MOLECULAR PHARMACOLOGY, 12, 731-737

Porphyria-Inducing Drugs: Comparative Effects on Nuclear Ribonucleic Acid Polymerases in Rat Liver

HAROLD L. MOSES,[1] THOMAS C. SPELSBERG,[2] JOSEF KORINEK,[3] AND FRANK CHYTIL

Departments of Pathology and Molecular Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901, and Departments of Pathology and Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

(Received August 1, 1975)

(Accepted April 19, 1976)

SUMMARY

MOSES, HAROLD L., SPELSBERG, THOMAS, C., KORINEK, JOSEF & CHYTIL, FRANK (1976) Porphyria-inducing drugs: comparative effects on nuclear ribonucleic acid polymerases in rat liver. *Mol. Pharmacol.*, 12, 731-737.

Changes in δ-aminolevulinic acid (ALA) synthetase activity, endogenous nuclear RNA polymerase activities (nucleolar and nucleoplasmic), and the availability of chromatin DNA to serve as template for RNA synthesis in rat liver in response to treatment with two porphyria-producing compounds, allylisopropylacetamide (AIA) and 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC), were determined. The alterations observed were compared with changes produced by two compounds that do not cause porphyria, phenobarbital (PB) and 3-methylcholanthrene (MC). Only AIA and DDC caused marked induction of ALA synthetase activity; PB caused a slight increase, and MC, no increase, in ALA synthetase. All four compounds caused some change in nuclear RNA synthesis in comparison with results in control animals given vehicle alone. AIA, DDC, and PB caused increased levels of RNA polymerase I (nucleolar) activity at time points later than 2 hr after injection, whereas MC resulted in a decreased level of RNA polymerase I activity at 8 and 12 hr. DDC, PB, and MC also caused prominent modulations in RNA polymerase II (nucleoplasmic) activity, the values 8 hr after injection being considerably greater than in control livers. An elevation in endogenous polymerase II activity persisted at 12 hr only in the DDC- and MC-treated animals. These alterations in polymerase II activity for the most part can be explained by changes in chromatin template capacity. DNA-dependent RNA synthesis using excess bacterial polymerase roughly paralleled the changes in polymerase II activity caused by DDC, PB, and MC treatment. Conversely, AIA caused only minimal changes in either polymerase II or chromatin template capacity. These data and those from other laboratories suggest that AIA and DDC may have different primary sites of action in the induction of excessive quantities of ALA synthetase, the first and rate-limiting enzyme in heme biosynthesis.

INTRODUCTION

Disorders resembling the human hepatic porphyrias can be produced in mammals by several chemicals (1). These compounds cause considerable increase in the

This investigation was supported in part by Research Grants AM-12977, AM-17360, CA-14920, HD-05384, and HL-15341 from the National Institutes of Health, United States Public Health Service.

[1] Recipient of National Institutes of Health Research Career Development Award AM-46415.

[2] Fellow of the National Genetic Foundation.

[3] Recipient of National Institutes of Health Special Fellowship GM-54746.

Copyright © 1976 by Academic Press, Inc.
All rights of reproduction in any form reserved.

activity of hepatic δ-aminolevulinic acid synthetase, the first and rate-limiting enzyme in porphyrin-heme biosynthesis (2–4). An increase in ALA[4] synthetase along with excessive porphyrin synthesis also can be produced in primary cultures of chick embryo liver cells by the same chemicals that are active *in vivo* (5, 6). The porphyria-producing chemicals form a heterogeneous group of exogenous compounds (1, 2), and the two most commonly studied are allylisopropylacetamide and 3,5-dicarbethoxy-1,4-dihydrocollidine. The only endogenous compounds known to be active are the $5\beta{:}H$ derivatives of the sex steroids, which were found to be active in chicken cells (5, 6) and in isolated rat liver cells (7), but not in rat or guinea pig liver *in vivo* (8).

Indirect evidence suggests that the chemically produced increase in ALA synthetase activity is due to induction of synthesis of enzyme *de novo* rather than to activation of an existing enzyme (3, 9). The increase in enzyme activity is prevented by two inhibitors of protein synthesis, cycloheximide and puromycin (3, 9). The inhibition of chemical induction of ALA synthetase by actinomycin D further suggests that the compounds may act at the transcriptional level, causing increased synthesis of ALA synthetase-specific mRNA (3, 9). The demonstration that α-amanitin, a potent inhibitor of the nucleoplasmic DNA-dependent RNA polymerase (polymerase II), blocks induction of ALA synthetase *in vivo* by AIA and the $5\beta{:}H$ metabolite of testosterone, etiocholanolone (10), adds further evidence that new mRNA synthesis is necessary for ALA synthetase induction. Recent studies by Incefy and Kappas (11) have shown an early increase in nuclear RNA synthesis and nucleoplasmic DNA-dependent RNA polymerase in chick embryo liver cell cultures after treatment with AIA or etiocholanolone. No comparable studies have been reported concerning the effects of porphyria-producing compounds on transcription and chromatin in mammalian liver cells *in vivo*.

[4] The abbrevations used are: ALA, δ-aminolevulinic acid; AIA, allylisopropylacetamide; DDC, 3,5-dicarbethoxy-1,4-dihydrocollidine; PB, phenobarbital; MC, 3-methylcholanthrene.

The purposes of this study were to examine transcriptional events during induction of hepatic ALA synthetase by the two porphyria-producing compounds, AIA and DDC, and to compare the changes observed with those produced by two compounds that do not cause porphyria, phenobarbital and 3-methylcholanthrene. The parameters examined include endogenous DNA-dependent RNA polymerase activities (nucleolar and nucleoplasmic) (12, 13) and chromatin template capacity for DNA-dependent RNA synthesis using bacterial polymerase (14). These studies showed that, whereas DDC and AIA caused similar alterations in polymerase I (nucleolar) activity, they had different effects on the other facets of nuclear transcription. DDC caused marked alteration of polymerase II (nucleoplasmic) activity and chromatin template capacity; AIA, however, had minimal effects on these factors, a feature that was unique to this compound in these studies.

MATERIALS AND METHODS

Animals. Male Charles River rats (CD strain) weighing 30–50 g were fasted for 24–30 hr and injected intraperitoneally with AIA (300 mg/kg), PB (100 mg/kg), DDC (300 mg/kg), or MC (50 mg/kg). Because of the varying solubility of these compounds, AIA and PB were dissolved in NaCl, while DDC and MC were dissolved in corn oil. Control animals received NaCl solution alone (1 ml) or corn oil (0.3 ml).

ALA synthetase assay. Animals were killed by decapitation, and pooled aliquots of livers from 7–10 animals were homogenized in 3 volumes (milliliters per gram) of a solution containing 0.5 mM EDTA, 10 mM Tris-HCl buffer (pH 7.4), and 30% glycerol (v/v). ALA synthetase was assayed according to a modification of the method of Marver *et al.* (4). The homogenate (0.5 ml) was added to 1.5 ml of incubation mixture containing 200 μmoles of glycine, 20 μmoles of EDTA, 150 μmoles of Tris-HCl (pH 7.2), 2 μmoles of pyridoxal 5′-phosphate, and 40 μl of partially purified succinyl-CoA synthetase prepared from a *Rhodopseudomonas spheroides* mutant deficient in ALA synthetase (15). After incubation at 37° for 30 min, the

reaction was terminated with 0.5 ml of cold 25% trichloracetic acid. After centrifugation, 3.0 ml of the supernatant were brought to pH 4.6 by the addition of 3 ml of 1.0 M sodium acetate buffer (pH 4.6) and 250 μl of 5 N NaOH. ALA and aminoacetone were then converted to the pyrroles by adding 200 μl of acetylacetone and heating in a boiling water bath for 20 min. After cooling to room temperature, this solution was poured over a 1 × 7 cm Dowex 1-acetate column to separate the ALA and aminoacetone pyrroles. Aminoacetone pyrrole was eluted with 10 ml of 1-butanol containing 0.01 M ammonium hydroxide. The column was washed with 10 ml of 1.0 M acetic acid, and the ALA pyrrole was eluted with 10 ml of a 1:2 mixture of glacial acetic acid and methanol. An equal volume of Ehrlich's reagent was added to aliquots of the eluate, and absorbance at 556 nm was read after 20–45 min (4).

Nuclear RNA polymerase assays. Aliquots of livers from 7–10 rats were pooled and homogenized in 25 volumes of 2.2 M sucrose in TKM buffer (0.01 M Tris-HCl, pH 7.5, 25 mM KCl, and 2 mM $MgCl_2$), using a Teflon pestle and glass homogenizer. Nuclei were isolated and purified as described previously (16). The homogenate was centrifuged for 60 min at 60,000 × g to pellet the nuclei. Nuclei to be used for RNA polymerase assays were resuspended in a small volume of a solution containing 25% (v/v) glycerol, 0.05 M Tris-HCl (pH 7.9), and 1 mM $MgCl_2$. The reactions for the assay of endogenous RNA polymerase have been described elsewhere (13, 16). Reactions were started by the addition of nuclei, and the mixtures (250 μl) were incubated for 10 min at 15°. Assays under low salt (0.01 M KCl) conditions contained 0.2 μg of α-amanitin, whereas those incubated under high salt [0.25 M $(NH_4)_2SO_4$] conditions contained no α-amanitin. The reactions were terminated by adding 1.0 ml of cold 10% trichloracetic acid, centrifuging, washing the pellet with 2.0 ml of cold 5% trichloracetic acid containing 1% (w/w) $Na_4P_2O_7$, and centrifuging again. The pellets, resuspended in the trichloracetic acid-$Na_4P_2O_7$ solution, were collected on Millipore filters, which were rinsed, dried, and counted in a liquid scintillation spectrometer. The filters were then removed from the vials and dried, and the DNA was hydrolyzed (16) followed by determination of DNA by the diphenylamine reaction (17).

Chromatin template capacity. Nuclei from pooled aliquots of 7–10 rat livers were isolated and purified in a manner similar to preparations for nuclear RNA polymerase assays. Nuclei were purified further by resuspension, using a Teflon-glass homogenizer, in 0.5 M sucrose and TKM buffer containing 0.1% (v/v) Triton X-100. The homogenate was filtered through organza cloth (100 mesh) and centrifuged for 10 min at 10,000 × g. The pellets were subjected to a series of hypoosmotic buffers to obtain chromatin as described elsewhere (18). The capacity of each of the chromatin preparations to serve as template for DNA-dependent RNA synthesis was determined using bacterial (*Escherichia coli*) polymerase under conditions wherein the template was rate-limiting (14). Bacterial polymerase was purified according to the method of Burgess (19) through the agarose 5.0m chromatography step.

Sources of materials. Allylisopropylacetamide was provided by Hoffmann–La Roche, and 3,5-dicarbethoxy-1,4-dihydrocollidine and 3-methylcholanthrene were purchased from Eastman Organic Chemicals. A culture of the *R. spheroides* ALA-requiring H-5 mutant was kindly provided by Dr. June Lascelles of the University of California at Los Angeles. Pyridoxal 5'-phosphate, 2-mercaptoethanol, ALA, and coenzyme A were purchased from Sigma Chemical Company. [5-^{3}H]Uridine 5'-triphosphate (19.8 Ci/mole) and [4-^{14}C]uridine 5'-triphosphate (48 mCi/mmole) were purchased from Amersham/Searle. GTP, CTP, and ATP were purchased from P-L Biochemicals. α-Amanitin was purchased from Boehringer.

RESULTS

ALA synthetase activity. The effects of two porphyria-producing compounds, AIA and DDC, were compared with the effects of two control compounds that do not cause porphyria. These latter compounds were PB, which causes a mild "physiological" increase in ALA synthetase but no over-

production of porphyrins or their precursors (1, 20), and MC, which causes no detectable increase in ALA synthetase.

Both AIA and DDC caused considerable induction of ALA synthetase, with a progressive increase up to 6 hr which was sustained until 12 hr after administration (Fig. 1), whereas PB caused only a slight increase in ALA synthetase activity. The enzyme activity after administration of MC, corn oil, and NaCl solution remained at baseline levels up to 12 hr (Fig. 1).

Characteristics of nuclear RNA polymerase reactions. The procedures utilized for assay of endogenous nuclear RNA polymerase I and II activities have given reproducible results with linear reactions for 15 min of incubation (16). The incorporation was shown to be DNA- and nucleotide-dependent, and the product was shown to be RNA. The conditions for incubation (15°) result in little or no detectable ribonuclease activity, in contrast to incubations at 25° (13). Reactions under low salt conditions in the presence of α-amanitin (0.8 μg/ml) would reflect both polymerase I and III activities (21); however, polymerase III is a minor component in rat liver (22). The low salt assay was shown to yield RNA with a ratio of uridine to guanine identical with that of ribosomal RNA (U:G $\sim$ 0.7). The high salt conditions in the absence of α-amanitin yielded a ratio of uridine to guanine similar to that of DNA (U:G $\sim$ 1.2) (13).

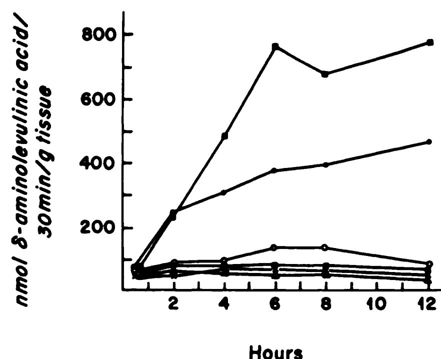

FIG. 1. *Liver δ-aminolevulinic acid synthetase activity*

Rats were injected intraperitoneally with allylisopropylacetamide (●——●), phenobarbital (○——○), 3,5-dicarbethoxy-1,4-dihydrocollidine (■——■), or 3-methylcholanthrene (□——□) at zero time. Control animals received NaCl solution alone (×——×) or corn oil (▲——▲). Pooled aliquots of livers from 7-10 animals were assayed for ALA synthetase activity as described in MATERIALS AND METHODS to determine each point.

The effects of the vehicles, corn oil and NaCl, on both polymerase I and polymerase II activities were determined. Both vehicles caused little or no alteration in activity at all time points examined, from 30 min to 12 hr. Control values ranged from 4 to 6 × 10^2 cpm of [^{3}H]UMP incorporated into RNA per milligram of DNA for polymerase I, and from 9 to 10 × 10^3 cpm for polymerase II. All polymerase data are expressed as a percentage of values from control animals injected with vehicle alone and killed at each time point (Figs. 2 and 3).

RNA polymerase I activity. DNA-dependent RNA polymerase I (A) is localized in the nucleolus, is active under low salt conditions, is insensitive to α-amanitin, and synthesizes ribosomal RNA (12). After an initial decrease, both AIA and PB caused a gradual increase in polymerase I activity, which was greater than in NaCl-treated controls at the 4- and 8-hr time points (Fig. 2). With AIA the increase was still present at 12 hr, by which time the PB-treated group had returned nearly to control levels. DDC and MC both had effects on polymerase I activity that were different from the effects caused by AIA and PB. While polymerase I activity was elevated at all time points examined after DDC administration, it was reduced in relation to control values after MC treatment (Fig. 2).

RNA polymerase II activity. DNA-dependent RNA polymerase II (B) is localized in the nucleoplasm, is active under high salt conditions, is sensitive to α-amanitin, and synthesizes DNA-like RNA (12). Since polymerase II is apparently responsible for synthesis of mRNA (12, 22), increases in this enzyme activity might cause overproduction of specific mRNA for the enzyme ALA synthetase (3, 9, 23, 24). However, AIA, a potent inducer of hepatic porphyria, caused virtually no change in the endogenous polymerase II activity of rat liver nuclei in comparison with controls treated with NaCl alone (Fig. 3). The

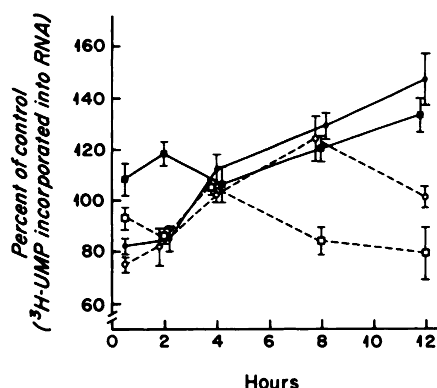

FIG. 2. *Endgoenous DNA-directed RNA polymerase I activity*

Rats were injected with allylisopropylacetamide (●——●), phenobarbital (○---○), 3,5-decarbethoxy-1,4-dihydrocollidine (■——■), or 3-methylcholanthrene (□---□) at zero time, and 7-10 animals receiving each compound were killed at each time point along with an equal number of animals receiving vehicle alone (NaCl for AIA and PB and corn oil for DDC and MC). The livers were pooled and the nuclei were isolated and assayed for RNA polymerase activity under low salt conditions in the presence of α-amanitin as described in MATERIALS AND METHODS. The values represent the means with ranges for three replicate analyses on the pooled samples and are expressed as percentages of the controls, handled concurrently.

activity showed an initial slight decrease at the 30-min time point. On the other hand, PB caused prominent modulations of polymerase II activity. Both DDC and MC had an effect on polymerase II similar to that of PB, except for the 12-hr time point, where the level remained elevated with DDC and MC (Fig. 3).

Chromatin template capacity. Under the assay conditions used, the endogenous RNA polymerase II activity has two rate-limiting factors: the quantity of active enzyme present and the amount of DNA available as a template for transcription (13). We therefore examined the chromatin template capacity for synthesis of DNA-like RNA, using bacterial polymerase in a system in which the only rate-limiting factor was the amount of template available for transcription. The vehicles, NaCl solution and corn oil, both caused a gradual, slight decrease in template capacity over the 12-hr period of observation. The control template capacity values ranged from 125 to 300 × 10^3 cpm of [^{14}C]UMP incorporated per milligram of DNA. To compensate for this variation, the treated animals were handled concurrently with the controls, and the data obtained are expressed as percentages of control values.

As with the endogenous polymerase II activity, AIA had little effect on template capacity except for a decrease at 12 hr (Fig. 4). PB, DDC, and MC all caused prominent, reproducible modulations in template capacity (Fig. 4) roughly parallel to changes noted in the endogenous polymerase II assay (Fig. 3), indicating that the alterations in the latter assay probably were secondary to changes in template capacity. Thus AIA is unique among the compounds tested because of its minimal effect on RNA polymerase II and on chromatin template capacity for DNA-dependent RNA synthesis.

DISCUSSION

Previous studies by other investigators have provided data suggesting that all compounds used in the present study cause increases in hepatic nuclear RNA synthesis (11, 25-28). Most of these studies with PB, MC, and DDC (26-28), however, did not distinguish between nucleolar and nu-

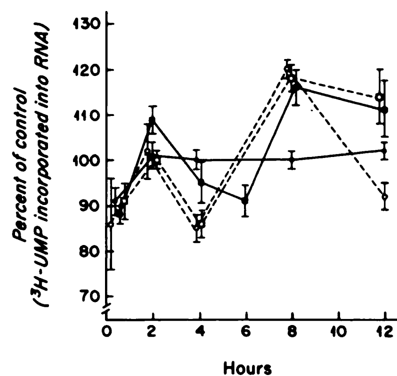

FIG. 3. *Endogenous DNA-directed RNA polymerase II activity*

Animals were treated as described for Fig. 2, and the nuclei from pooled livers were assayed for polymerase II under high salt conditions without α-amanitin as described in MATERIALS AND METHODS. The values are expressed in the same way as in Fig. 2. ●——●, allylisopropylacetamide; ○---○, phenobarbital; ■——■, 3,5-dicarbethoxy-1,4-dihydrocollidine; □---□, 3-methylcholanthrene.

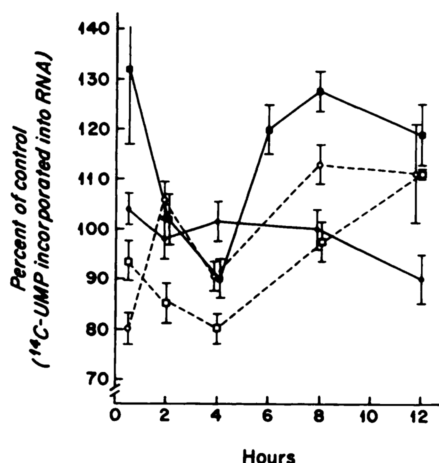

FIG. 4. *Chromatin template capacity*

Animals were treated as described for Fig. 2. Chromatin was isolated from pooled liver nuclei, and the capacity to serve as template for DNA-dependent RNA synthesis was determined as described in MATERIALS AND METHODS. The data are expressed as percentages of controls, handled concurrently, and each point represents the mean with range for three replicate analyses on the pooled samples from 7–10 livers. ●——●, allylisopropylacetamide; ○- - -○, phenobarbital; ■——■, 3,5-dicarbethoxy-1,4-dihydrocollidine; □- - -□, 3-methylcholanthrene.

cleoplasmic RNA synthesis, nor did they determine whether or not the increased synthesis was due to increased DNA template available for transcription, to increased DNA-dependent RNA polymerase activity, or to stabilization of nuclear RNA. In this study the effects of four compounds (AIA, DDC, PB, and MC) on several parameters of rat liver nuclear function, including nucleolar and nucleoplasmic RNA polymerase activities and chromatin template capacity for RNA synthesis, have been examined.

In these studies, as well as many others (1–4), it was shown that the drugs inducing hepatic porphyria (AIA and DDC) markedly enhanced ALA synthetase activity in rat liver. The non-porphyria-inducing drugs either failed to alter or only slightly increased the ALA synthetase activity. The only positive correlation between porphyria induction and changes in transcription was an elevation in RNA polymerase I activity relative to control levels 12 hr after administration of the two porphyria-producing compounds, AIA and DDC. PB and MC did not cause elevated polymerase I at the 12-hr time point. This observation is of doubtful significance, since the polymerase(s) measured with this assay would not transcribe the specific mRNA thought to be elevated in response to porphyria-inducing drugs. It is of interest that the elevated polymerase I seen with AIA correlates with an increase in nucleolar size observed in previous quantitative morphological studies of hepatocytes with AIA-induced porphyria (29).

DDC, PB, and MC all caused prominent reproducible, modulations in the levels of endogenous RNA polymerase II activity, whereas AIA produced minimal detectable change in this polymerase. Assays for chromatin template capacity for DNA-directed RNA synthesis showed drug effects which roughly paralleled those observed with polymerase II. This suggests that the observed RNA polymerase II changes were, for the most part, the result of alterations in template available for transcription. The assay for template capacity of isolated chromatin utilized in the present study does not necessarily represent the actual template capacity within the intact cell. However, this method is useful in the interpretation of changes in endogenous polymerase II activities, since the latter enzyme uses chromatin as a template.

The different effects of AIA and DDC on RNA polymerase II and the template capacity of isolated chromatin from rat liver may be interpreted as indicating that the two compounds have different primary sites of action or mechanisms of stimulation of synthesis of ALA synthetase. Sassa and Granick (23) arrived at the same conclusion, using primary cultures of chick embryo liver cells, in studies of the effects of actinomycin D and cycloheximide on ALA synthetase induction. They suggested that DDC acts at the transcriptional level whereas AIA has its ALA synthetase-inducing effect at a translational level. In more recent studies, Tyrrell and Marks (24) and Tomita *et al.* (30) carried out experiments similar to those of Sassa and Granick (23), using similar model systems. Their results were contradictory to those of Sassa and Granick and suggested

that both AIA and DDC act at a transcriptional level, although the possibility of post-transcriptional alteration of ALA synthetase and mRNA levels by AIA was not excluded (30).

The results of our studies concerning AIA influence on rat liver transcription are not in agreement with those reported by Incefy and Kappas (10, 11), who used the chick embryo liver cell system. They reported stimulation of nucleoplasmic RNA polymerase at 3, 6, 8, and 21 hr after treatment with AIA or etiocholanolone. These observations, together with their finding that α-amanitin inhibits the induction of ALA synthetase, suggest a correlation between increased polymerase II activity and the increased transcription of mRNA for ALA synthetase. While the inhibition of ALA synthetase induction by α-amanitin indicates a need for mRNA synthesis (10), our findings in rat liver show that an increase in polymerase II is not necessary for ALA synthetase induction by AIA. It is possible that the RNA polymerase II changes required for increased transcription of mRNA for synthesis of ALA synthetase might be small in relation to total nuclear function, and the procedures used therefore might be too insensitive to detect the changes induced. AIA may cause modulations of chromatin template with activation of some areas of the genome and repression of others, so that the net effect is no change in template capacity or RNA polymerase II activity as we measure it. The other compounds could induce the synthesis of many mRNA species, including those needed for induction of drug-metabolizing enzymes, and thereby have a more readily observable effect on transcription. Until more sophisticated probes such as mRNA isolation and quantitation are employed, these problems will remain speculative.

REFERENCES

1. Tschudy, D. P. & Bonkowsky, H. L. (1972) *Fed. Proc.*, 31, 147–159.
2. Granick, S. & Urata, G. (1963) *J. Biol. Chem.*, 238, 821–827.
3. Marver, H. S., Collins, A., Tschudy, F. P. & Rechcigl, M., Jr. (1966) *J. Biol. Chem.*, 241, 4323–4329.
4. Marver, H. S., Tschudy, D. P., Perlroth, M. G. & Collins, A. (1966) *J. Biol. Chem.*, 241, 2803–2809.
5. Granick, S. (1966). *J. Biol. Chem.*, 241, 1359–1375.
6. Kappas, A. & Granick, S. (1968) *J. Biol. Chem.*, 243, 346–351.
7. Edwards, A. M. & Elliott, W. H. (1975) *J. Biol. Chem.*, 250, 2750–2755.
8. Kappas, A., Song, C. S., Levere, R. D., Sachson, R. A. & Granick, S. (1968) *Proc. Natl. Acad. Sci. U. S. A.*, 61, 501–513.
9. Tschudy, D. P., Marver, H. S. & Collins, A. (1965) *Biochem. Biophys. Res. Commun.*, 21, 480–487.
10. Incefy, G. S., Rifkind, A. B. & Kappas, A. (1974) *Biochim. Biophys. Acta*, 361, 331–344.
11. Incefy, G. S. & Kappas, A. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 2290–2294.
12. Chambon, P. (1975) *Annu. Rev. Biochem.*, 44, 613–638.
13. Glasser, S. R., Chytil, F. & Spelsberg, T. C. (1972) *Biochem. J.*, 130, 947–957.
14. Spelsberg, T. C., Steggles, A. W. Chytil, F. & O'Malley, B. W. (1972) *J. Biol. Chem.*, 247, 1368–1374.
15. Burnham, B. F. (1963) *Acta Chem. Scand.*, 17, S123–S128.
16. Knowler, J. T., Moses, H. L. & Spelsberg, T. C. (1973) *J. Cell Biol.*, 59, 685–695.
17. Burton, K. (1956) *Biochem. J.*, 62, 315–323.
18. Spelsberg, T. C., Steggles, A. W. & O'Malley, B. W. (1971) *J. Biol. Chem.*, 246, 4188–4197.
19. Burgess, R. R. (1969) *J. Biol. Chem.*, 244, 6160–6167.
20. Marver, H. S. (1969) in *Microsomes and Drug Oxidations* (Gillette, J. R., Conney, A. H., Cosmides, G. J., Estabrook, R. W., Fouts, J. R. & Mannering, G. J., eds.), pp. 495–515, Academic Press, New York.
21. Weinmann, R. & Roeder, R. G. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 1790–1794.
22. Lindell, T. J., Weinberg, F., Morris, P. W. Roeder, R. G. & Rutter, W. J. (1970) *Science*, 170, 447–449.
23. Sassa, S. & Granick, S. (1970) *Proc. Natl. Acad. Sci. U. S. A.*, 67, 517–522.
24. Tyrrell, D. L. J. & Marks, G. S. (1972) *Biochem. Pharmacol.*, 21, 2077–2093.
25. Gelboin, H. V., Wortham, J. S. & Wilson, R. G. (1967) *Nature*, 214, 281–283.
26. Jacob, S. T., Scharf, M. B. & Vessel, E. S. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 704–707.
27. Nawata, H. & Kato, K. (1973) *Biochem. J.*, 136, 201–215.
28. Piper, W. N. & Bousquet, W. F. (1968) *Biochem. Biophys. Res. Commun.*, 33, 602–605.
29. Moses, H. L., Stein, J. A. & Tschudy, D. P. (1970) *Lab. Invest.*, 22, 432–442.
30. Tomita, Y., Ohashi, A. & Kikuchi, G. (1974) *J. Biochem. (Tokyo)*, 75, 1007–1015.