injection Metalcaptase® (Knoll AG, Ludwigshafen, FRG).

Results and discussion. Table 1 shows the enzyme activities and other parameters measured in liver homogenates from neonate rats treated with D-PA (T) as detailed in the Materials and methods section, and controls (C) of the same age.

Table 2 gives the corresponding data measured in organs (liver, kidney, lung, spleen, brain, heart and haemolyzate) of D-PA-treated and control adult rats.

The enzyme activities were calculated in all cases on 1 g wet liver weight (w.l.wt) or 1 g wet tissue weight (w.wt). The SOD activities are given in values of activity units per g w.l.wt or per g w.wt (data in the thousand range) and of U/mg protein (data in the range 3–6). As a consequence of the considerably increased protein values following D-PA treatment, the SOD activities calculated per mg protein are lower than the control values (see table 1). In contrast, the D-PA treatment leads to an increase in the liver P-ase, c. P-450, protein and Cu contents, and in the value of the C-ase activity on the 10th day. As already mentioned, the SOD activity and the LP of the treated liver are decreased.

The enzyme changes observed in the organs of the adult rats following D-PA treatment are not as uniform as those in the liver of the neonates. The SOD and the liver microsomal c.P-450 activities are higher (with the exception of the kidney and the lung), while the P-ase values and (apart from the brain homogenate) the C-ase and LP activities are lower.

We consider that our data point to important correlations in connection with the mechanism of the molecular action of D-PA, as regards the enzymes and other parameters studied here. The two tables reveal that, under the conditions employed, D-PA acts in different ways on the parameters in question in neonate and adult rats. A number of points may be emphasized:

1. In both cases D-PA was found to increase the liver microsomal c.P-450 activity and to decrease the LP. The former results in an increase of the drug metabolism via an enhancement of the mixed function oxidase activity, whereas the latter is connected with the membrane-stabilizing action of D-PA. These two findings provide a good explanation in terms of the molecular mechanism for the observations of Lakatos et al. that D-PA diminishes the hexobarbital sleeping time of rats[10], and for the important therapeutic effect observed in human neonatal jaundice[8]. The LP decrease promotes the rapid elimination of the haemoprotein decomposition products by conservation of the UDP-glucuronyl transferase activity.
2. The above statements also explain the observation that D-PA treatment prevents the development of retrolental hyperplasia, which frequently follows neonatal oxygen therapy[9].
3. The data illustrate well that D-PA does not influence uniformly the enzyme activities and other parameters examined in neonate and adult animals. More correctly, it may be assumed that the reactions of the enzymes differ as a function of the age.

1 "Properties of enzymes". Serial publication Part XVI.
2 Reprint requests should be addressed to: PO Box 539, Szeged 1, H-6701, Hungary.
3 Acknowledgment. We should like to take the opportunity to express our thanks to the Directorate of Knoll AG, Ludwigshafen. FRG, for the kind gift of Metalcaptase®.
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New non-photodynamic (type B) phototoxic molecules[1]

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Summary. This report completes the examination of the 3 butadiynes substituted with phenyl and thienyl groups, and their related thiophene molecules. With the exception of 2,5-diphenylthiophene, they are all non-photodynamic (type B) phototoxic compounds.

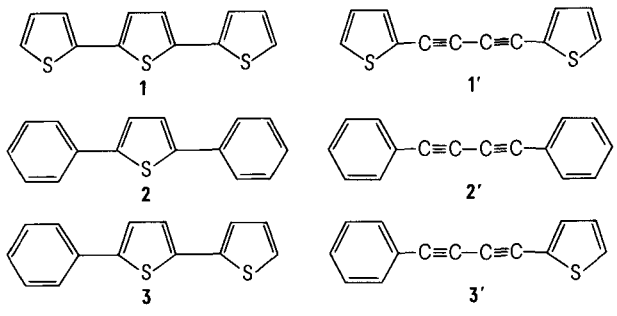
Photodynamic compounds[2] owe their phototoxicity to the conversion of molecular oxygen into singlet oxygen or superoxide ion which they mediate.

As can later be confirmed by observing the phototoxicity to a microorganism such as *E. coli* B or *Saccharomyces cerevisiae* under nitrogen, a simple test with *Candida utilis* accurately detects molecules which can be phototoxic in the absence of oxygen even if they are also efficient singlet oxygen sensitizers[3,4].

In response to a need for a simple nomenclature of phototoxic molecules, we designate by A and B the 2 general types of phototoxicity. Type A molecules generate an oxygen-dependent phototoxicity, whether through singlet oxygen or superoxide[5]. The molecules for which the phototoxicity in the absence of oxygen can be demonstrated belong to class B. The furocoumarins (psoralens) which have considerable importance in molecular biology and in medicine[6], were the only members of this latter category.

We recently reported that α-terthienyl (**1**)[4a,6] and 1,4-diphenylbutadiyne (**2′**)[7] were the first 2 new type B phototoxic compounds found outside the psoralens. We now disclose that 3 new type B phototoxic molecules have been discovered, namely 1,4-di(2-thienyl)butadiyne (**1′**), 2-phenyl-5-(2-thienyl)thiophene (**3**), and 1-phenyl-4-(2-thienyl)butadiyne (**3′**).

The close relationship between butadiynes and thiophenes in plants[8] suggested that these 2 types of molecules might have similar phototoxic properties. This hypothesis turned out to be incorrect since **2′** was phototoxic and **2** was not. However, the components of the other 2 pairs, **1** and **1′**, and **3** and **3′**, all exhibited type B phototoxicity, as demonstrated by their activity in the presence of UVA against *C. utilis*, and against both *E. coli* B and *S. cerevisiae* under nitrogen.



Because of the convenient screening procedures now available, it appears certain that the number of type B phototoxic molecules which are not furocoumarins will increase rapidly, and a structure-activity relationship will eventually be established. Fowlks et al.[9] suggested that there was a direct relationship between the diameter of the zone of inhibition and the activity of the compound tested. In general, this will not be true since the results will also depend on the light absorption characteristics and on the solubility of the compounds being tested, which cannot be expected to be identical in all compounds.

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Effect of liposomes containing cholesterol on a hepatic cholesterol-7a-hydroxylase and drug oxidation system

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Summary. A statistically significant increase ($p < 0.01$) in the activities of the microsomal enzymes cholesterol-7a-hydroxylase (EC 1.14.13.17), biphenyl-4-hydroxylase, aniline hydroxylase, NADPH-cytochrome-c-reductase (EC 1.6.2.4) and p-nitroanisole-O-demethylase, was shown in cholesterol-loaded rat liver microsomes. The data indicate that profound functional changes are induced in membrane proteins by exogenous cholesterol.

Cholesterol is a common component of biological membrane lipids, playing an important role in the control of the activity of membrane enzymes by modulating their structure[2-5]. Liver microsomal membrane cholesterol, which contributes up to 7% of the total lipid, may have both metabolic and structural functions[6]. Cholesterol from either 'aqueous solutions', or from lipid vesicles (liposomes), can be incorporated into the structure of biomembranes, producing profound functional changes in membrane-associated enzymes[3,4,7]. In the present study the effect of cholesterol on the activity of some microsomal enzyes was examined by incubating liver microsomes with cholesterol-rich liposomes.

Materials and methods. Liposomes of dipalmitoyl phosphatidyl choline (DPL) (Sigma) and cholesterol (Sigma) in the molar ratio 1:2, with (4-^{14}C)-cholesterol (sp. act. 57 mCi/mmole; Amersham, England) as a tracer, were prepared essentially as previously described[8] and suspended in 1.15% KCl-0.1 M potassium phosphate buffer, pH 7.4. Liposomes were extracted with $CHCl_3$:CH_3OH[9] and the extract chromatographed on silica gel G with appropriate standards, using $CHCl_3$:CH_3OH:CH_3COOH:H_2O (20:12:3:1 v/v) as the developing system. The content of lipid phosphorus was measured[10] in the scraped spots previously digested in 70% $HClO_4$ at 180 °C, and the cholesterol content by gas chromatography using cholestane as an internal standard. Microsomes were isolated from rat livers, according to Burke and Bridges[11], and suspended in 1.15% KCl-0.1 M potassium phosphate buffer, pH 7.4, at a concentration of 10 mg protein per ml. The microsomal lipids were quantified by $CHCl_3$:CH_3OH extraction[10] and the amount of phospholipids and cholesterol was estimated as stated above.

A liposomal suspension (40 ml), corresponding to 1.0 μmole of cholesterol, was incubated with 20 mg microsomal protein with continuous stirring at room temperature (23 °C), in a 1.15% KCl-phosphate buffer, 0.1 M, pH 7.4. Aliquots of the incubation mixture (4.0 ml) were removed at appropriate time intervals and after dilution with the same buffer were centrifuged for 30 min at 100,000 × g. The precipitate was resuspended in 20% (w/V) sucrose-phosphate buffer, 0.1 M, pH 7.4, and layered at the interface between 27% sucrose-phosphate buffer, 0.1 M, pH 7.4, and 1.15% KCl-phosphate buffer, 0.1 M, pH 7.4. The gradient was centrifuged for 2 h at 100,000 × g and the microsomes free of liposomes were recovered as a pellet at