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ON THE MODE OF ACTION OF DIALKYLDITHIOCARBAMATES ON MOULDS AND BACTERIA

by

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In the course of the last few years we have obtained a better insight into the mode of action of an important group of agricultural fungicides, the dialkyldithiocarbamates, and we should like to give here a general picture of the views we hold at present. Whereas the dialkyldithiocarbamates differ in their mode of action from the bisdithiocarbamates (17), we have found that there is a close parallel in the behaviour of dialkyldithiocarbamates, 8-hydroxyquinoline and pyridine-2 thiol-N-oxide (19,21).

When testing growth of a large variety of moulds on glucose mineral salts agar of pH 7.0, made up with tap water and containing different amounts of sodium dimethyldithiocarbamate (NaDDC) or tetramethylthiuram disulphide (TMTD, the oxidation product of NaDDC), one will find that according to their behaviour the moulds can be roughly divided into three groups (Table 1).

Group I: complete growth inhibition by *ca.* 0.2 p.p.m. of NaDDC. This picture of "sensitive moulds" is shown by *Glomerella cingulata* (21), *Botrytis allii*, *Botrytis cinerea*, *Ceratostomella ulmi*, *Glomerella fructigenum*, *Sclerotinia fructicola* and the yeast *Rhodotorula glutinis*.

Group II: growth is inhibited by low concentrations of NaDDC, *e.g.* 0.5-2 p.p.m. ("first zone of inhibition") but returns again at higher concentrations: 5, 10 and 20 p.p.m. ("zone of inversion growth"); inhibition follows again from 50 p.p.m. onwards ("second zone of inhibition"). The moulds showing this unusual pattern we have called "moulds with inversion growth". Examples: *As-*

TABLE 1.

Inhibition of moulds by NaDDC on a glucose mineral salts solution made up with tap water. (aneurin and biotin added when necessary).

	p.p.m. NaDDC										
	0	0.05	0.1	0.2	0.5	1	2	5	10	20	50
Group I *Glomerella cingulata* type	+	+	+	—	—	—	—	—	—	—	—
Group II *Aspergillus niger* type	+	+	+	+	—	—	—	±	+	+	—
Group III *Fusarium oxysporum* type	+	+	+	+	+	+	+	+	+	+	—

pergillus niger, Asp. oryzae, Asp. ruber, Alternaria solani, Alt. tenuis, Cladosporium cucumerinum, Clad. herbarum, Pen. brevicompactum.

Group III: growth is inhibited from *ca.* 50 p.p.m. of NaDDC upwards. Examples of these insensitive moulds are: *Fusarium oxysporum, Pen. expansum, Asp. fumigatus, Asp. nidulans, Asp. ochraceus, Asp. versicolor, Fusarium avenaceum, Fus. bulbigenum, Fus. coeruleum* and *Pen. citrinum.*

A behaviour intermediate between these groups can also be found: Slow growth in the zone of inversion growth [*e.g. Pen. italicum* (18) and *Saccharomyces cerevisiae*] or in the first zone of inhibition is seen then. The phenomenon of inversion growth has already been described by DIMOND, HEUBERGER and STODDARD (8) in 1941 for spore germination tests with *Macrosporium sarcinaeforme* and TMTD where they found a double maximum curve.

With the exception of *A. niger* the action of all moulds studied is independent of the pH value (16). This mould belongs to group II at pH above 6.5, whereas below a pH of *ca.* 6.5 a first zone of inhibition is not seen (group III).

A further study of these three groups has given us a better insight into their interrelationships and at the same time into the relation of growth inhibition by NaDDC and other dithiocarbamates, by 8-hydroxyquinoline (oxine) and by pyridine-2-thiol-N-oxide (PTO).

First zone of inhibition:

Our starting point has been the observation that on malt agar of pH 7.0 and on agar with 1% "casamino acids" (Difco) *Aspergillus niger* appears to grow in up to 20 p.p.m. of NaDDC, the first zone of inhibition which is shown on glucose agar, now being absent (16). Also the moulds of group I appear far less sensitive on these media than on glucose agar. For the insensitive moulds (group III) the medium does not influence the results. An analysis of the antagonistic activity of some probable constituents of "casamino acids" showed us (16) that of 18 amino acids, 9 growth factors, 4 purines and 2 pyrimidines, L-histidine is the only compound that is able to counteract NaDDC at pH 7.0. This effect is far more pronounced at this pH value than at pH 4, where cysteine was found to antagonize,

as was also reported by SMALE, COX and SISLER (31). Thus the histidine contained in malt is held responsible for the observed difference in growth response on malt agar as compared with that on glucose agar. Earlier results of KLÖPPING and VAN DER KERK (25) on the activities of dithiocarbamates are quite comparable to our present results if we consider that their experiments were carried out on malt agar of pH *ca.* 6.0.

Histidine as well as certain other imidazole derivatives (18) appeared to be competitive antagonists of NaDDC for moulds of group I and II, but only in the region up to 20 p.p.m. of NaDDC. At 50 p.p.m. and higher concentrations histidine did not show any effect. These results suggested strongly that for these two groups the mode of action of NaDDC at low concentrations differs from its mode of action at higher concentrations (from 50 p.p.m. onwards).

The role of metals:

We had found earlier that also oxine was less active on malt agar than on glucose medium. As it appeared that here too histidine as well as certain other imidazole derivatives were antagonists, we suggested a common factor in the mode of action of NaDDC and oxine.

It is a well-known fact that dialkyldithiocarbamates, oxine as well as histidine form complexes with metals. Moreover, the mode of action of oxine on bacteria was found by ALBERT (1) to be connected with complex formation of oxine with Fe and Cu; bactericidal activity was found to coincide with the presence in the medium of oxine and metal in a 2 : 1 ratio, whereas toxicity was less when either oxine or the metal was present in excess. Meanwhile BLOCK (5) has confirmed these findings for fungi. This suggested strongly that metals might also play a role in the fungitoxic action of NaDDC. A further confirmation was obtained by an investigation of the metal-binding ability of the various imidazol derivatives, which are active antagonists. We found that when Cu was taken as the metal, there was indeed a strong correlation between this property and the antagonistic activity (unpublished).

The role of metals was then further investigated especially with regard to the work of GOKSØYR (9) He stressed the importance of the two complexes of Cu and DDC in the inhibitory action of NaDDC on *Saccharomyces cerevisiae*. An equilibrium exists between these two possible complexes of Cu^{II} and DDC in the following way:

$$\underset{\text{1 : 1 complex}}{CuDDC^{+}} \underset{+Cu}{\overset{+DDC}{\rightleftharpoons}} \underset{\text{1 : 2 complex}}{CuDDC_2}$$

The 1 : 1 complex is water soluble and its concentration can be determined spectrophotometrically. The 1 : 2 complex is extremely insoluble (see later). GOKSØYR studied the combined effect of NaDDC and metal salts on the oxidation of acetate and glucose. A maximum in inhibition of acetate oxidation was found to coincide with a maximum in the amount of 1 : 1 complex present (namely in media containing 6.25 p.p.m. $CuSO_4.5\ H_2O$ and 0.9-1.5 p.p.m. NaDDC). At higher NaDDC concentrations the 1 : 1 complex is turned into the 1 : 2 complex and there was no inhibition of acetate oxidation. Also in a medium without added Cu the reversal phenomenon was seen in the inhibition of acetate oxidation; it was ascribed to the presence of NaDDC chelated with traces of metals adhering to the yeast cells. Under these latter circumstances the effect of NaDDC on growth was also studied and strict correlation between growth inhibition and inhibition of acetate oxidation was found, both showing a double maximum curve. Although no growth experiments were carried out with NaDDC and added Cu, it is inferred by GOKSØYR from the above experiments that growth inhibition in the first zone is due to 1 : 1 complex. Inversion growth occurs when all 1 : 1 complex has been transformed into the non-toxic 1 : 2 complex.

The role which Cu appeared to play urged us to apply henceforth basal media with an extremely low Cu content. To this end we used (21) a glucose mineral salts solution (pH 7.0) with a Cu content not exceeding 0.01 p.p.m.

The Cu content of the agar which we had used for our media was only very low. We had, however, generally washed this agar and it was now found that by washing with tap water the Cu content of agar increases considerably. An argument against using unwashed agar was the fact that for ás yet unknown reasons, the same amount of Cu was far more effective here than in liquid media.

By comparing growth results in liquid shaken medium (Table 2) to which Cu had been added, with the u.v. absorption spectra of similar media (20), we could confirm that for *A. niger* the first zone of inhibition coincided closely with the presence of certain amounts of 1 : 1 complex of Cu and dimethyldithiocarbamate. This amount is not yet reached in media with 3 p.p.m. of copper sulphate in con-

TABLE 2.

Effect of addition of copper sulphate on growth inhibition of *Aspergillus niger* by NaDDC.

Shake culture in glucose mineral salt solution; glass-distilled water; pH 7.0; aneurin and biotin added; 3 days 24°.

p.p.m. $CuSO_4.5H_2O$ added	p.p.m. NaDDC								
	0	0.2	0.5	1	2	5	10	20	50
0	+	+	+	+	+	+	+	+	—
3	+	+	+	+	+	+	+	+	—
10	+	+	—	—	—	±	+	+	—

trast to those with 10 p.p.m. copper sulphate (*cf.* also Table 4). With increasing NaDDC concentration the 1 : 1 complex is turned into the very insoluble 1 : 2 complex and toxicity disappears. When excess of NaDDC is present — inevitably together with a certain amount of 1 : 2 complex — toxicity returns again, but in this case it is due in our opinion to the free dithiocarbamate ion (*cf.* also SMALE (32)), and not to zinc or iron dithiocarbamates as GOKSØYR (9) suggests for his experiments with yeast. These latter compounds hydrolyze to a great extent. Moreover, the amount of free ions of these metals is greatly reduced due to the presence of other complexing agents as *e.g.* the phosphate ions.

To produce the first zone of inhibition Cu clearly is required. Of other heavy metals tested (Fe, Co, Mn, Zn) only Co appeared to have some action which, however, is less than that of Cu (20).

The action of CuDDC and $CuDDC_2$ on moulds of the three groups:

The fact that CuDDC is toxic in contrast to $CuDDC_2$ was remarkable with respect to ALBERTS finding that $Cuox_2$ was more toxic than Cuox. A further study shed more light upon this discrepancy. When in our Cu-free culture solution (21) *G. cingulata* was grown in the presence of different amounts of NaDDC and copper sulphate (Table 3), no inversion growth was witnessed at any combination of NaDDC (up to 50 p.p.m.) and $CuSO_4$ (up to 10 p.p.m.).

It appeared to us that the difference in response between *A.niger* and *G.cingulata* might be understandable if *G.cingulata* were sensitive to $CuDDC_2$ in contrast to *A.niger* which is not inhibited even by high amounts. This supposition proved to be true as *G.cingulata* was inhibited by 0.05 p.p.m. of $CuDDC_2$, 0.02 p.p.m. still giving growth.

TABLE 3.

Effect of addition of copper sulphate on growth inhibition of *Glomerella cingulata* by NaDDC.

Shake culture in glucose mineral salt solution.

p.p.m. copper sulphate added	minimal inhibiting conc. of NaDDC (p.p.m.)
0	0.1
0.03	0.05
0.1	0.1
0.3	0.1
1	0.2
3	0.2

TABLE 4.

Amount of CuDDC present (p.p.m.) in the medium (1% glucose, 0.5% K_2HPO_4, 0.1% $(NH_4)_2SO_4$, 0.05% NaCl, 0.05% $MgSO_4$, glass distilled water; pH 7.0).

p.p.m. $CuSO_4.5H_2O$ added	p.p.m. NaDDC								
	0.02	0.05	0.1	0.2	0.5	1	2	5	10
0									
0.03	0.01	0	0						
0.1	0.02	0.03	0.02	0					
0.3	0.02	0.04	0.05	0.04	0	0			
1	0.02	0.05	0.1	0.1	0.1	0.06	0		
3	0.02	0.05	0.1	0.2	0.2	0.2	0.1	0	0
10	0.02	0.05	0.1	0.2	0.4	0.34	0.32	0.30	0.2

——— Inhibition pattern of *Glomerella cingulata*.
--------- ,, ,, ,, *Aspergillus niger*.

The solubility of this compound is only *ca.* 0.01 p.p.m. [(15), *cf.* Table 8]. Probably this concentration is just inhibitory, but the rate of dissolution is slow; therefore somewhat more seems to be required to build up quickly an inhibitory concentration.

The amounts of CuDDC and $CuDDC_2$ present in each instance of our experiments could be calculated (Table 4, 5) starting from the known values for the stability constants for the complexes and the solubility.

TABLE 5.

Amount of $CuDDC_2$ present in solution (p.p.m.) in the medium.

p.p.m. $CuSO_4.5H_2O$ added	p.p.m. NaDDC				
	0.02	0.05	0.1	0.2	0.5
0					
0.03	S	S	S	S	S
0.1	0.002	S	S	S	S
0.3	0.001	0.004	S	S	S
1		0.001	0.005	S	S
3			0.001	S	S

——— Inhibition pattern of *Glomerella cingulata*.
S = saturated solution of $CuDDC_2$ (*ca.* 0.01 p.p.m.).

In the calculations the partial hydrolysis of Cu^{++} ions at pH 7 was taken into account. No allowance was made for the action of other complexing agents, but since their concentrations are small, it is assumed that their influence will be negligible. Of course the calculated values of the Tables 4 and 5 are approximate. A comparison of these with Table 2 confirms that *A.niger* is not inhibited by $CuDDC_2$, but by the 1 : 1 complex, namely when the amount of this latter substance surpasses *ca.* 0.3 p.p.m.; this leads to the first zone of inhibition. For *G.cingulata*, however, $CuDDC_2$ had proved to be toxic. At all Cu concentrations sufficient $CuDDC_2$ seems to be present to explain growth inhibition, though at several instances the concentration of the 1 : 1 complex may at the same time be inhibitory as well (15). This cannot be proved since sufficient $CuDDC_2$ is invariably present to explain inhibition. Yet, similar experiments with diethyldithiocarbamate (see later) gave evidence that the 1 : 1 complex will be inhibitory as well. Inhibition in flasks without added Cu must be due to complex formation of dithiocarbamate with Cu present in the medium or adhering to the cells.

The difference between moulds with and without inversion growth (groups II and I) thus seems to be solely that the former are somewhat less sensitive than the latter so that a toxic concentration of the very insoluble compound $CuDDC_2$ cannot be reached. Further confirmation will be given below.

The distinction between group II and III equally is not fundamental. Moulds of the latter group do in fact also show a first zone of inhibition – followed by inversion growth – if only sufficient Cu is

added. For instance with *F.oxysporum* on glucose agar when 10 p.p.m. of copper sulphate is added. For *A.niger* only 1 p.p.m. of copper sulphate is required to give the same effect on agar (20). Thus the difference between these groups appears to be a gradual one which means that the distinction in two groups is more or less deliberate. It was only on our, arbitrarily chosen, glucose agar medium made up with tap water and inevitably containing a certain amount of Cu that we could distinguish these two groups.

In view of the three types of response to NaDDC, it seems a happy coincidence that TMTD or the dimethyldithiocarbamates can be used at all as a fungicide. If the solubility of $CuDDC_2$ had only been slightly less, these compounds would have proved completely useless. We now know several important plant parasites which are sensitive to $CuDDC_2$ as *e.g. Venturia inaequalis*, and which can thus be combatted by dithiocarbamates; *Phytophthora infestans*, another ill-famed plant parasite, cannot be combatted by these compounds; $CuDDC_2$ in fact, was here not inhibitory *in vitro*.

Antagonists:

For the second zone of inhibition (NaDDC content 50 p.p.m. or more) we know no antagonists. But in the first zone of inhibition of *A.niger* growth can be restored (20): a) by the addition of agents that bind Cu more readily than NaDDC does, *i.e.* by higher dithiocarbamates, or b) by addition of a large excess of agents that bind Cu less readily than NaDDC, *i.e.* ethylene diaminetetraacetic acid (EDTA) or c) by certain imidazole derivatives. The latter bind Cu less readily than NaDDC and EDTA but they are believed to compete with the enzyme for the toxic 1 : 1 complex by formation of imidazole: Cu : DDC complexes. As exposed above the antagonistic activity of imidazole derivatives runs closely parallel to their Cu-binding ability. Also the fact that the antagonistic activity of imidazole derivatives decreases with lowering of the pH is an argument for metal binding being involved.

In general the pH dependence of the action of the fungitoxic agents as well as of the antagonists is in accordance with expectations. Dithiocarbamic acids which are completely ionized at pH about 7 show no effect of pH, whereas oxine and imidazole derivatives which are partially ionized do show the effect.

The same compounds are also antagonistic for other moulds; for those of group II in the first zone of inhibition; in group I up to about 20 p.p.m. of NaDDC (21).

α-Keto acids antagonize in the first zone of inhibition, but only when *A.niger* is used (18). We cannot give an explanation for this.

anomalous behaviour. Yet, on the other hand it seems possible that the remarkable fact, that this same mould shows no first zone of inhibition at low pH values, could be due to a production of α-keto acids by spores and mycelium leading to antagonism of NaDDC.

In general we can say that for moulds the action of NaDDC in various media is determined by the Cu content of the medium and by the histidine content of organic additions. The barrier of 50 p.p.m. of NaDDC can, however, not be surpassed since from here onwards the free dithiocarbamate ion is inhibitory.

Action of NaDDC on bacteria:

Though Gram+ bacteria seem to be very sensitive to NaDDC in contrast to Gram— bacteria, further confirmation of this is required. We found that the inhibition of *Bac.subtilis* by NaDDC follows the same pattern as that of the fungi of group I; there is no inversion growth. Complete inhibition is obtained by *ca.* 0.2 p.p.m. of NaDDC, and Cu probably is the only metal involved in inhibition.

Homologues of NaDDC:

If we now turn to the higher dithiocarbamates we find that the ethyl compound does not show a first zone of inhibition with *A.niger*; this mould is only inhibited by 50 p.p.m. of the diethyl compound irrespective of the amount of Cu present (21). On the other hand *G.cingulata* shows (21) with diethyldithiocarbamate more or less the pattern of *A.niger* with NaDDC (see Fig. 2). This difference between the dimethyl and the diethyl compound can be fully explained by the fact that the solubility of the 1 : 2 complex with Cu is far less for the latter compound than for the first namely only 0.002 p.p.m. (15); *G.cingulata* obviously is not completely inhibited by this concentration (Table 8). Spectrophotometric measurements have shown that the first zone of inhibition coincides with the presence of maximal amounts of 1 : 1 complex. However, because of the low solubility of the 1 : 2 complex the concentration of 1 : 1 complex present (*ca.* 0.15 p.p.m. when 0.2-5 p.p.m. diethyldithiocarbamate are combined with 10 p.p.m. copper sulphate) is less than the corresponding concentration when NaDDC is used. This fact explains why *A.niger* does not show a first zone of inhibition with the diethyl compound.

Because the 1 : 1 complex of the diethyl compound is toxic for *G.cingulata* we suggested above that the dimethyl complex is toxic

too. It seems impossible, however, to tell which of the two 1 : 1 complexes is the most toxic, since the toxic $CuDDC_2$ always accompanies the CuDDC.

Bac. subtilis follows again the pattern of *G. cingulata* showing a first zone of inhibition and a zone of inversion growth. CRUICKSHANK (7) in a paper on the differentiation of three *Brucella* species describes that, when a disc containing diethyldithiocarbamate is placed on a plate which then is incubated with the bacteria, *Brucella melitensis* and *Br. abortus* develop around the disc a ring of growth between two rings of inhibition, whereas *Br. suis* shows only one large zone of inhibition. This points to the same differentiation as we have found above between moulds of group II and of group I for NaDDC.

With the propyl and dibutyl compounds the concentration of the 1 : 1 complexes is still less than with the diethyl compound (21) because of the lower solubility of the 1 : 2 complexes (15). Even *G.cingulata* does not show a first zone of inhibition now. *Bac. subtilis* however, appears to be more sensitive: on glucose agar with 5 p.p.m. of copper sulphate one finds inhibition with 1, 2, 5 and 10 p.p.m. dipropyl derivative and incomplete inhibition with 1 and 2 p.p.m. dibutyl derivative.

The f r e e diethyl compound is as toxic as the f r e e dimethyl compound (21), *i.e.* it acts at *ca.* 50 p.p.m. The free dipropyl compound acts at *ca.* 500 p.p.m. whilst the dibutyl compound is not yet inhibitory at this concentration. The same was found for *Bac.subtilis*. As we know that the chelating ability of the dithiocarbamates increases from methyl to butyl (14) it seems unlikely that the action of the free dithiocarbamates is due to chelation, unless permeability of these free compounds differs considerably.

We know only few compounds with an 1 : 2 complex having the "high" solubility of the dimethyl or diethyl compound. Na-cyclopentamethylenedithiocarbamate shows an activity of the 1 : 1 complex which is somewhat less than that of the diethyl compound; also the solubility of its 1 : 2 complex with Cu was somewhat less than that of the diethyl compound (15). The activity of the 1 : 2 complexes seems to be determined rather by solubility than by structure. But another factor is also involved, since bis (hydroxyethyl) dithiocarbamate is not toxic, yet it can be expected to have a higher solubility than the dialkyl derivatives. It has been found that the 1 : 2 complex with Cu rapidly decomposes with formation of a complex of univalent copper. So the picture becomes fairly complicated although

up till now all facts can be related to the chemical and physicochemical properties of the dithiocarbamates.

The conclusion from these investigations seems to be that in general the activity of the dialkyldithiocarbamates at low concentrations is determined by the solubility of the 1 : 2 complex with Cu and by the sensitivity of the mould.

Comparison of the action of 8-hydroxyquinoline and pyridine-2-thiol--N-oxide with that of the dithiocarbamates:

We have already mentioned that the action of oxine can also be antagonized by histidine and other imidazole derivatives. This can also be affected by EDTA and dibutyldithiocarbamate, antagonism never going beyond 5 p.p.m. of oxine (19). This suggested that oxine has the same mode of action as NaDDC. ALBERT (1,3) has shown that the bactericidal action of oxine is effected by the 1 : 2 complex which is supposed to penetrate rapidly into the cells and to be transformed there into the actual toxic agent: the 1 : 1 complex. The latter is supposed to penetrate slowly and thus to act only when high concentrations are present in the outside medium. BLOCK (5) confirmed this for fungi.

We carried out the same experiments with oxine and $CuSO_4$ as we did with NaDDC (19); equally the amounts of 1 : 1 complex and 1 : 2 complex were calculated (Table 6 and 7).

The inhibition picture of *A.niger* is drawn in these tables. It appeared that moulds of all three groups showed the same picture now and this was the picture which we found for *G.cingulata* and NaDDC; there was no inversion growth. Accordingly $Cuox_2$ appeared inhibitory to moulds of all three groups (Table 8). Clearly the figures in Tables 6 and 7 show that for *A.niger* there is no correlation between inhibition and amount of Cuox in the medium unless perhaps at high Cu concentrations. The amounts of $Cuox_2$ show, however, a correlation with inhibition, 0.03 p.p.m. being about the inhibitory concentration. This is just about the value which we find inhibitory when $Cuox_2$ is added as such to the medium. Only when Cuox occurs in amounts $\geqslant 0.5$ p.p.m. (*e.g.*, in the combination of 0.5 p.p.m. oxine sulphate and 10 p.p.m. copper sulphate where only 0.02 p.p.m. of $Cuox_2$ is present) it seems to have a definite effect of its own.

Similar growth experiments have been described for bacteria by SORKIN, ROTH and ERLENMEYER (33). From their results it appeared that *Staphylococcus aureus* and *Mycobacterium tuberculosis* are inhi-

TABLE 6.

Amount of Cuox present (p.p.m.) in the medium.

p.p.m. copper sulphate added	p.p.m. oxine sulphate			
	0.05	0.1	0.2	0.5
0	0	0	0	0
0.03	0	0	0	0
0.1	0.03	0.04	0	0
0.3	0.05	0.08	0.1	0
1	0.05	0.1	0.2	0.3
3		0.1	0.2	0.5
10			0.2	0.5

-------- = Inhibition pattern of *Aspergillus niger*.

TABLE 7.

Amount of $Cuox_2$ present (p.p.m.) in the medium.

p.p.m. copper sulphate added	p.p.m. oxine sulphate			
	0.05	0.1	0.2	0.5
0	0	0	0	0
0.03	0.04	0.04	0.04	0.04
0.1	0.02	0.06	0.14	0.14
0.3	0.007	0.03	0.09	0.4
1	0.002	0.008	0.03	0.2
3		0.003	0.01	0.07
10			0.004	0.02

----- = Inhibition pattern of *Aspergillus niger*.

TABLE 8.

Solubility and inhibitory concentration of various complexes.

	solubility in p.p.m.	min. inh. conc. in p.p.m.		
		G. cingulata	*A. niger*	*F. oxysporum*
$Cuox_2$	1	0.02-0.05	0.1	0.2
$CuPTO_2$	0.2	0.1	0.2	
$CuDDC_2$	0.01	0.05	no inh.	no inh.
Cu diethyl-dithiocarbamate	0.002	partial inh.	no inh.	no inh.

bited by $Cuox_2$ but the activity increased by the addition of more Cu. Histidine must have been present in these media and since it also combines with Cu, it seems difficult to evaluate the amounts of Cuox and $Cuox_2$ actually present.

In Table 8 some figures are also given for pyridine-2-thiol-N-oxide (PTO) a compound that shows the same response as oxine in the presence of various amounts of Cu (19). ALBERT (3) reported that the mode of action of PTO on bacteria is comparable to that of oxine.

Oxine and PTO, like NaDDC, can be antagonized by stronger Cu binders as *e.g.* dibutyl dithiocarbamate. EDTA and imidazole derivatives are also antagonistic and will act as exposed above for NaDDC. Antagonistic activity never surpasses the barrier of 5 p.p.m. of oxine or PTO, and we suppose that from here onwards the free compound is inhibitory. α-Keto acids have no antagonistic activity.

Thus there is a very close correlation in the mode of action of dialkyldithiocarbamates, oxine and PTO.

Theories on the site of action of NaDDC:

It will be clear that since NaDDC can act in three different ways (1 : 1 complex, 1 : 2 complex and dithiocarbamate ion) each of these forms could have a different mode of action. We think it, however, most likely at present that the two complexes both act within the cells as the 1 : 1 complex, much in the same way as ALBERT suggests for the complexes of oxine: The lipophilic 1 : 2 compound penetrates better than the hydrophilic 1 : 1 compound, but the latter is the actual toxic agent which combines with an essential enzyme. The 1 : 2 complex then must be transformed into the 1 : 1 complex within the cells. The mode of transformation is as yet unknown. The dithiocarbamate ion acts probably on a quite different enzyme and there is reason to believe that the complexes act more fungicidally whereas the ion acts more fungistatically, but conclusive experiments are still lacking.

From Warburg experiments described by VAN DER KERK and KLÖPPING (23) it follows that O_2 consumption of *A.niger* in malt is left undisturbed by the free dithiocarbamate ion up to about 500 p.p.m. GOKSØYR (9) found that O_2 consumption of *Saccharomyces cerevisiae* with glucose as a substrate was only slightly inhibited by concentrations of 1 : 1 complex of NaDDC which did inhibit growth. With acetate as a substrate, inhibition of respiration was much

decreased. Moreover, acetate incorporation was strongly inhibited by the 1 : 1 complex. The acetate-activating properties of a yeast extract were inhibited by *ca.* 20 p.p.m. TMTD, but not by NaDDC with or without addition of Zn (5). GOKSØYR (10) found, moreover, that in cell-free homogenates, but not in intact cells 2-20 p.p.m. NaDDC caused strong inhibition of succinic acid dehydrogenase, especially in the presence of *ca.* 15 p.p.m. zinc sulphate (9). The Cu complexes were inactive. SISLER and COX (23) described that the anaerobic fermentation of glucose by *Saccharomyces cerevisiae* was strongly inhibited by 20 p.p.m. of TMTD and this was due to inhibition of the oxidation of glyceraldehyde-3-phosphate.

Some other enzyme complexes are known to be inhibited by low concentration of NaDDC or TETD (tetraethylthiuram disulphide, the oxidation product of sodium diethyldithiocarbamate). Thus KEILIN and HARTREE (22) reported that *ca.* 1-3.5 p.p.m. TETD caused strong inhibition of the succinic dehydrogenase of heart muscle. We found that CuDDC, but not $CuDDC_2$, gave rise to some accumulation of pyruvic acid by *A.niger*, whereas the dithiocarbamate ion caused much stronger accumulation of this metabolic product (20). Acetaldehyd oxidase of liver homogenate appeared to be inhibited by 0.06 p.p.m. TETD (KJELDJAARD (24)) and by 1 p.p.m. TETD (GRAHAM (11)). NYGAARD and SUMNER (26) reported that 1 p.p.m. TETD inhibited D-glyceraldehyde 3-phosphate dehydrogenase from muscle.

These reports seem sometimes contradictory and the role played by metals is far from clear. Yet, when one considers the enzyme systems which can be inhibited by low concentration of NaDDC, TMTD or TETD, it is striking that with the exception of glyceraldehyde dehydrogenase all are known to contain an essential dithiol group; the dithiol compound dihydrolipoic acid functions in pyruvate oxidase (12), in the acetate-activating system of animal tissues (27) and yeast (28); in addition dithiol groups act in acetaldehyde oxidases (13) and their presence in succinic acid dehydrogenase was made acceptable (30). Arsenite is known to act on all these enzyme systems (12, 13, 18, 30) and this is due to interference with the dithiol group. This means that there is a parallel between enzyme inhibition by arsenite and dithiocarbamate, though arsenite is not growth-inhibiting in concentrations which produce strong effects on the enzymes mentioned.

Thus there seems to be much reason to suggest that NaDDC acts

in general on enzymes which contain dihydrolipoic acid as coenzyme.

We know that an analogue of dihydrolipoic acid causes growth inhibition of bacteria and a yeast (6). The compound appeared to inhibit pyruvate oxidase and phosphotransacetylase (4). Growth inhibition by the analogue is thought to be due to interference with one of the functional forms of lipoic acid concerned in acetyl transfer, and in our opinion it may well be that growth inhibition by NaDDC is due to interference with the same system.

Little is known about the role which copper plays in the inhibition by NaDDC of the enzymes mentioned, but it seems acceptable that dithiols react rather with the 1 : 1 complex than with the dithiocarbamate ion.

Knowledge about the action of oxine or PTO and their metal complexes on enzyme systems with essential dithiol groups is very scanty. We have, however, found that pyruvic acid accumulation can be effected by oxine and by its 1 : 1 complex with Cu, in the same way as described above for NaDDC.

Summary.

In the action of sodium dimethyldithiocarbamate on moulds and bacteria the formation of its 1 : 1 and 1 : 2 complexes with Cu^{++} from the medium plays a decisive role. These Cu complexes on the whole have a higher toxicity than NaDDC itself. The response pattern varies, however, according to the sensitivity of the organism used.

The activity of higher homologues of NaDDC is determined by the solubility of their 1 : 2 complexes.

The mode of action of the dialkyldithiocarbamates is closely comparable with that of oxine and pyridine-2-thiol-N-oxide.

It is suggested that dithiocarbamates act by interference with some function of lipoic acid.

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