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# THE INFLUENCE OF PHENOBARBITAL ADMINISTRATION UPON THE "SOLUBLE" NADP-REQUIRING ENZYMES IN LIVER*

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**Abstract**—Phenobarbital pretreatment effected a twofold increase in the activities of D-glucose-6-phosphate:NADP-oxidoreductase and 6-phospho-D-gluconate:NADP-oxidoreductase (G-6-P and 6-PG oxidoreductases). No change was observed in the activities of isocitrate or dihydrouracil oxidoreductases. The minimal effective dose for a detectable increase in G-6-P oxidoreductase was 1 mg/kg body weight of phenobarbital twice daily for 4 days; maximal stimulation was obtained with 20 mg/kg of the drug. In addition, aminopyrine, barbital, and diphenhydramine increased G-6-P oxidoreductase activity, whereas 3,4-benzpyrene and 3-methylcholanthrene were ineffective. The phenobarbital-induced increase in G-6-P oxidoreductase was inhibited by *p*-fluoro-DL-phenylalanine; prevention of this inhibition was achieved by the simultaneous administration of L-phenylalanine. Ethionine administration did not significantly reduce the enzymatic activity. The increase in enzymatic activity resulting from pretreatment with phenobarbital was also prevented by 6-mercaptopurine and 5-fluorouracil. A comparison of the pH optimum, $K_m$ for G-6-P and NADP, storage denaturation at −5°, and heat denaturation at 50° revealed no significant difference between the control supernatant fractions and the preparations obtained from the livers of phenobarbital-pretreated rats.

PRETREATMENT of rats with phenobarbital results in an increase in the drug-metabolizing enzymes located within the endoplasmic reticulum.[1–4] Although these enzymes catalyse a variety of reactions, they do require a common cofactor, nicotinamide adenine dinucleotide phosphate (NADP). The observed stimulation is not entirely restricted to microsomal enzymes, for an increased activity of the 'soluble' enzyme, UDP-glucose:NAD-oxidoreductase,† has also been reported.[5, 6] It was of interest, therefore, to ascertain the effect of phenobarbital upon other 'soluble' enzymes, e.g. D-glucose-6-phosphate:NADP-oxidoreductase; 6-phospho-D-gluconate:NADP-oxidoreductase (decarboxylating); 4,5-dihydrouracil:NADP-oxidoreductase; and L$_S$-isocitrate:NADP-oxidoreductase. The results of this investigation are reported herein.

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† In accordance with the new regulations of the International Union of Biochemistry Commission on Enzymes, the following nomenclature will be employed throughout: NAD, diphosphopyridine nucleotide; NADP, triphosphopyridine nucleotide; UDP-glucose; NAD-oxidoreductase, UDPG dehydrogenase; L$_S$-isocitrate: NADP-oxidoreductase, isocitrate dehydrogenase; 6-phospho-D-gluconate: NADP-oxidoreductase, 6-phosphogluconate dehydrogenase; D-glucose-6-phosphate: NADP-oxireductase, glucose-6-phosphate dehydrogenase; 4,5-dihydrouracil:NADP-oxidoreductase, uracil reductase.

## EXPERIMENTAL

Male Holtzman rats, 40 to 50 g in weight, were injected intraperitoneally twice daily (9.30 a.m. and 5.30 p.m.) with phenobarbital (40 mg/kg body weight) for 4 days. Control rats were treated similarly with 0·9% saline. All rats were sacrificed on the day 5; the livers were removed, washed in 0·9% saline, and a 20% homogenate prepared in cold 0·25 M sucrose. The homogenate was centrifuged at 100,000*g* for 60 min and the resultant supernatant fraction was employed in the enzyme assays.

### *Enzyme Assays*

D-Glucose-6-phosphate:NADP-oxidoreductase (G-6-P oxidoreductase) activity was assayed by a modification of the method described by Glock and McLean.[7] The method in our hands did not distinguish between G-6-P oxidoreductase activity and 6-phospho-D-gluconate:NADP-oxidoreductase (6-PG oxidoreductase) activity at pH 7·6; however, the total contribution of the latter to the change in optical density at 340 mμ ($\Delta OD_{340}$) did not materially affect the results presented herein.

6-PG Oxidoreductase was also assayed according to the method of Glock and McLean[7] with 6-phosphogluconate as substrate, at its pH optimum (i.e. 9·0), and at the pH optimum for G-6-P oxidoreductase (pH 7·6).

L-Isocitrate:NADP-oxidoreductase (isocitrate oxidoreductase) activity was determined by the method of Plaut.[8] The activities of the NADP-requiring enzymes have been expressed in terms of millimicromoles NADPH produced per gram protein per minute with a molar absorbancy of $6{\cdot}2 \times 10^3$ for NADPH;[9] at 340 mμ, $5{\cdot}62 \times 10^{-5}$ M NADPH yields an OD = 0·330.

The ability of the supernatant fractions from control and phenobarbital-stimulated livers to catabolize uracil-6-$^{14}$C to $^{14}CO_2$ was determined by employing the assay system proposed by Reichard and Skold.[10]

The protein concentration was determined by the method of Lowry *et al.*[11] with crystalline bovine serum albumin as the standard.

## RESULTS

Phenobarbital pretreatment of Holtzman rats resulted in an increase of 41% in the per cent liver weight body/weight ratio and a twofold increase in the activity of G-6-P oxidoreductase (Table 1). The magnitude of the stimulation of the liver weight and enzymatic activity was reduced in the rats obtained from the Cheek-Jones Co., Houston, although the latter animals stemmed originally from the Holtzman line in Wisconsin. Of interest is the much larger per cent liver weight/body weight in the Cheek-Jones animals.

The dose–response data (Table 2) indicate the effectiveness of phenobarbital as an inducer of G-6-P oxidoreductase; a 20% increase in activity was observed after treatment with the drug at 1 mg/kg body weight. No further response was manifested above a dose of 20 mg/kg body weight.

The phenobarbital-induced effect became pronounced at day 2 after treatment of the rats with phenobarbital (40 mg/kg), and the maximal increase in enzymatic activity occurred after administration twice daily for 3 days (Fig. 1). The per cent liver weight/body weight increased progressively during the administration period.

The effect of phenobarbital pretreatment upon 6-PG oxidoreductase activity and isocitrate oxidoreductase activity is depicted in Table 3. At the pH optimum for

TABLE 1. EFFECT OF PHENOBARBITAL PRETREATMENT UPON THE LIVER WEIGHT AND LIVER G-6-P OXIDOREDUCTASE*

| Source of rats | Pretreatment | % Liver wt. / Body wt. | G-6-P Oxidoreductase (mμmoles NADPH produced/g protein/min) |
|---|---|---|---|
| Holtzman | Saline | 3·58 ± 0·13 (12)† | 4·4 ± 0·3 |
| | Phenobarbital | 5·06 ± 0·19 (13) | 8·3 ± 0·4 |
| Cheek-Jones | Saline | 5·09 ± 0·12 (4) | 7·5 ± 0·4 |
| | Phenobarbital | 6·42 ± 0·35 (6) | 10·2 ± 0·8 |

* Male rats, 40 to 50 g in weight, were obtained from either the Holtzman Rat Co., Madison, Wis., or the Cheek-Jones Co., Houston, Texas, and were injected intraperitoneally with either 0·9% saline or phenobarbital (20 mg/kg body weight) twice daily for 4 days. The rats were sacrificed on the day 5, the livers were removed, washed in saline, blotted dry, weighed, and assayed for G-6-P oxidoreductase as described in the experimental section. The livers obtained from 3 rats were grouped for a single determination.

† Values for all tables are Mean ± standard error, and number of determinations is indicated within the parentheses. Standard error = $\{\Sigma(X - \bar{x})^2 n(n-1)\}^{\frac{1}{2}}$

G-6-P oxidoreductase, pH 7·6, no change in activity of 6-PG oxidoreductase was observed, although an enhancement of activity was apparent when assayed at its own pH optimum (pH 9·0). The maximal effect occurred after a dose of 10 to 20 mg/kg, albeit the increase was apparent after only 1 mg phenobarbital/kg. Isocitrate oxidoreductase, on the other hand, did not respond consistently to the administration of the drug. The initial activity of the latter enzyme, however, was 7 to 10 times higher than that of the other oxidoreductases. Dihydrouracil oxidoreductase activity was not influenced by phenobarbital pretreatment.

TABLE 2. EFFECT OF DOSAGE OF PHENOBARBITAL UPON LIVER G-6-P OXIDOREDUCTASE*

| Expt. | Phenobarbital (mg/kg body weight) | G-6-P Oxidoreductase (mμmoles NADPH produced/g protein/min) |
|---|---|---|
| 1 | 0 | 5·7 ± 0·5 (4) |
| | 10 | 9·1 ± 0·8 (5) |
| | 20 | 10·8 ± 0·9 (5) |
| | 40 | 9·5 ± 0·4 (5) |
| | 60 | 9·5 (2) |
| | 80 | 10·1 (1) |
| 2 | 0 | 6·0 ± 0·4 (4) |
| | 1 | 7·2 ± 0·7 (6) |
| | 5 | 8·0 ± 0·8 (6) |
| | 10 | 10·2 ± 0·7 (6) |
| | 20 | 12·0 ± 1·3 (6) |

* Male Holtzman rats were injected intraperitoneally with phenobarbital or saline twice daily at the doses shown, for 4 days. The rats were sacrificed on the day 5, and the liver G-6-P oxidoreductase was determined as described in the experimental section. Each determination was performed on a single liver.

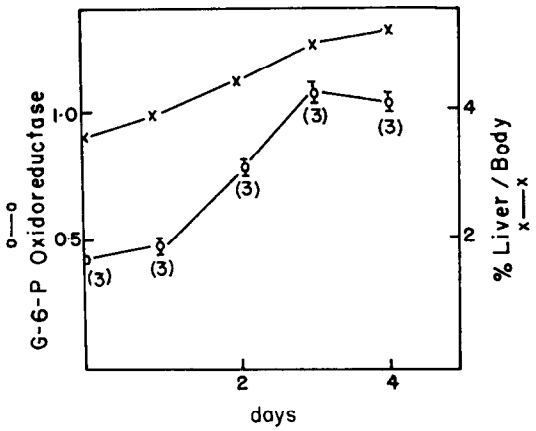


FIG. 1. Dosage schedule vs. liver weight and G-6-P oxidoreductase. Male Holtzman rats were pretreated with phenobarbital twice daily (20 mg/kg) for the number of days indicated on the abscissa and were sacrificed on the day 5. The number of determinations is indicated within the parentheses, and the specific deviation is depicted at each point. Enzymatic activity (left ordinate) is expressed as mμmoles/100 mg protein/min.

The effects upon G-6-P oxidoreductase of a group of drugs which have been shown to increase microsomal enzymatic activity[3] are presented in Table 4. The dosages employed in this study were adopted from those used by Conney *et al.*[3] Phenobarbital, aminopyrine, diphenhydramine (Benadryl), and barbital increased G-6-P oxidoreductase activity, whereas 3,4-benzpyrene and 3-methylcholanthrene proved ineffective.

The inhibition of the phenobarbital-induced effect by the amino acid antagonists, DL-ethionine and *p*-fluoro-DL-phenylalanine, was investigated (Table 5). Ethionine, an inhibitor of the incorporation of methionine and glycine into liver protein,[12] did not influence the activity of G-6-P oxidoreductase in either control or phenobarbital-stimulated supernates. On the other hand, *p*-fluorophenylalanine effectively inhibited the phenobarbital-induced increase of enzymatic activity without altering control activity. Neither ethionine nor *p*-fluorophenylalanine significantly altered enzymatic activity *in vitro*.

TABLE 3. EFFECT OF PHENOBARBITAL UPON OTHER NADP-REQUIRING ENZYMES*

| Phenobarbital (mg/kg) | 6-PG Oxidoreductase | | Isocitrate oxidoreductase |
|---|---|---|---|
| | pH 7·6 (mμmoles NADPH produced/g protein/min) | pH 9·0 | (mμmoles NADPH produced/g protein/min) |
| 0 | 3·2 ± 0·7 (4) | 6·5 ± 0·6 (5) | 47·0 ± 4·0 (5) |
| 1 | 3·1 ± 0·6 (6) | 9·0 ± 1·0 (6) | 60·0 ± 5·0 (6) |
| 5 | 3·2 ± 0·2 (6) | 9·6 ± 0·5 (6) | 64·0 ± 4·8 (6) |
| 10 | 3·4 ± 1·8 (6) | 9·9 ± 0·8 (6) | 55·1 ± 5·6 (6) |
| 20 | 3·6 ± 0·9 (6) | 12·3 ± 1·3 (6) | 64·1 ± 9·0 (6) |

* Male Holtzman rats were pretreated for 4 days, twice daily, with phenobarbital at the prescribed dosages. The enzyme assays were conducted as described in the experimental section. Each determination was performed on a single liver.

TABLE 4. EFFECT OF DRUGS UPON G-6-P OXIDOREDUCTASE ACTIVITY*

| Drug | Daily dose (mg/kg) | G-6-P Oxidoreductase (mμmoles NADPH produced/g protein/min) |
|---|---|---|
| Control | | 4·7 ± 0·6 (4) |
| Safflower control | | 4·3 (2) |
| 3,4-Benzpyrene | 25 | 4·6 (2) |
| 3-Methylcholanthrene | 125 | 4·9 ± 0·9 (6) |
| Phenobarbital | 60 | 13·6 |
| Aminopyrine | 125 | 7·2 ± 0·2 (3) |
| Diphenhydramine | 50 | 8·0 ± 0·1 (3) |
| Barbital | 125 | 7·5 ± 0·8 (3) |

* Male Holtzman rats were injected intraperitoneally twice daily for 4 days with phenobarbital, barbital, aminopyrine, or diphenhydramine; 3,4-benzpyrene or 3-methylcholanthrene was administered once intraperitoneally, in safflower oil, and the animals sacrificed 24 hr later. Enzymatic activity was determined as described in the experimental section.

Prevention of the inhibition of the phenobarbital-induced increase of G-6-P oxidoreductase may be achieved by the simultaneous administration of L-phenylalanine with the amino acid antagonist (Table 6). Neither L-phenylalanine nor *p*-fluoro-DL-phenylalanine, alone or in combination, had any effect upon the control enzymatic activity.

The effect of a purine and pyrimidine antimetabolite upon G-6-P oxidoreductase was also investigated (Table 7). 6-Mercaptopurine (either one or four administrations) and 5-fluorouracil had little effect upon control enzymatic activity, but both analogs did significantly reduce the activity of G-6-P oxidoreductase in supernatant fractions

TABLE 5. EFFECT OF AMINO ACID ANTAGONISTS UPON THE PHENOBARBITAL-INDUCED INCREASE IN G-6-P OXIDOREDUCTASE*

| Conditions | G-6-P Oxidoreductase (mμmoles NADPH produced/g protein/min) |
|---|---|
| Control (3) | 5·9 ± 0·5 |
| Phenobarbital (5) | 10·3 ± 0·7 |
| Phenobarbital + DL-ethionine (5) | 11·0 ± 0·2 |
| DL-Ethionine (5) | 6·0 ± 0·5 |
| Phenobarbital + *p*-fluoro-DL-phenylalanine (4) | 7·5 ± 0·2 |
| *p*-Fluoro-DL-phenylalanine (5) | 6·1 ± 0·8 |

* Male Holtzman rats were injected intraperitoneally for 4 days, twice daily, with phenobarbital (20 mg/kg) and/or DL-ethionine (200 mg/kg) or *p*-fluoro-DL-phenylalanine (130 mg/kg). The ethionine and *p*-fluorophenylalanine were dissolved in 3% $NaHCO_3$ and were given only once to the rats 30 min prior to the first phenobarbital injection for 4 days. All animals were sacrificed on day 5 and the enzyme assay was conducted as described in the text.

TABLE 6. PREVENTION OF THE *p*-FLUORO-DL-PHENYLALANINE INHIBITION OF THE PHENOBARBITAL-INDUCED INCREASE IN G-6-P OXIDOREDUCTASE*

| Conditions | G-6-P Oxidoreductase (mμmoles NADPH produced/g protein/min) |
|---|---|
| Control | 5·8 (2) |
| *p*-Fluoro-DL-phenylalanine | 6·4 ± 0·4 (4) |
| L-Phenylalanine | 5·5 ± 0·5 (5) |
| *p*-Fluoro-DL-phenylalanine + L-phenylalanine | 6·8 ± 0·5 (6) |
| Phenobarbital | 11·3 ± 0·8 (6) |
| Phenobarbital + *p*-fluoro-DL-phenylalanine | 7·0 ± 0·3 (6) |
| Phenobarbital + L-phenylalanine + *p*-fluoro-DL-phenylalanine | 9·3 ± 0·5 (6) |

* Male Holtzman rats were treated with phenobarbital (20 mg/kg, twice daily), L-phenylalanine (90 mg/kg, once daily) or *p*-fluoro-DL-phenylalanine (90 mg/kg, once daily). The amino acids were given 30 min prior to the first injection of phenobarbital. The enzyme assay was conducted as described in the text.

obtained from the livers of phenobarbital-treated rats (PB-S preparation). The purine derivative was less potent as an inhibitor than 5-fluorouracil; four doses of the former were required for maximal reduction in activity. It is interesting to note the lack of effect of the analogs upon the per cent liver weight/body weight in the phenobarbital-treated animals.

The minimal effective dose of 5-fluorouracil and dosage schedule eliciting this effect in the PB-S preparations was investigated (Table 8). One injection of 5-fluorouracil (20 mg/kg body weight) reduced enzymatic activity by 26% in the PB-S preparation. Four administrations at 2 mg/kg were ineffective, although at 10 mg/kg a 38% reduction in activity was observed.

TABLE 7. EFFECT OF 6-MERCAPTOPURINE AND 5-FLUOROURACIL UPON THE PHENOBARBITAL-INDUCED INCREASE IN G-6-P OXIDOREDUCTASE*

| Conditions | % Liver wt. / Body wt. | G-6-P Oxidoreductase (mμmoles NADPH produced/g protein/min) |
|---|---|---|
| Control (2) | 4·22 | 6·6 |
| 6-MP-1 (5) | 4·05 ± 0·05 | 6·1 ± 0·5 |
| 6-MP-4 (4) | 4·32 ± 0·08 | 6·5 ± 0·5 |
| Phenobarbital (4) | 5·62 ± 0·05 | 10·0 ± 0·6 |
| Phenobarbital + 6-MP-1 (5) | 5·51 ± 0·04 | 8·2 ± 0·3 |
| Phenobarbital + 6-MP-4 (4) | 5·57 ± 0·05 | 7·0 ± 0·4 |
| 5-Fluorouracil (5) | 4·85 ± 0·03 | 6·4 ± 0·8 |
| Phenobarbital + 5-fluorouracil (5) | 5·95 ± 0·07 | 6·2 ± 0·5 |

* Male Holtzman rats were injected with phenobarbital (20 mg/kg, twice daily for 4 days); 6-mercaptopurine (60 mg/kg, once either on day 1: 6-MP-1; once daily for 4 days: 6-MP-4), or 5-fluorouracil: (20 mg/kg, once daily for 4 days). The inhibitors were injected 30 min prior to the first administration of phenobarbital.

TABLE 8. EFFECT OF DOSAGE OF 5-FLUOROURACIL UPON THE PHENOBARBITAL-INDUCED INCREASE IN G-6-P OXIDOREDUCTASE*

| Conditions | $\frac{\text{Liver wt.}}{\text{Body wt.}}$ % | G-6-P Oxidoreductase (mμmoles NADPH produced/g protein/min) |
|---|---|---|
| Phenobarbital (3) | 5·65 ± 0·03 | 11·9 ± 0·4 |
| Phenobarbital + 5-fluorouracil, 2 mg/kg; 4 injections (5) | 5·44 ± 0·04 | 10·7 ± 0·5 |
| Phenobarbital + 5-fluorouracil, 10 mg/kg; 1 injection (5) | 5·52 ± 0·06 | 10·4 ± 0·4 |
| Phenobarbital + 5-fluorouracil, 10 mg/kg; 4 injections (5) | 5·34 ± 0·05 | 7·4 ± 0·5 |
| Phenobarbital + 5-fluorouracil, 20 mg/kg; 1 injection (5) | 5·10 ± 0·03 | 8·9 ± 1·0 |

* See footnote to Table 7 for dose and dosage schedule for 5-fluorouracil.

TABLE 9. COMPARISON OF G-6-P OXIDOREDUCTASE ACTIVITY IN THE SUPERNATES FROM CONTROL AND PHENOBARBITAL-STIMULATED LIVER*

| | Control | Phenobarbital |
|---|---|---|
| pH optimum | 7·6 | 7·6 |
| $K_m$–Glucose-6-phosphate | 1·2–1·5 × $10^{-4}$ M | 1·0–1·1 × $10^{-4}$ M |
| $K_m$–NADP | 1·3 × $10^{-5}$ M | 1·1–1·4 × $10^{-5}$ M |
| Storage denaturation, $T_{1/2}$ | 14 days | 16 days |
| Heat denaturation, $T_{1/2}$ (at 50°) | 10 min | 15 min |

* The $K_m$ for glucose-6-phosphate and for NADP was determined twice on different samples; the two values are recorded in the table.

*Properties of G-6-P Oxidoreductase in P-BS and Control Preparations*

Enzymatic activity was labile to heat (i.e. 50°). The times required for a 50% reduction in activity for control and PB-S preparations were 10 and 15 min respectively.

G-6-P oxidoreductase activity also decreased upon storage at −5°. The intervals required for a 50% reduction in activity of the control and PB-S preparations were 14 and 16 days respectively.

Some of the properties of the G-6-P oxidoreductase activity in control and PB-S preparations are compared in Table 9.

The 'apparent' Michaelis constants for glucose-6-phosphate and for NADP were determined by the method of Lineweaver and Burk.[13] No significant difference existed in the pH optimum, $K_m$ for glucose-6-phosphate, $K_m$ for NADP, storage denaturation, or heat denaturation.

## DISCUSSION AND CONCLUSIONS

The results presented herein show that phenobarbital (and other drugs) increases the activity of NADP-requiring enzymes located not only in the endoplasmic reticulum

but in the cytoplasm as well. The exact mechanism underlying this increase is unknown, although presumptive evidence (reviewed in Ref. 14) is suggestive of induced enzyme synthesis. This evidence is based upon (1) the prevention of induction of microsomal enzyme activity by the amino acid antagonists, ethionine and β-2-thienylalanine; (2) the appearance of enhanced enzyme activity only after treatment of the rats with the drug *in vivo*; and (3) the marked increase in the liver weight of the pretreated rats. Some insight, however, into the mechanism has been gained from the studies of von der Decken and Hultin[15] who have reported that the activity of enzyme systems that incorporate amino acids into microsomal protein, isolated from the livers of rats pretreated with 3-methylcholanthrene is, increased. Further information has been supplied by Gelboin and Loeb[16] whose studies suggest the increased availability or synthesis of messenger-RNA as a result of pretreatment with 3-methylcholanthrene.

All these studies have been hampered by the lack of any definitive establishment of an increased enzyme synthesis. Hitherto it has not been possible to obtain purified preparations of the drug-metabolizing enzymes from endoplasmic reticulum; however, procedures do exist for the purification of the 'soluble' enzyme, G-6-P oxidoreductase.[17–22] It is feasible, therefore, to obtain an antibody to G-6-P oxidoreductase, and by means of antibody–antigen precipitation techniques, to measure quantitatively the amount of enzyme present in a given preparation. This would supply an experimental approach for the establishment of an increased synthesis of enzyme in phenobarbital-treated rats.

The data reported in this manuscript conform to the above hypothesis. Although ethionine does not influence enzymatic activity in the phenobarbital-treated animals, this amino acid analog does possess multiple mechanisms of action,[23] the least of which may involve the inhibition of protein synthesis by virtue of its structural relationship to methionine. Ethionine, however, did prevent the stimulation of the activity of the drug-metabolizing enzymes resulting from phenobarbital pretreatment.[3, 4, 24] *p*-Fluoro-DL-phenylalanine, an inhibitor of protein synthesis in bacterial systems[25–28] significantly depressed G-6-P oxidoreductase activity in our hands, although the amino acid analog was ineffective in preventing the rise in activity of the drug-metabolizing enzymes resulting from phenobarbital pretreatment.[4]

The results obtained from the experiments employing the antimetabolites, 6-mercaptopurine, and 5-fluorouracil appear to implicate nucleic acid biosynthesis in the stimulation of G-6-P oxidoreductase, although here too, Kato *et al.*[4] have reported that 6-mercaptopurine is without effect in the drug-metabolizing enzyme system. The difference, however, may be a matter of dosage schedule. Although the mechanism of action of 6-mercaptopurine has been sought by a number of investigators,[29–31] the manner by which 6-mercaptopurine suppresses induced enzyme formation[32] or suppresses antibody formation[33] has not been elucidated.

In bacterial systems, induced enzyme formation is also blocked by the administration of 5-fluorouracil,[34, 35] and as reported herein, the induced synthesis of G-6-P oxidoreductase is very effectively inhibited by this analog.

The data here reported suggest that phenobarbital induces an increase in G-6-P oxidoreductase, identical in the properties examined with that which is found in control rat liver. On the other hand, Conney *et al.*[5] and Touster and Hefter[6] have observed in control rats that the 'soluble' enzyme, UDP glucose:NAD oxidoreductase is unstable, whereas the enzyme from chlorobutanol-treated rats is stable in storage.

The latter phenomenon suggests either the production of an enzyme protector by the drug or the production of an enzyme with a different conformation.

It is clear that phenobarbital does not simply affect *all* NADP-requiring 'soluble' enzymes, since no increase in the activities of isocitrate or dihydrouracil oxidoreductase was observed. It is also evident that the mechanism of action of phenobarbital is not identical with that of the polycyclic hydrocarbons–i.e. 3-methylcholanthrene. The latter was ineffective as an inducer of G-6-P oxidoreductase. The mechanism behind the selective action of phenobarbital and 3-methylcholanthrene must await further study.

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