Biochimica et Biophysica Acta, 673 (1981) 225—233

BBA 29530

CHARACTERIZATION OF THE INDUCTION OF DRUG-METABOLIZING ENZYMES BY 2-ACETYLAMINOFLUORENE

ANDERS ÅSTRÖM and JOSEPH W. DePIERRE

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

(Received October 6th, 1980)

Key words: Enzyme induction; Drug metabolism; 2-Acetylaminofluorene

Summary

Changes in hepatic drug-metabolizing enzymes after intraperitoneal treatment of rats with 2-acetylaminofluorene have been investigated. This treatment was found to increase microsomal epoxide hydrolase to 762%, cytochrome *P*-450 to 143%, NADPH-cytochrome *c* reductase to 160%, cytochrome b_5 to 171%, cytoplasmic DT-diaphorase to 229% and soluble glutathione *S*-transferase activities to 200—250% of control values. These increases were time- and dose-dependent, being maximal after injection of 50 mg 2-acetylaminofluorene/kg body wt. once daily for 5 days. Enzyme markers for the plasma membrane, mitochondria, lysosomes and the soluble cytoplasm were not affected by treatment with 2-acetylaminofluorene. The present study indicates that this induction is different from that obtained with phenobarbital and 3-methylcholanthrene and more closely resembles that seen with *trans*-stilbene oxide.

Introduction

The development of preneoplastic nodules in the livers of rats which receive 2-acetylaminofluorene (2-AAF) in their diet is a well-established and promising model system for studies of chemical carcinogenesis [1—3]. The initial step in this tumorigenesis may occur soon after exposure to 2-AAF is begun and it is therefore of interest to examine early effects of this exposure. In the light of the widely accepted hypothesis that it is the metabolism of many xenobiotics which gives rise to the actual carcinogenic species, it may be of special importance to determine the effect of short-term treatment with 2-AAF on drug-metabolizing systems.

It is known that treatment of rats with 2-AAF induces the microsomal

N-hydroxylation of this compound [4]. Certain investigators have reported that this induction is accompanied by increased levels of microsomal cytochrome *P*-450 [5], while others maintain that cytochrome *P*-450 is unaffected [4]. It has also been demonstrated that treatment of rats with the classical inducer 3-methylcholanthrene increases the microsomal oxidation of 2-AAF [4,6,7].

On the other hand, there have been very few reports on the effect of short-term treatment with 2-AAF on other drug-metabolizing enzymes. One investigation showed that such treatment also results in increased microsomal epoxide hydrolase activity [8] while another demonstrated that cytoplasmic DT-diaphorase activity is increased [9]. Here, we have characterized the response of microsomal epoxide hydrolase, cytoplasmic glutathione *S*-transferase activities, cytochrome *P*-450, NADPH-cytochrome *P*-450 reductase, cytochrome b_5, NADH-cytochrome b_5 reductase and cytoplasmic DT-diaphorase in the livers of rats treated intraperitoneally with different doses of 2-AAF for varying lengths of time. In addition, we have studied the specificity of this effect and the time course of return to control levels after cessation of 2-AAF treatment.

Materials and Methods

Chemicals. 2-Acetylaminofluorene (m.p. 192—193°C) was purchased from Fluka AG (Buchs, Switzerland) and was essentially pure as judged by TLC (chloroform, chloroform/methanol (97 : 3) and petroleum ether (b.p. 40—60°C)/acetone (7 : 3)). Poly(ethylene glycol) was obtained from Kebo (Stockholm, Sweden).

All other chemicals were of reagent grade and purchased from common commercial sources.

Animals. Male Sprague-Dawley rats weighing 180—200 g were used in all experiments. The animals were maintained on pellets and water ad libitum and housed in open steel cages with no contact with excrement. The animal room had a 12-h dark and 12-h light cycle with constant temperature (23°C).

Induction. 2-AAF was dissolved in poly(ethylene glycol) 300 and 0.5 ml of this solution was injected intraperitoneally into the rats once daily. Control rats received the same amount of poly(ethylene glycol) 300. At the highest doses used (50 and 100 mg/kg) body wt.), the rats showed some bleeding from the nose, had shortened whiskers and decreased in body weight. The decrease in body weight was very likely a result of reduced appetite. These toxic effects were not seen at the lower doses of 2-AAF.

Preparation of microsomes and the supernatant fraction. The animals were starved overnight in order to reduce liver glycogen before decapitation and preparation of microsomes according to Ernster et al. [10]. The supernatant fraction from the 100 000 × *g* centrifugation was used without further treatment to measure cytoplasmic enzymes.

Enzyme assays. Cytochrome *P*-450 content was determined by the procedure of Omura and Sato [11], while NADPH-cytochrome *P*-450 reductase was assayed as NADPH-cytochrome *c* reductase [12]. Epoxide hydrolase was measured using a modification [13] of the method of Oesch et al. [14] with [7-^{3}H]styrene oxide as substrate. Glutathione *S*-transferase activity was quan-

titated spectrophotometrically at 30°C using 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene as substrates [15,16]. DT-diaphorase was determined with NADH as the electron donor and 2,6-dichlorophenolindophenol as electron acceptor [17]. Cytochrome b_5 [11], NADH-ferricyanide reductase activity (which is catalyzed by NADH-cytochrome b_5 reductase) [18], cytochrome oxidase [19], lactate dehydrogenase [20], acid phosphatase [21], AMPase [22] and glucose-6-phosphate dehydrogenase [23] were all assayed according to reported procedures. Homogenates were sonicated twice for 30 s while immersed in an ice-water bath in order to obtain maximal enzyme activity.

Protein was measured using a modification of the method of Lowry et al. [24] with bovine serum albumin as standard. Phospholipid was extracted by the Folch procedure [25] and the content of lipid phopshorus determined according to Bartlett [26].

Results

Induction of hepatic drug-metabolizing enzymes by 2-acetylaminofluorene

In Table I the effects of treating rats intraperitoneally with 50 mg 2-AAF/kg body wt. once daily for 5 consecutive days can be seen. The most dramatic effect is a greater than 7-fold increase in the specific activity of microsomal epoxide hydrolase. In addition, cytoplasmic glutathione *S*-transferase activity measured with two different substrates increases 2—2.5-fold and cytoplasmic DT-diaphorase activity demonstrates a similar increase.

Injection of 2-AAF also induces the components of the cytochrome *P*-450 system. After treatment, the microsomal cytochrome *P*-450 content is 43% higher than the control level and no spectral shift in the absorption maximum of the reduced carbon monoxide complex is observed. NADPH-cytochrome *c* reductase activity increased somewhat more than cytochrome *P*-450 itself. Cytochrome b_5, another microsomal enzyme apparently involved in drug metabolism (see Discussion), is induced 71% whereas the enzyme which reduces this cytochrome with electrons from NADH is not affected. All of these differences are relatively small but highly significant.

As a simple control that 2-AAF was not directly affecting these drug-metabolizing enzymes, cytochrome *P*-450, epoxide hydrolase, glutathione *S*-transferase and DT-diaphorase activities from control animals were measured in the presence of added 2-AAF. Concentrations of this substance were employed which corresponded to that which might be found in the liver if everything accumulated in this organ and no metabolism occurred (i.e., up to 1 mmol/mg protein for the microsomal enzymes and up to 10 μM (in the assay) for cytoplasmic enzymes). None of these enzymes is affected directly by 2-AAF, an observation which, together with the time course of the effects demonstrated (see below), supports the conclusion that the changes seen actually reflect an induction.

As also shown in Table I, intraperitoneal treatment of rats with 2-AAF does not affect the total amount of protein per gram liver, nor are the levels of microsomal and supernatant protein altered. Microsomal phospholipid is also unchanged, suggesting that, in contrast to phenobarbital, 2-AAF does not

TABLE I

EFFECT OF TREATMENT WITH 2-ACETYLAMINOFLUORENE ON VARIOUS HEPATIC ENZYMES AND PARAMETERS

	Control	Induced [a]	Induced/control
Body weight (g)	200 ± 12 (9)	166 ± 9 (9)	0.83 ***
Liver weight (g)	7.5 ± 0.5 (9)	6.8 ± 0.5 (9)	0.91 *
Total protein per g liver (mg)	159 ± 21 (3)	167 ± 4 (3)	1.05
Microsomal protein (mg/g liver)	11.5 ± 1.0 (3)	11.3 ± 1.6 (3)	0.98
Supernatant protein (mg/g liver)	58.4 ± 5.8 (3)	58.7 ± 6.4 (3)	1.01
Microsomal phospholipid [b]	0.68 ± 0.02 (3)	0.73 ± 0.03 (3)	1.07
DT-diaphorase [c]	1.01 ± 0.24 (3)	2.31 ± 0.33 (3)	2.29 *
Glutathione *S*-transferase			
with CDNB [b]	0.90 ± 0.10 (9)	1.83 ± 0.27 (9)	2.03 ***
with DCNB [e]	28.6 ± 1.2 (3)	70.4 ± 5.9 (3)	2.46 **
Epoxide hydrolase [f]	3.49 ± 0.71 (9)	26.6 ± 5.2 (9)	7.62 ***
Cytochrome *P*-450 [g]	0.49 ± 0.04 (9)	0.70 ± 0.04 (9)	1.43 ***
NADPH-cytochrome *c* reductase [h]	76.5 ± 8.7 (9)	129 ± 21 (9)	1.69 ***
Cytochrome b_5 [g]	0.28 ± 0.01 (3)	0.48 ± 0.03 (3)	1.71 **
NADH-ferricyanide reductase [i]	4.89 ± 0.10 (3)	4.66 ± 0.23 (3)	0.95
Cytochrome *c* oxidase [j]	45.6 ± 13.1 (3)	49.6 ± 11.8 (3)	1.09
Lactate dehydrogenase [k]	2.48 ± 0.08 (3)	2.81 ± 0.16 (3)	1.13
Glucose-6-phosphate dehydrogenase [l]	19.6 ± 2.1 (3)	18.5 ± 0.7 (3)	0.94
AMPase [m]	1.85 ± 0.06 (3)	1.86 ± 0.02 (3)	1.01
Acid phosphatase [m]	1.36 ± 0.14 (3)	1.25 ± 0.12 (3)	0.92

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; using Student's *t*-test.

[a] Injected intraperitoneally once daily with 50 mg/kg for 5 days and killed 24 h after the final injection.

[b] μmol phosphorus per mg microsomal protein.

[c] μmol 2,6-dichlorophenolindophenol reduced/min per mg supernatant protein.

[d] μmol 1-chloro-2,4-dinitrobenzene conjugated/min per mg supernatant protein.

[e] nmol 1,2-dichloro-4-nitrobenzene conjugated/min per mg supernatant protein.

[f] nmol styrene glycol formed/min per mg microsomal protein.

[g] nmol/mg microsomal protein.

[h] nmol cytochrome *c* reduced/min per mg microsomal protein.

[i] μmol ferricyanide reduced/min per mg microsomal protein.

[j] μmol cytochrome *c* oxidized/min per g liver.

[k] μmol NADH oxidized/min per mg supernatant protein.

[l] nmol $NADH^+$ reduced/min per mg supernatant protein.

[m] μmol inorganic phosphate released/min per g liver.

Figures are means ± S.D. for the number of animals indicated in parentheses.

cause a proliferation of the membrane of the endoplasmic reticulum. Administration of 2-AAF also results in a decrease in body weight. The liver weight decreases almost proportionally, suggesting that 2-AAF does not cause liver hypertrophy. Indeed, such hypertrophy is not to be expected, since 2-AAF is a suspected inhibitor of cellular mitosis [27].

Only a very minor portion of the changes in microsomal cytochrome *P*-450 and epoxide hydrolase caused by 2-AAF administration can be attributed to changes in the composition of the microsomal fraction obtained. As illustrated in Table II, the percentage of the inner mitochondrial membranes, lysosomes and plasma membrane fragments — the three major contaminants of rat liver microsomes [28] — recovered in the 100 000 × *g* pellet is not altered by induction. On the other hand, recovery of the endoplasmic reticulum (judging from the distribution of NADPH-cytochrome *c* reductase activity) in this fraction

TABLE II

EFFECT OF TREATMENT WITH 2-ACETYLAMINOFLUORENE ON THE COMPOSITION OF THE LIVER MICROSOMAL FRACTION

Figures are the means ± S.D. for three rats. Induction was carried out as described in footnote (a) to Table I.

Enzyme	Organelle for which it is taken to be marker	Percent of total homogenate activity recovered in total microsomal fraction from livers of	
		Control rats	Induced rats
NADPH-cytochrome *c* reductase	Endoplasmic reticulum	29.3 ± 1.5	44.5 ± 9.4
Cytochrome *c* oxidase	Inner mitochondrial membrane	2.59 ± 0.07	1.98 ± 0.36
Acid phosphatase	Lysosome	9.7 ± 0.7	9.5 ± 0.7
AMPase	Plasma membrane	12.4 ± 1.6	13.2 ± 1.2

was increased after 2-AAF treatment. A similar observation was made after induction with *trans*-stilbene oxide [29] and we do not at present have any explanation for this change. At most, 80% of the protein in control rat liver microsomes originates from the endoplasmic reticulum, while the remaining protein originates from contaminating organelles. One can easily calculate from these figures and from the recoveries of NADPH-cytochrome *c* reductase shown in Table II that about 14% of the protein in liver microsomes from rats treated with 2-AAF is present on contaminating organelles. Consequently, at most a 6% increase in the content of enzymes present on the endoplasmic reticulum can be explained on the basis of this change in composition.

Specificity of the induction by 2-AAF

Table I also demonstrates that the specific activity of markers for the soluble cytoplasm (lactate dehydrogenase and glucose-6-phopshate dehydrogenase), lysosomes (acid phosphatase), the plasma membrane (AMPase) and the inner mitochondrial membrane (cytochrome oxidase) are not affected by intraperitoneal treatment of rats with 2-AAF.

Time course of the induction by 2-AAF

Fig. 1 illustrates the time course of the induction of hepatic cytochrome *P*-450, NADPH-cytochrome *c*-reductase, microsomal epoxide hydrolase and glutathione *S*-transferase activities by 2-AAF. It can be seen that all of these enzymes have attained their maximal values after 3—5 days treatment with 50 mg/kg body wt. The time course is much the same in all cases, with the exception of cytochrome *P*-450, where a 2-day 'lag time' can be seen. Similar lag times in the induction of epoxide hydrolase and glutathione *S*-transferase by *trans*-stilbene oxide have been observed earlier [29].

Fig. 2 documents the return of the drug-metabolizing enzymes to control values after cessation of treatment with 2-AAF. All of the enzymes show a delay of about 3 days before the activities start decreasing. This effect might result from retention of a 'depot' of 2-AAF in the rats or from induction by some metabolite which is only slowly excreted. All of the enzymes return to essentially control levels within 7 days after the final administration of 2-AAF.

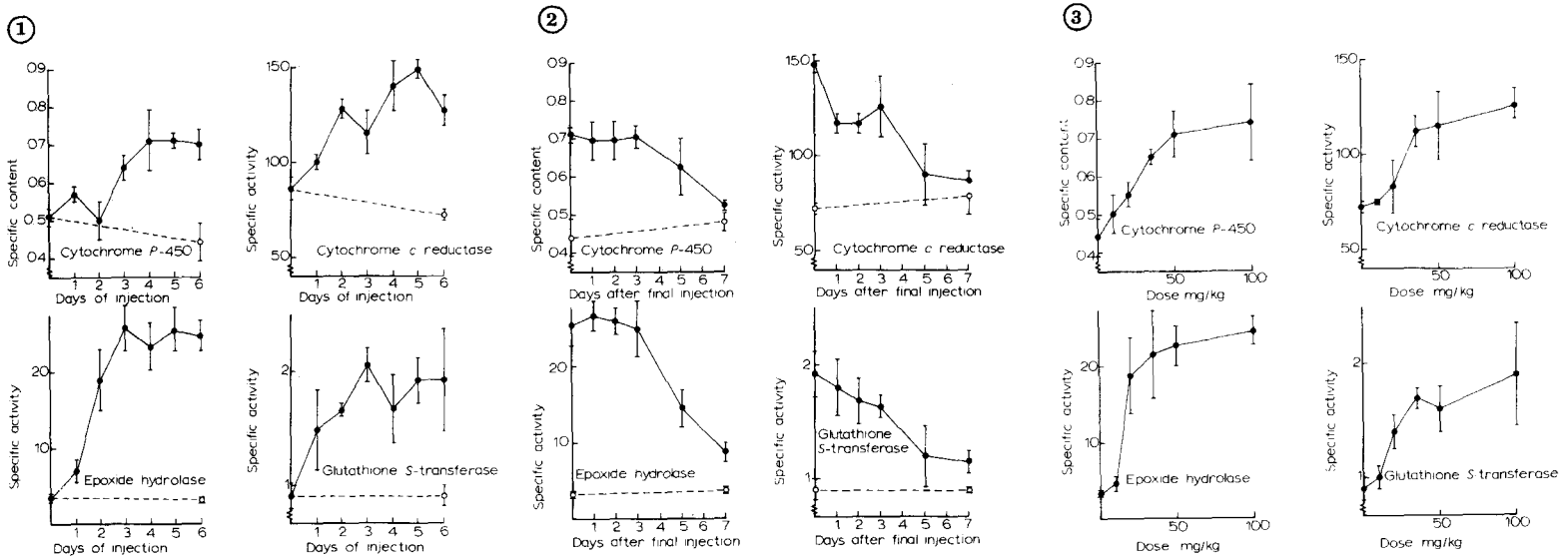

Fig. 1. (Left-hand set of four figures.) Time course of the changes in hepatic drug-metabolizing enzymes brought about by treatment of rats with 2-AAF. Rats were injected intraperitoneally once daily for 1—6 days with 50 mg 2-AAF/kg body wt. dissolved in poly(ethylene glycol) 300 (●) or with poly(ethylene glycol) alone (○) and killed 24 h after the final injection. The specific content of cytochrome *P*-450 is expressed in nmol/mg microsomal protein and the specific activities of NADPH-cytochrome *c* reductase in nmole cytochrome *c* reduced/min per mg microsomal protein, of epoxide hydrolase in nmol styrene glycol formed/min per mg microsomal protein, and of glutathione *S*-transferase in μmol 1-chloro-2,4-dinitrobenzene conjugated/min per mg supernatant protein. Each point represents the mean value ±S.D. for three rats.

Fig. 2. (Middle set.) Time course of the return of drug-metabolizing enzymes to control levels after cessation of treatment of rats with 2-AAF. Rats were injected intraperitoneally once daily for 5 days with 50 mg 2-AAF/kg body wt. dissolved in poly(ethylene glycol) 300 (●) or with poly(ethylene glycol) alone (○) and killed at different times after the final injection. The specific content and activities of the various enzymes are as explained in the legend to Fig. 1. Each point represents the mean value ±S.D. for three rats.

Fig. 3. (Right-hand set.) Response of drug-metabolizing enzymes to different doses of 2-AAF. Rats were injected intraperitoneally once daily for 6 days with different doses of 2-AAF and killed 24 h after the final injection. The specific content and activities of the various are as expressed in the legend to Fig. 1. Each point represents the mean value ±S.D. for three rats.

Response of the hepatic drug-metabolizing enzymes to different doses of 2-AAF

Fig. 3 shows the effects of different doses of 2-AAF on hepatic drug-metabolizing enzymes in the rat. 35—50 mg/kg body wt. apparently results in maximal response for the six enzymes measured. This dose lies between those required for maximal induction with 3-methylcholanthrene (20 mg/kg body wt.) and phenobarbital (80 mg/kg body wt.).

Discussion

The results presented here strongly suggest that 2-acetylaminofluorene is an inducer of drug-metabolizing enzymes. A less likely explanation is that 2-AAF or its metabolites activate certain drug-metabolizing systems. 2-AAF does not itself affect activities from control animals, but because of the wide variety of metabolites which can be formed from this substance in the liver [30], it is very difficult to test all of these products in the same manner.

One indication that induction has occurred is the time course of the increases in enzyme activities, as well as of the return to control levels. In addition, the Coomassie blue-staining band corresponding to epoxide hydrolase in SDS polyacrylamide gel electrophoretic patterns obtained with liver microsomes is strongly increased in intensity after treatment of the rats with 2-AAF. The most definitive method for demonstrating that increases in the total amounts of enzyme protein have occurred is through the use of quantitative immunoelectrophoresis. We plan to carry out such determinations.

It is of interest to compare our findings to those reported by other laboratories. We have observed that intraperitoneal injection of 2-AAF into male Sprague-Dawley rats induces liver microsomal epoxide hydrolase activity measured with styrene oxide as substrate to 762% of control values. Levin and his coworkers [8] found that epoxide hydrolase measured with octene 1,2-oxide and benz[*a*]-pyrene 4,5-oxide as substrates was increased 5—6-fold in the liver of male Holtzman rats maintained for 3 weeks on a diet containing 0.05% 2-AAF.

There is more disagreement in the case of cytochrome *P*-450. We have observed that 2-AAF induces cytochrome *P*-450 to 143% and cytochrome *c* reductase to 169% of control levels, while Malejka-Giganti et al. [4] report no induction for these enzymes. The same strain of rats was used in both cases and it is difficult to explain this discrepancy. Malejka-Giganti and coworkers did use a lower dose of 2-AAF (22 mg/kg) for a shorter time (a single intraperitoneal injection). In addition their control values for cytochrome *P*-450 are much higher than ours, indicating that their animals may be unintentionally induced by something in their environment. Cameron et al. [5] have reported an induction of cytochrome *P*-450 to 139% of control values with male Fischer rats maintained for 4 days on a diet containing 0.05% 2-AAF. We are now in the process of determining whether the cytochrome *P*-450 induced by 2-AAF is an isozyme not previously identified.

It is difficult to explain functionally why 2-AAF acts as an inducer of epoxide hydrolase and glutathione *S*-transferase activities, since neither of these enzymes is thought to be involved in the metabolism of this carcinogen [8,31].

One suggestion is that the glutathione *S*-transferases (e.g., ligandin) bind reactive metabolites of 2-AAF without actually conjugating them with glutathione. Nor is it clear why DT-diaphorase, which is postulated to function as a quinone reductase in the metabolism of polycyclic hydrocarbons [32], is induced by treatment with 2-AAF. On the other hand, there is an impressive and growing body of evidence indicating the involvement of cytochrome b_5 in cytochrome *P*-450-catalyzed monooxygenase reactions. Thus, induction of cytochrome b_5 may partially explain the large increase in *N*-hydroxylation observed after treatment with 2-AAF (Ref. 4, Åström and DePierre, unpublished results).

Both 2-AAF and *trans*-stilbene oxide [29] induce liver microsomal epoxide hydrolase activity to somewhat more than 700% of control values. This induced level may represent some sort of a 'ceiling', i.e., a maximal level of epoxide hydrolase activity which can be attained in the hepatocyte. At the same time, *trans*-stilbene oxide induces glutathione *S*-transferase activity to a greater extent than does 2-AAF [29]. It is not impossible that the type or induction obtained with *trans*-stilbene oxide and 2-AAF, i.e., larger increases in the 'phase II' than in 'phase I' activities, may actually be more typical than the type of induction obtained with phenobarbital and 3-methylcholanthrene.

We have demonstrated here that the induction obtained with 2-AAF is relatively selective for drug-metabolizing enzymes. Markers for mitochondria, lysosomes, plasma membranes and the soluble cytoplasm are not affected by this treatment. Nor is there any increase in the amount of microsomal phospholipid obtained per gram liver, indicating that no proliferation of the endoplasmic reticulum such as that seen after administration of phenobarbital is occurring in this case. On the other hand, the recovery of endoplasmic reticulum in the microsomal fraction is increased after induction with 2-AAF. A similar observation was made in connection with induction by *trans*-stilbene oxide and we have no explanation for these findings at the present.

We have also carried out detailed dose vs. response studies and time courses of the induction brought about by 2-AAF and of the return of induced enzyme levels to control values. These time courses are very similar to those seen in connection with induction by *trans*-stilbene oxide [29] or phenobarbital [33, 34].

Our future experiments will be designed to determine whether there is any connection between the induction of drug-metabolizing enzymes obtained by short-term intraperitoneal treatment with 2-AAF and the preneoplastic nodules which arise in the livers of rats maintained for several months on a diet containing this xenobiotic.

Acknowledgement

These studies were supported by a grant from the Swedish Medical Research Council.

References

1 Solt, D.B., Medline, A. and Farber, E. (1977) Am. J. Pathol. 88, 545—818
2 Solt, D.B. and Farber, E. (1976) Nature 263, 701—703
3 Emmelot, P. and Scherer, E. (1980) Biochim. Biophys. Acta 605, 247—304

4 Malejka-Giganti, D., McIver, C., Glacebrook, A.L. and Gutman, H.R. (1978) Biochem. Pharmacol. 27, 61—69
5 Cameron, R., Sharma, R.N., Sweeney, G.D., Farber, E. and Murray, R.K. (1976) Biochem. Biophys. Res. Commun. 71, 1054—1061
6 Gutman, H.R. and Bell, P. (1977) Biochim. Biophys. Acta 498, 229—243
7 Lotlikar, P.D., Hong, Y.S. and Baldy, W.J. (1978) Toxicol. Lett. 2, 135—139
8 Levin, W., Lu, A.H.Y., Thomas, P.E., Ryan, D., Kizer, D.E. and Griffin, M.J. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 3240—3243
9 Short, N.A. and Boh, E. (1977) Res. Commun. Chem. Pathol. Pharmacol. 16, 179—182
10 Ernster, L., Siekevits, P. and Palade, G.E. (1962) J. Biol. Chem. 239, 2379—2385
11 Omura, T. and Sato, R. (1964) J. Biol. Chem. 239, 2379—2385
12 Strobal, H.W. and Dignam, J.D. (1978) Methods Enzymol. 52, 89—96
13 Seidegård, J., DePierre, J., Moron, M.S., Johannesen, K.A.M. and Ernster, L. (1977) Cancer Res. 37, 1075—1082
14 Oesch, F., Jerina, D.M. and Daly, J. (1971) Biochim. Biophys. Acta 227, 685—691
15 Askelöf, P., Guthenberg, C., Jakobson, I. and Mannervik, B. (1975) Biochem. J. 147, 513—522
16 Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974) J. Biol. Chem. 249, 7130—7139
17 Höjeberg, B., Blomberg, K., Stenberg, S. and Lind, C. (1981) Arch. Biochem. Biophys., in the press
18 Mihara, K. and Sato, R. (1978) Methods Enzymol. 52, 102—108
19 Sottocasa, G., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) J. Cell. Biol. 32, 415—438
30 Schwert, G.W. and Winer, A.D. (1963) in The Enzymes (Boyer, P.D., Lardy, J. and Myrbäck, K., eds.), 2nd edn., Vol. 7, pp. 127—148, Academic Press, New York
21 Applemans, F., Wattiaux, R. and deDuve, C. (1955) Biochem. J. 59, 438—445
22 Song, C.S. and Bodansky, O. (1967) J. Biol. Chem. 242, 694—699
23 Taketa, K. and Watanabe, A. (1971) Biochim. Biophys. Acta 235, 19—26
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
25 Folch, J., Lees, M. and Stanly, G.H.S. (1957) J. Biol. Chem. 226, 497—509
26 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
27 Farber, E. (1973) Methods Cancer Res. 73, 345—375
28 DePierre, J. and Dallner, G. (1976) in Biochemical Analysis of Membranes (Maddy, A.H., ed.), pp. 79—131, Chapman and Hall, London
29 Seidegård, J., Morgenstern, R., DePierre, J.W. and Ernster, L. (1979) Biochim. Biophys. Acta 586, 10—21
30 Miller, E.C. (1978) Cancer Res. 38, 1479—1496
31 Dybing, E., Søderlund, E., Timm-Haug, L. and Thorgeirsson, S.S. (1979) Cancer Res. 39, 3268—3275
32 Lind, C., Vadi, H. and Ernster, L. (1976) FEBS Lett. 68, 219—224
33 Ernster, L. and Orrenius, S. (1965) Fed. Proc. 24, 1190—1199
34 DePierre, J.W. and Ernster, L. (1976) FEBS Lett. 68, 219—224