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Polychlorinated Biphenyls as Unjustified or Gratuitous Inducers in *Aspergillus flavus* Cultures

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The toxic effects of polychlorinated biphenyls (PCBs), particularly those derived from chronic exposures to subletal levels, seems more related to subtle alterations of metabolic balances than to dramatic interferences with primary vital functions. The PEAKALL review (1975) on the incidence of chlorinated hydrocarbons on the reproductive mechanisms of birds, which also deals with a toxicological problem that may be commonly generalized to vertebrates, is highly illustrative in this respect (LITTERST et al. 1972, ZEPP et al. 1974).

This paper summarizes a study of the variations of some global physiological parameters in Aspergillus flavus batch cultures dosed with different amounts of five Aroclor whose chlorine content were variously 32, 42, 48, 54 and 60 per cent. The qualitative and quantitative relationships of the various aspects of the microorganism responses to the xenobiotics suggest an explanation of the toxic effects found, which involves certain mechanisms analogous in form to others described for higher animals; their adaptative value is discussed.

MATERIAL AND METHODS

Methods for microorganism culture, PCBs dosification, analysis, and RNA estimation were described in a previous paper (MURADO et al. 1976). Oxygen consumption was determined according to the direct manometric method of Warburg (UMBREIT et al. 1972) on 2.5 ml of a spore suspension in a fresh sterile medium with 0.01 % of Tween-20 added. Three separate replications for each experiment were conducted, the growth for all cultures being homologated in solid media wherefrom the spores were taken, as well their density in the suspensions, which were then adjusted by dilution and hemacytometer counting to 10 x 10^6 spores / ml.

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The transformation of aldrin and DDT, purposely studied to investigate the influence of PCBs on the conversion, was measured by GLC-EC on the extracts of medium and mycelium according to techniques already described for the quantitation of PCBs (MURADO et al. 1976). Fractionation of extracts by pretreatment in Florisil columns (REYNOLDS 1969) allows the separation of PCBs, aldrin, DDT, and their transformed products, in such a way that they do not overlap in the chromatogram profiles obtained.

The hydroxilation of pentobarbital, studied in conection with the previous transformations, was estimated by the method of BRODIE et al. (1953). The presence of the cytochrome P-450 was investigated in the supernatant obtained by the centrifugation (9000 g/ 20 min., 0-4 º C) of mycelium homogenates in 0.1 M phosphate buffer, pH 7.4 (OMURA and SATO 1967).

RESULTS AND DISCUSSION

Microorganism response as a function of PCBs dose and chlorine content - The dose-response relationships were studied, after 60 hours incubation, on cultures exposed to initial doses of 5, 10, 25, and 50 ppm of Aroclor-1254 (bearing in mind that this Aroclor shows an analogous chromatographic profile to those obtained from extracts of our environmental samples).

The variations in the dry weight of crops and the relative levels of RNA, expressed as percentages of inhibition (I) and stimulation (S) respectively, on the basis of the control values obtained, are plotted against the logarithm of the initial dose (D) (Fig. 1). Whilst there is a linear correlation between both variables in the dry weight, the relative levels of RNA (i.e. relationship between RNA content and dry weight) appear to increase exponentialy with the logarithm of the initial concentration. The corresponding regression equations

$$I = 68.1 \log D - 18.9 \quad (>99\ \%\ \text{significance})$$

$$S = 66.2\ e^{1.1 \log D} \quad (>99.9\ \%\ \text{significance})$$

give values of 10.3 for ID_{50} and 10.0 for SD_{200} (coventional term for the stimulation dose that doubles the relative levels of RNA with respect to controls).

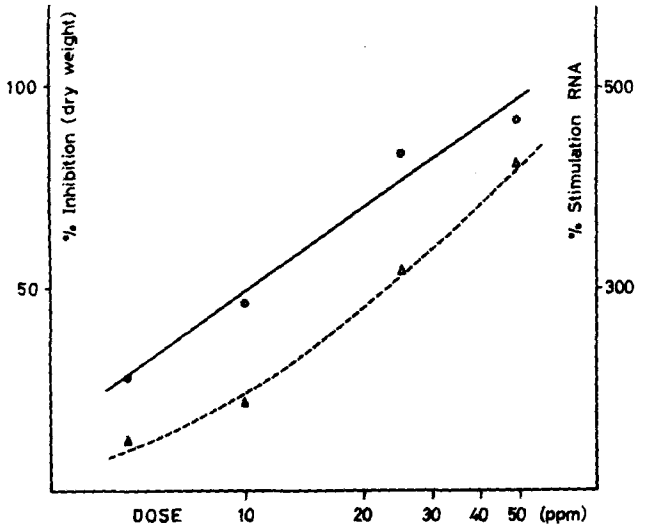

Fig. 1 - Dose-response relationship in A. flavus cultures Aroclor treated. (o) dry weight depression; (Δ) stimulation of RNA biosynthesis. Exposure time 60 h.

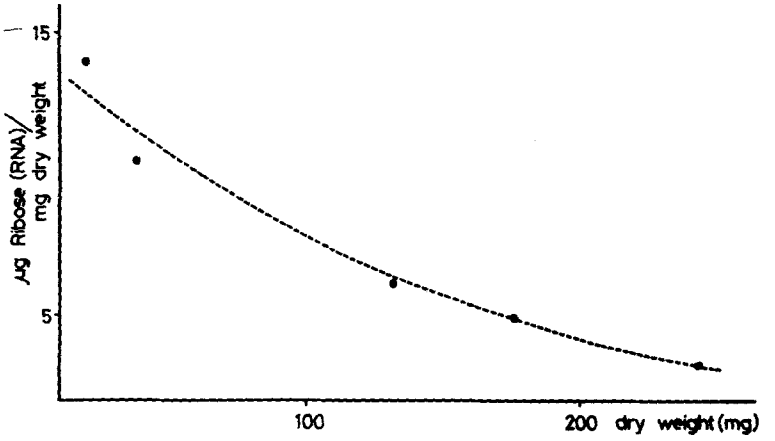

Fig. 2 - Exponential correlation between the weight of dry mycelium and the relative levels of RNA in A. flavus cultures grown in the presence of different dose of Aroclor-1254. Exposure time 60 hours.

The exponential correlation (Fig. 2) defined by the equation

$$RNA^{*} = 14.3\ e^{-0.006\ P} \quad (> 99.9\ \%\ \text{significance})$$

between the dry weight of mycelium (P) and the relative levels of RNA (RNA^{*}) was consequently deduced when the microorganism was exposed to an increasing dose of Aroclor-1254.

On the other hand, when the cultures were grown in the presence of 25 ppm of the various Aroclor assayed, these effects increased as the percentage of the chlorine content was decreased (Fig. 3). The abnormally strong effects of Aroclor-1254 may well be attributable to impurities of greater toxic incidence; probably polychlorinated dibenzofurans.

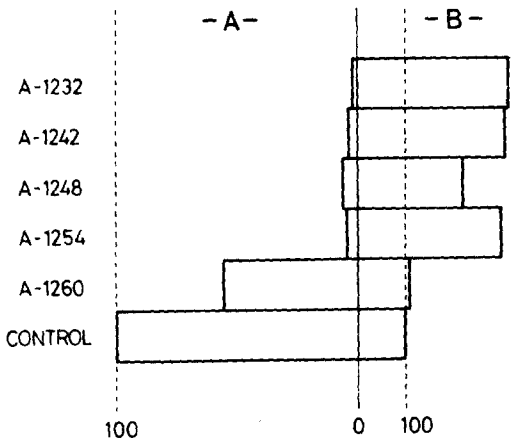

Fig. 3 - Effects of 25 ppm of each of the Aroclor assayed on the dry mycelium weight (A) and the relative levels of RNA (B). (Percentage of controls).

Oxygen consumption - Accumulative values of the oxygen consumption for 19 hours by suspensions of germinating spore of A. flavus in the presence of 25 ppm of

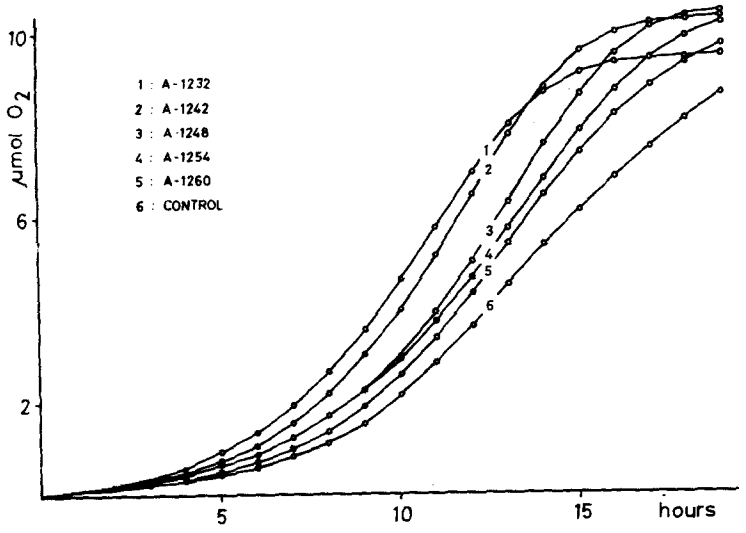

Fig. 4 - Accumulative oxygen consumption by germinating spore of A. flavus in the presence of 25 ppm of each of the Aroclor assayed.

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each of the Aroclor assayed are shown in Fig. 4. The results indicate that PCBs significantly enhance oxygen consumption; again this effect is greater the lower the percentage of PCBs chlorination.

The high density of the spore suspension at the beginning of these experiments involved a rapid exhaustion of nutrients in the medium, whose limiting effects come into play during the exposed period studied, and condition, therefore, the configuration of the resulting sigmoidal curves. The evolution process can be assimilated to one followed by a conventional batch culture, and it may be described by the logistical equation

$$\frac{dx}{dt} = rx\left(\frac{K-x}{K}\right)$$

where x is the variable to be studied, r is the rate of exponential increase of x, K is the maximum value of x (upper asymptote or "carry capacity") and t is time.

When the experimental values are adjusted to the above equation the stimulations determined by the presence of PCBs are essentialy reflected in the increase of the exponential rate r (see Table 1), where a discontinuity produced by Aroclor-1254 can also be appreciated.

TABLE 1

Equation parameters ($dx/dt = rx\,[(K-x)/K]$) to which the oxygen consumption is adjusted ($x = \mu l\ O_2$; t = hours).

	r	K
CONTROL	0.446	234.7
A-1232	0.605	239.1
A-1242	0.558	263.2
A-1248	0.506	270.6
A-1254	0.511	260.7
A-1260	0.500	248.8

These results, once the growth depressions previously indicated have been taken into account, show a remarkable drop in the energetic metabolism yield of the microorganism as a consequence of PCBs effects. This event becomes in some way complementary to another described for yeast, Saccharomyces cerevissiae (TEJEDOR et al., in press), in which the PCBs, which hindered electron transport in the respiratory chain, depressed oxygen consumption and fundamentally affected the "carry capacity".

Effects on the metabolism of other xenobiotics - The rise in the relative content of RNA has been repeatedly quoted as a characteristic of the response of different biological entities to various foreign compounds. This response was found to be related with the induced synthesis of enzymes catabolizing inductive structures.

In spite of the scarce information about this process in microorganisms, the response in this case may be supposed to hold an analogous meaning. In effect, the results summarized in Table 2 show that PCBs enhance the aldrin epoxidation, pentobarbital hydroxilation, and

TABLE 2

Metabolism of aldrin, DDT, and pentobarbital separately added (µg transformed/ mg dry mycelium weight) in the presence of 25 ppm of each of the Aroclor assayed. a: addition at the beginning of the incubation period and estimation after 70 hours; b: addition after 45 hours incubation; c: initial concentration in the medium.

	Aldrin[a] (5 ppm)[c]	DDT[a] (7 ppm)[c]	Pentobarbital[a] (45 ppm)[c]	Pentobarbital[b] (45 ppm)[c]
CONTR.	0.30	0.07	5.47	5.20
A-1232	1.22	0.54	55.03	28.46
A-1242	0.93	--	8.92	7.39
A-1248	0.51	--	7.45	5.82
A-1254	0.90	--	7.78	6.80
A-1260	0.48	--	6.47	6.31

DDT dehydrochlorination after each of the unchanged products was added to the corresponding culture medium. On the other hand, as demostrated in the experiments performed with the various Aroclor assayed, the influence of their chlorine content on conversion is also parallel with their influence on the RNA levels. In the case of aldrin there is a significant linear correlation (correl. coeff. = 0.897) between both effects (Fig. 5), but the scarce significance obtained with pentobarbital, whose meaning is very similar, could perhaps be attributed to the variable recovery attained by the method used, as was confirmed through the standard solutions of barbituric acid.

All the evidence suggests, then, that PCBs induce on the microorganism the synthesis of an enzyme system involved in the metabolism of xenobiotic compounds, its meaning analogous to the mixed function oxidases, which are mainly studied in relation to the liver of mammals, but have also been detected performing similar functions in many biological entities.

The cytochrome P-450, a characteristic component in many of these enzyme systems, could not be confirmed however in the microsomal fraction of A. flavus.

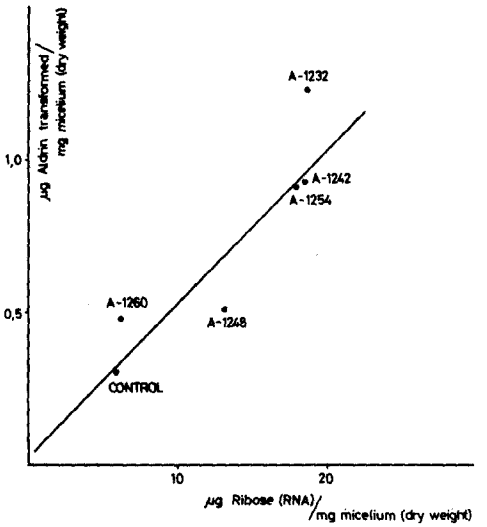

Fig. 5 - Correlation between the relative levels of RNA and epoxidated aldrin per unit weight of dry mycelium in A. flavus cultures grown in the presence of 25 ppm of each of the Aroclor assayed.

CONCLUSIONS

From the toxicological premises discussed the main aspect appears to be the contrasting signs of metabolic activation (high RNA levels, increased oxygen consumption) that the microorganism develops in the presence of PCBs, and the fact that such signs are not accompanied by a higher rate of biomass synthesis but quite the opposite.

At least a partial explanation of the problem may be deduced from two main aspects of the inductive process:

Firstly, as the PCBs are not transformed by interaction with the microorganism (MURADO et al. 1976) their inductive activity must be considered unjustified or gratuitous.

Secondly, taking into account that enhanced metabolism of aldrin, DDT, and pentobarbital is determined by the presence of structures that operate as unjustified inducers, we suggest such metabolism may be qualified as "allo-inductive".

It appears obvious, then, that PCBs as "allo-inducers" are able to generate a considerable change in the energetic economy of the microorganism. Even supposing that the induced enzymatic system does not act on the endogen fungal components, it must be borne in mind that the inductive PCBs, on resisting the attack of the induced system, compels the microorganism in a sustained way to divert a fraction of its own disponsable energy towards the synthesis of a series of highly complex components.

If, from the point of view of its formal biological meaning, the response developed by A. flavus is a consequence of a detoxifying function, its inefficacy against the PCBs may be identified as a stereotyped and non-specific reaction, whilst if the toxic effects increase in parallel with the response the reaction can be considered prejudicial.

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