zunächst nur in vivo beobachtete Abnahme des Cytochroms P450 lässt sich auch in vitro nach Zugabe eines NADPH-regenerierenden Systems wiederholen. Durch den Abbau des Tetrachlorkohlenstoffs wird wahrscheinlich ein «aktiver» Metabolit gebildet, der den Gehalt an Cytochrom P450 vermindert und teilweise in das Cytochrom P420, der inaktiven, teilweise von der Membran losgelösten Form umwandelt. Nach Phenobarbitalvorbehandlung ist die Wirkung des Tetrachlorkohlenstoffs wesentlich verstärkt.

Ein Nachweis des vermuteten «aktiven» Metaboliten des Tetrachlorkohlenstoffs ist bisher noch nicht gelungen (SASAME[4], REMMER[14], HENI und REMMER[5,15]). GARNER und MCLEAN[16] konnten durch Nachweis von $^{14}CO_2$ in der Atemluft mit $^{14}CCl_4$ behandelter Tiere lediglich nachweisen, dass Tetrachlorkohlenstoff in vivo abgebaut wird. Eine Aktivierung des mikrosomalen Arzneimittel-metabolisierenden Enzymsystems durch DDT oder Phenobarbital steigert die Umwandlung von CCl_4 in CO_2 und verstärkt die toxische Wirkung des Tetrachlorkohlenstoffs, während Substanzen, die dieses Enzymsystem hemmen, eine Abschwächung der Schädigung bewirken (GARNER und MCLEAN[16]). Wie eigene Vorversuche ergeben haben, bewirkt eine direkte Zugabe des CCl_4 zur Mikrosomenfraktion ohne gleichzeitige Zugabe eines NADPH-regenerierenden Systems keine Beeinflussung des Cytochroms P450[10]. Es ist anzunehmen, dass von dem mikrosomalen Arzneimittel-metabolisierenden Enzymsystem ein möglicherweise sehr kurzlebiger Metabolit des CCl_4 gebildet wird, der bisher noch nicht nachgewiesen werden konnte (SASAME[4], REMMER[14], HENI und REMMER[5,15]).

Tabelle III. Abnahme der Aktivität der mikrosomalen Desmethylase nach in-vitro-Zugabe von 5 µl CCl_4/5 ml

Vorbehandlung	nMol Formaldehyd/ mg Protein/min	(%)
Kontrolltiere	7,51 ± 0,52	100
Kontrolltiere + 5 µl CCl_4	5,22 ± 0,41	69
Phenobarbital	20,11 ± 0,95	100
Phenobarbital + 5 µl CCl_4	9,19 ± 0,61	46

$n = 4$; $\bar{x} \pm s$.

Summary. Isolated damage of cytochrome P450 and partial conversion to cytochrome P420 appears after incubation of carbon tetrachloride with a NADPH-regenerating enzyme system and the microsomale fraction. This effect enlarges after phenobarbital pretreatment.

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The Influence of Deoxycorticosterone, Digitoxigenin and Cyanide Ions on the Respiration of *Fusarium lini* (BOLLEY)[1]

It has been demonstrated that growing cultures of *Fusarium lini* (BOLLEY) (Fungi imperfecti) are able to hydroxylate cardenolides[3-5] and bufadienolides[6-8] in 12β-position whereas steroids of the androstane and pregnane series[9,10] are oxygenated in 15α-position. Most likely a single enzyme is responsible for the various hydroxylations[11]. The site of the attack seems to be determined by the stereochemistry of the substrate. It was also observed that upon simultaneous incubation of deoxycorticosterone and digitoxigenin which served as models the rate of the hydroxylation of the former retarded whereas the oxygenation of the latter is enhanced[11] (cf.[12]). Furthermore it is known that cyanide ions influence the course of steroid hydroxylations[13,14]. In the case of *F. lini* KCN enhances the enzymatic activity of the microorganism at low concentrations while it inhibits it at higher concentrations[11].

Since cyanide is a poison of respiration, it was interesting to investigate the dependence of the respiration of growing cultures of *Fusarium lini* in the presence of both cyanide ions at various concentrations and the steroid substrates which are hydroxylated. In the first experiment the respiration of the micro-organism was measured only in the presence of cyanide ions of various concentrations in the Warburg apparatus using ROBBIE's technique[15]. Figure 1 shows that CN^- inhibits the respiration of *F. lini* proportional to its concentration.

In the presence of only either deoxycorticosterone or digitoxigenin used again as model substrates, the respiration was normal before the hydroxylation reaction took place. During the hydroxylation of deoxycorticosterone the respiration was diminished as observed by ČAPEK et al.[16] in other cases. However, in the presence of digitoxigenin the O_2-uptake was surprisingly enhanced during the hydroxylation. In the presence of equimolar amounts of both substrates the respiration was enhanced again, even before the hydroxylation took place. The results of these experiments are summarized in the Table.

As previously mentioned, the presence of cyanide ions in $10^{-3}M$ concentration reduces the respiration during the first hours of the microbial growth. However, in the following period cyanide intensifies the consumption of O_2.

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Consumption of molecular oxygen of growing cultures of *F. lini* in the presence of various substrates and of cyanide ions

Substrate	Period of O_2-determination	Difference of O_2-uptake of normal respiration (%)
$10^{-3}M$ KCN	1–8 h after addition	−25
$10^{-4}M$ KCN		−24
$10^{-5}M$ KCN		−11
Deoxycorticosterone	Before hydroxylation	0
Digitoxigenin	Before hydroxylation	0
Deoxycorticosterone	During hydroxylation	−32
Digitoxigenin	During hydroxylation	+12
Deoxycorticosterone + digitoxigenin	During hydroxylation of deoxycorticosterone	+9[a] to +23[b]
Deoxycorticosterone + digitoxigenin	During hydroxylation of digitoxigenin	+41

[a] Beginning of the hydroxylation. [b] End of the hydroxylation.

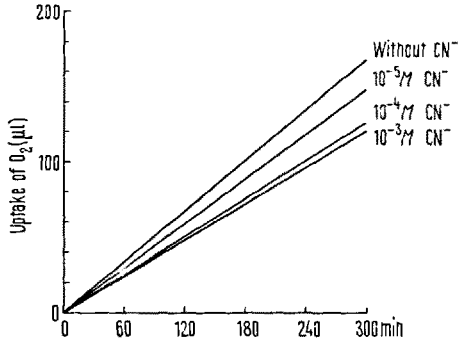

Fig. 1. Respiration of growing cultures of *Fusarium lini* in the presence of KCN^+ (Conditions of culture cf.[11]). The curves are based on the mean values of a great number of independent incubation experiments.

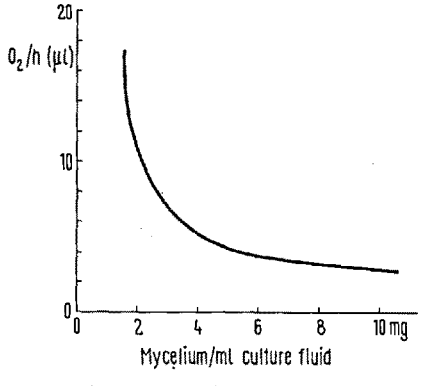

Fig. 2. O_2-consumption of *F. lini* and concentration of mycelium in the culture fluid after 6–8 days of incubation.

This enhancement is also observed when deoxycorticosterone or digitoxigenin are added to the cultures of *F. lini*. It takes place during the period of the hydroxylation the rate of which is also enhanced at this moment. The maximum of O_2-consumption is encountered if cyanide, deoxycorticosterone and digitoxigenin are simultaneously present.

In the course of these investigations it became apparent that the respiration of the micro-organism depends upon the quantity of molecular oxygen dissolved in the culture fluid and the amount mycelium formed. A semiquantitative estimation of this relationship is demonstrated by the curve of Figure 2 in which the O_2-uptake is plotted against the weight of dried mycelium per volume culture fluid. It shows that the amount of O_2 consumed decreases with the increasing quantity of mycelium present.

The experiments described demonstrate that the respiration of *F. lini* is influenced by both deoxycorticosterone and digitoxigenin during their hydroxylation. Addition of KCN effects inhibition of the O_2-consumption of the micro-organism, the extent being dependent of the concentration of the cyanide ions. However, with $10^{-3}M$ KCN an enhancement of respiration is observed which is also maintained in the presence of the steroidal substrates. The results are in agreement with the view that the extraneous steroids added to the culture inhibit the respiration of the micro-organism. By the hydroxylation the extraneous substance is converted into a more water soluble form and can thus be removed more rapidly from the closer environment of the organism. By this process of detoxification normal respiration is achieved[17]. The enhancing effect of cyanide can be explained by their blocking action on the cytochromoxidase of the mitochondria[18,19].

Zusammenfassung. Die Atmung von wachsenden Kulturen von *Fusarium lini* (BOLLEY) wird durch Cortexon und Digitoxigenin während ihrer Hydroxylierung erhöht. 0,001-molares Kaliumcyanid, allein und in Gegenwart von Cortexon und Digitoxigenin, bewirkt ebenfalls eine Erhöhung des Sauerstoffverbrauchs. In kleineren und grösseren Konzentrationen hingegen hemmt Kaliumcyanid die Atmung. Die vom Mikroorganismus aufgenommene Sauerstoffmenge nimmt mit dessen Wachstum ab.

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Myofibrillar ATP-Splitting in the Elementary Contractile Cycle of an Insect Flight Muscle

The elementary contractile cycle in force generation is probably a cyclic attachment and detachment of a cross-bridge between thick and thin myofilaments, which may be associated with the splitting of ATP[1]. The possibility of cross-bridge synchronization was recently considered by SCHÄDLER et al.[2], when they obtained oscillations of the isometric tension in glycerinated flight muscles subjected to quick stretch, for oscillation frequency roughly corresponded to the molecular turnover-number of the actomyosin ATPase. On the assumption that one such isometric oscillation period does correspond to the period of a cross-bridge cycle, it may be possible