Accelerated Biliary Thyroxine Secretion in Rats Treated with 3-Methylcholanthrene[1] (36015)

W. C. NEWMAN,[2] R. C. FERNANDEZ, R. M. SLAYDEN, AND R. C. MOON

Department of Physiology and Biophysics, University of Tennessee Medical Units, Memphis, Tennessee 38103

In an earlier investigation (1) we found that administration of the carcinogenic polycyclic hydrocarbon 3-methylcholanthrene (MCA) to rats markedly altered the metabolism of exogenous radioactively labeled thyroxine (T_4). Our data suggested that the decreased rate of iodine excretion and increased rate of fecal T_4 excretion which was observed in MCA-treated rats was secondary to accelerated hepatic conjugation and biliary secretion of T_4. In the experiment reported herein, we have examined this possibility by investigating the influence of MCA upon the biliary secretion of ^{131}I derived from a tracer dose of ^{131}I-labeled L-thyroxine (^{131}I-T_4).

Materials and Methods. Virgin female Sprague-Dawley rats received a single dose of 10 mg of MCA in 1 ml of sesame oil by intragastric intubation at age 60 days. Control rats of the same age, sex, and strain received a single intragastric administration of only 1 ml sesame oil. Twenty-four hours later the rats were anesthetized with urethan and the bile duct was exposed and cannulated. One-half microcurie of ^{131}I-T_4 was administered iv. Bile was collected in hourly samples during the 4 hr period following injection of ^{131}I-T_4. A blood sample was taken from the jugular vein 30 min after injection of ^{131}I-T_4.

The total radioactivity in each hourly bile sample and in a 50 μl aliquot thereof was counted in a well-type scintillation counter. Bile flow rate (ml/hr) was calculated from the ratio of these counts. The radioactivity in a 50 μl aliquot of each plasma sample was also counted. Biliary clearance of plasma ^{131}I ($C_{^{131}I}$) was calculated for the first hour collection period according to the equation:

$$C_{^{131}I} = \frac{(\text{cpm/ml of bile})\,(\text{ml of bile})}{\text{cpm/ml of plasma}}.$$

Biliary radioactivity was analyzed by ascending one-dimensional paper chromatography combined with radioautography. Chromatograms were developed in a solvent system consisting of collidine:water: NH_4OH (2) and applied to Kodak Blue-Brand X-ray film for radioautography.

Results. The average total biliary secretion of ^{131}I by MCA-treated rats during the 4 hr collection period was approximately twice that by control rats (Table I). The average volume flow rate of bile was also slightly higher, but not significantly so, in MCA-treated rats. Biliary clearance of plasma ^{131}I in MCA-treated rats was more than three times that observed in control rats during the first hour of bile collection. Chromatographically, the major fraction of biliary ^{131}I for both MCA-treated and control rats appeared to be in the form of T_4-glucuronide.

Discussion. These data clearly indicate that the biliary secretion of radioactivity derived from a tracer dose of ^{131}I-T_4 was accelerated in MCA-treated rats and suggest that this was due to the presence of a greater quantity of T_4-glucuronide in the bile of the carcinogen-treated rats. The slight increase in bile flow rate in carcinogen-treated rats is not sufficient to account for the observed difference in biliary ^{131}I secretion. Furthermore, the biliary clearance of plasma ^{131}I in carcinogen-treated rats during the first hour was increased out of proportion to the differ-

[1] This investigation was supported by U.S. Public Health Service Grants CA-05105 and CA-10323.

[2] Present address: Department of Physiology, Tulane University Medical School, New Orleans, Louisiana.

TABLE I. Effect of MCA on the Biliary Secretion of ^{131}I Derived from a Tracer Dose of ^{131}I-Labeled L-Thyroxine.[a]

	Control	MCA
Biliary ^{131}I secretion (% dose/hr)		
1st hr	2.11 ± 0.37[b]	5.46 ± 0.47
2nd hr	2.63 ± 0.36	5.40 ± 0.43
3rd hr	2.17 ± 0.32	4.37 ± 0.44
4th hr	2.32 ± 0.32	5.06 ± 0.56
Total (% dose/4 hr)	9.23 ± 1.11[cd]	20.30 ± 1.76[e]
Bile vol flow rate (ml/hr)	1.64 ± 0.13[f]	1.90 ± 0.14[g]
Plasma ^{131}I clearance (ml/hr) (1st hr)	0.50 ± 0.08[h]	1.69 ± 0.21[i]

[a] Twelve rats in each group. Each rat received a single intragastric administration of either 1 ml of sesame oil or 1 ml of sesame oil containing 10 mg of MCA. One-half microcurie of ^{131}I-labeled L-thyroxine was administered iv 24 hr later. Bile was collected in hourly samples during 4 hr period following ^{131}I-labeled L-thyroxine.

[b] Mean ± SE.

[c] Student's t test, level of significance: [de] .001; [fg] not significant; [hi] .025.

ence in bile flow rate. Thus, the results of this study are in general agreement with those of Goldstein and Taurog (3) who found that the carcinogenic hydrocarbon 3, 4-benzpyrene stimulates biliary secretion of T_4-glucuronide by increasing liver glucuronyl transferase.

In recent years, increasing attention has been focused on the ability of MCA to alter the activity of a group of closely related microsomal enzymes which function in the metabolism of drugs and other foreign compounds (4–8). The stimulatory effect of MCA is specific to certain enzymes (9), is not limited to the liver (10, 11), and is apparently due to stimulation of enzyme synthesis (12). The ability of MCA to increase the activity of drug metabolizing enzymes assayed *in vitro* is paralleled *in vivo* by accelerated rates of drug metabolism and by shortened duration of drug action. The enzyme systems affected by MCA may also function in the metabolism of other hormonal substrates such as estrone (13), hydrocortisone (14), and testosterone (15), as well as T_4. Our data are consistent with the hypothesis that the rate of hepatic conjugation of T_4 was accelerated in MCA-treated rats, which resulted in an increased rate of biliary secretion and an increased fecal excretion of T_4.

Summary. Rats received a single dose of 10 mg of 3-methylcholanthrene (MCA). Twenty-four hours later each rat received a 0.5 μCi of ^{131}I-labeled L-thyroxine (^{131}I-T_4). Bile was collected in hourly samples for 4 hr following ^{131}I-T_4. Average total (4 hr) biliary secretion of ^{131}I by MCA-treated rats was approximately twice that in control rats. Average volume flow rate of bile was not significantly different in the two groups. During the first hour of bile collection, biliary clearance of plasma ^{131}I in MCA-treated rats was more than 3 times that in control rats. The major fraction of biliary ^{131}I in both groups appeared to be T_4-glucuronide.

1. Newman, W. C., and Moon, R. C., Endocrinology **80**, 896 (1967).

2. Taurog, A., Tong, W., and Chaikoff, I. L., J. Biol. Chem. **184**, 83 (1950).

3. Goldstein, J. A., and Taurog, A., Biochem. Pharmacol. **17**, 1049 (1968).

4. Conney, A. H., Miller, E. C., and Miller, J. A., Cancer Res. **16**, 450 (1956).

5. Conney, A. H., Miller, E. C., and Miller, J. A., J. Biol. Chem. **228**, 753 (1957).

6. Cramer, J. W., Miller, J. A., and Miller, E. C., J. Biol. Chem. **235**, 250 (1960).

7. Arcos, J. C., Conney, A. H., and Buu Hoi, N. P., J. Biol. Chem. **236**, 1291 (1961).

8. Inscoe, J. K., and Axelrod, J., J. Pharmacol. Exp. Ther. **129**, 128 (1960).

9. Conney, A. H., Gillette, J. R., Inscoe, J. K., Trama, E. R., and Posner, H. S., Science **130**, 1478 (1959).

10. Wattenberg, L. W., and Leong, J. L., J. Histochem. Cytochem. **10**, 412 (1962).

11. Gillman, A. G., and Conney, A. H., Biochem. Pharmacol. **12**, 591 (1963).

12. Gelboin, H. V., Biochim. Biophys. Acta **91**, 130 (1964).

13. Jellinck, P. H., and Irwin, L., Nature (London) **198**, 787 (1963).

14. Glenn, E. M., Lyster, S. C., Bowman, B. J., and Richardson, S. L., Endocrinology **64**, 419 (1959).

15. Conney, A. H., and Klutch, A., J. Biol. Chem. **238**, 1611 (1963).

Received July 8, 1971. P.S.E.B.M., 1971, Vol. 138.