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Comparison of the effects of the hypolipidaemic agents ICI 53072 and clofibrate with those of phenobarbitone on liver size, blood flow and DNA content in the rat

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1 The effects of the hypolipidaemic agents ICI 53072 and clofibrate on cardiac output and its distribution to the hepatosplanchnic bed were determined by the use of radioactive microspheres in the rat. The effects of these agents on hepatic DNA content were compared with those of phenobarbitone. Also the effects of ICI 53072 on hepatic microsomal enzymes and bile flow were determined together with the effects of phenobarbitone.

2 ICI 53072 and clofibrate both increased liver size and liver blood flow. A daily dose of 25 mg kg^{-1} ICI 53072 for 5 days increased liver weight by 55% and liver blood flow by 43%, the latter by enhancing the proportion of cardiac output passing to the hepatosplanchnic bed. The increased liver blood flow with clofibrate (480 mg kg^{-1} daily for 5 days) was the result of greater cardiac output but the change (35%) was half the increase in liver weight.

3 Phenobarbitone (80 mg kg^{-1} daily for 5 days) produced a fall in DNA content per unit mass of liver but no change in hepatic DNA relative to body weight. ICI 53072 (25 mg kg^{-1} daily) increased hepatic DNA relative to body weight but by a lesser extent than it increased liver weight as a proportion of body weight; hence DNA content per unit mass of liver decreased. Clofibrate at three dose levels increased hepatic DNA relative to body weight but only one dose significantly decreased DNA content as a proportion of liver weight.

4 Phenobarbitone (80 mg kg^{-1} daily) increased bile flow whereas ICI 53072 (25 mg kg^{-1} daily) had no effect. Both treatments increased hepatic cytochrome P450 content and cytochrome c reductase activity.

5 It is concluded that phenobarbitone increases liver size by hepatocyte enlargement rather than cellular proliferation but that the hepatomegaly produced by the hypolipidaemic agents, at least at some doses, is due to a mixture of both processes.

6 It is further concluded that there is no simple relationship between the mechanism of hepatic enlargement resulting from drug treatment and changes in liver blood flow.

Introduction

Clofibrate and other hypolipidaemic agents cause liver enlargement and proliferation of hepatic smooth endoplasmic reticulum and peroxisomes in the rat (Best & Duncan, 1964; Hess, Stäubli & Riess, 1965). Clofibrate also increases both hepatic cytochrome P450 and cytochrome c reductase levels (Orton & Higgins, 1980). The increase in cytochrome P450 content of the liver is due to the formation of specific haemoproteins which appear to differ catalytically from those induced by other agents such as phenobarbitone or 3-methylcholanthrene. This is shown by the failure of clofibrate to increase the activity of some model metabolic routes such as aminopyrene demethylation and benzo(a)pyrene hydroxylation (Odum & Orton, 1980; Orton & Higgins, 1980). ICI 53072, an analogue of clofibrate (Figure 1), has hypolipidaemic and hepatomegalic actions similar to clofibrate but is considerably more potent (Platt & Cockrill, 1967 and 1969).

In the study of some drugs producing hepatic en-

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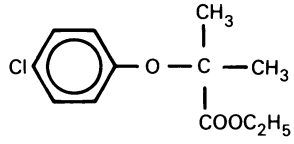
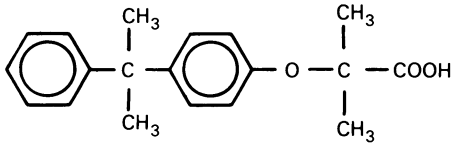


Clofibrate



ICI 53072

Figure 1 Structures of clofibrate and ICI 53072.

largement in rats it has been shown that phenobarbitone produces an increase in hepatosplanchnic blood flow (Ohnhaus & Locher, 1975; Nies, Wilkinson, Rush, Strother & McDevitt, 1976; Yates, Hiley, Roberts, Back & Crawford, 1978) and microsomal drug metabolizing activity as well as the increase in liver size. However, it has also been shown that other drugs such as phenytoin, antipyrine and chlordiazepoxide, whilst also increasing liver size and microsomal enzyme activity, fail to increase hepatic blood flow (Yates *et al.*, 1978). Thus neither enzyme induction nor liver size themselves appear to be the factor determining hepatic blood flow.

In view of the enhanced potency of ICI 53072 as a hepatomegalic agent relative to clofibrate, a substance not increasing model substrate metabolism, we have investigated the effects of both these agents on liver blood flow and some aspects of liver function. A further purpose of the study was to compare the changes brought about by ICI 53072 and clofibrate with those produced by phenobarbitone.

Methods

Animals

Male Wistar rats weighing 200–280 g (Tucks Ltd., Rayleigh, Essex), were maintained on standard laboratory chow (Labsure, C. Hill Ltd., Poole, Dorset) in drop-through cages on a 12 h light/dark cycle. Phenobarbitone (BDH, Poole, Dorset) was given by intraperitoneal (i.p.) injection at a dose of 80 mg kg^{-1} daily for 5 days in divided doses in a volume of 2 ml kg^{-1} physiological saline. ICI 53072 (ICI Pharmaceuticals, Macclesfield, Cheshire) was given orally for 5 days in a volume of 4 ml kg^{-1} saline with an appropriate amount of NaOH to produce the salt. Clofibrate (ICI Pharmaceuticals) was suspended in 1% (w/v) aqueous gum tragacanth (Sigma, Poole, Dorset) and given orally for 5 days in a volume of 4 ml kg^{-1}. Animals were deprived of food for 18 h before operative procedures.

Liver blood flow determination

The procedure used has been described in detail elsewhere (Nies *et al.*, 1976; Yates *et at.*, 1978). Briefly, animals were anaesthetized with 40–60 mg kg^{-1} i.p. pentobarbitone (Sagatal; May and Baker Ltd., Dagenham, Essex) and the right femoral artery was cannulated with polyethylene tubing (PP25) for the withdrawal of blood at a constant rate of 0.6 ml min^{-1} during, and for 70 s after, injection of microspheres. Carbonised microspheres of 15 $\mu m \pm 1$ μm diameter labelled with ^{85}Sr (3M Cp., St. Paul, MN, USA) were injected into the left ventricle over 15 s through a cannula (PP25) passed down the right carotid artery. The microspheres were suspended in 0.3 ml physiological saline containing 0.01% (v/v) Tween 80 (BDH, Poole, Dorset). Cardiac output and liver blood flow were calculated as described by Nies *et al.* (1976).

Bile flow

Bile flow was measured following cannulation of the bile duct with PP10 polyethylene tubing while the rat was anaesthetized with 40 mg kg^{-1} pentobarbitone. Body temperature was maintained at 37° ± 0.5°C by a homeothermic blanket. After discarding the first 15 min sample a further 15 min sample was collected into a tared microcentrifuge tube and weighed. The volume of bile was calculated from the weight assuming the density of bile to be identical to that of water.

Microsomal enzyme assays

Rats, treated as described above, were killed by cervical dislocation and the livers rapidly removed and homogenized in ice-cold 1.15% KCl with a Teflon-in-glass homogeniser. A microsomal fraction was then prepared as described by Yates *et al* (1978). Microsomal protein, cytochrome P450 and cytochrome c reductase were determined respectively by the methods of Lowry, Rosebrough, Farr & Randall (1951), Omura & Sato (1964) and Williams & Kamin (1962).

Table 1 Effect of ICI 53072 administered orally for 5 days at a dose of 25 mg kg^{-1} daily on cardiac output, its distribution to the hepatosplanchnic bed, liver weight and liver blood flow in anaesthetized rats

	Saline (4 ml kg^{-1} daily p.o.) ($n=8$)	*ICI 53072* (25 mg/kg^{-1}) ($n=9$)
Cardiac output (ml min^{-1} per 100 g b.wt)	20.7 ± 0.9	21.3 ± 0.9
Liver blood flow (ml min^{-1})	11.5 ± 0.4	15.3 ± 0.8***
Liver blood flow (ml min^{-1} per 100 g b.wt)	4.80 ± 0.26	6.88 ± 0.43***
Liver blood flow (ml min^{-1} per g liver)	1.31 ± 0.09	1.28 ± 0.07
% cardiac output received by hepatosplanchnic bed	22.3 ± 2.1	32.1 ± 1.0***
Liver weight (g per 100 g b.wt)	3.46 ± 0.07	5.38 ± 0.08***

Values are expressed as the mean ± s.e.mean. Statistical significance was determined by Student's *t* test: $^{**}P<0.01$; $^{***}P<0.001$.

Determination of hepatic DNA

Following treatment, rats were killed by cervical dislocation and samples of liver (200–300 mg) homogenized in 4 ml of ice-cold 6% trichloroacetic acid (TCA) in a Teflon-in-glass homogeniser. The homogenate, plus a 3 ml TCA rinse of the homogenizer tube were pooled and spun at 4°C for 20 min at 20000 *g*. After discarding the supernatant, the pellet was resuspended in 1 ml 1M perchloric acid. The remainder of the assay followed the diphenylamine reaction described by Burton (1956) and modified by Bevan, Van Marthens & Bevan (1976). Analytical reagent grade glacial acetic and diphenylamine (Fisons, Loughborough, Leicestershire) were used in the assay.

Results

Effects of ICI 53072 and clofibrate on liver blood flow

Table 1 shows the effect of ICI 53072 at a daily dose of 25 mg kg^{-1} on hepatic blood flow and liver weight. In this experiment liver weight was increased by 55% and total liver blood flow by 43% when both are expressed relative to body weight. There was no significant change in cardiac output and thus the increase in hepatic blood flow was due to the increase in the fraction of cardiac output received by the hepatosplanchnic bed. Furthermore, this change was entirely due to increased flow to the organs draining into the hepatic portal vein and not to increased flow in the hepatic artery.

Table 2 Effect of ICI 53072 administered orally for 5 days at doses of 5 mg kg^{-1} and 10 mg kg^{-1} daily on cardiac output, its distribution to the hepatosplanchnic bed, liver weight and liver blood flow in anaesthetized rats

	Saline (4 ml kg^{-1} daily p.o.) ($n=9$)	*ICI 53072 (daily dose)* (5 mg kg^{-1}) ($n=6$)	(10 mg kg^{-1}) ($n=8$)
Cardiac output (ml min per 100 g b.wt)	23.6 ± 0.9	28.2 ± 1.6*	29.9 ± 1.1***
Liver blood flow (ml min^{-1})	15.5 ± 0.7	16.3 ± 0.8	19.3 ± 1.2*
Liver blood flow (ml min^{-1} per 100 g b.wt)	7.53 ± 0.26	7.98 ± 0.39	8.77 ± 0.56
Liver blood flow (ml min^{-1} per g liver)	2.17 ± 0.11	1.89 ± 0.05	2.05 ± 0.13
% cardiac output received by hepatosplanchnic bed	32.1 ± 1.0	28.4 ± 0.6	29.7 ± 2.4
Liver weight (g per 100 g b.wt)	3.52 ± 0.15	4.20 ± 0.11**	4.29 ± 0.07***

Values are expressed as the mean ± s.e.mean. Statistical significance was determined by analysis of variances: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

Table 3 Effect of various doses of clofibrate administered orally for 5 days on cardiac output, its distribution to the hepatosplanchnic bed, liver weight and liver blood flow in anaesthetized rats

	1% gum tragacanth (4 ml kg^{-1} daily p.o.) ($n=14$)	*Clofibrate (daily dose)* (120 mg kg^{-1}) ($n=9$)	(240 mg kg^{-1}) ($n=10$)	480 mg kg^{-1}) ($n=11$)
Cardiac output (ml min^{-1} per 100 g b.wt)	18.2 ± 1.1	18.4 ± 1.7	19.9 ± 1.2	23.3 ± 1.4**
Liver blood flow (ml min^{-1})	13.9 ± 0.9	15.8 ± 1.2	16.0 ± 2.0	17.7 ± 1.2*
Liver blood flow (ml min^{-1} per 100 g b.wt)	4.77 ± 0.35	5.06 ± 0.34	5.24 ± 0.67	6.45 ± 0.46**
Liver blood flow (ml min^{-1} per g liver)	1.47 ± 0.08	1.31 ± 0.09	1.11 ± 0.13*	1.21 ± 0.08*
% cardiac output received by hepatosplanchnic bed	26.4 ± 1.4	28.8 ± 2.4	26.2 ± 2.8	27.7 ± 0.8
Liver weight (g per 100 g b.wt)	3.22 ± 0.07	3.89 ± 0.11***	4.66 ± 0.11***	5.50 ± 0.08***

Values are expressed as the mean ± s.e.mean. Statistical significance was determined by analysis of variance: *$P<0.05$; **$P<0.01$; ***$P<0.001$

A second experiment, using ICI 53072 at daily doses of 5 and 10 mg kg^{-1}, was carried out in order to determine whether or not the response to this hypolipidaemic agent was dose-related (Table 2). Liver weight relative to body weight increased by 19% and 22% for 5 and 10 mg kg^{-1} respectively but the total liver blood flow was significantly greater than control only at the higher dose. Liver blood flow relative to body weight showed a tendency to increase but the values did not reach statistical significance. There was no significant change in the proportion of the cardiac output passing to the organs of the hepatosplanchnic bed but both doses produced increases in cardiac output.

In view of the unusual observation that ICI 53072 appeared to increase cardiac output at the two lower doses but not at the higher dose, a similar experiment was carried out with the parent compound clofibrate and the results are shown in Table 3. Again, this compound produced dose-related increases in liver weight relative to body weight; daily doses of 120, 240 and 480 mg kg^{-1} gave increases in hepatic weight of 21%, 45% and 71% respectively. Liver blood flow relative to body weight was only significantly greater than control at the highest dose used, representing an increase of 35%, and this was due to an increase in cardiac output rather than its redistribution in favour of the organs of the hepatosplanchnic bed. This increase was insufficient to maintain liver blood flow per unit weight of liver which was reduced not only by the largest dose but also by pretreatment with a daily dose of 240 mg kg^{-1}.

Table 4 Effects of phenobarbitone (80 mg kg^{-1} daily i.p.) and ICI 53072 (25 mg kg^{-1} daily, orally.) given for 5 days upon hepatic DNA and microsomal protein contents in the rat

	Saline (2 ml kg^{-1} daily i.p.) ($n=4$)	*Phenobarbitone* ($n=4$)	*Saline* (4 ml kg^{-1} daily p.o.) ($n=4$)	*ICI 53072* ($n=4$)
Liver weight (g per 100 g b.wt)	3.44 ± 0.09	4.50 ± 0.07***	4.99 ± 0.13	6.80 ± 0.28***
Total hepatic DNA (mg)	27.9 ± 0.5	29.3 ± 1.3	25.7 ± 1.2	30.6 ± 1.9*
Hepatic DNA content (mg per g liver)	3.76 ± 0.12	2.88 ± 0.09***	2.58 ± 0.15	1.97 ± 0.06**
Hepatic DNA content (mg per 100 g b.wt)	12.9 ± 0.2	13.0 ± 0.5	11.5 ± 0.5	13.4 ± 0.7*
Hepatic microsomal protein (mg per g liver)	32.2 ± 1.0	36.3 ± 1.2**	45.7 ± 5.4	65.3 ± 2.8**
Protein: DNA ratio	8.6 ± 0.6	12.7 ± 0.9**	19.1 ± 2.0	33.3 ± 2.0**

Values are expressed as the mean ± s.e.mean. Statistical significance was determined by Student's *t* test between the experimental and the appropriate control: * $P< 0.05$; ** $P< 0.01$; *** $P< 0.001$

Table 5 Effects of ICI 53072 administered orally for 5 days at doses of 5 mg kg^{-1} and 10 mg kg^{-1} daily on hepatic DNA and microsomal protein contents in the rat

	Saline (4 ml kg^{-1} daily p.o.) ($n = 4$)	*ICI 53072 (daily dose)* (5 mg kg^{-1}) ($n = 4$)	(10 mg kg^{-1}) ($n = 4$)
Liver weight (g per 100 g b.wt)	3.63 ± 0.07	3.79 ± 0.23	4.41 ± 0.10***
Total hepatic DNA (mg)	24.1 ± 0.9	25.9 ± 0.9	28.4 ± 1.3*
Hepatic DNA content (mg per g liver)	3.27 ± 0.09	3.22 ± 0.06	3.14 ± 0.08
Hepatic DNA content (mg per 100 g b.wt)	11.9 ± 0.4	12.2 ± 0.8	13.9 ± 0.6*
Hepatic microsomal protein (mg per g liver)	23.7 ± 0.7	36.8 ± 1.1***	37.4 ± 1.7***
Protein: DNA ratio	7.3 ± 0.3	11.5 ± 0.3***	11.9 ± 0.6***

Values are expressed as the mean ± s.e.mean. Statistical significance was determined by analysis of variance: *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.

Effects of phenobarbitone, ICI 53072 and clofibrate on hepatic DNA and microsomal protein

Table 4 shows the effect of daily administration of 80 mg kg^{-1} phenobarbitone and 25 mg kg^{-1} ICI 53072; liver weights were increased respectively by 31% and 36% relative to the appropriate controls and with both drugs DNA content per unit weight of liver was decreased. In the case of phenobarbitone this was because there was no change in hepatic DNA content relative to body weight. However, ICI 53072 did increase both this parameter and total hepatic DNA but only by 17% and 19% respectively, approximately half the increase in liver mass and hence the fall in DNA relative to liver weight. Both treatments increased hepatic microsomal protein, phenobarbitone by 13% and ICI 53072 by 43%, which resulted in enhanced protein: DNA ratios.

The effects of the two lower daily doses of ICI 53072, 5 and 10 mg kg^{-1}, are shown in Table 5. The hepatomegalic response to the lower dose was less than in the previous experiment and the change in liver weight did not attain statistical significance. However, there was an increase of 21% in liver weight relative to body weight after treatment with 10 mg kg^{-1} daily. This dose, like 25 mg kg^{-1} daily, also significantly increased total hepatic DNA and DNA content relative to body weight, but DNA content per unit weight of liver was unchanged. Both 5 and 10 mg kg^{-1} daily significantly increased microsomal protein content, by 55% and 58% respectively, with a consequential increase in protein: DNA ratio.

From the results given in Table 6 it may be seen that, in this experiment, clofibrate increased liver weight by 41%, 54% and 63% at daily dose levels of 120, 240 and 480 mg kg^{-1}. Total hepatic DNA was also increased by the three doses, the highest and

Table 6 Effect of varying doses of clofibrate administered orally for 5 days on hepatic DNA and microsomal protein contents in the rat

	1% gum tragacanth (4 ml kg^{-1} daily p.o.) ($n = 4$)	(120 mg kg^{-1}) ($n = 4$)	*Clofibrate (daily dose)* (240 mg kg^{-1}) ($n = 4$)	(480 mg kg^{-1}) ($n = 4$)
Liver weight (g per 100 g b.wt)	3.53 ± 0.12	4.98 ± 0.37*	5.42 ± 0.30**	5.77 ± 0.24***
Total hepatic DNA (mg)	24.5 ± 1.7	37.3 ± 1.8**	33.0 ± 2.2*	36.3 ± 3.9*
Hepatic DNA content (mg per g liver)	2.52 ± 0.16	2.61 ± 0.16	2.03 ± 0.10*	2.23 ± 0.13
Hepatic DNA content (mg per 100 g b.wt)	8.8 ± 0.6	12.9 ± 0.8**	11.0 ± 0.6*	12.9 ± 1.1*
Hepatic microsomal protein (mg per g liver)	27.2 ± 1.0	26.2 ± 0.8	22.9 ± 0.8*	22.1 ± 1.1*
Protein: DNA ratio	11.0 ± 0.9	10.3 ± 0.9	10.9 ± 0.4	10.0 ± 0.6

Values are expressed as the mean ± s.e.mean. with n the number of animals in the group. Statistical significance was determined by analysis of variance: *$P < 0.05$; **$P < 0.001$; ***$P < 0.001$

Table 7 Effects on bile flow and microsomal enzyme activity in rats of saline (4 ml kg^{-1} daily, i.p.), ICI 53072 (25 mg kg^{-1} daily, orally) or phenobarbitone (80 mg kg^{-1} daily, i.p.), each given for 5 days

	Saline ($n = 6$)	*ICI 53072* ($n = 8$)	*Phenobarbitone* ($n = 7$)
Bile flow			
$\mu l\ min^{-1}$ per 100 g b.wt	7.90 ± 0.49	9.08 ± 0.61	12.80 ± 0.90***
$\mu l\ min^{-1}$ per g liver	2.06 ± 0.12	1.64 ± 0.12	2.86 ± 0.26*
$\mu l\ min^{-1}$ per mg hepatic DNA	0.65 ± 0.03	0.70 ± 0.06	1.04 ± 0.06**
Microsomal enzyme activity	($n = 4$)	($n = 4$)	($n = 4$)
Cytochrome P450 (nmol per g liver)	14.6 ± 1.7	22.8 ± 2.1*	39.0 ± 3.9**
Cytochrome c reductase (nmol min^{-1} per g liver)	2079 ± 190	2908 ± 127*	5404 ± 261***

Values are given as mean ± s.e.mean with n the number of animals in the group.
Statistical significance was determined by analysis of variance: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

lowest doses by similar extents, 48% and 52% respectively, whereas the change given by the intermediate dose was somewhat less at 35%. Hepatic DNA content relative to body weight was increased by all three treatments but, relative to liver weight, 240 mg kg^{-1} daily produced a significant reduction in DNA content. Hepatic microsomal protein content per unit weight of liver was significantly reduced by the two higher doses but, unlike ICI 53072, none of the clofibrate doses produced a change in protein: DNA ratio.

Effects of phenobarbitone and ICI 53072 on bile flow and hepatic microsomal enzyme activity

Table 7 shows that cytochrome P450 content and cytochrome c reductase activity increased to 166% and 155% respectively above corresponding control values with phenobarbitone. After ICI 53072 at a daily dose of 25 mg kg^{-1} the cytochrome P450 and cytochrome c reductase levels increased only to 56% and 40% respectively above control values.

Table 7 also gives the results of bile flow measurements. Bile production relative to body weight increased by 62% following phenobarbitone treatment and bile flow per unit weight of liver and relative to DNA content also increased significantly. In rats that received ICI 53072 there was no statistically significant change in bile flow expressed in terms of body weight although the mean value was slightly greater. When bile flow is related to liver weight there was no statistically significant change but the mean was decreased to 80% of control levels. After ICI 53072, bile flow in terms of hepatic DNA did not change.

Discussion

Many compounds causing hepatomegaly have been tested by now for their effect on liver blood flow and their number includes barbiturates and non-barbiturates (Nies *et al.*, 1976; Yates *et al.*, 1978). Only phenobarbitone of those compounds previously investigated has been shown to produce an unequivocal increase in liver blood flow which was found to be dose-dependent although in all cases the hepatomegaly was accompanied by increases in hepatic microsomal enzyme activity. Tables 1–3 show that both the hypolipidaemic agents studied here, ICI 53072 and clofibrate, can increase liver blood flow as well as produce hepatomegaly. Only the highest doses used of these two agents produced statistically significant increases in blood flow relative to body weight although the mean blood flows with the intermediate doses appeared to increase above control values in parallel with dose. With ICI 53072 the increase in liver weight and liver blood flow, when both are expressed relative body weight, were sufficiently similar (55% and 44% respectively) that liver perfusion per unit mass was unchanged. However, in the case of the highest dose of clofibrate the increase in liver weight relative to body weight (71%) was twice as great as the change in blood flow (an increase of 35%) with the result that perfusion of each unit weight of liver fell significantly below the control values. There was also a significantly lower rate of perfusion in the animals subjected to treatment with a daily dose of 240 mg kg^{-1} clofibrate.

The increase in liver blood flow relative to body weight produced by 25 mg kg^{-1} daily ICI 53072 was due to an increased distribution of cardiac output to the hepatosplanchnic bed. This is the same basis as for the increased liver blood flow produced by phenobarbitone pretreatment (Nies *et al.*, 1976; Yates *et al.*, 1978) and with both agents the increase is due to increased blood flow through the organs draining into the hepatic portal vein. The results with ICI 53072 are paradoxical in that the experiment with the two lower doses, 5 and 10 mg kg^{-1} daily, showed it to increase cardiac output and to have no

effect on the proportion of cardiac output passing through the hepatosplanchnic bed. There is no obvious explanation for this observation although it must be noted that the fraction of the cardiac output passing to the organs of the hepatosplanchnic bed was considerably different in the two control groups. This basal level may exert some effect on the type of response that occurs in response to the hepatomegaly if, indeed, the increased liver blood flow is a consequence of the hepatic enlargement.

There is also some evidence for a dose-dependent response of liver blood flow relative to body weight with clofibrate. However, the only significant change was observed with the highest dose used and it was due to enhanced cardiac output rather than its redistribution. It is interesting to note that the increase in liver blood flow relative to body weight produced by the daily dose of 480 mg kg^{-1} was insufficient to maintain blood flow per unit mass of liver, unlike the changes observed here with ICI 53072 and previously with phenobarbitone (Nies *et al.*, 1976; Yates *et al.*, 1978).

The effects of phenobarbitone, ICI 53072 and clofibrate upon hepatic DNA content were determined in order to see if the hepatic blood flow changes might correlate with the means by which these agents bring about hepatic enlargement, whether by increase in cell number or increase in cell size. With the single dose of phenobarbitone used, 80 mg kg^{-1} daily, hepatic DNA relative to body weight was unchanged while DNA relative to liver weight declined to 76% of the control value. These observations are consistant with the total number of cells in the liver remaining constant but with the size of the cells increasing which would reduce the amount of DNA in a given volume; hence, since hepatic density is unchanged by phenobarbitone pretreatment (Hiley, Wilson & Yates, 1981), there is a reduction in DNA content per unit mass of liver.

Clofibrate, on the other hand, presents a somewhat different picture in that hepatic DNA relative to body weight was increased by all three doses used, suggesting that there was an increase in the number of hepatocyte nuclei and thus, presumably, of hepatocytes. The intermediate daily dose of 240 mg kg^{-1} also resulted in a significant reduction, to 81% of the control value, of DNA relative to liver weight and the mean value for the higher dose was 88% of the control but the result did not reach statistical significance. Therefore it would appear there is a degree of hypertrophy of the hepatocytes with this compound as well as an increase in their number.

With ICI 53072 there was a similar pattern with the highest dose used producing both an increase in DNA content relative to body weight and a decrease relative to liver weight. Therefore a mix of hyperplasia and hypertrophy would seem to be occurring at a daily dose of 25 mg kg^{-1}. At the intermediate dose of 10 mg kg^{-1} daily, the predominant effect appears to be an increase in hepatocyte since DNA content relative to body weight was increased whilst the mean DNA content per unit mass of liver, though lower than, was not significantly different from the mean control value.

The nature of the increased liver mass produced by phenobarbitone was originally described as cellular proliferation although cellular hypertrophy was also noticed (Herdson, Garvin & Jennings, 1964; Stäubli, Hess & Weibel, 1969). Evidence has now accumulated using measurements of both DNA and hepatocyte size that suggests cellular hypertrophy as the principle change occurring during phenobarbitone treatment (Shenoy & Peraino, 1977; Sweeney, Jones & Krestynski, 1978). The results shown in Table 4 confirm this with DNA concentration per unit of liver decreasing to 76% of control values. A recent study by Miner & Gaito (1979) also showed such a decrease although, in their experiments, this was not statistically significant.

Previous studies with clofibrate and other hypolipidaemic agents have, as with phenobarbitone, left some uncertainty as to the mechanism of hepatic enlargement. Leighton, Coloma & Koenig (1975) used one clofibrate analogue, 2-methyl-2-(*p*[1,2,3,4-tetrahydro-1-naphthyl]phenoxy) proprionic acid, and reported on the basis of an unchanged DNA concentration per unit mass of liver that the hepatomegaly in the rat was the result of hyperplasia, although a previous study of clofibrate in mice had suggested the presence of both hyperplasia and hypertrophy (Beckett, Weiss, Stitzel & Cenedella, 1972). Our results for both clofibrate and ICI 53072 show that hyperplasia at lower doses is admixed with hepatocyte enlargement at higher rates of administration and thus might explain the previous uncertainty in the literature.

The differential effect of ICI 53072 on hepatic DNA at various doses is interesting in view of its different effects upon cardiac output and its distribution to the hepatosplanchnic bed according to the dose administered to the animals. Coupled with the observations with phenobarbitone, it would be possible to suggest that the occurrence of an increased distribution of cardiac output to the splanchnic bed, and hence an increase in liver blood flow, was associated with cellular enlargement rather than proliferation. However, the failure of the highest dose of clofibrate to produce a redistribution of cardiac output indicates that hepatocyte hypertrophy alone cannot provide the basis for this means of increasing blood flow. Since previous studies have suggested that hepatic cellular activity, at least as regards microsomal enzymes, does not correlate with liver blood flow (Nies *et al.*, 1976; Yates *et al.*, 1978) the cause of

cardiac output redistribution in favour of the hepatosplanchnic bed remains elusive.

Further evidence showing lack of correlation between hepatocyte microsomal enzyme activity and blood flow has been produced by this study. ICI 53072, at a daily dose of 25 mg kg^{-1} gave a comparable increase in liver weight to 80 mg kg^{-1} daily phenobarbitone and the increase in blood flow, 43% is slightly greater than the 32% previously reported for this dose of phenobarbitone (Yates *et al.*, 1978). However, at these doses ICI 53072 produced a 56% increase in cytochrome P450 content whereas phenobarbitone gave an increase of 160% (Table 7). This dissimilarity in the potency of enzyme induction is interesting as recent studies (Orton & Higgins, 1980; Odum & Orton, 1980) with clofibrate reveal induction of a species of cytochrome P450 that differs catalytically from those induced by either 3-methylcholanthrene or phenobarbitone as observed by its failure to increase the metabolism of certain model substrates. The profiles of hypolidaemic agents have been well characterized with respect to the proliferation of peroxisomes and their associated enzymes (Azarnoff, Reddy, Hignite & Fitzgerald, 1976; Lazarow, 1977; Masters & Holmes, 1978) and it is likely that the predominant effect of ICI 53072 on hepatocyte activity occurs through action on this system. Indeed electron microscopy has shown peroxisomal enlargement after treatment with a daily dose of 25 mg kg^{-1} (Wilson, 1981). The results with clofibrate, which showed no tendency to increase splanchnic blood flow by cardiac output redistribution, suggest there is no relationship between enhancement of total peroxisome activity and the redistribution caused by 25 mg kg^{-1} ICI 53072.

Further dissimilarities between the effects of ICI 53072 and phenobarbitone on liver function are revealed by our bile flow measurements (Table 7). ICI 53072 did not produce a significant increase in bile flow expressed relative to body weight. This is in contrast to a recent report which indicates that clofibrate increases bile volume, although in this case the doses were rather high with the diet including 0.5–1.5% clofibrate for 30 days (Mehendale, Desaiah & Mishra, 1980). Comparison between such a high dose chronic treatment with the acute dosing regime of a clofibrate analogue described here may be inappropriate and may explain the differing results. Bile flow expressed relative to liver weight gives a better indication of unit function of the liver and shows that, while phenobarbitone increases bile flow significantly ICI 53072, in contrast, decreases it (Table 7). This is due to the hepatomegaly caused by ICI 53072 not being accompanied by a large increase in bile volume. When bile flow is related to hepatic DNA the results show no difference following ICI 53072 treatment which is to be expected if hyperplasia, without changes in unit cellular activity or bile production, is primarily the response of the liver to this drug. Phenobarbitone, in contrast, increases bile flow however expressed, indicating a greater degree of bile formation within the hepatocytes and these results are comparable to those of Klaassen (1969). The increase in bile production does not appear to be linked with microsomal enzyme induction as inducers of cytochrome P450 do not always have this effect (Boyer, 1980). Bile flow is affected by such factors as liver blood flow, metabolic activity, uptake and secretion of bile into the canaliculi and the precise mechanisms are not known.

Our results show that the hypolipidaemic agents and phenobarbitone have markedly different effects on liver growth, enzyme activities and bile flow whilst both types of drug increase hepatosplanchnic blood flow. However, our observations do not give any indication that there is a common cause of the enhanced liver blood flow and the fact that clofibrate brought about its increase by changing cardiac output underlines the complexity of this aspect of drug action.

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