# Effects of Gonadectomy on the Uptake of Polycyclic Hydrocarbons by Rat Liver Nuclei, DNA, and Histones (35853)

J. S. SCHWEPPE, E. KOT, AND R. A. JUNGMANN

*Departments of Biochemistry and Medicine, Northwestern University Medical School, Chicago, Illinois 60611*

Administration of polycyclic hydrocarbons to rats is followed by an increase in the activities of several of the mixed function oxidases present in the liver. Under certain conditions polycyclic hydrocarbons are metabolized by these enzymes to metabolites of high carcinogenic potency (1). The development of tumors induced by chemical carcinogens is subject to some elements of endocrine control (2–4), but the mechanisms by which endocrine factors affect chemical carcinogenesis are not understood. It remains a possibility that during the early phases of carcinogen–tissue interaction hormones modify the metabolic activity of liver and thus increase or decrease the extent of metabolic modification of administered polycyclic hydrocarbons. The effective level of proximate carcinogens or the modification of the interaction between carcinogen and macromolecules of liver nuclei may be among the biochemical parameters which can be affected by hormones. In the present investigation, we have studied some of the initial phases of the interaction of 7,12-dimethylbenz[*a*]anthracene-$^3$H (DMBA) and of benz[*a*]-anthracene-$^3$H (1,2-BA) with hepatic DNA and histones from intact and gonadectomized male and female rats.

*Materials and Methods.* Intact and gonadectomized male and female Sprague-Dawley rats (180 to 200 g) were used throughout the experiments. The time lapse between operation and killing was 21 days. The animals were guillotined, after which the livers were cannulated and perfused with ice-cold 0.9% NaCl. Slices were prepared by means of a Stadie-Riggs microtome. The slices (500 mg of wet wt) were placed in a 25-ml Erlenmeyer flask and suspended in 5 ml of 0.05 *M* sodium phosphate buffer, pH 7.9, containing 2 $\mu$Ci of 7,12-dimethylbenz-[*a*]anthracene-$^3$H (G) (sp act 750 $\mu$Ci/mmole), or 2 $\mu$Ci of benz[*a*]anthracene-$^3$H (G) (sp act 750 $\mu$Ci/mmole) dissolved in 0.1 ml of propylene glycol. Incubations were carried out at 37° in a Dubnoff metabolic incubator in air. At the end of the incubation time the slices were removed from the incubation medium and washed 5 times in 4 ml of ice-cold 0.05 *M* sodium phosphate buffer, pH 7.9, containing the respective nonradioactive carcinogens (dissolved in propylene glycol) at a concentration of $10^{-4}$ *M*. The slices were homogenized in 8 ml of 0.05 *M* sodium phosphate buffer, pH 7.9, containing 0.25 *M* sucrose in a Potter-Elvehjem glass homogenizer. The nuclear fraction was isolated and purified by sedimentation through 2.3 *M* sucrose as described previously (5).

For the isolation of the total histone fraction, nuclei were extracted for 6 hr with three 5-ml portions of ice-cold 0.25 *N* HCl (6). The combined acid extract was clarified by centrifugation and contained the total acid-extractable histone fraction. DNA was extracted from the purified nuclei by a modification of the Kirby procedure (7). DNA was determined with diphenylamine (8). Protein was determined by the method of Lowry *et al.* (9). For the determination of radioactivity, the samples were dissolved in 2 ml of NCS solubilizer (NCS Tissue Solubilizer—Amersham/Searle, Chicago, Illinois). After the addition of 15 ml of liquid scintillation solution (6 g of PPO, 250 mg of dimethyl-POPOP in 1 liter of toluene), the samples were counted in a liquid scintillation spectrometer.

*Results.* The rate of uptake of radioactive DMBA and 1,2-BA by rat liver slices at time intervals from 1 to 30 hr is shown in Fig. 1.

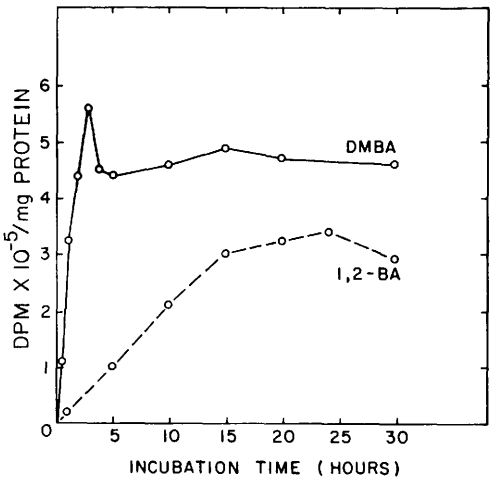


FIG. 1. Time course of 7,12-dimethylbenz[*a*]anthracene-$^3$H and benz[*a*]anthracene-$^3$H uptake by rat liver slices: Liver slices from normal male rats were incubated with labeled DMBA and 1,2-BA. After incubation, the slices were homogenized and the homogenate was assayed for protein content and radioactivity; see "Methods" for details. Each point represents the mean value of 4 incubations. Standard error of mean was for all time points less than 10% (not plotted).

The initial rate of uptake of radioactivity was significantly higher in the incubations with DMBA than in similar incubations with 1,2-BA. The 1,2-BA was only slowly incorporated into the incubating liver slices and maximum specific activities were not obtained until 20 to 25 hr after the start of the incubation. In the subsequent experiments in which the effects of sex differences and gonadectomy on the uptake of labeled hydrocarbons are compared, the length of the incubation time was chosen to obtain maximal uptake of radioactivity by the liver slices (3 hr for incubations with DMBA; 20 hr for incubations with 1,2-BA).

Liver nuclei from intact male rats showed a significantly lower specific activity than nuclei from the liver of intact female rats after incubation of liver slices with DMBA (Table I). Ovariectomy had little or no effect on the uptake of DMBA by liver nuclei from female rats, whereas the uptake of DMBA by liver nuclei from male rats increased approximately 99% after gonadectomy. Table I further shows that incubation of liver slices with 1,2-BA led to a relatively higher labeling of liver nuclei from intact male rats than those in intact female rats. Gonadectomy of the male did not affect the degree of uptake of radioactive 1,2-BA by liver nuclei, but nuclei from the liver of female rats exhibited a significant increase of specific activity after ovariectomy.

Quantitatively, only small amounts of radioactivity were detected in the DNA fractions after incubation of liver slices with labeled DMBA and 1,2-BA (Table II), and it is conceivable that the radioactivity was actually bound to a protein contaminant in the DNA preparation. The lack of significant labeling of hepatic DNA under these conditions seems to be characteristic of *in vitro* experiments, since a much higher degree of labeling of DNA is observed after *in vivo* administration of radioactive DMBA and 1,2-BA (10, 11).

The specific activities of the total histone fractions isolated from nuclei after incubation of liver slices with radioactive DMBA and 1,2-BA are listed in Table II. No significant differences are found in the specific activities

TABLE I. The Effect of Sex Differences and Gonadectomy on the Uptake of 7,12-Dimethylbenz[*a*]anthracene-$^3$H and Benz[*a*]anthracene-$^3$H by Isolated Nuclei from Rat Liver.[a]

| Group | Polycyclic hydrocarbon incubated (dpm $^3$H × $10^{-3}$/mg of protein) | |
|---|---|---|
| | DMBA | 1,2-BA |
| Male | | |
| Intact | 16.8 ± 1.5[b] | 48.7 ± 3.3 |
| Gonadectomized | 31.6 ± 2.8 | 49.3 ± 5.0 |
| Female | | |
| Intact | 30.5 ± 3.2 | 18.9 ± 2.5 |
| Ovariectomized | 37.1 ± 3.5 | 36.1 ± 2.7 |

[a] Liver slices from intact and gonadectomized male and female rats were incubated for 3 hr with labeled DMBA and for 20 hr with labeled 1,2-BA. After incubation, nuclei were isolated from the homogenized liver slices and assayed for protein content and radioactivity; see "Methods" for details.

[b] Values represent the mean ± standard error of four experiments.

TABLE II. The Effect of Sex Differences and Gonadectomy on the Uptake of 7,12-Dimethylbenz[*a*]anthracene-$^3$H and Benz[*a*]anthracene-$^3$H by Nuclear DNA and Total Histone from Rat Liver.[a]

| | Polycyclic hydrocarbon incubated | | | |
|---|---|---|---|---|
| | (dpm $^3$H/mg of DNA) | | (dpm $^3$H/mg of histone) | |
| Group | DMBA | 1,2-BA | DMBA | 1,2-BA |
| Male | | | | |
| Control | 23 ± 0.3[b] | 4 ± 0.02 | 1237 ± 80 | 1291 ± 160 |
| Gonadectomized | 29 ± 0.3 | 2 ± 0.02 | 1114 ± 111 | 2435 ± 210 |
| Female | | | | |
| Control | 49 ± 0.4 | 5 ± 0.08 | 1500 ± 160 | 915 ± 31 |
| Ovariectomized | 12 ± 0.1 | 2 ± 0.04 | 2922 ± 250 | 2709 ± 91 |

[a] Liver slices from intact and gonadectomized male and female rats were incubated for 3 hr with labeled DMBA and for 20 hr with labeled 1,2-BA. After incubation, nuclei were isolated from the homogenized liver slices. The total acid-soluble histone fraction and DNA were extracted and their specific activity was determined; see "Methods" for details.

[b] Values represent the mean ± standard error of four experiments.

of histones from the liver of intact male or female rats, and radioactive DMBA as well as 1,2-BA were bound to histones to the same extent. Gonadectomy had no effect on the uptake of DMBA by total liver histones of male rats but it led to an increased uptake of radioactivity by liver histones in all other experiments listed.

*Discussion.* The binding of polycyclic hydrocarbons to DNA, RNA, and cytoplasmic proteins has been demonstrated previously by Brookes and Heidelberger (12) and other investigators (10, 11). In a preliminary communication we have demonstrated the binding of DMBA and *p*-dimethylaminoazobenzene-$^{14}$C to rat liver histone fractions *in vivo* (13). It is evident from the present study that the uptake *in vitro* of DMBA and 1,2-BA by rat liver nuclear components can be influenced by sex differences and by gonadectomy. It would appear, in general, that the uptake of DMBA and 1,2-BA by rat liver nuclear histones is greatly enhanced after gonadectomy. The binding of DMBA to histone was responsive to gonadectomy only in the female rat.

The biological significance of these findings remains obscure. However, there is a distinct possibility that interaction of administered polycyclic hydrocarbons with DNA and nuclear proteins may alter the genetic expression and information release in a cell. The presence or the lack of male or female hormone could play a permissive or promoting role in this process.

*Summary.* The effects of sex difference and of gonadectomy on the uptake of 7,12-dimethylbenz[*a*]anthracene-$^3$H and benz[*a*]anthracene-$^3$H by rat liver slices, hepatic DNA, and histones were studied. Liver slices from the intact male rat incorporated 7,12-dimethylbenz[*a*]anthracene-$^3$H at a higher rate than benz[*a*]anthracene-$^3$H. The uptake of 7,12-dimethylbenz[*a*]anthracene-$^3$H by intact isolated liver nuclei increased after gonadectomy of the male rat, whereas the uptake of benz[*a*]anthracene-$^3$H increased in liver nuclei from female rats after ovariectomy. Relatively small amounts of radioactive hydrocarbons were bound to DNA under the *in vitro* experimental conditions. Liver histones were found to bind radioactivity after incubation of liver slices with 7,12-dimethylbenz[*a*]anthracene-$^3$H and benz[*a*]anthracene-$^3$H. Gonadectomy led to an increase in the extent of binding of benz[*a*]anthracene-$^3$H to liver histones from both male and female rats. Uptake of 7,12-dimethylbenz[*a*]anthracene-$^3$H by histones was increased only in liver of ovariectomized female rats.

The investigation was supported by the Steroid

Hormone Research Fund, Chicago Wesley Memorial Hospital.

1. Boyland, E., and Sims, P., Int. J. Cancer **2**, 500 (1967).

2. Weisburger, J. H., Yamamoto, R. S., Korzis, J., and Weisburger, E. K., Science **154**, 673 (1966).

3. Dao, T. L., Cancer Res. **22**, 973 (1962).

4. Noble, R. L., Hormones **5**, 559 (1964).

5. Jungmann, R. A., Schweppe, J. S., and Lestina, F. A., J. Biol. Chem. **245**, 4321 (1970).

6. Bonner, J., Chalkley, G. R., Dahmus, M., Fambrough, D., Fujimura, F., Huang, R. C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivera, B., and Widholm, J., Methods Enzymol. **12** (Pt. B.), 20 (1968).

7. Irving, C. C., and Veazey, R. A., Biochim. Biophys. Acta **166**, 246 (1968).

8. Burton, K., Methods Enzymol. **12** (Pt. B), 163 (1968).

9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

10. Brookes, P., and Lawley, P. D., Nature (London) **202**, 781 (1964).

11. Brookes, P., and Lawley, P. D., J. Cell. Comp. Physiol. **64** (Suppl. 1), 111 (1964).

12. Brookes, P., and Heidelberger, C., Cancer Res. **29**, 157 (1969).

13. Jungmann, R. A., and Schweppe, J. S., Proc. Amer. Ass. Cancer Res. **12**, 50 (1971).

Received April 20, 1971. P.S.E.B.M., 1971, Vol. 138.