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DRUG-INDUCED PORPHYRIN BIOSYNTHESIS—XVII. EVALUATION OF THE PORPHYRIN-INDUCING ACTIVITY OF ALIPHATIC MONO- AND DIESTERS BEFORE AND AFTER BIS(*p*-NITROPHENYL) PHOSPHATE TREATMENT OF CHICK EMBRYO LIVER CELLS*

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Abstract—The porphyrin-inducing activity of a series of aliphatic mono- and diesters was measured in chick embryo liver cells. Sterically unhindered diesters, which are inactive in the absence of a carboxylesterase inhibitor, bis-(*p*-nitrophenyl) phosphate (BNPP), are markedly active in the presence of BNPP. Sterically hindered diesters do not require pretreatment with BNPP to exhibit activity, but show greater potency in its presence. After BNPP pretreatment, the potency of diesters paralleled their lipophilicity, supporting the conclusion that porphyrin-inducing activity of esters and amides depends upon lipophilicity and resistance to rapid metabolism to compounds of lower lipophilicity. Aliphatic monoesters were found to be inactive; moreover, in contrast to diesters, they remained inactive after BNPP pretreatment. The inactivity of monoesters after BNPP pretreatment could not be attributed to hydrolysis by BNPP-insensitive microsomal or lysosomal esterases, but was found to be the result of evaporation of these compounds from the surface of the culture medium in which the cells were incubated.

Allylisopropylacetamide (AIA) and 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) are two chemicals which arewidely used in the study of drug-induced porphyrin biosynthesis. Since the former chemical is an amide and the latter a diester, the porphyrin-inducing activity of a series of esters, diesters and amides has been measured [1–3] in order to elucidate the structural features required for activity. Studies have been conducted in chick embryo liver cells, a system which is highly sensitive to drug-induced porphyrin biosynthesis. Sterically unhindered aromatic esters and aliphatic amides were found to be inactive in the absence of a carboxylesterase inhibitor, bis-(*p*-nitrophenyl) phosphate (BNPP), but markedly active in the presence of BNPP. On the other hand, the potency of sterically hindered aromatic esters and aliphatic amides was shown to be similar in the presence and absence of BNPP. In contrast to the aromatic esters, both sterically hindered and unhindered aromatic amides were found to be active in the absence of BNPP. A high correlation was shown between porphyrin-inducing activity and lipophilicity of aromatic amides and esters after inhibition of aromatic ester hydrolysis by BNPP. Similarly, a good correlation was found between porphyrin-inducing activity and lipophilicity of aliphatic amides after inhibition of aliphatic amide hydrolysis by BNPP. On the basis of the above data it was concluded that the porphyrin-inducing activity of aromatic esters and aromatic and aliphatic amides depends upon lipophilicity and resistance to rapid metabolism to compounds of lower lipophilicity.

In this study, the porphyrin-inducing activity of a series of aliphatic mono- and diesters has been examined in the presence and absence of BNPP. The objective of this study was to determine whether the porphyrin-inducing activity of these compounds could similarly be explained by lipophilicity and resistance to rapid metabolism to compounds of lower lipophilicity.

METHODS

Source of compounds

The following compounds were purchased from Aldrich Chemical Co., Milwaukee, WI: diethyl glutarate, 3,3-dimethylglutaric acid, 3-methyl-glutaric acid and ethyl butyrate. Insulin (bovine pancreas, 24 I.U./mg), L-thyroxine sodium pentahydrate (T_4), bovine serum albumin (insulin-free), hexanoic acid, heptanoic acid, decanoic acid, ethyl octanoate and ethyl nononoate were purchased from Sigma Chemical Co. (St. Louis, MO). 2,4-Dimethylglutaric acid was obtained from Sapon Chemical Co. (Oceanside, NY). *N*-decanoic acid[^{14}C] was purchased from Amersham/Searle, Arlington Heights, IL. The ethyl esters were prepared from the appropriate acids by methods previously described [3], BNPP was prepared by the procedure of Corby *et al.* [4]. Waymouth MD 705/1 medium was purchased in powder form from Grand Island Biological Co, Grand Island, NY.

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Estimation of steric hindrance to hydrolysis of aliphatic diesters

The degree of steric hindrance to chemical hydrolysis was estimated by Newman's rule of six [5]. In the diethyl glutarate series, the degree of hindrance from enzymic hydrolysis parallels the degree of hindrance from chemical hydrolysis, viz. diethyl-3,3-dimethylglutarate > diethyl-3-methylglutarate > diethyl glutarate or diethyl-2,4-dimethylglutarate [6].

Cell culture technique

The details of the cell culture technique have been previously described [7–9]. The cells were maintained in 60-mm-diameter disposable plastic Petri dishes (Falcon Plastics, Oxnard, CA) containing 5ml of Waymouth MD 705/1 medium supplemented with 60 mg penicillin G, 100 mg streptomycin sulfate, 1.0 mg insulin and 1.0 mg T_4/liter. After an initial incubation period of 24 hr, the medium was discarded and replaced with fresh medium. BNPP (50 μg) in 95% redistilled ethanol (10 μl) or ethanol alone (10 μl) was added to cell cultures which were then returned to the incubator for 1 hr. Chemicals, dissolved in 95% ethanol (10 μl), were added to the cell cultures, and the dishes re-incubated. Porphyrin and protein content of cells and medium were measured 24 hr later [10]. Results are expressed as ng porphyrins/mg of protein.

Determination of chick embryo liver carboxylesterase activity

Preparation of microsomes. Microsomes were prepared by the method of Omura and Sato [11]. Chick embryos (17-day-old) were decapitated and the livers removed and homogenized with a Potter–Elvehjem homogenizing apparatus using a Teflon pestle (six strokes) in 4 vol. of ice-cold buffered 1.15% KCl solution (0.0001 M potassium phosphate buffer, pH 7.4, containing 1.04% KCl, solution A). The homogenate was centrifuged for 20 min at 9000 *g* at 4° in a refrigerated Sorval centrifuge and the supernatant fraction containing microsomes and soluble proteins was removed with a Pasteur pipette. The pellet containing nuclei and broken cells was discarded. The supernatant fraction was centrifuged in a Beckman preparative ultracentrifuge at 102,000 *g* for 1 hr at 4°. The supernatant fraction was removed and the pellet (containing microsomes) was washed by resuspending it in solution A (ice-cold). The suspension was centrifuged for 1 hr at 102,000 *g*. The supernatant fraction was discarded and the pellet suspended in a suitable volume of CO_2-free saline. Kinetic parameters were determined using a 0.2-ml aliquot as described below.

Preparation of lysosomes. Lysosomes were prepared according to the method of Lee and Fritz [12]. A known weight of liver was homogenized with a Potter–Elvehjem homogenizing apparatus (Teflon pestle; six strokes) in 10 vol. of ice-cold 0.25 M sucrose containing 10 mM Tris–HCl, pH 7.5 (solution B). The homogenate was centrifuged for 20 min at 800 *g* at 4°. The pellet containing nuclei, trapped mitochondria and unbroken cells was discarded. The supernatant fraction was centrifuged for 20 min at 40,000 *g* at 4°. The pellet containing mainly mitochondria and lysosomes was then suspended in solution B and centrifuged as above. The pellet was suspended in an appropriate volume of solution B and kinetic parameters were determined using a 0.2-ml aliquot as described below.

Kinetic measurements. Kinetic measurements were made using a Radiometer pH stat with various aliphatic mono- and diesters as substrates. Suitable concentrations of each substrate were prepared in 0.2 ml ethanol and this volume of ethanol was kept constant in all experiments. Hydrolysis reactions were carried out in a closed chamber, previously flushed by a stream of nitrogen to remove CO_2, at 25° with constant stirring. The substrate was added to CO_2-free saline (7.6 ml) and titrated automatically to pH 7.8 with 0.001 N NaOH. When constant pH was achieved, the enzyme solution (0.2 ml in CO_2-free saline) was added to initiate the reaction. The order of addition of reactants did not affect the reaction. When BNPP (final concn 1.25×10^{-4} M) was used, it was added in a volume of 10 μl in 95% redistilled ethanol, together with the enzyme solution (0.2 ml), to CO_2-free saline (7.6 ml) and titrated automatically to pH 7.8, at which point the substrate was added. By measuring the amount of NaOH consumed by the hydrolysis reaction with time, the initial velocity was calculated. The data were analyzed by the method of Hofstee [13] and V_{max} values were determined for each substrate and expressed as μmoles hydrolyzed/min/mg of protein. Protein content of the various enzyme solutions was determined by the method of Lowry *et al.* [14] as modified by Miller [15].

Fate of [1-^{14}C]*ethyl decanoate and* [1-^{14}C]*decanamide in chick embryo liver cell culture*

Synthesis of [1-^{14}C]*ethyl decanoate and* [1-^{14}C]*decanamide.* Labeled ethyl decanoate and decanamide were synthesized from [1-^{14}C]decanoic acid by standard procedures [3]. The labeled ester was diluted with unlabeled drug and dissolved in 95% redistilled ethanol to give a final concentration of 15.0 mg/ml (sp. act. = 0.605 μCi/mg). The labeled amide was diluted with unlabeled drug to give a final concentration of 15 mg/ml (sp. act. = 0.605 μCi/mg).

Extraction of labeled ethyl decanoate from the medium. The method used was that of Racz and Moffat [16]. However, since the ester was very susceptible to alkaline hydrolysis, the procedure was modified as follows: 10 μl of the above solution of [1-^{14}C]ethyl decanoate was added to each of twenty Petri dishes containing 5 ml Waymouth medium. At various time periods (0, 0.5, 1, 4 and 12 hr), the media were removed from four dishes and placed in separatory funnels; the Petri dishes were washed with 10 ml ether which was added to the media. The separatory funnels were then shaken and the ether phase was removed. The aqueous phases were extracted twice more with two 10-ml portions of ether. The ether extracts (3 × 10 ml) were combined and 1-ml aliquots of the ether extracts were added to counting vials containing 10 ml of a toluene scintillation solution (6 g of 2,5-diphenyloxazole (PPO);

Amersham/Searle) and 100 mg of 1,4-bis-[2(5-phenyloxazolyl)]benzene (POPOP; Amersham/Searle) in 1 liter toluene). Aliquots (1 ml) of the ether-extracted aqueous phases were added to counting vials containing 10 ml Aquasol (New England Nuclear, Boston, MA). The above experiment was repeated with the modification that [1-^{14}C]-ethyl decanoate was added to media alone which were then incubated at 37° for 0, 0.5, 1, 4 and 12 hr prior to extraction. The percentage of unchanged drug was determined by dividing the counts (dis./min) in the ether phase by the counts (dis./min) added to the cultures.

Extraction of [1-^{14}C]*decanamide from the medium.* The proceudure used was that of Racz and Moffat [16] and differs from that described above for the extraction of the labeled ester in that 0.1 ml of 1 N NaOH was added to the aqueous phase prior to extraction with ether. The percentage of unchanged drug was determined by dividing the counts (dis./min) in the ether phase by the counts (dis./min) added to the cell cultures.

Studies with BNPP. BNPP (50 μg) in 95% redistilled ethanol (10 μl) was added to cell cultures which were then returned to the incubator for 1 hr. The solution of radioactive drug in 95% ethanol (150 μg/10 μl) was added and the dishes were returned to the incubator. The cell cultures were treated as described above.

Determination of radioactivity in cells. At appropriate time periods (0, 0.5, 1, 4 and 12 hr) after the addition of labeled ethyl decanoate or decanamide, medium was removed from cell culture dishes and 2 ml of Earle's calcium- and magnesium-free solution was added to the cells and the cells were removed with a rubber policeman into centrifuge tubes. The cells were sedimented by centrifugation at 300 *g* for 5 min, the supernatant fraction was removed and 0.5 ml of NCS (Nuclear Chicago Solubilizer) added to the cells, which were maintained at 50° for 6–8 hr with occasional shaking. Fresh benzoyl peroxide (0.5 ml, 20% benzoyl peroxide in toluene) was added to the sample. After 18 hr, the sample was transferred to a glass counting vial, and 0.2 ml of glacial acetic acid and 10 ml Aquasol were added to the partially decolorized sample and the sample was counted.

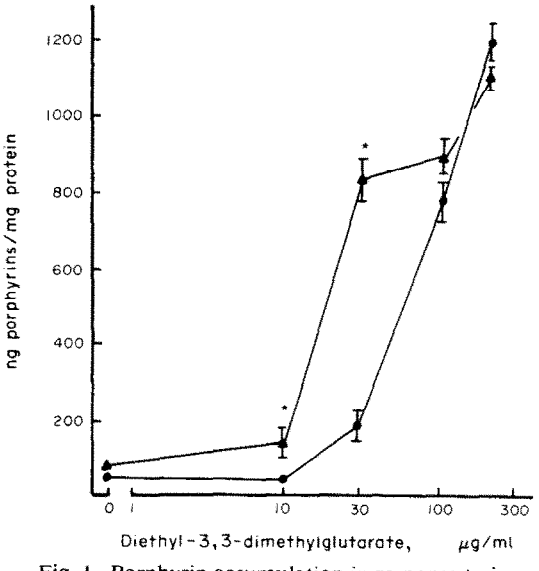

Fig. 1. Porphyrin accumulation in response to increasing doses of diethyl-3,3-dimethylglutarate in the presence (▲) and absence (●) of BNPP (10 μg/ml). Asterisks indicate a significant difference in porphyrin accumulation between cells pretreated with BNPP and those untreated at the same dose of diester. Each point represents the mean of four determinations ± S. E. M.

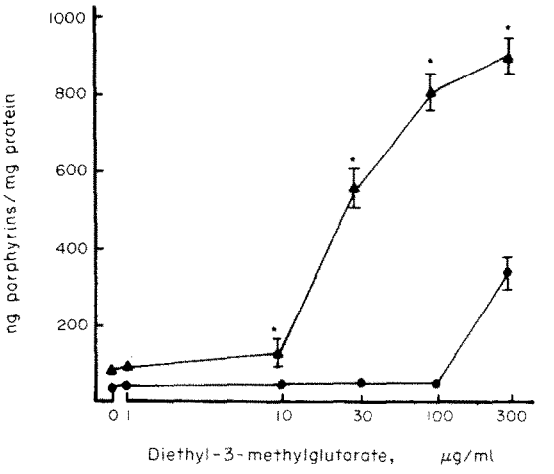

Fig. 2. Porphyrin accumulation in response to increasing doses of diethyl-3-methylglutarate in the presence (▲) and absence (●) of BNPP (10 μg/ml). Asterisks indicate a significant difference in porphyrin accumulation between cells pretreated with BNPP and those untreated at the same dose of diester. Each point represents the mean of four determinations ± S. E. M.

Counting of radioactive samples. All radioactive samples were counted in a Beckman liquid scintillation counter, using an external standard channel ratio to correct for quenching; background counts were subtracted.

RESULTS AND DISCUSSION

In the absence of BNPP, the porphyrin-inducing activity of the aliphatic diesters in chick embryo liver cells maintained in serum-free medium (Figs. 1–3) was found to be similar to the activity previously reported by Schneck and Marks [6], who used a qualitative technique for measuring porphyrins in cells maintained in serum-containing medium. The sterically hindered diester, diethyl-3,3-dimethylglutarate, was markedly active (Fig. 1), while diethyl-3-methylgutarate, which is less hindered from hydrolysis, was considerably less active (Fig. 2). Diethyl glutarate, which is unhindered from hydrolysis, was inactive (Fig. 3). In the presence of BNPP, the activity of the sterically hindered diethyl-3,3-dimethylglutarate (Fig. 1) was unchanged either at 100 or 300 μg/ml but its activity was increased significantly at lower doses (10–30 μg/ml). The activity of the partially hindered diethyl-3-methylglutarate and the unhindered diethyl glutarate was markedly increased after BNPP pretreatment (Figs. 2 and 3). Similar results were obtained in serum-containing

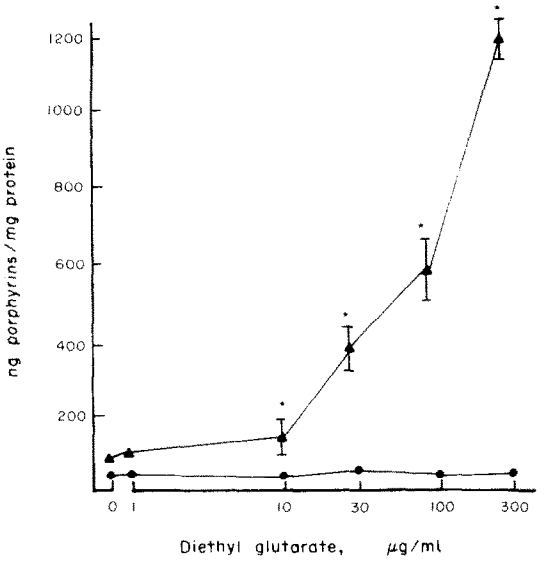

Fig. 3. Porporphyrin accumulation in response to increasing doses of diethyl glutarate in the presence (▲) and absence (●) of BNPP (10 μg/ml). Asterisks indicate a significant difference in porphyrin accumulation between cells pretreated with BNPP and those untreated at the same dose of diester. Each point represents the mean of four determinations ± S. E. M.

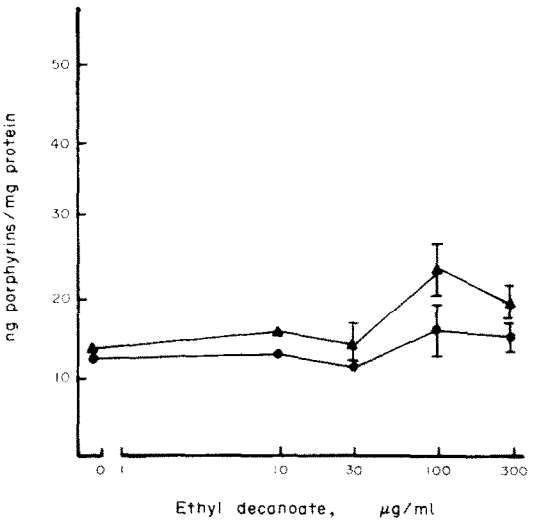

Fig. 4. Porphyrin accumulation in response to increasing doses of ethyl decanoate in the presence (▲) and absence (●) of BNPP (10 μg/ml). Each point represents the mean of eight to twelve determinations ± S. E. M.

media. Diethyl-2,4-dimethylglutarate, which is unhindered from hydrolysis, was inactive in inducing porphyrin accumulation in serum-containing medium, but became markedly active in the presence of BNPP. It was of interest to attempt to correlate the porphyrin-inducing activity of aliphatic diesters with their lipophilicity after blockade of their hydrolysis with BNPP. Hansch *et al.* [17–20] have been able to correlate the relative biological activity of chemicals in a series of congeners with their lipophilicity. As a measure of lipophilicity, Hansch has used log *P*, where *P* is defined as the octanol–water partition coefficient of a drug. The log *P* values of the diesters were available [20]: diethyl-3,3-dimethylglutarate, 2.7; diethyl-3-methylglutarate, 2.2; and diethyl glutarate, 1.9. We defined the porphyrin accumulation observed 24 hr after the addition of 30 μg/ml of AIA as the standard response. The concentrations of diethyl-3,3-dimethylglutarate, diethyl-3-methyl-glutarate and diethyl glutarate in the presence of BNPP required to give the standard response in cells maintained in serum-free medium were 17, 27 and 70 μg/ml. Clearly, therefore, the potency of these aliphatic diesters parallels their lipophilicity after BNPP pretreatment and, as with aromatic esters and aromatic and aliphatic amides [1, 2], their activity is dependent upon lipophilicity and resistance to metabolism to compounds of lower lipophilicity.

A series of aliphatic monoesters, viz. ethyl butyrate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl nononoate and ethyl decanoate, was shown to be inactive in inducing porphyrin accumulation in chick embryo liver cells cultured in serum-free medium supplemented with insulin and T_4. The results obtained with a representative aliphatic monoester, viz. ethyl decanoate, are shown in Fig. 4. It was anticipated that BNPP pretrea ment would prevent the hydrolysis of the sterically unhindered monoesters, and allow them to exhibit porphyrin-inducing activity, as was observed with aliphatic diesters. However, this was not the case, as shown with a representative monoester (ethyl decanoate) in Fig. 4. There appeared to be at least three possible explanations for the inactivity of aliphatic monoesters after BNPP pretreatment: (1) monoesters may be hydrolyzed by a BNPP-insensitive esterase, (2) monoesters may be more rapidly hydrolyzed than are aliphatic diesters by a BNPP-sensitive esterase and BNPP may be unable to totally block hydrolysis of the monoesters, and (3) monoesters may be rapidly degraded to compounds which are used in other cellular processes. Our next experiments were directed to examining the validity of the first two explanations. The major portion of hepatic carboxylesterase activity is located in microsomes [21] and an investigation was carried out of the rates of hydrolysis of various aliphatic mono- and diesters by microsomes and the effect of BNPP on enzymic activity. In order to examine the third possibility, viz. that monoesters are rapidly degraded to products used in other cellular processes, the fate of radioactive ethyl decanoate was followed in cell culture in the presence and absence of BNPP.

The V_{max} values, determined by the method of Hofstee [13] for the hydrolysis of diesters by a microsomal preparation of chick embryo liver carboxylesterase, are shown in Table 1. As anticipated, the sterically hindered diester, diethyl-3-methylglutarate, had a significantly lower V_{max} than the two sterically unhindered diesters, viz. diethyl glutarate and diethyl-2,4-dimethylgutarate. No hydrolysis of the most highly hindered diester, diethyl-3,3-dimethylglutarate, was detectable. The V_{max} values for the hydrolysis of aliphatic monoesters obtained by the Hofstee method are shown in Table 1 and in general are higher than the values observed with the diesters. The addition of BNPP (1.25×10^{-4} M) was effective in inhibiting the hydrolysis of

Table 1. Hydrolysis of various aliphatic mono- and diesters by a microsomal preparation of carboxylesterase

Substrate	V_{max}*	S. E.	Log P_{oct} [20]
Diethyl glutarate	0.2161	0.0401	1.9
Diethyl-2,4-dimethylglutarate	0.2043	0.0351	2.7
Diethyl-3-methylglutarate	0.0317	0.0118	2.2
Diethyl-3,3-dimethylglutarate	0		2.7
Ethyl butyrate	0.2982	0.0879	1.73
Ethyl hexanoate	0.5218	0.1310	2.73
Ethyl heptanoate	0.4703	0.0629	3.23
Ethyl octanoate	0.6196	0.0610	3.73
Ethyl nononoate	0.4703	0.0511	4.23
Ethyl decanoate	0.4234	0.0471	4.73

* Expressed as μmoles/min/mg of protein.

all aliphatic mono- and diesters to greater than 90 per cent.

It is generally assumed that the esterase activity of liver is mainly associated with the microsomal fraction [22–26]. However, histochemical studies have emphasized the importance of carboxylesterase activity in lysosomes [27, 28]. Several workers [29–31] have suggested that the microsomal esterase is the non-specific esterase, inhibited by organophosphates, and that the lysosomal esterase is an "A" type esterase, and therefore not inhibited by organophosphates such as BNPP. It was therefore thought that the monoesters may be susceptible to hydrolysis by this BNPP—"insensitive" lysosomal carboxylesterase, therefore accounting for their inactivity in inducing porphyrin accumulation in cell culture after BNPP pretreatment. Lysosomes were prepared according to the method of Lee and Fritz [12] to separate the lysosomal fraction from the microsomal fraction. The lysosomal fraction hydrolyzed ethyl octanoate at a rate approximately twice that of the microsomal fraction (V_{max} = 1.0960 ± 0.0845 μmoles/min/mg of protein). However, the addition of BNPP (1.25×10^{-4} M) inhibited hydrolysis of the monoester by the lysosomal esterase to an extent greater than 90 per cent.

In summary, we have shown that BNPP blocks the major esterases of the liver cells, viz. those in the microsomes and lysosomes. It was therefore unlikely, although not impossible, that the inactivity of monoesters after BNPP pretreatment of liver cells could be attributed to their hydrolysis by BNPP-insensitive esterases in other subcellular fractions. To satisfactorily resolve this problem would have required an exhaustive analysis of the BNPP sensitivity of esterase activity in other cell fractions. Such a study was rendered unnecessary by experiments described below on the fate of a [^{14}C]-labeled monoester in the cell culture system. We have also shown that monoesters are more rapidly hydrolyzed by a BNPP-sensitive microsomal esterase than are diesters (Table 1). This observation, however, proved of minor importance to the understanding of the inactivity of monoesters after BNPP pretreatment of hepatic cells once a study of the fate of a [^{14}C]-labeled monoester had been carried out.

Our next objective was to determine whether monoesters were rapidly degraded to compounds used in other cellular processes by following the fate of a radioactive monoester, viz. [1-^{14}C]ethyl decanoate, in cell culture in the presence and absence of BNPP. In the presence or absence of BNPP, a rapid decrease of ether-extractable radioactivity, representing unchanged drug, occurred in the media (Fig. 5). This suggested that the drug had been converted to a metabolite which remained in the medium and which was not ether-extractable. For this reason, the radioactivity remaining in the medium was measured. However, less than 2 per cent of the radioactivity added to the cell culture dishes was detected in the medium. It therefore appeared that the drug had entered the cells. To examine this possibility, the cells were solubilized with NCS and radioactivity was determined. To our surprise, no radioactivity was detectable in the cells. These experiments were repeated in the presence and

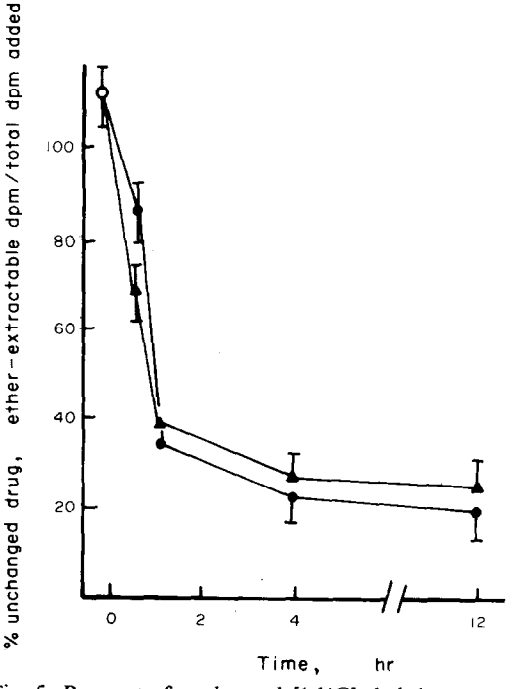

Fig. 5. Per cent of unchanged [1-^{14}C]ethyl decanoate in the medium after different periods of incubation of the monoester with a monolayer of chick embryo liver cells in the presence (▲) and absence (●) of BNPP (10 μg/ml). Each point represents the mean of twenty determinations. ± S. E. M.

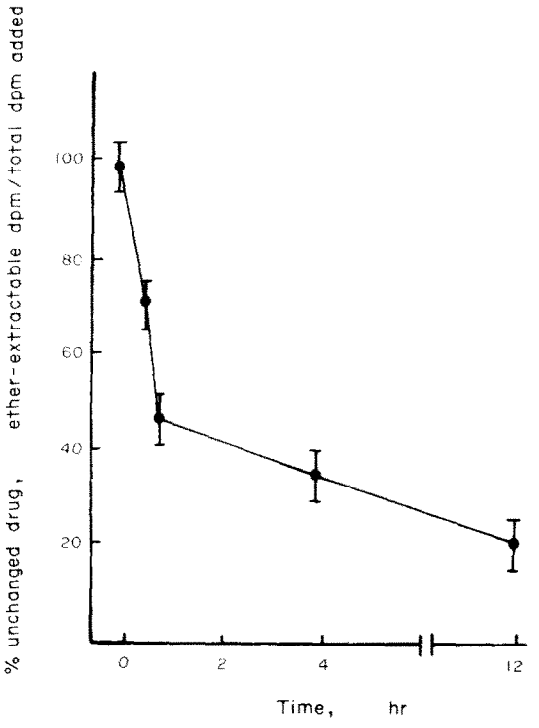

Fig. 6. Per cent of [1-^{14}C]ethyl decanoate in the medium after different periods of incubation of the monoester with the medium alone. Each point represents the mean of sixteen determinations ± S. E. M.

absence of BNPP in media alone and it was observed that the levels of ether-extractable drug decreased rapidly (Fig. 6), following the same time course as that observed in the presence of cells (Fig. 5). This result was totally unexpected and the possibility was considered that the use of serum-free medium, in place of serum-containing medium used in earlier studies with other drugs, was the source of difficulty. We therefore repeated earlier studies on the fate of [1-^{14}C]decanamide that had been carried out in serum-containing media [1], in serum-free media. No essential difference was observed. In addition, the above experiments with [1-^{14}C]ethyl decanoate were repeated in serum-free media supplemented with 0.2% bovine serum albumin. The addition of protein had no effect on the amount of radioactivity recovered from media alone or from media in the presence of cells with or without BNPP. As a further control on our methodology, the fate of [1-^{14}C]-decanamide in media alone was compared to that of [1-^{14}C]ethyl decanoate. In contrast to results with the ester, where only 35 per cent of the drug could be recovered after 4 hr of incubation, 100 per cent of the amide was recoverable at this time period. The inactivity of the monoester must therefore be ascribed to its physico-chemical properties, which result in its evaporation from the culture medium in the stream of 95% air and 5% CO_2 in which the culture dishes were incubated. It is of interest that ethyl decanoate (25 mg/0.1 ml of dimethylsulfoxide) was found to be devoid of activity [no effect on δ-aminolevulinic acid (ALA) synthetase levels after 6 hr] when injected into the fluids surrounding the embryo in the presence or absence of BNPP. In the same experiment, allylisopropylacetamide produced a 25-fold elevation of the enzyme level.

Thus the inactivity of aliphatic monoesters in a monolayer of chick embryo liver cells is explained by evaporation from the surface of the medium so that insufficient drug is left to induce porphyrin accumulation. The activity of aliphatic diesters cannot be explained by lipophilicity alone, but can be explained by a combination of lipophilicity and steric factors. This study supports the previous conclusion [1, 2] that porphyrin-inducing activity of esters and amides depends upon lipophilicity and resistance to metabolism to compounds of lower lipophilicity. It is of interest to consider how the property of lipophilicity may be related to porphyrin-inducing activity. Jain *et al.* [32] have shown that lipophilic drugs induce a transition of lipid acyl chains from an organized gel to a randomized liquid crystalline phase and suggest that drug-induced changes in the lipid phase of a bilayer may affect the function of various membrane-bound proteins. It is therefore possible that porphyrin-inducing drugs may interact with membrane lipids and thereby modulate the activity of a key membrane-bound enzyme involved in the control of ALA synthetase, the rate-limiting enzyme in heme biosynthesis.

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