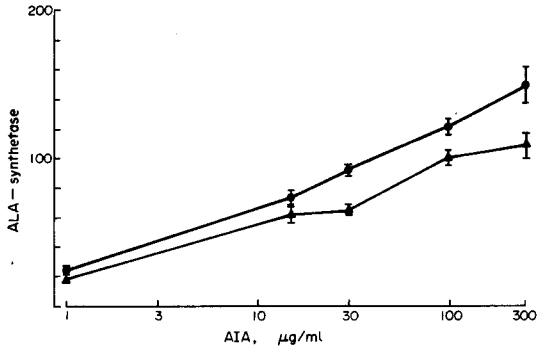


Fig. 4. ALA-synthetase activity in cells maintained in serum-free Waymouth MD 705/1 medium in the presence (●——●) and absence (▲——▲) of 100 ng $T_4$/ml, 16 hr after the addition of various doses of AIA. The results are expressed as the mean of from 8 to 13 determinations ± S. E. M.

doses of AIA in chick embryo liver cells maintained in Waymouth MD 705/1 in the presence and absence of $T_4$ (1000 ng/ml) is shown in Fig. 3. Enhancement of AIA-induced porphyrin biosynthesis by $T_4$ is considerably greater at low dose levels of AIA than at higher doses. Thus, at a dose of 10 μg AIA/ml there is an enhancement of approximately 6-fold as compared to a 2-fold enhancement at a dose of 300 μg/ml. Moreover, AIA (10 μg/ml) when present in a medium containing $T_4$ produces an accumulation of porphyrins similar to the accumulation of porphyrins produced by AIA (300 μg/ml) in a medium lacking $T_4$. When the AIA dose–response curve (Fig. 3) was compared with the AIA dose–response curve previously obtained [6] using Eagle's Basal Medium supplemented with 10% donor calf serum, it was found that, at a dose of 10 μg/ml of AIA, porphyrin accumulation was three to four times greater in the $T_4$-supplemented Waymouth medium (Fig. 3) than in serum-containing medium. On the other hand, the levels of porphyrins accumulating in response to higher doses of AIA (300 μg/ml) in the $T_4$-supplemented Waymouth medium (Fig. 3) were approximately two-thirds those observed in the serum-containing medium.

Goodridge [4] added 1% serum albumin to Waymouth medium in order to protect $T_3$ and $T_4$ from degradation. It, therefore, appeared possible that the addition of serum albumin might enhance the effectiveness of $T_3$ and $T_4$ in our system. When 0.2% bovine serum albumin was added to the medium, the levels of porphyrins which accumulated at doses of 100 and 300 μg/ml of AIA were greater than the levels observed previously in serum-containing media. Increasing the quantity of bovine serum albumin above 0.2% resulted in less cells attaching to the Petri dishes.

The induction of ALA-synthetase by various doses of AIA in chick embryo liver cells maintained in Waymouth MD 705/1 in the presence and absence of $T_4$ (100 ng/ml) is shown in Fig. 4. The presence of $T_4$ in the medium enhanced AIA-induced ALA-synthetase activity, although less than was anticipated from the marked enhancement of AIA-induced porphyrin biosynthesis by $T_4$ (Fig. 3). It is possible that thyroid hormone influences the heme pathway through increased ALA-synthetase activity and by other means. For example, since the oxygen consumption of tissues such as liver is stimulated by thyroid hormone [9], it is possible that the environment of the liver cell under the influence of thyroid hormone might facilitate the irreversible oxidation of porphyrinogens to porphyrins. Insulin has been shown [6] to enhance AIA-induced porphyrin biosynthesis in serum-free Waymouth medium. It was of interest to determine whether insulin was required for optimal activity in a $T_4$-supplemented Waymouth medium. The results in Table 1 show that insulin is required for optimal activity. In summary, our present and previous results [6] show that, when Waymouth medium 705/1 is supplemented with insulin and thyroid hormone ($T_3$ or $T_4$), the AIA-induced porphyrin accumulation is greater at low doses (10 μg/ml) and approximately two-thirds at higher doses (300 μg/ml) of that previously observed in serum-containing media.

In recent years, specific nuclear binding sites have been recognized for $T_3$ and $T_4$ which might be important for the initiation of hormonal action. The nuclear binding sites are nonhistone nucleoproteins and it is suggested that the interaction of $T_3$ with its receptors results in an augmented transcription of DNA followed by an increase in protein synthesis [10–12]. Porphyrin-inducing drugs such as AIA are believed to act by increasing the levels of a specific RNA for ALA-synthetase [7, 13]. Thus, thyroid hormone, by augmenting transcription of DNA and increasing protein synthesis, may enhance the ability of AIA to increase ALA-synthetase levels.

It has been reported that, in abnormal thyroid states, the human metabolism of some drugs is altered [14]. Gillette [15] and Kato and Gillette [16] reported that, after administration of thyroxine to male rats, the rate of aniline hydroxylation by their liver microsomes increased and the rate of aminopyrine *N*-demethylation decreased. It is possible that, in the presence of thyroid hormone, the rate of metabolism of AIA by chick embryo liver cells is decreased and the concentration of the active drug is thus increased.

Table 1. Biosynthesis of porphyrins by cells maintained in serum-free Waymouth medium containing 100 ng $T_4$/ml in the presence and absence of 1 ng insulin/ml, 24 hr after the addition of 300 μg AIA/ml*

| Medium | Porphyrin (ng/mg protein) |
|---|---|
| Waymouth medium + $T_4$ + insulin | 532.1 ± 20.1 (14) |
| Waymouth medium + $T_4$ − insulin | 156.3 ± 6.4 (13) |

* Results are expressed as the average of the number of determinations shown in parentheses ± S. E. M.

The effect of thyroid hormone on ALA-synthetase activity is not confined to chicken liver cells nor to cell culture systems. Matsuoka *et al.* [17] showed that the AIA-induced increase of ALA-synthetase was stimulated when rats were given $T_3$; $T_3$ administration had no effect by itself. A question that arises from the following study is: Could the level of $T_3$ or $T_4$ in liver cells of patients with porphyria contribute, at least in part, to their sensitivity to a series of drugs [18]? The thyroid status of patients with acute intermittent porphyria has been investigated [19, 20] and it has been shown that the protein-bound iodine, total thyroxine iodine and thyroxine-binding globulin are increased in many of these patients. The increase was most pronounced and occurred most often in females. The free thyroxine in the circulation was normal and the patients were in a euthyroid state. Since disordered hypothalamic function is frequently observed in patients with acute intermittent porphyria, the possibility has been considered that disordered thyrotropic activity might occur [18, 19]. The results in Fig. 3 show that a small dose of AIA, which would not cause porphyrin accumulation in $T_4$-free Waymouth medium, would cause marked porphyrin accumulation in $T_4$-containing Waymouth medium. Thus, if the increased thyroxine binding to thyroxine-binding globulin in acute intermittent porphyria is accompanied by an increase in thyroxine (or $T_3$) binding to hepatic cell thyroxine-specific binding sites, then it is possible to envisage a mechanism to explain increased hepatic sensitivity to drugs. The possibility is also raised that treatment of acute intermittent porphyria patients with antithyroid drugs may cause a lowered hepatic sensitivity to porphyrin-inducing stimuli (exogenous or endogenous) and thus exert a beneficial effect.

*Acknowledgement*—The authors wish to thank Mrs. Frances Taylor for her excellent technical assistance.

## REFERENCES

1. S. Granick, *J. biol. Chem.* **241,** 1359 (1966).
2. D. W. Schneck, D. L. J. Tyrrell and G. S. Marks, *Biochem. Pharmac.* **20,** 2999 (1971).
3. P. R. Sinclair and S. Granick, *Ann. N.Y. Acad. Sci.* **244,** 509 (1975).
4. A. G. Goodridge, *Fedn Proc.* **34,** 117 (1975).
5. P. A. Kitos, R. Sinclair and C. Waymouth, *Expl Cell Res.* **27,** 307 (1962).
6. P. W. F. Fischer, R. O. Morgan, V. Krupa and G. S. Marks, *Biochem. Pharmac.* **25,** 687 (1976).
7. D. L. J. Tyrrell and G. S. Marks, *Biochem. Pharmac.* **21,** 2077 (1972).
8. S. Refetoff, N. I. Robin and V. S. Fang, *Endocrinology* **86,** 793 (1970).
9. A. G. Gilman and F. Murad, in *The Pharmacological Basis of Therapeutics* (Eds. L. S. Goodman and A. Gilman), 5th Edn, p. 1406. Macmillan, New York (1975).
10. J. H. Oppenheimer, *New Engl. J. Med.* **292,** 1063 (1975).
11. J. H. Oppenheimer and M. I. Surks, *Med. Clins N. Am.* **59,** 1055 (1975).
12. R. J. Tata, *Nature, Lond.* **257,** 18 (1975).
13. S. Sassa and S. Granick, *Proc. natn. Acad. Sci. U.S.A.* **67,** 517 (1970).
14. M. Eichelbaum, G. Bodem, R. Gugler, C. Schneider-Deters and H. J. Dengler, *New Engl. J. Med.* **290,** 1040 (1974).
15. J. R. Gillette, *Ann. N.Y. Acad. Sci.* **123,** 42 (1965).
16. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150,** 285 (1965).
17. T. Matsuoka, B. Yoda and G. Kikuchi, *Archs Biochem. Biophys.* **126,** 530 (1968).
18. D. P. Tschudy, in *Diseases of Metabolism* (Eds. P. K. Bondy and L. E. Rosenberg), 7th Edn, p. 775 Saunders, Philadelphia (1974).
19. E. S. Hellman, D. R. Tschudy, J. Robbins and J. E. Rall, *J. clin. Endocr. Metab.* **23,** 1185 (1963).
20. C. S. Hollander, R. L. Scott, D. P. Tschudy, M. Perlroth, A. Waxman and K. Sterling, *New Engl. J. Med.* **277,** 995 (1967).