(fig. 2A). In contrast, hepatocytes cultured in the absence of glucocorticoids for the same period of time adopted a flattened shape and were very poorly granulated (fig. 2B). Our data confirm the previous report of Sirica et al.[5] on the increase of GGT activity which occurs in adult rat hepatocytes maintained in primary cultures. Although these authors found no decrease in the number of cells exhibiting GGT activity when hepatocytes were cultured in the presence of 10^{-5}M dexamethasone, we found that this glucocorticoid practically blocked the rise in GGT activity. There is no clear explanation for this apparent discrepancy, but it is possible that the histochemical method used by Sirica et al. for the detection of GGT activity, was not adequate or sufficiently sensitive to detect the partial block in the rise of this enzyme activity produced by the glucocorticoids.

The biochemical basis of the known beneficial effect of glucocorticoids on the survival of cultured hepatocytes[13-18] has been poorly studied. It appears that these hormones delay the substitution of aldolase B by aldolase A in primary cultures of adult rats hepatocytes[24,25]. These findings, in connection with the blocking effect of the glucocorticoids on the rise of GGT activity, could suggest a role for these hormones in the control of the spontaneous dedifferentiation of liver cells in culture.

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Interaction of organic dyes with hepatic microsomal drug-metabolizing monoöxygenases in vitro

F.E. Beyhl[1,2]

Hoechst AG, D-6230 Frankfurt am Main 80 (Federal Republic of Germany), 11 November 1980

Summary. Organic dyes such as malachite green, methylene blue, fuchsin, safranine T, neutral red, phenazine methosulphate, riboflavin, dichlorophenolindophenol, phenolphthalein, and fluorescein, inhibit hepatic microsomal mixed-function oxidases and, partly, enhance, partly, inhibit hepatic microsomal NADPH-dependent cytochrome c and neotetrazolium reductases, in contrast to other inhibitors of drug metabolism which do not affect cytochrome c reductase but only interact with cytochrome P-450.

Most inhibitors of the biotransformation of drugs and other xenobiotics by liver microsomal monoöxygenases interact with cytochrome P-450, the terminal oxidase of the microsomal NADPH-dependent electron transport system[3] and thus inhibit drug monoöxygenation, but they do not interact with microsomal NADPH-cytochrome c reductase, the other constituent of this electron transport system[4-9]. This paper shows that, on the other hand, some organic dyes behave in a distinct way insofar as they not only inhibit the overall mixed-function oxidation reactions but have effects on the reductase, too. Parts of these findings have been previously reported, in a preliminary way[6,10].

Experimental. The following dyes were used: dichlorophenolindophenol (sodium salt), fluorescein (sodium salt), fuchsin (NB), malachite green, methylene blue (B), phenolphthalein, and riboflavin, from Merck AG, (Darmstadt, FRG), neutral red and safranine T, from Riedel-De Haën (Seelze, FRG), phenazine methosulphate and neotetrazolium chloride from Schuchardt (München, FRG). Aminopyrine, anisic acid N,N-diethylaminoethyl ester hydrochloride ('anisic ester'), methylayapanine, and 4-nitroanisole were from Hoechst AG (Frankfurt, FRG), 4-methylumbelliferone from EGA-Chemie (Steinheim, FRG). All biochemicals came from Boehringer (Mannheim, FRG), and buffer substances, reagents, and solvents were purchased from Merck (Darmstadt, FRG) and Riedel-De Haën (Seelze, FRG).

For the enzyme studies, rat liver 13,000 × g supernatants were prepared from crude potassium chloride homogenates by centrifugation, and rat and guinea-pig liver microsomes

Effect of organic dyes on drug-metabolizing enzymes of liver microsomes in vitro

	Aminopyrine N-demethylase	Nitroanisole O-demethylase	Anisic-ester-O-demethylase	Methylayapanine O-demethylase[a]	NADPH-cytochrome c reductase	Neotetrazolium reductase
Control	100.0 ± 6.5% (N = 11)	100.0 ± 9.5% N = 22)	100.0 ± 8.4% (N = 14)	100.0 ± 0.9% (N = 16)	100.0 ± 4.3% (N = 18)	100.0 ± 4.5% (N = 11)
Malachite green 0.1 mM	8.0 ± 3.0% (N = 7)	18.8 ± 16.6% (N = 8)	49.1 ± 21.2% (N = 7)	91.9 ± 5.9% (N = 12)	70.9 ± 13.9% (N = 11)	34.5 ± 26.2% (N = 8)
Methylene blue 0.01 mM	93.5 ± 2.4% (N = 9)	67.4 ± 14.4% (N = 12)	53.3 ± 24.0% (N = 5)	51.8 ± 13.2% (N = 12)	556.9 ± 167.9% (N = 18)	102.4 ± 9.1% (N = 8)
Fuchsin 0.3 mM	84.9 ± 6.1% (N = 9)	66.9 ± 18.7% (N = 7)	62.5 ± 24.3% (N = 8)	33.5 ± 5.5% (N = 12)	0.0 ± 0.0% (N = 14)	
Safranine T 0.1 mM	15.6 ± 14.7% (N = 8)	169.4 ± 48.1% (N = 10)	36.0 ± 23.6% (N = 8)	56.2 ± 2.9% (N = 12)	217.3 ± 50.9% (N = 14)	54.7 ± 18.8% (N = 8)
Neutral red 0.1 mM	71.6 ± 5.8% (N = 7)	42.0 ± 20.5% (N = 12)	69.7 ± 14.4% (N = 8)	27.9 ± 2.3% (N = 8)	− 129.4 ± 5.3%[b] (N = 10)	85.4 ± 9.2% (N = 8)
PMS 0.001 mM	65.8 ± 14.2% (N = 7)	53.0 ± 24.1% (N = 13)	29.5 ± 16.0% (N = 8)	1.2 ± 1.6% (N = 12)	− 189.4 ± 51.6%[b] (N = 24)	250.0 ± 66.5% (N = 8)
Riboflavin 0.1 mM	92.3 ± 6.1% (N = 9)	98.4 ± 10.2% (N = 10)	15.9 ± 12.0% (N = 6)	79.6 ± 15.5% (N = 10)	50.6 ± 15.4% (N = 16)	68.3 ± 33.3% (N = 8)
DCIP 0.3 mM	89.4 ± 4.4% (N = 8)	50.4 ± 12.0% (N = 6)	66.9 ± 16.5% (N = 6)	1.8 ± 1.6% (N = 12)	− 1404.9 ± 827.8%[b] (N = 18)	− 72.5 ± 10.0%[b] (N = 8)
Phenolphthalein 0.1 mM	55.2 ± 10.1% (N = 8)	60.3 ± 20.8% (N = 6)	64.0 ± 18.7% (N = 6)	83.5 ± 2.4% (N = 12)	68.0 ± 19.7% (N = 20)	85.3 ± 14.9% (N = 8)
Fluorescein 0.1 mM	74.9 ± 9.4% (N = 5)	74.3 ± 15.9% (N = 8)	82.0 ± 10.0% (N = 5)	71.4 ± 13.9% (N = 12)	93.0 ± 14.4% (N = 20)	89.9 ± 14.5% (N = 8)

Enzyme activities (mean values ± SD) are given as a percentage of the uninhibited control. Abbreviations: DCIP, dichlorophenolindophenol; PMS, phenazine methosulphate. [a]in guinea-pig, all other enzymes in rat. [b]Negative signs denote that the substrate was oxidized instead of reduced.

by the $CaCl_2$ precipitation method as cited earlier[7,9]. For all assays, livers of untreated animals were used. In the supernatants, the enzymic activities of aminopyrine N-demethylase, NADPH-cytochrome c reductase, and NADPH-specific neotetrazolium reductase were measured as described earlier[7], and those of anisic ester O-demethylase by our own method[11]. In rat liver microsomes, the activities of nitroanisole O-demethylase were determined as described below (Netter and Seidel[12]), and in guinea-pig liver microsomes, the activities of methylayapanine O-demethylase were measured by the method of Beyhl and Sinharay[10].

For the nitroanisole O-demethylase assay, 220 µmoles of tris(hydroxymethyl)aminomethane HCl buffer (pH 8.0), 15 µmoles of $MgSO_4$, 10 µmoles of 4-nitroanisole (added as a 0.20 molar solution in acetone), 0.68 µmoles of acetone (as the solvent for nitroanisole), 0.10 ml of a NADPH-regenerating system[10], and microsomal suspension equivalent to 50 mg of liver fresh weight, were incubated in a total volume of 3 ml, at a temperature of 37 °C, in open centrifuge tubes. After 20 min of incubation, the enzyme reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid solution, and after centrifugation, the supernatant was made alkaline by adding a 0.5% solution of disodium ethylenediamine tetraacetate (EDTA) in 0.2 M NaOH solution, and absorptions were read at 400 nm, in a 2-cm cuvette. In reference incubations, the nitroanisole solution was added to the mixture after the acidification. Under these conditions, reaction rates were proportional to time and enzyme amount.

All enzyme assays were run in the absence of inhibitors and in the presence of the dyes in concentrations given in the table.

Results and Discussion. The results are listed in the table. The demethylases are inhibited by the dyes (with $p < 5\%$ as calculated with the t-test); only riboflavin does not inhibit aminopyrine N-demethylase and nitroanisole O-demethylase, and safranine T seems to promote nitroanisole O-demethylase. Inhibition of demethylases by methylene blue is in agreement with observations that this dye inhibits naphthalene hydroxylation[13] and trichloroethylene biotransformation[14]. Demethylase inhibition by dichlorophenolindophenol fits with the inhibition of acetanilide hydroxylase by this dye[15].

These inhibition effects of demethylation reactions as caused by organic dyes cannot be simply interpreted as effects at the cytochrome P-450 level only, as is often done with other substances[4-9], since these dyes cause severe changes in the microsomal electron transfer at the cytochrome reductase level, too. These changes range from mere enzyme inhibition to increase of electron transport (resulting in elevations of enzymic activity), and even to inversion of the electron flow from substrate reduction to substrate oxidation, as is to be seen with NADPH-cytochrome c and neotetrazolium reductases. Only fluorescein has no effect on NADPH-cytochrome c reductase, and neotetrazolium reductase is unaffected by methylene blue, riboflavin, phenolphthalein, and fluorescein. The microsomal NADPH-cytochrome c reductase is a flavoprotein and therefore susceptible to interactions with these organic dyes which have redox properties themselves[16]. Phenazine methosulphate, for instance, is known to act as an electron carrier from reduced pyridine nucleotides to - as one example - tetrazolium salts; this effect is used for the visualization of NAD(P)H-dependent oxidoreductases in gel electrophoresis[17]. Methylene blue is known to increase the oxidation rate of NADPH in microsomal preparations[18] and it is used as a redox indicator and hydrogen acceptor in the well-known Thunberg technique[19].

Another possible mechanism by which at least some of these dyes could inhibit microsomal enzymes is by photoöxidation[20,21]. Which mechanism is actually effective in the inhibition of microsomal enzyme activities has to be investigated in the future, for each of these organic dyes individually.

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Changes in high density lipoprotein subfractions during alimentary lipaemia

M. Mattock, A. Salter, T. Omer and H. Keen

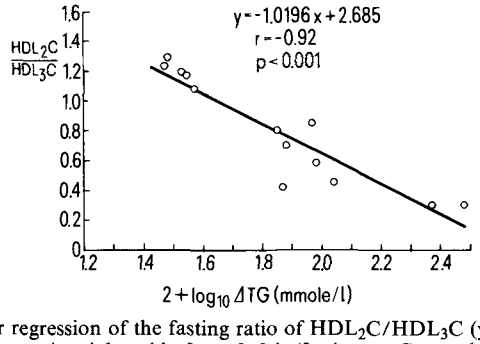
Unit for Metabolic Medicine, Guy's Hospital Medical School, London Bridge SE1 9RT (England), 8 December 1980

Summary. Significant changes in high density lipoprotein subfractions accompanied alimentary lipaemia in normal subjects. Results emphasized the importance of using fasting subjects in HDL studies but did not support an in vivo transformation of HDL_3 to HDL_2.

Low concentrations of plasma high density lipoprotein cholesterol (HDL-C) are associated with increased incidence of coronary heart disease (CHD)[1,2]. Two major subclasses of HDL, HDL_2 and HDL_3 can be isolated in the ultracentrifuge at density (d) intervals of 1.063–1.125 and 1.125–1.21 g/ml respectively[3]. Additional fractions of HDL_2, HDL_{2b} (d 1.063–1.100) and HDL_{2a} (d 1.100–1.125) have also been described[4].

The lipolysis of triglyceride-rich lipoproteins is associated with rapid transfer of lipids and peptides to HDL[5,6]. Patch et al.[7] showed that in vitro lipolysis of very low density lipoprotein (VLDL) triglyceride by lipoprotein lipase is associated with transfer of VLDL components to HDL_3 with subsequent conversion of HDL_3 to HDL_2.

We have sought in vivo evidence for this precursor-product relationship by examining the lipid content of HDL subfractions during the lipolysis accompanying alimentary lipaemia in normal subjects.



Linear regression of the fasting ratio of HDL_2C/HDL_3C (y) on (x) the change in triglyceride from 0–3 h ($2+\log_{10}$ TG mmole/l). The regression equation $y=mx+c$ is given and r is the correlation coefficient.

Methods. 13 normal subjects (6 males, 7 females, age range 22–50 years) from whom informed consent had been obtained, attended the Metabolic Ward after a 12–14 h overnight fast. With the subject resting supine, blood was drawn from an indwelling venous cannula into EDTA tubes with avoidance of venous stasis. Samples were taken fasting and then at intervals to 6 h, following the consumption of a saturated fat-rich meal (1.1 g of fat as double cream and 0.75 g of carbohydrate as canned fruit per kg b.wt). The meal was well tolerated by all subjects.

Duplicate 4-ml plasma samples were adjusted to d = 1.063 background density by addition of 2 ml NaBr solution (d = 1.177) in cellulose nitrate tubes and centrifuged at 115,000 × g 20 h in an MSE.50 preparative ultracentrifuge with No. 2582 angle-head rotor (MSE, West Sussex, Great Britain). The top fraction, containing triglyceride-rich and low density lipoproteins, was removed by tube slicing and the bottom fraction (HDL d > 1.063) quantitatively recovered. The duplicate HDL fractions were then adjusted to d = 1.100 (tube 1) and d = 1.125 (tube 2) by addition of solid NaBr and overlaid with solutions of equivalent densi-

Table 1. Serum triglycerides and cholesterol (mmole/l, mean ± SD) following the fat-rich meal

Time	0 h (fasting)	3 h	6 h
Triglycerides[a]	0.76 ± 0.60	1.75 ± 1.53***	1.09 ± 1.12**
Cholesterol	4.70 ± 1.02	4.89 ± 1.18*	5.03 ± 1.13**

[a]Triglycerides were $\log_{10}$ transformed for statistical analysis. Significantly above fasting levels by paired t-test, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.