Biochemical Pharmacology, Vol. 28, pp. 3533–3538.

0006-2952/79/1215–3533 $02.00/0

DRUG-INDUCED PORPHYRIN BIOSYNTHESIS—XIX

POTENTIATION OF THE PORPHYRIN-INDUCING EFFECTS OF SKF 525-A IN THE CHICK EMBRYO LIVER BY 3,5-DIETHOXYCARBONYL-1,4-DIHYDRO-2,4,6-TRIMETHYLPYRIDINE, AN INHIBITOR OF FERROCHELATASE*

SUSAN P. C. COLE, ELIZABETH J. VAVASOUR and GERALD S. MARKS
Department of Pharmacology, Queen's University, Kingston, Ontario, Canada K7L 3N6

(*Received* 21 *February* 1979; *accepted* 13 *June* 1979)

Abstract—Pretreatment of 17-day-old chick embryos with 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) resulted in enhancement of hepatic δ-aminolevulinic acid (ALA)-synthetase activity and porphyrin accumulation induced by 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC). The levels of [^{14}C]DDC and its metabolites in chick embryo livers were measured at different times after administration of [^{14}C]DDC in the presence and absence of SKF 525-A. It is concluded that the magnitude of inhibition of DDC metabolism following SKF 525-A pretreatment is too small to account for the enhanced inducing effects of DDC. DDC was found to inhibit ferrochelatase in chick embryo liver at doses considerably less than those required to induce ALA-synthetase activity. A dose of DDC was selected for administration to the chick embryo large enough to produce 95 per cent inhibition of ferrochelatase without affecting ALA-synthetase activity. When SKF 525-A was then administered, a marked synergistic effect was observed on ALA-synthetase activity and porphyrin accumulation. It is concluded that DDC, by inhibiting ferrochelatase, enhances the ability of SKF 525-A to induce ALA-synthetase activity and porphyrin accumulation.

A practical problem which arises in deciding which drugs may be safely administered to patients with hereditary hepatic porphyria is the choice of an appropriate system in which to test the drugs. Variability of drug response in different species is a well known phenomenon. Two reasons have been suggested by Brodie *et al.* [1] to account for this variation: (1) variation in drug metabolism, resulting in differences in the amounts of drug at the site of action, and (2) variation in sensitivity of receptor sites. Different rates of drug metabolism are thought to account largely for the species variation [2]. Consequently, it has been suggested by Mannering that the use of 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A), an inhibitor of hepatic drug metabolism, in conjunction with a new drug being investigated, would eliminate or minimize variability of drug response in different species. On the basis of these considerations, Taub *et al.* [3] examined the possibility of using SKF 525-A in conjunction with porphyrin-inducing drugs to minimize the variability of response observed previously in different test systems.

Taub *et al.* [3] showed that the ability of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) to induce δ-aminolevulinic acid (ALA)-synthetase activity and porphyrin accumulation in chick embryo livers was greatly enhanced by pretreatment with SKF 525-A. They attributed the increase in porphyrin-inducing ability of DDC to elevated hepatic levels of the unchanged drug, resulting from an SKF 525-A-mediated blockade of metabolism of DDC to inactive metabolites. However, an alternative explanation is possible. SKF 525-A induces ALA-synthetase activity in the 17-day-old chick embryo liver [3,4] but does not induce porphyrin accumulation. If DDC inhibits chick embryo liver ferrochelatase, as it has been shown to do in mouse and rat liver [5,6], then heme formation would decrease and heme-mediated feedback repression of ALA-synthetase would diminish. As a result, the SKF 525-A-mediated increase in ALA-synthetase would be enhanced and porphyrins would accumulate. This alternative explanation appears plausible in view of the synergistic effects observed previously when DDC was used in combination with other porphyrin-inducing drugs [7–9]. The objective of the present study was to determine which of the two explanations of the synergism between DDC and SKF 525-A is correct.

EXPERIMENTAL

Experimental animals. Fertilized eggs used were of the White Leghorn strain, obtained from Archer's Poultry Farm, Brighton, Ontario. They were stored at 10° for no longer than 7 days prior to incubation at 38°. The age of the embryo was taken as the number of days from onset of incubation.

Male Wistar rats (approximately 250 g) were obtained from Bio-Breeding Laboratories, Ottawa. The rats were housed in wire cages for at least 5 days before being killed and were fed Purina Laboratory Chow for mice, rats and hamsters (Ralston Purina Co., Woodstock, Ontario). The rats were starved overnight prior to killing; water was available *ad lib.*

* Supported by the Medical Research Council, Canada

Source of compounds. [2,6-^{14}C]-3,5-Diethoxycarbonyl - 1,4 - dihydro - 2,4,6 - trimethylpyridine-([^{14}C]DDC) was prepared from ethylacetoacetate-3-[^{14}C] according to the method of Loev and Snader [10], as modified by Racz and Marks [11]. DDC was prepared as described by Marks *et al.* [12], and 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) was obtained from Smith, Kline & French, Montreal. Mesoporphyrin IX dihydrochloride was obtained from Porphyrin Products, Logan, UT. Tween 80 (polyoxyethylene sorbitan mono-oleate), Tween 20 (polyoxyethylene sorbitan monolaurate), dithioerythritol (DTE), iodoacetamide and ferrous sulfate heptahydrate were obtained from the Sigma Chemical Co., St. Louis, MO.

Administration of drugs to 17-day-old chick embryos. A small hole was made in the egg shell above the air sac. Drugs were injected through this hole into the fluids surrounding the embryo with a sterile 1 inch 20 gauge needle attached to a 1 cm^3 tuberculin syringe. The hole in the shell was sealed with Cello-tape and the eggs were returned to the incubator.

Effect of SKF 525-A on [^{14}C]DDC metabolism. One group of chick embryos was injected with SKF 525-A (0.3 mg) dissolved in saline (0.1 ml), while a control group was injected with saline (0.1 ml). The eggs were returned to the incubator for 1 hr. [^{14}C]DDC (0.2 mg) (sp. act. 200 μCi/mmole) in dimethylsulfoxide (DMSO; 0.1 ml) was then injected into all chick embryos and the eggs were again returned to the incubator. The embryos were killed and the livers were removed for extraction of DDC and metabolites after further incubations of 1, 3, 6 and 12 hr.

The method used for extraction of [^{14}C]DDC and its metabolites from the livers of chick embryos was that of Racz and Marks [11], who reported a recovery of 99 per cent of total radioactivity from the liver. Chick embryos were decapitated and the livers were removed, blotted and homogenized in methanol (3 ml), using a Potter–Elvehjem apparatus with a teflon pestle. After centrifugation of the homogenate, the supernatant fraction was removed, and the residue was resuspended in methanol (3 ml) for a second homogenization and centrifugation. This process was repeated once again and the three supernatant fractions were combined, made up to 10 ml with methanol, and stored at 4°. The methanolic solutions were centrifuged to remove precipitated material which interfered with subsequent chromatography. Two 1-ml aliquots were placed in counting vials, Aquasol (10 ml) was added, and the samples were counted in a Beckman LS-335 liquid scintillation counter for 10 min. An external standard method was used to correct for quenching, and background counts were subtracted. The remaining extract (8 ml) was evaporated under a stream of nitrogen at 37° to a volume of approximately 0.3 ml. The concentrated extract was spotted in a horizontal band on a thin-layer chromatographic plate which was developed in a solvent mixture containing benzene–methanol in a ratio of 14:1. In this system, DDC has an R_f of 0.45 and the metabolite(s) remain at the origin. The silica gel was divided into ten bands of equal width (approximately 1.0 to 1.5 cm wide), and each portion was placed in a counting vial containing water (3 ml). Aquasol (10 ml) was added to form a gel suspension and the samples were shaken thoroughly and counted.

Effect of DDC on ferrochelatase activity. Seventeen-day-old chick embryos were injected with DDC dissolved in DMSO (0.1 ml). Controls received DMSO (0.1 ml). The animals were killed 9 hr later and the livers of eight similarly treated embryos were pooled. All procedures subsequent to decapitation took place at 4°. The method used to obtain a solubilized ferrochelatase preparation was a modification of that used by Porra *et al.* [13]. The pooled livers were homogenized in 9 vol. of a solution containing 0.25 M sucrose, 0.05 M Tris–HCl and 1 mM EDTA at pH 8.2, and the homogenate was centrifuged at 1200 *g* for 10 min. The supernatant fraction was centrifuged at 11,400 *g* for 10 min and the resulting mitochondrial pellet was washed twice with 0.02 M Tris–HCl buffer, pH 8.2. The washed mitochondria were suspended in 0.1 M Tris–HCl buffer (pH 10.4) (1.5 ml/g of liver) and stirred vigorously, while 0.1 vol. of aqueous 10% (w/v) Tween 20 solution followed by 0.1 vol. of ethanol was added dropwise. Gentle stirring was continued for 2.5 hr and then the mixture was centrifuged for 10 min at 20,000 *g*. The supernatant fraction was dialyzed overnight (approximately 16 hr) against two changes of 30 vol. of 0.02 M Tris–HCl buffer, pH 8.2. The dialysate was centrifuged at 100,000 *g* for 60 min and the supernatant fraction was stored at −20°. Ferrochelatase activity was assayed within 5 days. Protein concentrations were determined by the method of Lowry *et al.* [14], and varied from 1.5 to 2.9 mg/ml. Rat liver ferrochelatase was similarly prepared using a single liver; protein concentrations varied from 2.7 to 3.1 mg/ml.

Ferrochelatase activity was measured anaerobically in Thunberg tubes by a modification of the pyridine hemochromogen method of Porra *et al.* [13]. A mesoporphyrin IX solution was made up, as described by Porra *et al.* [13]. A 10-μl aliquot of the solution was diluted 300-fold with 0.1 N HCl, and its absorbance was measured at 399 nm. This absorbance was used [15] to calculate its concentration ($E^{399}_{mM} = 445$). Reagents were added to each tube in the following order: mesoporphyrin (120 nmoles), 1% (w/v) Tween 80 (0.3 ml), ethanol (0.3 ml), 0.2 M Tris–HCl buffer (pH 8.2) (1.5 ml), 0.2 M DTE (60 μl) and 1 mM $FeSO_4$ (120 μl). Chick embryo liver ferrochelatase preparation (equivalent to 1.25 mg protein) was placed in the side arm. The Thunberg tubes were alternately evacuated and flushed with oxygen-free nitrogen ten times and finally sealed under nitrogen. The tubes were preincubated at 37° for 5 min and the reaction was started by tipping the enzyme into the reaction mixture. At the end of the incubation period 0.4 M iodoacetamide (0.5 ml) was rapidly added and the tube vortexed. Pyridine (1.0 ml) and 1 N NaOH (0.5 ml) were added, the tube was vortexed, and within 5 min the spectrum of reduced minus oxidized pyridine hemochromogen was recorded from 600 to 500 nm on a Unicam SP800 ultraviolet spectrophotometer. The mesoheme formed was calculated [16] by using $\Delta E_{mM} = E^{547\,nm}_{mM} - E^{531\,nm}_{mM} = 21.7$. Rat liver ferrochelatase was similarly measured, except that the amount of

enzyme used was 0.5 ml, which varied in protein content from 1.35 to 1.55 mg.

Effects of DDC and SKF 525-A on ALA-synthetase activity and porphyrin accumulation. DDC and SKF 525-A were coadministered to the 17-day-old chick embryos in the following manner: one control group received saline (0.1 ml) and DMSO (0.1 ml), a second control group received saline (0.1 ml) and DDC (10 μg) in DMSO (0.1 ml), while a third control group received SKF 525-A (1.0 mg) in saline (0.1 ml) and DMSO (0.1 ml). Five experimental groups received SKF 525-A (1.0 mg) in saline (0.1 ml) and 0.1, 1, 3, 6 or 10 μg DDC in DMSO (0.1 ml) respectively. The eggs were returned to the incubator and removed 9 hr later for ALA-synthetase and porphyrin estimation [17]. The experiment was repeated using SKF 525-A (0.3 mg) and 10 μg DDC; ALA-synthetase and porphyrins were estimated 12 hr later [17].

RESULTS

After injection of [^{14}C]DDC into chick embryos, total radioactivity in the liver was measured at various time intervals. This radioactivity represented unchanged DDC and metabolite(s) and is referred to as "total drug". Results were converted from dis./min/g of liver into nmoles DDC/g of liver. The portion of DDC that had not undergone metabolic transformation is referred to as "unchanged drug". The amounts of total DDC and unchanged DDC in chick embryo livers at different times following injection of [^{14}C]DDC into SKF 525-A pretreated and control chick embryos are shown in Fig. 1. The total DDC reached a maximum between 1 and 3 hr and then declined slowly to approximately 20 nmoles/g of liver after 12 hr in both the control and SKF 525-A-pretreated chick embryos. The amount of unchanged DDC was significantly higher at 1 hr in SKF 525-A-pretreated, as compared with control, chick embryos; however, at 3, 6 and 12 hr neither the amount of unchanged DDC nor the total drug differed significantly between control and SKF 525-A-pretreated embryos.

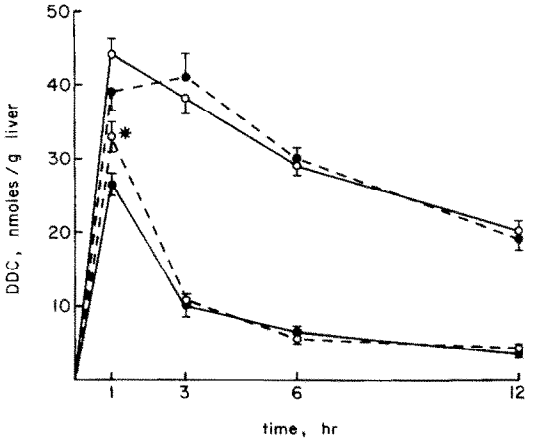

Fig. 1. Amounts of total DDC and unchanged DDC in the livers of 17-day-old chick embryos at different times after injection of 0.2 mg [^{14}C]DDC (333,000 dis./min) into SKF 525-A-pretreated and control chick embryos. Key: (●---●) total [^{14}C]DDC control; (○——○) total [^{14}C]DDC, SKF 525-A-pretreated; (●——●) unchanged [^{14}C]DDC, control; and (○- - -○) unchanged [^{14}C]DDC, SKF 525-A-pretreated. Each point represents the mean of at least four determinations (± S.E.M.). The asterisk denotes significance at the 0.05 level.

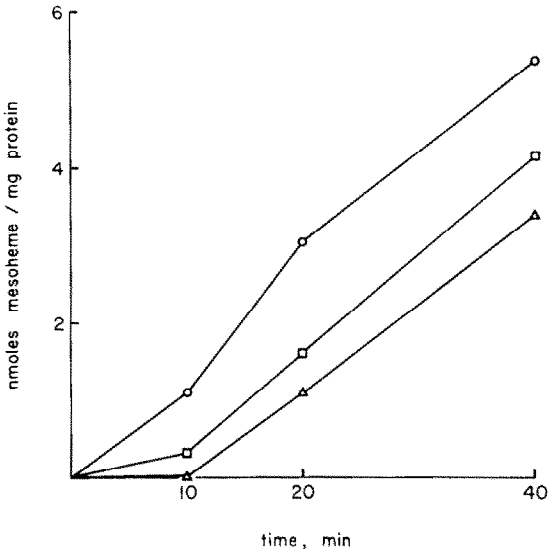

Fig. 2. Time course of ferrochelatase activity in the livers of 17-day-old chick embryos 9 hr after administration of DDC. Key: (○——○) control; (□——□) 1 μg DDC/egg; and (△——△) 10 μg DDC/egg. Each point represents the average of duplicate determinations and is representative of results obtained in two experiments.

Ferrochelatase activity was measured as the rate of formation of mesoheme/mg of protein. In the rat, the ferrochelatase activity was 0.81 ± 0.15 nmole mesoheme/min/mg of protein and was linear up to 8 min. Control chick embryo liver ferrochelatase activity was 0.13 ± 0.01 nmole mesoheme/min/mg of protein and the reaction proceeded approximately linearly with respect to time for 60 min (Fig. 2).

The time course of ferrochelatase activity in 17-day-old chick embryo livers 9 hr after administration of 1 and 10 μg DDC is shown in Fig. 2. The degree of inhibition of ferrochelatase produced by DDC is dependent upon the time at which measurements were taken. The percent inhibition of ferrochelatase was calculated by taking control ferrochelatase activity at 10 min as 100 percent and subtracting the percent enzyme activity of DDC-treated embryos. The percent inhibition of ferrochelatase 9 hr after the administration of increasing doses of DDC is shown in Fig. 3. Some inhibition of ferrochelatase was seen at DDC concentrations as low as 0.1 μg/egg, while 90–100 percent inhibition was achieved at 10 μg DDC/egg. The degree of inhibition of ferrochelatase exerted by 10 μg DDC/egg was the same at 0.5 and 3 hr as at 9 hr after administration of the drug. The effect of increasing doses of DDC on ALA-synthetase activity 9 hr after administration is shown in Fig. 3. No significant increase in activity was detected until amounts of DDC greater than 100 μg/egg were administered to the embryo.

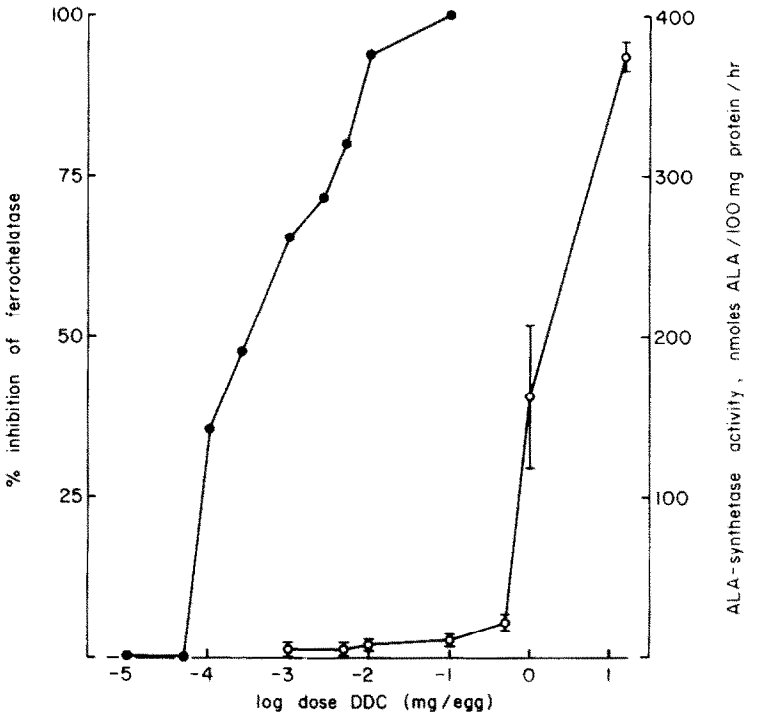

Fig. 3. ALA-synthetase activity (○——○) and percent inhibition of ferrochelatase (●——●) in the livers of chick embryos 9 hr after administration of increasing doses of DDC. For ferrochelatase inhibition, each point represents the average of duplicate determinations and is representative of results obtained in two to four experiments. For ALA-synthetase activity, each point represents the mean of four determinations (± S.E.M.).

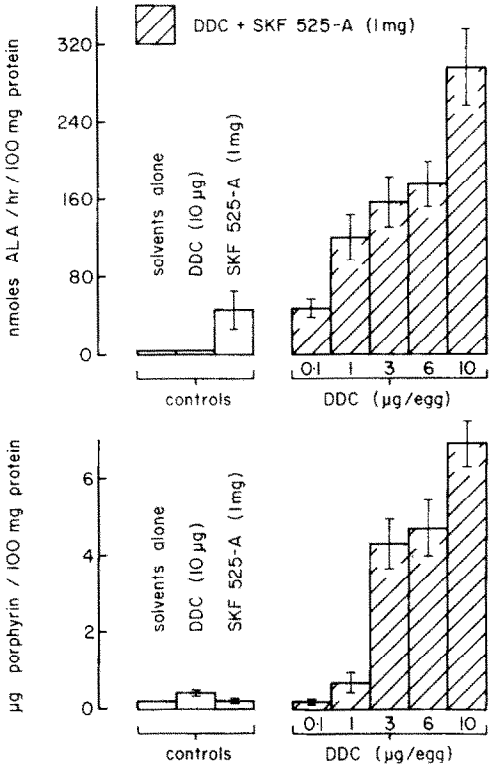

Fig. 4. ALA-synthetase activity and porphyrin accumulation in chick embryo livers 9 hr after administration of increasing doses of DDC in the presence of SKF 525-A (1 mg) (hatched bars) and in corresponding controls (open bars). Each bar represents the mean of four determinations (± S.E.M.).

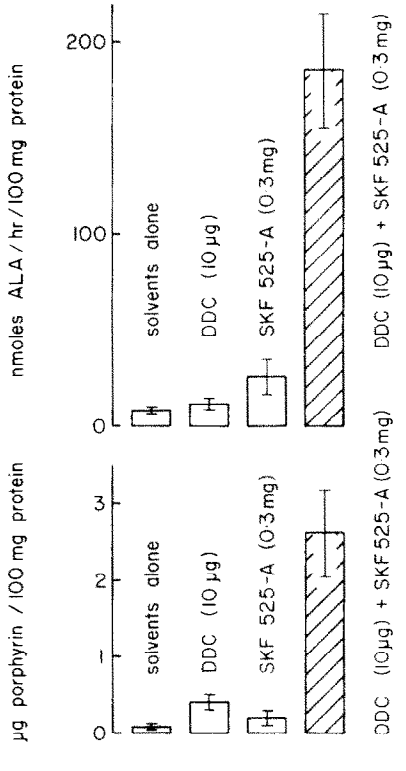

Fig. 5. ALA-synthetase activity and porphyrin accumulation in chick embryo livers 12 hr after the administration of DDC (10 µg) in the presence of SKF 525-A (0.3 mg) (hatched bar) and in corresponding controls (open bars). Each bar represents the mean of four to five determinations (± S.E.M.).

The effects of DDC and SKF 525-A on ALA-synthetase activity and porphyrin accumulation 9 hr after drug administration are shown in Fig. 4. Control values of ALA-synthetase activity and porphyrin levels in 17-day-old chick embryos receiving saline (0.1 ml) and DMSO (0.1 ml) were 5.53 ± 0.31 nmoles ALA/100 mg of protein/hr and 0.18 ± 0.02 μg porphyrin/100 mg of protein. Administration of 10 μg DDC/egg in DMSO (0.1 ml) and saline (0.1 ml) caused no increase in ALA-synthetase activity (6.34 ± 0.37 nmoles ALA/100 mg of protein/hr), although a slight increase in porphyrins was detected (0.42 ± 0.04 μg porphyrin/100 mg of protein). Administration of SKF 525-A (1 mg) in saline (0.1 ml) and DMSO (0.1 ml) resulted in a significant increase in ALA-synthetase activity (46.95 ± 19.50 nmoles ALA/100 mg of protein/hr), while porphyrins remained at control levels (0.22 ± 0.04 μg porphyrin/100 mg of protein). DDC markedly enhanced the ALA-synthetase activity and the porphyrin accumulation observed with SKF 525-A alone, in a dose-related manner. For example, coadministration of DDC (10 μg/egg) in DMSO (0.1 ml) and SKF 525-A (1 mg) in saline (0.1 ml) elevated the levels of ALA-synthetase to 296.92 ± 41.57 nmoles ALA/100 mg of protein/hr in addition to increasing porphyrin accumulation to 6.89 ± 0.60 μg porphyrin/100 mg of protein. Similarly, DDC (10 μg) markedly enhanced the ALA-synthetase activity and porphyrin accumulation observed with SKF 525-A (0.3 mg) alone (Fig. 5).

DISCUSSION

Taub *et al.* [3] showed that the ability of DDC to induce ALA-synthetase activity and porphyrin accumulation in chick embryo livers was greatly enhanced by pretreatment with SKF 525-A. These authors attributed the enhancement to elevated levels of unchanged DDC resulting from SKF 525-A-mediated blockade of metabolism of DDC to inactive metabolites. To determine whether this interpretation was correct we measured the levels of unchanged DDC and its metabolites in chick embryo livers at various time intervals after the administration of [^{14}C]DDC in the presence and absence of SKF 525-A. The results shown in Fig. 1 do not support the interpretation previously offered [3], since the slightly elevated levels of unchanged DDC noted at 1 hr cannot account for the marked elevation of ALA-synthetase and porphyrin levels previously observed.

We next investigated an alternative explanation for the synergistic effect noted earlier with SKF 525-A and DDC. According to this explanation, DDC exerts an inhibitory effect on ferrochelatase, resulting in decreased heme formation and diminished heme-mediated feedback repression of ALA-synthetase activity. As a result, the SKF 525-A-mediated increase in ALA-synthetase would be enhanced and porphyrins would accumulate.

In order to investigate the validity of this alternative explanation, our studies were next directed toward the development of an assay for ferrochelatase in chick embryo livers. A modified version of the pyridine hemochromogen method used by Porra *et al.* [13] in the rat was found to be suitable (Fig. 2). Inhibition of hepatic ferrochelatase by DDC was first reported by Onisawa and Labbe [5] in mice and by Tephly *et al.* [6] in rats. Out studies (Fig. 2) show that DDC also inhibits the enzyme from chick embryo liver. This observation has been confirmed recently by Anderson [9] who measured ferrochelatase in chick embryo liver mitochondria and showed that DDC exerts an inhibitory effect. In our studies (Fig. 2), the liver ferrochelatase activity from untreated chick embryos was approximately linear with respect to time. However, after DDC treatment, a lag phase or initial inhibition period of about 10 min was followed by a linear rate of activity. An apparently similar effect has been reported previously for rat liver enzyme [6]. We have chosen a 10-min time period for determination of ferrochelatase activity. However, it is clear from Fig. 2 that the degree of inhibition produced by DDC is dependent upon the time period selected for determination of ferrochelatase activity. The relationships among the dose of DDC, ferrochelatase inhibition, and ALA-synthetase activity were investigated (Fig. 3). An inhibitory effect on ferrochelatase was observed with a dose of DDC as low as 0.1 μg/egg. Almost complete inhibition of the enzyme was observed with 10 μg DDC/egg. Approximately 50 percent inhibition of the enzyme was achieved with about 1 μg DDC/egg. On the other hand, at least 1 mg DDC/egg was required to induce ALA-synthetase activity to half of its maximal level. Thus, the inhibitory effect of DDC on ferrochelatase activity appears to be dissociated from its ability to induce ALA-synthetase activity. It should be noted that this dissociation may not be as great as it appears in view of the possibility that the degree of inhibition of ferrochelatase by DDC may be exaggerated by our choice of a 10-min time period for measurement of enzyme activity.

With the above information we were able to test our alternative explanation for the synergism between SKF 525-A and DDC. Doses of DDC were selected which produced approximately 35, 65, 75, 85 and 95 percent inhibition of ferrochelatase and were injected into chick embryos together with SKF 525-A. Nine hours later hepatic ALA-synthetase activity and porphyrin accumulation were measured. The results (Fig. 4) show that DDC, used at doses which did not on their own affect ALA-synthetase activity, greatly enhanced SKF 525-A-induced ALA-synthetase activity. The synergistic effect of these two drugs on porphyrin accumulation is even more marked (Fig. 4). It is clear that the enhancement of SKF 525-A-induced ALA-synthetase activity and porphyrin accumulation is related to the degree of ferrochelatase inhibition exerted by DDC. A synergistic effect was also observed when the experiment was repeated using a lower dose of SKF 525-A (0.3 mg) and a 10 μg dose of DDC which produces approximately 95 percent inhibition of ferrochelatase (Fig. 5). These results thus support the alternative explanation for the synergism, viz. that ferrochelatase inhibition exerted by DDC results in decreased heme formation and diminished heme-mediated feedback repression of ALA-synthetase activity. As a result, the SKF 525-A-mediated increase in ALA-

synthetase activity is enhanced and porphyrins accumulate. These results also explain some puzzling observations made earlier [3], viz. that SKF 525-A greatly enhances the porphyrin-inducing activity of DDC but fails to enhance or only minimally enhances the porphyrin-inducing activity of allylisopropylacetamide, propylisopropylacetamide and 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine. Clearly a synergistic effect should be expected only when a combination of a ferrochelatase inhibitor (e.g. DDC) is used together with a drug which induces ALA-synthetase activity [7–9]. A similar synergistic effect has been observed by Maxwell and Meyer [18] in rat liver, using a combination of lead and phenobarbital. These workers interpreted the synergism as follows: lead produces a partial block in heme synthesis by inhibiting ferrochelatase and other enzymes of the pathway. While the partial inhibition of heme synthesis results in only a small elevation of ALA-synthetase activity, it greatly enhances the ability of phenobarbital and similar drugs to induce ALA-synthetase activity. Maxwell and Meyer [18] suggest that the lead treatment resulting in partial block of heme synthesis produces a condition resembling that seen in the hereditary hepatic porphyrias and provides an explanation for the sensitivity of these patients to induction of ALA-synthetase activity and porphyrin accumulation by a variety of drugs.

It is of interest to compare the sensitivity of rat, mouse and chick embryo liver ferrochelatase to DDC inhibition. Seventy percent inhibition of ferrochelatase occurs with a DDC dose of 25 mg/kg in rats [19], 5 mg/kg in mice [19], and 0.075 mg/kg in the chick embryo. The order of sensitivity of ferrochelatase to DDC inhibition is, therefore, chick embryo > mouse > rat.

It has been reported that the order of sensitivity of various test animals to porphyrin-inducing drugs is: 17-day-old chick embryo > chicken > rat [20]. De Matteis [8] has suggested that the enhanced sensitivity of chicken liver, as compared to rat liver, is due to the fact that ferrochelatase levels in chicken liver are 25 percent of those found in rat liver. In the present study, levels of ferrochelatase in the chick embryo liver were found to be 15 percent of those found in rat liver. This finding may explain, at least in part, the high sensitivity of the chick embryo liver to porphyrin-inducing drugs.

De Matteis *et al.* [19] have shown that in both rats and mice administration of DDC in doses larger than those required to produce maximal inhibition of ferrochelatase resulted in a gradual increase in ALA-synthetase activity and in porphyrin accumulation. These findings, which agree with our findings in the chick embryo, indicate that the effects of DDC on ferrochelatase and ALA-synthetase are dissociated from each other. Moreover, these findings raise the possibility that analogues of DDC might be synthesized which would inhibit ferrochelatase activity but which would not affect ALA-synthetase. Clearly a comparison of the structural requirements in DDC analogues for ferrochelatase inhibition and ALA-synthetase induction will be valuable. A DDC analogue which is a specific inhibitor of ferrochelatase should, by inhibiting heme formation, reduce cytochrome P-450 formation and thus serve as a valuable tool in drug metabolism studies.

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