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FURTHER STUDIES ON THE INVOLVEMENT OF SELENIUM IN PEROXISOME PROLIFERATION IN RAT LIVER

COMPARISON OF EFFECTS WITH CLOFIBRIC ACID AND PERFLUOROOCTANOIC ACID AND THE PHARMACOKINETICS OF [^{14}C]CLOFIBRATE

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Abstract—Most effects of the peroxisome proliferator clofibrate on rat liver are marginal or absent in selenium (Se) deficiency. The purpose of the present study was to determine whether the uptake or distribution of clofibrate is altered by Se deficiency. Rats were fed a Se-adequate or -deficient diet for 10–11 weeks and then these same diets with 0.5% (w/w) clofibric acid (the direct acting hydrolysis product of clofibrate) or 0.02% (w/w) perfluorooctanoic acid (PFOA) for 10 days. Other groups of rats received radiolabeled clofibrate by intubation. Clofibric acid was as ineffective as clofibrate in producing effects (i.e. decreased body weight gain, increases in liver somatic index and protein content of the mitochondrial fraction, and increased activities of catalase and peroxisomal fatty acid beta-oxidation) in the liver of Se-deficient rats. Microsomal omega-hydroxylation was, however, equally induced in both dietary groups. In contrast to clofibric acid, the biological effects of PFOA were not affected by Se status. Furthermore, neither the tissue distribution (plasma, liver and kidney) nor the urinary excretion of ^{14}C was affected by Se deficiency. These results demonstrate that the hydrolysis of clofibrate to clofibric acid is not impaired in the Se-deficient rat. In addition, the involvement of Se in the effects of peroxisome proliferators differs for different members of this structurally heterogenous group of compounds. It is concluded that the Se-deficient rat may provide valuable information concerning the biochemical mechanism(s) underlying peroxisome proliferation.

Selenium (Se§) is an essential micronutrient necessary for the maintenance of high activity of the Se-dependent enzyme glutathione peroxidase (GSH-Px; EC 1.11.1.9) [1]. In recent years several additional selenoproteins have been characterized, including type I iodothyronine 5′-deiodinase and the liver type of fatty acid-binding protein [1]. In addition, Se plays a role for the activities of several enzymes involved in xenobiotic metabolism [2, 3] and is regarded as a cancer preventive dietary factor [4]. Thus, Se would seem to play important roles in the metabolism of both endogenous and exogenous compounds.

We recently reported that most hepatic effects (16 examined parameters, including morphometric analysis of the number and volume density of hepatic peroxisomes) of treatment with the peroxisome proliferator clofibrate [2-(4-chlorophenoxy)-2-methylpropanoic acid ethyl ester] failed to appear in the Se-deficient rat or were significantly smaller than those seen in Se-adequate controls [5]. The present study was undertaken to determine whether the pharmacokinetics of clofibrate might be altered in the Se-deficient rat, such that a lower concentration of this peroxisome proliferator reaches the liver. The background to this possibility is that the glucuronide or free form of clofibric acid [2-(4-chlorophenoxy)-2-methylpropanoic acid] is the dominating metabolite of clofibrate in tissues and urine, while the unmetabolized substance cannot be detected [6, 7]. This is to be expected, since clofibrate is known to be rapidly hydrolysed by tissue and plasma esterases [8]. Thus, hydrolysis of clofibrate to the immediate peroxisome proliferator clofibric acid is necessary for these biological effects of this compound.

Rats were fed diets containing clofibric acid in order to determine whether hydrolysis of clofibrate might be inhibited with Se deficiency. The reasoning was that if the biological effects of clofibric acid were similar in Se-adequate and -deficient rats, Se deficiency might be proposed to inhibit the hydrolysis of clofibrate.

Perfluorooctanoic acid (PFOA) resembles clofibric acid in that it is also an immediate peroxisome proliferator, which is readily absorbed by the intestine without the need for hydrolysis. PFOA was, therefore, included in this study for comparison. Thus, it is unlikely that Se deficiency affects the pharmacokinetics of clofibric acid or of PFOA via effects on the uptake and distribution of these compounds. To examine this question further, the

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§ Abbreviations: Se, selenium; GSH, glutathione; GSH-Px, selenium-dependent glutathione peroxidase; PFOA, perfluorooctanoic acid; L-FABP, liver-type fatty acid-binding protein.

distribution and excretion of [^{14}C]clofibrate in Se-adequate and Se-deficient rats was also determined.

MATERIALS AND METHODS

Chemicals. Uniformly ring-labeled [^{14}C]clofibrate (sp. act. 12.2 μCi/mg) was a generous gift from I.C.I. Pharmaceuticals Division (Alderley Park, U.K.). Hydrogen peroxide (30%) (E. Merck, Darmstadt, Germany), clofibrate, NAD, NADPH, palmitoyl-CoA, GSH, GSH reductase (the Sigma Chemical Co., St Louis, MO, U.S.A.), [^{14}C]lauric acid (New England Nuclear, Dreieich, Germany), PFOA (Aldrich Chemie, Steinheim, Germany) and Lumasolve and Lumagel (Chemical Instruments AB, Lidingö, Stockholm, Sweden) were all purchased from the sources indicated. All other chemicals were at least of analytical grade and obtained from common commercial sources.

Animals and diets. Newly weaned male Wistar rats, 20–22 days old, were fed Torula yeast-based pellets for 10 weeks. The Se content of the Se-deficient basal diet was less than 0.01 mg/kg, while that of the Se-adequate diet was 0.2 mg/kg (added as sodium selenite). Rats and diets are described in more detail elsewhere [5]. The rats were housed in a room with controlled temperature (20–22°) and a 12-hr light–dark cycle. Diet and tap water were provided *ad lib.*, unless otherwise stated.

Dietary treatment with peroxisome proliferators. Rats were divided into groups immediately prior to dietary treatment with peroxisome proliferator, such that the mean body weights of each of the three groups of Se-adequate and Se-deficient rats were as similar as possible (about 357 g and 330 g, respectively).

Diets containing peroxisome proliferator were prepared by dissolving the substance in 20 mL acetone (PA) and mixing with 100 g of the respective powdered diet to give a dose of 0.5% (w/w) clofibric acid or 0.02% (w/w) PFOA. These diets were subsequently dried for 12–24 hr in a ventilated hood (after which no smell of acetone was detectable) and stored for a few days in the refrigerator before use. Rats were fed the powdered diets, with or without peroxisome proliferator, during the last 10 days before being killed. To decrease the hepatic level of glycogen, which interferes with subcellular fractionation, rats were starved for 16 hr (overnight) prior to killing.

Subcellular fractionation, protein quantification and enzyme analysis. Preparation of the subcellular fractions, quantification of protein content and assays of GSH-Px, catalase (EC 1.11.1.6), peroxisomal palmitoyl-CoA oxidation and microsomal omega-hydroxylation were performed as described previously [5].

Treatment with [^{14}C]clofibrate. Rats were starved and maintained in wire-bottomed cages for 16 hr (overnight) before peroral administration, by intubation, of 1.4 μCi [^{14}C]clofibrate plus 96.6 mg unlabeled clofibrate/kg body weight. These compounds were dissolved in propyleneglycol and 0.5 mL was administered per 300 g body weight.

In the distribution study rats were killed about 6 hr after intubation. Blood was collected in heparinized tubes and centrifuged for 10 min at 3000 rpm to obtain plasma. The livers were perfused with 0.9% NaCl until uniformly pale and then blotted under slight pressure to remove excess perfusion media. The kidneys were removed, rinsed in saline and blotted. Tissues were placed between aluminium foil sheets and clamped between two blocks of carbon dioxide ice and then transferred into a mortar with crushed carbon dioxide ice and pulverized with a pestle. These samples were stored at −20° until analysis.

In the excretion study, rats were placed in metabolic cages, after intubation, in order to collect urine and feces during periods of 0–24, 24–48 and 48–72 hr after treatment. Samples were stored at −20° until analysis.

Measurement of radioactivity in tissues and urine. One milliliter Lumasolve was added to 250 μL plasma and 2 mL to 150 mg liver or kidney sample and the samples incubated at 50°, with intermittent shaking, until completely dissolved. After the mixtures had reached room temperature, 300 μL isopropanol were added and the vials mixed by shaking. Hydrogen peroxide (300 μL) was then added to decolorize the samples. When the initial reaction ceased, the samples were incubated at 40° for 3 hr to complete the reaction. After the samples had again reached room temperature, 10 mL Lumagel were added. In the case of urine, 10 mL Lumagel were added directly to 0.50 mL sample. The sample vials were vortexed, allowed to equilibrate overnight and scintillation counted the following day using a liquid scintillation counter, model 1211 Rackbeta, LKB (Sweden). Quench correction was by external standardization. All samples were duplicates and counted three times.

Statistics. The data are presented as means ± SE for the number of rats indicated. Analysis of statistical significance was performed using Student's *t*-test.

RESULTS

Biological effects of dietary treatment with peroxisome proliferators

No significant differences were noted in the food consumption of the six groups of rats during the 10 days of treatment with peroxisome proliferator (Table 1). Whereas dietary treatment with clofibric acid significantly decreased the body weight gain (g/day) and increased the liver somatic index and protein content of the mitochondrial fraction in the Se-adequate group, these effects were absent in Se deficiency. In contrast, PFOA caused significant changes in these same parameters independent of Se status (Table 1).

As shown in Table 2, Se-dependent GSH-Px activity in Se-deficient rat liver cytosol was less than 0.4% of the corresponding control value, thus demonstrating the efficiency of the dietary regimens employed. The activities of the marker enzymes for peroxisome proliferation, catalase and cyanide-insensitive palmitoyl-CoA oxidation were significantly increased when Se-adequate rats were treated with clofibric acid or PFOA. However, in Se deficiency these same activities were increased

Table 1. Effects of treatment with clofibric acid or PFOA on basal parameters in Se-deficient and -adequate rats

Parameter and treatment	Se-adequate	Se-deficient	Se-dependent difference
Food consumed (g/day)‖			
Control	23.3 ± 1.2	19.4 ± 1.6	NS
Clofibric acid	16.5 ± 3.2	18.3 ± 0.6	NS
PFOA	16.6 ± 2.2	16.0 ± 2.0	NS
Body weight gain (g/day)‖			
Control	2.96 ± 0.27	1.11 ± 0.36	$P < 0.02$
Clofibric acid	−2.22 ± 1.20†	1.52 ± 0.08	$P < 0.05$
PFOA	−3.89 ± 0.86‡	−2.59 ± 0.26‡	NS
Liver somatic index¶			
Control	2.58 ± 0.13	2.65 ± 0.05	NS
Clofibric acid	3.38 ± 0.12†	2.91 ± 0.11	$P < 0.05$
PFOA	4.04 ± 0.14‡	4.08 ± 0.04§	NS
Mitochondrial protein (mg/g)**			
Control	17.1 ± 1.4	15.4 ± 3.1	NS
Clofibric acid	30.6 ± 1.5‡	22.8 ± 0.6	$P < 0.01$
PFOA	37.9 ± 1.7§	36.0 ± 2.1‡	NS

Rats were fed a Se-adequate or Se-deficient diet for 10 weeks and received peroxisome proliferator in their diet during the last 10 days. Values are means ± SE for three rats. Values that are statistically significant from comparable controls are indicated by: *$0.02 < P < 0.05$, †$0.01 < P < 0.02$, ‡$0.001 < P < 0.01$ or §$P < 0.001$. The same denotations are used in Tables 2 and 3. NS, not significant.

‖ During the last 10 days.

¶ The liver to body weight ratio × 100.

** Of the liver mitochondrial fraction, containing peroxisomes.

Table 2. Effects of treatment with clofibric acid or PFOA on enzyme activities in liver subcellular fractions from Se-deficient and -adequate rats

Enzyme and treatment	Se-adequate	Se-deficient	Se-dependent difference
GSH-Px‖			
Control	574 ± 26	2 ± 0	$P < 0.001$
Clofibric acid	534 ± 73	2 ± 0.3	$P < 0.001$
PFOA	741 ± 53*	1 ± 0.3*	$P < 0.001$
Catalase¶			
Control	0.38 ± 0.06	0.39 ± 0.05	NS
Clofibric acid	0.71 ± 0.04†	0.42 ± 0.06	$P < 0.02$
PFOA	0.74 ± 0.03‡	0.63 ± 0.03†	NS
Palmitoyl-CoA oxidation**			
Control	8.0 ± 1.2	11.3 ± 1.4	NS
Clofibric acid	29.0 ± 0.0§	17.7 ± 3.3	$P < 0.05$
PFOA	59.7 ± 4.4§	67.7 ± 0.3§	NS
Omega-hydroxylation††			
Control	0.79 ± 0.10	0.78 ± 0.06	NS
Clofibric acid	5.60 ± 0.52§	4.52 ± 0.94†	NS
PFOA	7.25 ± 0.97‡	6.39 ± 0.36§	NS

For a description of rats, diets, treatments and statistical significances, see legend to Table 1.

‖ Se-dependent activity in liver cytosol (nmol/min/mg protein).

¶ Cytosolic activity (nmol/min/mg protein).

** Peroxisomal activity in mitochondrial fraction (nmol/min/mg protein).

†† Microsomal activity (nmol/min/mg protein).

Table 3. Effects of treatment with clofibrate‖ on some basal parameters and enzyme activities in Se-deficient and -adequate rats

Parameter or enzyme and treatment	N	Se-adequate	Se-deficient	Se-dependent difference
Food consumed				
Control	7	25.0 ± 0.6	19.8 ± 0.5	$P < 0.001$
Clofibrate	7	19.2 ± 1.4‡	21.4 ± 0.7	NS
Body weight gain				
Control	7	2.76 ± 0.39	1.39 ± 0.24	$P < 0.02$
Clofibrate	7	0.33 ± 0.28§	2.05 ± 0.18*	$P < 0.001$
Liver somatic index				
Control	11	2.57 ± 0.09	2.76 ± 0.06	NS
Clofibrate	11	3.42 ± 0.10§	3.02 ± 0.06‡	$P < 0.01$
Mitochondrial protein				
Control	11	17.8 ± 1.5	14.1 ± 1.3	NS
Clofibrate	11	36.4 ± 2.9§	21.9 ± 1.9‡	$P < 0.001$
Catalase				
Control	8	0.38 ± 0.03	0.45 ± 0.08	NS
Clofibrate	8	0.74 ± 0.10‡	0.35 ± 0.05	$P < 0.01$
Palmitoyl-CoA oxidation				
Control	11	17.8 ± 1.4	33.0± 4.7	$P < 0.01$
Clofibrate	11	85.3 ± 15.8§	34.4 ± 5.7	$P < 0.01$
Omega-hydroxylation				
Control	4	1.42 ± 0.33	1.48 ± 0.46	NS
Clofibrate	4	7.8 ± 1.82†	4.70 ± 0.96*	NS

‖ Except for the cytosolic catalase activities all data have been published previously [5]. These data are given for comparison with the present results.

Rats were fed a Se-adequate or -deficient diet for 10 weeks and received clofibrate in their diet during the last 10 days. Values are means ± SE of N rats.

For a description of units and statistical significances, see the legends to Tables 1 and 2.

only in rats treated with PFOA (Table 2). Microsomal omega-hydroxylation was, on the other hand, significantly and similarly induced in both Se-adequate and Se-deficient animals treated with either clofibric acid or PFOA.

To allow easy comparison with the Se-dependent effects of clofibrate, selected data from our previous publication [5] are presented in Table 3. These data demonstrate the great similarities in effects obtained with clofibric acid (Tables 1 and 2) and clofibrate (Table 3).

Tissue distribution and urinary excretion of radiolabeled clofibrate

The distribution of radioactivity (clofibrate hydrolysed to clofibric acid, and to some extent glucuronidated, 6 hr after treatment) between plasma, liver and kidneys was independent of Se status (Table 4). A significantly higher ($P < 0.05$) per cent of administered radioactivity (per g tissue) was detected in plasma than in liver and kidneys 6 hr after treatment, while the liver and kidney values were almost identical. The total amount of radioactivity detected in whole plasma plus liver plus kidneys 6 hr after administration was about 10% of the total dose.

More than 90% of the radioactivity administered was excreted in the urine within 48 hr after intubation (Table 4). In the urine collected 0–24 hr after treatment Se-deficient rats excreted 14% more of the total dose (reflecting one high value of 121% of the dose) than did the Se-adequate controls. This difference was, however, not statistically significant.

DISCUSSION

Peroxisome proliferators constitute a vast group of chemically heterogenous compounds, which cause many common biological effects in rodents, including increases in the number and volume of hepatic peroxisomes, decreased body weight gain, increased liver somatic index and protein content of the hepatic mitochondrial fraction and increases in hepatic activities of catalase, cyanide-insensitive palmitoyl-CoA oxidation and omega-hydroxylation [10]. We previously reported that, except in the case of omega-hydroxylation, these effects are absent or small when Se-deficient rats are treated with the peroxisome proliferator clofibrate. We suggested that hydrolysis of clofibrate to the immediate peroxisome proliferator clofibric acid and/or the tissue distribution of this metabolite might be affected by Se deficiency [5].

However, we have found here that clofibric acid is as ineffective as clofibrate [5] in causing peroxisome proliferation and related changes in the Se-deficient rat. Furthermore, our experiments with radiolabeled clofibrate are well in line with the results of dietary treatment with clofibric acid, since no Se-dependent differences in the tissue distribution or urinary excretion of ^{14}C were noted. This clearly indicates that the pharmacokinetics of clofibrate are not

Table 4. Distribution and excretion of ^{14}C after administration of [^{14}C]clofibrate to Se-deficient and -adequate rats

% of dose recovered	Se-adequate	Se-deficient
Per g tissue†		
Plasma	0.58 ± 0.08	0.44 ± 0.04
Liver	0.31 ± 0.03*	0.27 ± 0.03*
Kidneys	0.32 ± 0.03*	0.29 ± 0.03*
Total†‡		
Plasma	8.8 ± 1.3	6.6 ± 0.5
Liver	2.8 ± 0.3	2.5 ± 0.3
Kidneys	0.60 ± 0.09	0.66 ± 0.05
Plasma + liver + kidneys	12.1 ± 1.6	9.8 ± 0.8
In the urine		
0–24 hr after treatment	80.5 ± 9.5	94.5 ± 9.5
0–48 hr after treatment	91.4 ± 8.4	101.2 ± 9.5
0–72 hr after treatment	92.9 ± 8.4	102.4 ± 9.6

Rats were fed a Se-adequate or Se-deficient diet for 10–11 weeks before administration of 1.4 μCi [^{14}C]clofibrate plus 96.6 mg unlabeled clofibrate/kg body weight by intubation. Values are the means ± SE for four rats. Statistically significant differences from comparable plasma values are indicated by * for $P < 0.05$.

† Six hours after peroral administration.

‡ The total plasma volume was taken to be 15 mL (50% of the total blood volume), which is an approximation from data for rats of this age and weight [9]. The mean liver weights in these rats were 8.96 ± 0.09 and 9.33 ± 0.43 g for the Se-adequate and -deficient groups, respectively, and the respective mean weights of both kidneys together were 1.88 ± 0.07 and 2.22 ± 0.09 g. The GSH-Px activity in liver cytosol was 630 ± 39 and 2.3 ± 0.4 U for the Se-adequate and Se-deficient groups, respectively (1 U = 1 nmol NADPH oxidized/min/mg protein).

affected by the Se status. We also found that our pharmacokinetic results are quite comparable to those of others using rats fed a standard diet [6], indicating that our semisynthetic diet did not affect the findings. It has, thus, been reported that 97.7% of a single peroral dose of [^{14}C]clofibrate was excreted in the urine within 72 hr after administration [6], a value which is exactly the mean of the Se-adequate and -deficient rats in the present study.

Maximal radioactivity in plasma after administration of [^{14}C]clofibrate has been reported to appear after 90 min and 70% of the maximal level was still present in plasma 6 hr after administration [6]. Based on these observations, we decided to analyse tissue samples of rats killed 6 hr after treatment with [^{14}C]-clofibrate, i.e. at a time when the plasma level of ^{14}C was still high and when the level of radioactivity in the liver and kidneys could be expected to be significant.

We recovered about 10% of the administered ^{14}C in the plasma, liver and kidneys together 6 hr after treatment and no Se-dependent differences were noted for any of these compartments. Thus, most of the dose was either not absorbed, excreted or present in other compartments at this time-point. It seems probable that a significant amount of ^{14}C was present in bile, since it has been reported that about 48% of the total radioactivity after ^{14}C(-clofibrate) administration was recovered in bile within 6 hr [6]. It has been suggested that the low level of clofibric acid in plasma, the high level in bile and the low recovery of ^{14}C in feces indicate that enterohepatic recirculation plays a major role in the pharmacokinetics of this drug [6].

The level of ^{14}C per g was significantly higher in plasma than in liver and kidneys and the low amounts of ^{14}C per g found in liver and kidney were the same. Thus, there were no signs of accumulation of clofibric acid in the liver, even though this tissue is the major target organ for peroxisome proliferators.

Taken together, our present results strongly suggest that decreased hydrolysis of clofibrate cannot explain the low degree of response to this peroxisome proliferator by the Se-deficient rat [5]. The question as to why clofibrate and clofibric acid do not cause peroxisome proliferation and related effects in the Se-deficient rat must have another explanation than impaired uptake and distribution. Possible involvement of the liver form of fatty acid-binding protein (L-FABP) and/or of thyroid hormones should be considered, since both L-FABP [11, 12] and the enzyme type I iodothyronine 5′-deiodinase [13], which metabolizes thyroid hormones, are selenoproteins. Furthermore, there are certain indications that L-FABP [14, 15], as well as thyroid hormones [16–18], might be involved in peroxisome proliferation.

L-FABP plays an important role in the cytosolic transfer of fatty acids and their CoA- and carnitine esters between intracellular organelles [19]. For example, the transport of fatty acids and acyl-CoAs to peroxisomes, for subsequent oxidation there, is performed by L-FABP [19]. In addition, there is a strong correlation between the level of L-FABP present and the degree of induction of peroxisomal beta-oxidation caused by several different peroxisome proliferators [14]. It should be noted, however, that clofibrate does not bind to L-FABP [20], although certain other peroxisome proliferators are able to displace fatty acids from FABP [15, 21].

Although L-FABP is classified as a selenoprotein, due to its strong binding of Se [11, 12], the liver content of this protein does not vary with the dietary content of Se [11, 12]. Nonetheless, it is reasonable to assume that Se plays a role in the function(s) of L-FABP, although such a role remains to be established. One tentative possibility is that Se affects the binding capacity and/or dissociation constant of certain specific fatty acids. If so, the intracelluar transport of these fatty acids could be significantly affected in Se deficiency, despite the fact that the amount of L-FABP protein is not decreased.

It was noted, 4 years before L-FABP was identified as a selenoprotein [11], that Se deficiency is associated with a 50% reduction in the incorporation of fatty acids into, as well as in the mobilization of triacylglycerols in rat liver [22]. It is, however, not known whether these Se-dependent effects are specific for certain types of triacylglycerols or of a more general nature. One obvious possible explanation for this phenomenon is that Se plays a role in the transport functions of L-FABP.

One important conclusion from our previous [5] and present findings is that the Se-deficient rat might

prove a valuable model to explore further the action of different groups of peroxisome proliferators. Studies on Se-deficient rats might provide clues concerning the mechanism of peroxisome proliferation, where events outside the peroxisomes are probably of crucial importance. For example, induction of microsomal omega-hydroxylation is thought to be invariably associated with peroxisome proliferation [23]. This is obviously not the case in Se deficiency, since clofibrate induces omega-hydroxylation without affecting peroxisomal number and volume or fatty acid beta-oxidation [5]. As shown in the present study, this uncoupling of omega-hydroxylation and peroxisomal enzyme activities was also found for Se-deficient rats treated with clofibric acid, while PFOA induces peroxisomal and other marker effects in both Se-adequate and -deficient rats. Therefore, there must be some Se-dependent difference in the mechanism by which clofibrate–clofibric acid, on the one hand, and PFOA, on the other, bring about these biological effects. It is possible that peroxisome proliferators fall into two distinct subgroups whose biological effects are or are not dependent on the Se status.

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REFERENCES

1. Sunde RA, Molecular biology of selenoproteins. *Annu Rev Nutr* **10**: 451–474, 1990.
2. Burk RF, Biological activity of selenium. *Annu Rev Nutr* **3**: 53–70, 1983.
3. Reiter R and Wendel A, Selenium and drug metabolism—I. Multiple modulations of mouse liver enzymes. *Biochem Pharmacol* **32**: 3063–3067, 1983.
4. Medina D and Morrison DG, Current ideas on selenium as a chemo-preventive agent. *Pathol Immunopathol Res* **7**: 187–199, 1988.
5. Olsson U, Garberg P, Lundgren B, Andersson K, Hultenby K, Bergstrand A, Messing-Eriksson A, Högberg J and DePierre JW, The involvement of selenium in peroxisome proliferation caused by dietary administration of clofibrate to rats. *Chem Biol Interact* **85**: 49–67, 1992.
6. Baldwin JR, Witiak DT and Feller DR, Disposition of clofibrate in the rat. Acute and chronic administration. *Biochem Pharmacol* **29**: 3143–3154, 1980.
7. Emudianughe TS, Caldwell J, Sinclair KA and Smith RL, Species differences in the metabolic conjugation of clofibric acid and clofibrate in laboratory animals and man. *Drug Metab Dispos* **11**: 97–102, 1983.
8. Thorp JM, Experimental evaluation of an orally active combination of androsterone with ethyl chlorophenoxyisobutyrate. *Lancet* **i**: 1323–1326, 1962.
9. Flecknell PA, Non-surgical experimental procedures. In: *Laboratory Animals* (Ed. Tuffery AA), pp. 225–260. John Wiley and Sons Ltd, Chichester, 1987.
10. Hawkins JM, Jones WE, Bonner FW and Gibson GG, The effect of peroxisome proliferators on microsomal, peroxisomal and mitochondrial activities in the liver and kidney. *Drug Metab Rev* **18**: 441–515, 1987.
11. Bansal MP, Cook RG, Danielson KG and Medina D, A 14-kilodalton selenium-binding protein in mouse liver is fatty acid-binding protein. *J Biol Chem* **264**: 13780–13784, 1989.
12. Bansal MP, Mukhopadhyay T, Scott J, Cook RG, Mukhopadhyay R and Medina D, DNA sequencing of a mouse liver protein that binds selenium: implications for selenium's mechanism of action in cancer prevention. *Carcinogenesis* **11**: 2071–2073, 1990.
13. Behne D, Kyriakopoulos A, Meinhold H and Köhrle J, Identification of type I iodothyronine 5'-deiodinase as a selenoenzyme. *Biochim Biophys Res Commun* **173**: 1143–1149, 1990.
14. Kawashima Y, Nakagawa S, Tachibana Y and Kozuka H, Effects of peroxisome proliferators on fatty acid-binding protein in rat liver. *Biochim Biophys Acta* **754**: 21–27, 1983.
15. Cannon JR and Eacho PI, Interaction of LY171883 and other peroxisome proliferators with fatty-acid-binding protein isolated from rat liver. *Biochem J* **280**: 387–391, 1991.
16. Just WW and Hartl FY, Rat liver peroxisomes. II. Stimulation of peroxisomal fatty acid beta-oxidation by thyroid hormones. *Hoppe Seylers Z Physiol Chem* **364**: 1541–1547, 1983.
17. Kawashima Y, Katoh H and Kozuka H, Differential effects of altered hormonal state on the induction of acyl-CoA hydrolases and peroxisomal beta-oxidation by clofibric acid. *Biochim Biophys Acta* **750**: 365–372, 1983.
18. Hertz R, Aurbach R, Hashimoto T and Bar-Tana J, Thyromimetic effect of peroxisomal proliferators in rat liver. *Biochem J* **274**: 745–751, 1991.
19. Veerkamp JH, Peeters RA and Maatman RGHJ, Structural and functional features of different types of cytoplasmic fatty acid-binding proteins. *Biochim Biophys Acta* **1081**: 1–24, 1991.
20. Bass NM and Ockner RK, Interaction of lipid-lowering drugs with fatty acid-binding proteins. In: *Drugs Affecting Lipid Metabolism* (Ed. Paoletti R), p. 105. Springer, Berlin, 1987.
21. Brandes R, Kaikaus RJ, Lysenko N, Ockner RK and Bass NM, Induction of fatty acid binding protein by peroxisome proliferators in primary hepatocyte cultures and its relationship to the induction of peroxisomal beta-oxidation. *Biochim Biophys Acta* **1034**: 53–61, 1990.
22. Olsson U, Impaired ketone body metabolism in the selenium deficient rat. Possible implications. *Metabolism* **34**: 993–998, 1985.
23. Gibson GG, Orton TC and Tamburini PP, Cytochrome P-450 induction by clofibrate. *Biochem J* **203**: 161–168, 1992.