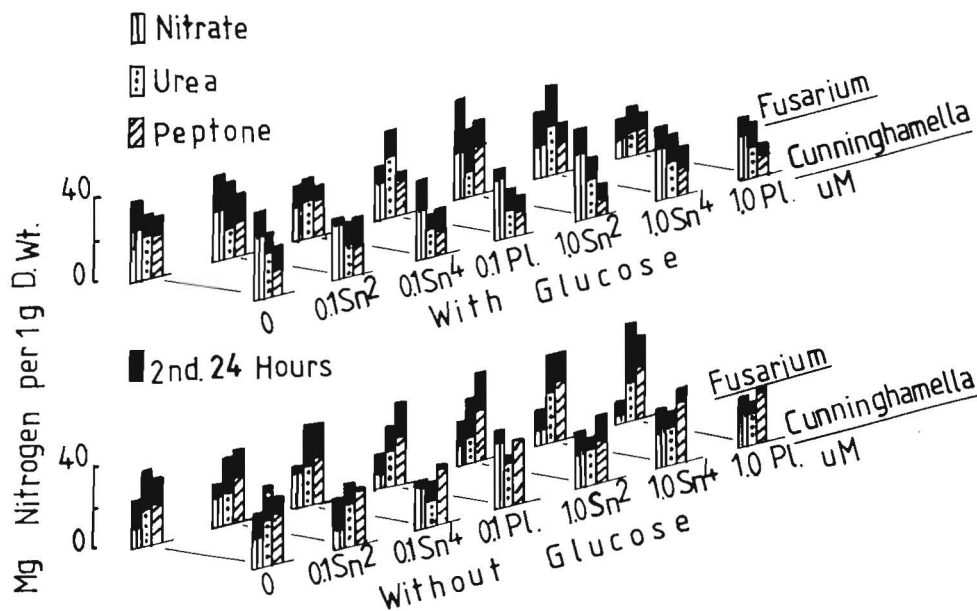


Fig. 4. Effect of various concentrations of tin compounds on nitrogen uptake by mycelial felts of *Cunninghamella echinulata* and *Fusarium oxysporum* supplied with different nitrogen sources in presence or absence of glucose. Pl. = Plictran.

had little or no effect on nitrogen absorption by *Cunninghamella*, except Plictran which increased nitrate absorption at 0.1 μM concentration.

However, all tin compounds severely reduced urea absorption. Conversely, tin compounds retarded nitrate and enhanced peptone absorption by *Fusarium*, most prominently in presence of stannic or Plictran. Uptake of urea was lowered by stannous and low doses of Plictran, but was stimulated by the high dose of stannic or Plictran.

Feeding with glucose had little effect on urea uptake, but stimulated nitrate and attenuated peptone absorption to almost the same extent by both *Cunninghamella* and *Fusarium*.

Stannic appeared to have little effect on nitrate uptake by glucose-fed *Cunninghamella*, but the other tin compounds were inhibitory. However, urea or peptone uptake was suppressed, most prominently with administration of Plictran. Nitrate uptake by *Fusarium* was stimulated by stannous but inhibited by stannic or Plictran, the latter being most effective. The low doses of stannous or Plictran also favoured better urea absorption by *Fusarium*, whereas 0.1 μM stannic induced lower uptake;

a phenomenon that was reversed with increased concentration of either tin compounds. Further, peptone absorption was accelerated by stannous and retarded by either Plictran or stannic.

Discussion

The results reported in this investigation clearly establish the difference in biologic behaviour, as well as response towards the tin compounds, between *Cunninghamella* and *Fusarium*. In absence of external carbon source, the loss of weight was faster from *Fusarium* than *Cunninghamella* when supplied with nitrate, though carbon dioxide output was almost similar.

In spite of lower glucose uptake, carbon dioxide output and nitrogen uptake by *Cunninghamella* from peptone than from nitrate media, the percent gain in dry weight with glucose administration was almost similar in both cases; an indication that the carbon skeleton of peptone was mostly incorporated in building up organic matter, whereas most of the sugar absorbed by nitrate-fed samples was consumed in obtaining energy required for assimilation of inorganic nitrogen. The latter deduction may apply to *Fusarium* which absorbed the largest amount of glucose and nitrate nitrogen and had the highest production of carbon dioxide, thus showing the same percent gain in dry matter. On the other hand, in spite of lower sugar absorption coupled with higher carbon dioxide evolution and almost equal nitrogen absorption by *Cunninghamella* and *Fusarium* when cultured on urea media, the gain in dry weight by the former was higher than by the latter organism; an indication that *Cunninghamella* can utilize both carbon and nitrogen of urea in a more efficient manner than *Fusarium* when supplemented with glucose.

In spite of comparatively higher sugar and nitrogen uptake by *Fusarium* than *Cunninghamella* with peptone feeding, the gain in dry weight was much less on a percentage basis. This may be attributed to the remarkably high carbon dioxide output in the former, and a much lower output in the latter organism.

The results, further, show the diversity in response of either organism to the various tin concentrations of the different tin compounds, dependent on the nutritive medium. Thus, although the three chemicals seemed to exert no effect on growth of *Cunninghamella* when supplied with nitrate alone, stannous ion stimulated growth on either urea or peptone media; a phenomenon that was apparent only at the low dose of stannic ion or Plictran when the organism was fed with urea. However, the high dose of both chemicals was inhibitory in the peptone media. On the contrary, the three compounds were toxic to *Fusarium* when supplied with urea and had little or no effect on nitrate or peptone, except for the high Plictran dose which inhibited growth with the latter nitrogen source. With an adequate supply of carbon, the inorganic tin enhanced growth of *Cunninghamella* on all nitrogen media at their low dose, but exerted no effect at higher levels

(inhibited growth with peptone feeding). On the contrary, the organic tin was inhibitory in the three media tested. Further, all three compounds were toxic to *Fusarium* in the presence of external carbon supply, particularly with urea feeding.

These observations suggest that a change in permeability may occur to the fungi tested when glucose is added to the medium. It appears that the sugar facilitated the permeation of *Fusarium* cells with either of the tin compounds, but allowed for such effect with *Cunninghamella* in the presence of Plictran only.

In this connection, it is important to note that Zlochevskaya and Galimova (1975) reported that organic tin compounds are more toxic to fungi than inorganic forms. They reported that 0.0001% triethyl tin chloride inhibited growth of *Penicillium cyclopium*.

The results, further, indicate (Fig. 2) that during the first 24 hr, stannic and Plictran were suppressive to glucose uptake by *Cunninghamella* fed with either nitrogen sources. On the other hand, stannous exerted almost no effect with nitrate or urea feeding but was suppressive with peptone. The tin compounds also hindered glucose uptake by *Fusarium*, with their effect more prominent at their high concentration; a phenomenon that was alleviated or even reversed with urea feeding. With lapse of time, the inhibitory effect of the low dose was counteracted or even reversed (nitrate feeding) but remained highly apparent in *Cunninghamella* with the high dose, particularly with Plictran.

These observations indicate that the organisms could overcome the deleterious effects of tin compounds on sugar absorption depending on the prevailing nitrogen source, with nitrate being most efficient.

The results further show (Fig. 3) that the respiratory carbon dioxide output was lowered when either organism was supplied with organic nitrogen, with peptone exerting the greatest effect. This indicates that such readily available building blocks (carbonyl phosphate from urea or amino acids produced from peptone) directly supported the metabolism of both organisms, with least energy consumption, compared with inorganic media, free of sugars, where energy is required to reform the storage carbon compounds into the appropriate structures necessary for metabolism.

Increased respiration, coupled with increased nitrogen uptake from nitrate media, when the organism received an external carbon supply, indicates the dependence of absorption and utilization of nitrate nitrogen on the available carbon skeleton. On the other hand, the comparatively lower rise in carbon dioxide output, concomitant with lowered sugar uptake and its minor effect on nitrogen uptake from urea media, points to the independence of urea absorption and metabolism on energy and/or carbon supply. Still repression by glucose is possible. *Cunninghamella* differed from *Fusarium*, in this respect, when fed with peptone and glucose. Glucose lowered nitrogen uptake by *Cunninghamella* with peptone media

and was coupled with least absorption of sugar and carbon dioxide output, an indication that glucose restricted peptone metabolism by *Cunninghamella* and tended towards increased gain of dry matter within the cells, producing the highest economic coefficient. On the other hand, the considerably high carbon dioxide output by *Fusarium* in peptone media, accompanied by less sugar absorption and coupled with minor changes in nitrogen uptake, points out the increased decarboxylation activities of *Fusarium* under such conditions.

Figure 3 also reveals that inorganic tin was stimulatory in nitrate and inhibitory in urea to respiration of *Cunninghamella*, whereas Plictran was inhibitory in both media. The three compounds had almost the same inhibitory effect in peptone media. On the other hand, the three compounds enhanced respiration of *Fusarium* regardless of the nitrogen source; a phenomenon that continued with urea and was reversed with the other nitrogen sources in the presence of glucose. Still, the presence of glucose in the tin media attenuated respiration of *Cunninghamella* (with few exceptions) regardless of the nitrogen source. These observations revealed the differences in response of the experimental organisms to tin compounds.

The results further show (Fig. 4) that nitrogen absorption depends on the prevailing source and/or carbon supply. In the absence of sugar, nitrogen absorption was greatest from urea and least from nitrate. Glucose slightly suppressed nitrogen absorption from urea and peptone, but significantly increased nitrogen uptake from nitrate media. If these observations are coupled with the observed increased rate of respiration accompanying sugar administration, one may conclude that inorganic nitrogen absorption requires an external energy supply.

In most cases, the various tin compounds inhibited nitrogen absorption by *Cunninghamella* with urea media (in the absence of sugar), with the inhibition being more prominent with an increase in concentration. The same applied to nitrate absorption by *Fusarium*, as well as with urea or peptone when glucose was supplied to the media. In the absence of glucose, the tin compounds stimulated urea and peptone absorption.

It should be noted that Singh and Bragg (1979) attributed the inhibition of absorption by *E. coli*, in the presence of tributyltin chloride, to enhancement of the transmembrane OH-anion exchange reactions by this substance.

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