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ON THE OBSERVED LOGARITHMIC FACTOR IN DOSE-RESPONSE RELATIONSHIP, ILLUSTRATED WITH THE EFFECT OF SOME HERBICIDES ON PHOTOSYNTHESIS

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SUMMARY

It has been observed that in many processes a logarithmic relation exists between dosage and response. To explain this a simple model is proposed departing from the fact that the occupancy of target molecules by poison molecules depends on the number of effective contacts. The fraction of poisoned target molecules is determined by the equation $E(p) = 1-e^{-r/n}$, where n is the total number of target molecules and r the total number of poison molecules that are in contact with the target molecules. It appears from this model that, within limits, the range of inhibition will be proportional to log dose.

This relationship has indeed been observed for the effects of three herbicides on photosynthetic reactions in the alga *Scenedesmus*, where the gradual degree of inhibition has been measured in various dosage steps. Here also a log dose relation was found.

For DCMU and simeton the slope of the lines is in accordance with the model. Differences in sensitivity to the poison under various conditions were discussed. No explanation could be given for the less steep slope in one of the three conditions for both DCMU and simeton. When the poisoning is reversible the line will be less steep when plotting log dose against response on normal scale. When elimination is important, the line will shift to the right when the same method of plotting is used.

The model simulates the logarithmic factor in dose-response graphs, except in the region of very high or very low occupancy, where in the model and in the experiments the lines bend.

1. INTRODUCTION

BLISS (1934, 1935) introduced the transformation of the sigmoid dose-response regression line by plotting the dose on a logarithmic scale and the response (mortality) on a probit scale. With few exceptions the transformed regression is well represented by a straight line. This may be interpreted by assuming variable sensitivity of the organism and increase of response proportional to the logarithm of the poison dose. Logarithmic relations often appear in biological processes (KOCH 1966, 1969, and WASSINK 1971). One wonders whether this logarithmic relation between response and dosage is characteristic only for poisons studied, e.g. insecticides, herbicides (RIEPMA 1960) and fungicides (HORSFALL 1955), or whether there is some fundamental reason for this phenomenon.

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A model for gradual increase of occupation of target molecules by a poison was presented in this study. It was tested for the action of some herbicides on photosynthesis to determine if it could explain the logarithmic factor and the slope of the response line.

2. MODEL

One may suppose that poisoning is caused by contacts between poison molecules and target molecules in the organism, e.g. the molecules of some vital enzyme being inactivated by the poison in a vulnerable centre. At some level of poisoning a response will be observed, e.g. an organism will be killed. The number of unpoisoned target molecules, a stochastic variable, will depend on the total number of target molecules n and the number r of poison molecules in contact with them. The process may be described by the classical occupancy problem where r balls are distributed at random over n boxes. It is shown that the expectation of the fraction of poisoned target molecules $E(p)$ may be approximated by $E(p) = 1-e^{-r/n}$ so that the proportion r/n is in fact the determining feature.* For the derivation we refer to Feller's well-known text book (1950). n can be defined here as the total number of target molecules and r as the total number of poison molecules in contact with the target molecules. Though proportional to the dose, r will only be a small fraction of the total amount of poison applied.

In discussing the Weber-Fechner law, PFEFFER (1884, cited by WASSINK 1946, 1954) recognized a logarithmic factor in the relation between stimulus and reaction in plants. CHICK (1908, cited by WASSINK 1954) was the first to introduce chance considerations into the explanation of the behaviour of micro-organisms with regard to external conditions. RAHN (1930) pointed out that the relation between the reaction of micro-organisms and inhibitory agents depends on the number of initial processes, influenced by the inhibitor; when this number is 1, the plot showing log number of micro-organisms that have survived, against dose or duration of the agent, is a straight line.

Our formula has also been used by THOMPSON (1924) with reference to superparasitism, NICHOLSON & BAILY (1935) on a similar subject, JUSTESEN & TAMMES (1960) referring to damage by pests and by KLOMP *et al.* (1964) for the relation of number of fertilizations to population density. With the above formula the percentage of poisoned target molecules can be plotted against the mean number of contacts of the target molecules, when only the first contact is effective (*fig. 1*) (see also KLOMP *et al.* 1964).

The mean number of contacts can be regarded as proportional to the applied dose. *Fig. 2* shows the same relationship when using a logarithmic scale on the abscissa. Over a long range, a straight line is obtained which can be supposed to lie within the range between e.g. no mortality and total mortality in a population.

For the model it was assumed that the elimination of the poison during the time between application and response was so small that it could be neglected.

* (e is the base of natural logarithm.)

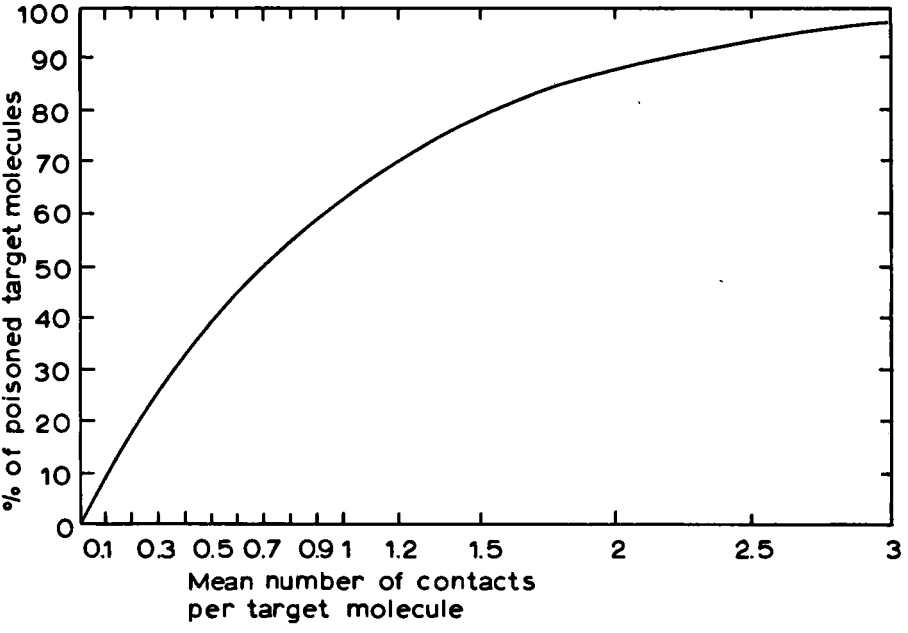


Fig. 1. Percentage of poisoned target molecules plotted against the mean number of contacts with the target molecules when only the first contact is effective. Linear scale.

When the elimination is considerable, e.g. in chronic poisoning, an additional phenomenon will occur. It is known from pharmacology that elimination is nearly always proportional to the concentration in the organism. There is a half time for the poison or its poisonous or non–poisonous metabolites.

In case of elimination a smaller number of poison molecules (r) will be available, and this will be proportional to the dose. For the same response all those values will therefore be higher by the same factor. The response line will then shift parallel to the right, when plotted log dose against response on normal scale. The same will occur when it is more difficult for the poison molecules to reach the targets, e.g. in some cases of lower sensitivity.

When the poisoning process is reversible, the number of non-poisoned target molecules (n) will be increased. In that case the relation on the ordinate (normal scale) will change in proportion to the response, e.g. when 80% occupation becomes 40%, then 2% will be 1%. In case of reversible poisoning the slope of the line will thus be less steep when plotted log dose against response on normal scale.

According to the model it can be expected that either elimination of poison or lower sensitivity will lead to a parallel shifting of the response line to the right when plotted dose-log against response normal. Reversible poisoning will, however, lead to a slope that is less steep, when plotting is the same.

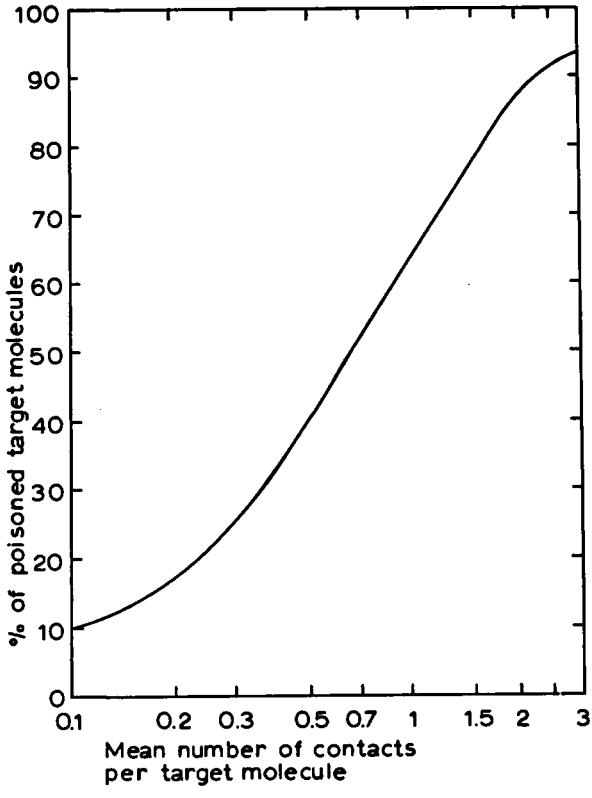


Fig. 2. Percentage of poisoned target molecules plotted against the mean number of contacts with the target molecules when only the first contact is effective. Logarithmic scale.

The above model covers only the inhibition (thus loss) of the target molecules by a poison and not the velocity of a reaction between enzyme and substrate. This is a double logarithmic relation that can be expressed either by the equation of Freundlich & Langmuir's adsorption isotherm or the Michaelis & Menten's equation for enzyme activity, which are intrinsically the same. Such processes, e.g. the response of a plant to auxin, are best fit by a straight line on a double log scale of response against concentration, up to the optimum (BOTTELIER 1959).

If the model is in accordance with the facts, it can be used to make predictions on the range of effective doses and the percentage of inhibition. In the model between 10% and 95% not 9.5 times the dose will be necessary as in a direct relationship (*fig. 1*), but 30 times (*fig. 2*), between e.g. 50 and 95% not 1.9 but 4.3 times. The range of the model covers the range observed in mortality experiments or spore germination tests, though the range may be larger when the reaction between target and poison molecules is reversible or when elimination of poison is important.

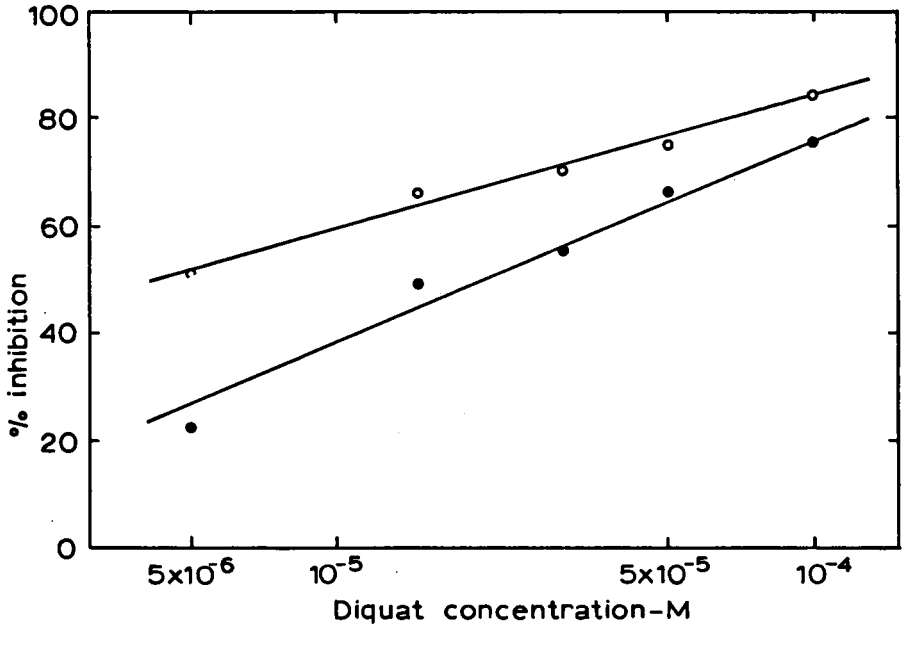
Dose-response graphs are usually based on a plus or minus response, e.g. the organism is killed or is not killed; a spore germinates or it does not. It could be that the variability in sensitivity of a population sample is asymmetric and the log probit transformation is needed to obtain straight lines. It would be an advantage if a gradual degree of inhibition by a poison could be measured quantitatively for a range of dosage steps.

3. EXPERIMENTS

With this in mind, the dose-response curves obtained earlier for three photosynthesis inhibiting herbicides were examined. With the aid of the Warburg technique the effects of diquat (VAN RENSEN 1969), DCMU and simeton (VAN RENSEN & VAN STEEKELENBURG 1965) on oxygen evolution in the unicellular alga *Scenedesmus* had been investigated. Also, the effect of DCMU (VAN RENSEN 1969*a*) and simeton on cyclic photophosphorylation *in vivo* had been measured under different conditions.

Fig. 3 shows the effect of different diquat concentrations on oxygen evolution at 60 and 90 minutes after addition of the herbicide. The inhibition increases with time. When the diquat concentration is plotted on a logarithmic scale and the response on a linear scale, straight lines are obtained. In this way, also for

Fig. 3. Effect of diquat on oxygen evolution in *Scenedesmus*, 60 (●) and 90 (○) minutes after addition of the herbicide.



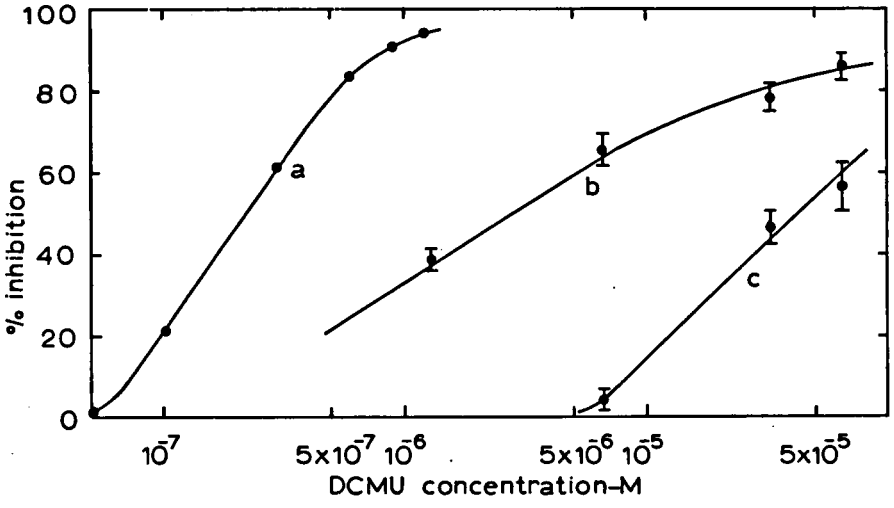


Fig. 4. Effect of DCMU on oxygen evolution in white light (curve a) and on cyclic photophosphorylation in white (curve c) and in far red light (curve b).

the effect of DCMU and simeton on oxygen evolution straight lines are obtained (*fig. 4* curve *a* and *fig. 5* curve *a*).

In a previous paper (VAN RENSEN 1969*a*) a hypothesis on the mode of action of DCMU was presented. According to this hypothesis DCMU affects the oxidized state of a substance X, which is, or is very close to, the primary substrate of Photosystem II. Moreover, it was assumed that X takes part in the cyclic electron transport chain. So there is a competition for oxidized X between DCMU and electron donors, arriving from Photosystem II and Photosystem I (via cyclic electron transport). Later (VAN RENSEN 1971) it was suggested that X might be plastoquinone.

In terms of the model advanced in the present paper the target molecules are the oxidized plastoquinone molecules. Their concentration varies according to conditions as may be demonstrated below.

1. Plastoquinone is more oxidized at limiting light intensities than at saturating ones. So, the inhibition of oxygen evolution by DCMU is greater in the light-limited part than in the light-saturated part of the curve (VAN RENSEN & VAN STEEKELENBURG 1965). Moreover, the effect on cyclic photophosphorylation is higher at low light intensities than at high ones (VAN RENSEN 1969*a*). The same reasoning can be applied for inhibition by simeton (VAN RENSEN 1971).

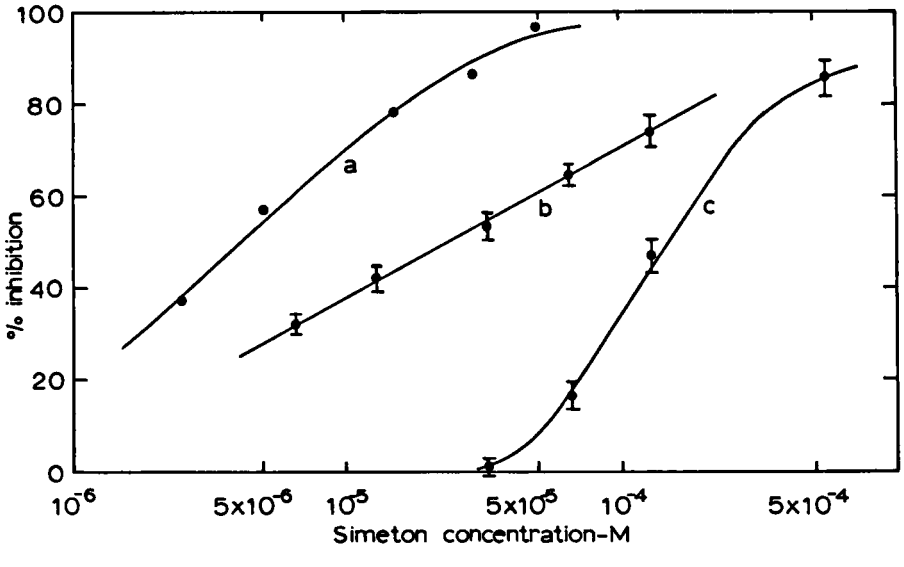
2. In cyclic photophosphorylation in white light there is only cyclic electron transport; however, since Photosystem II still absorbs light, there is an electron pressure from Photosystem II on plastoquinone. This results in an enhanced electron pressure on plastoquinone coming from both Photosystem II and cyclic electron transport. Thus the fraction of plastoquinone in the oxidized state

will be smaller than in the case of oxygen evolution, when only a single electron donor for plastoquinone is in operation. Consequently more DCMU is needed to obtain the same degree of inhibition. This is shown in *fig. 4*, in which curve *c* is situated at much higher concentrations than the inhibition curve for oxygen evolution (curve *a*). In *fig. 5*, curves *a* and *c* show that simeton behaves like DCMU in the same experiments. When the concentration of the herbicides is plotted on a logarithmic and the response on a linear scale, both curves are more or less straight lines.

3. With cyclic photophosphorylation in light, preferently absorbed by Photosystem I, the photoreductive capacity of Photosystem II is decreased. In far red light (obtained with Schott RG N9 filters) the activity of Photosystem II is only 2.5% of that in white light. However, cyclic photophosphorylation proceeds at the same rate as in white light (VAN RENSEN 1969*a*). Owing to the decreased electron pressure from Photosystem II, the fraction of plastoquinone in the oxidized state will be greater than in the case mentioned above under 2, and less DCMU would be needed to obtain inhibition. Indeed, in *fig. 4*, curve *b* is situated at lower concentrations than curve *c*, the former showing inhibition of cyclic photophosphorylation in far red light, and the latter showing inhibition in white light. The same holds for simeton (*fig. 5* curve *b*). Again, both curves are straight lines. The reason for the less steep slope of the *b*-curves in *figs. 4* and *5* is not clear.

From these experiments it has been concluded that the mechanism of action of simeton probably is the same as that for DCMU. Simeton is only about ten

Fig. 5. Effect of simeton on oxygen evolution in white light (curve a) and on cyclic photophosphorylation in white (curve c) and in far red light (curve b).



times less effective, which can depend on partition characteristics. The inhibition depends to a high extent on reaction conditions (VAN RENSEN 1971).

In all curves presented the log dose-response regression curves are best approached by straight lines, which demonstrates that the model describes the observed effects. Also the range fits the model for DCMU and simeton (*figs. 4* and *5*) but not for diquat (*fig. 3*). The latter poison acts in a catalytical way and is therefore alternately bound and freed from the site of action, which results in a broader range, and thus in a slope that is less steep. In case of high or low occupancy the line of the model bends (*fig. 2*) and this was also observed in the experiments.

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