for eubacteria. DNA from the Eaton agent possesses the same average base composition as certain Lactobacillaceae and *Hemophilus* species; this finding is of special interest because both PPLO and *Hemophilus* species are specific agents of interstitial pneumonias, and characteristic pleuropneumonias.

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Comparative Metabolism of ^{14}C-Estrone in the Rat and Guinea Pig.* (29894)

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It is well established(1,2,3) that estrogens are excreted mainly as sulfate or glucosiduronate conjugates and that the formation of the latter requires uridine diphosphate glucuronic acid (UDPGA) present in the soluble fraction of liver cells and a microsomal glucuronyl transferase. Previous studies(4,5), however, have indicated that the water-soluble estrogen metabolites formed by rat liver may differ from the normal conjugates produced by other mammalian species and it was therefore decided to verify this by carrying out parallel incubations with rat and guinea pig liver preparations. In addition, the comparative *in vivo* metabolism of estrone-16-^{14}C was investigated in these two species.

Materials and methods. In vivo studies. Estrone-16-^{14}C (0.98 μc in 25 μg) dissolved in 0.25 ml of ethanol was added to 5 ml of isotonic saline and given intraperitoneally to 2 young female albino guinea pigs (470-490 g). The same amount of radioactive estrogen was administered to 2 female Wistar rats (155-160 g) and the first 24-hour collection of urine was pooled for each species. After adjusting the pH to 6.8, aliquots (0.2 ml) of the urine were counted in a Packard Tri-Carb Scintillation Spectrometer before and after extraction with ether. In addition, 3 ml samples of the rat and guinea pig urine were incubated for 48 hours at 37°C with 2.5 mg (187 units) of bacterial β-glucuronidase in the presence of one drop of chloroform (to retard bacterial growth) and extracted with ether. Other samples (8 ml) were subjected to acid hydrolysis by refluxing with 2 N HCl for 2 hours and the amount of ^{14}C removed by subsequent ether extraction was determined. The radioactive estrone was purchased from Amersham (England) and was shown by chromatography and autoradiography(4,6) to be virtually free of radioactive impurities.

In vitro studies. Subcellular fractions derived from 100 mg of rat and guinea pig liver

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TABLE I. *In vivo* Metabolism of ^{14}C-Estrone in Rat and Guinea Pig. Distribution of urinary radioactivity after acid and enzymatic hydrolysis.

Treatment of urine	% of excreted radioactivity remaining in urine after treatment	
	Rat*	Guinea pig†
Extracted with ether	74.8	63.4
Incubated with β-glucuronidase and extracted with ether	67.8	10.8
Refluxed with 2N HCl and extracted with ether	51.8	9.6

Estrone-16-^{14}C (10^6 counts/min in 25 μg) given intraperitoneally to 2 animals; urine collected for 24 hr.

* 1.41×10^5 counts/min in rat urine.

† 5.72×10^5 counts/min in guinea pig urine.

were prepared as described previously(6) from female litter mates of the animals used for the *in vivo* experiments. The microsomes (100,000 × *g* pellet) or the microsomes + soluble fraction (8000 × *g* supernatant) were incubated in an atmosphere of O_2 at 37°C for 1 hour with 10 μg (0.39 μc) of estrone-16-^{14}C and NAD and NADPH (0.5 mM) in 0.1 M potassium phosphate buffer, pH 7.4; total volume: 3 ml. In some cases (Table II) UDPGA (0.1 μmole) was also added. After extraction with ether, each volume was made up to 20 ml with 0.1 M phosphate buffer, pH 6.8, and duplicate 3 ml portions hydrolyzed with acid or β-glucuronidase (75 units in 1 mg) as described above. The amount of ^{14}C remaining in the aqueous fraction after extraction with ether was then determined. Control tubes with β-glucuronidase omitted were also incubated and the activity of the enzyme was checked by adding estradiol-17β-glucosiduronate (50 μg) to the ether-extracted rat and guinea pig incubation media and carrying out an enzymatic hydrolysis. The liberated estrogen was identified by paper chromatography(4). A system consisting of n-butanol, acetic acid, water (13:3:5) was used for the chromatographic analysis of estradiol glucosiduronate and both free and conjugated estrogens were detected with Folin-Ciocalteu phenol reagent.

Results and discussion. The *in vivo* results (Table I) show that a larger proportion of the injected ^{14}C was excreted in the urine of the guinea pig than of the rat and that in the latter case virtually none was conjugated with glucuronic or sulfuric acid. Thus, incubation of rat urine with β-glucuronidase failed to release any radioactivity into ether and even after refluxing with strong mineral acid which would certainly cleave sulfates or glucosiduronates, over 50% of the ^{14}C still remained in the aqueous fraction. In contrast, in the guinea pig, both procedures released most of the urinary activity into the organic phase.

These findings were supported by the *in vitro* incubation results (Table II) which showed that although rat liver microsomes are able to conjugate estrone in the presence

TABLE II. *In vitro* Metabolism of ^{14}C-Estrone in Rat and Guinea Pig. Conjugate formation by rat and guinea pig liver preparations in presence and absence of UDPGA.

Liver preparation	UDPGA (0.1 μmole)	% of total radioactivity in aqueous fraction	% of radioactivity in aqueous fraction remaining water-soluble after treatment	
			Incubated with β-glucuronidase	Refluxed with 2N HCl
Rat				
Microsomes	—	12.3	91.5	92.3
"	+	23.0	48.7	48.9
Microsomes + soluble fraction	—	25.2	94.8	83.3
" " "	+	32.2	92.9	62.7
Guinea pig				
Microsomes	—	46.0	94.1	77.3
"	+	71.3	26.9	31.8
Microsomes + soluble fraction	—	68.5	37.1	38.8
" " "	+	71.3	21.4	22.1

Tissue preparation derived from 100 mg liver incubated with estrone-16-^{14}C (4×10^5 counts/min in 10 μg), NADPH (0.5 mM) and NAD (0.5 mM).

of UDPGA, this reaction did not occur if the soluble fraction of the cell was also present. This confirms the results of earlier experiments(4,5) with rat liver slices. Yet, under identical conditions, marked glucosiduronate formation took place with the 8000 $\times$ *g* supernatant (microsomes + soluble fraction) of guinea pig liver, even without addition of UDPGA. Furthermore, it was shown by chromatography and autoradiography that a compound with the R_f of estradiol-17β-glucosiduronate was present in those guinea pig and rat liver fractions where conjugation was indicated (Table II) and that this radioactive product disappeared after hydrolysis with β-glucuronidase.

Thus, although rat liver microsomes are able to conjugate estrogens with glucuronic acid, no such reaction occurs when the soluble fraction of the cell is also present and a different type of compound must be postulated to account for the water-soluble ^{14}C-metabolites that appear under these conditions. They could be very polar polyhydroxylated estrogen derivatives, degradation products in which ring opening has occurred or else unhydrolyzed conjugates of unknown nature. This species difference must be exerted at the microsomal level because the same products were formed by rat liver microsomes irrespective of whether the soluble fraction was derived from rats or guinea pigs, and there is some evidence(7) that glutathione may be involved.

Work is in progress to identify these unknown metabolites and to determine whether any other species metabolize estrogens in this atypical manner since it may have some bearing on the ease with which mammary tumors can be induced in the rat(8) by estrone pellets.

Summary. A marked species difference in estrogen metabolism has been observed between the rat and the guinea pig, both *in vivo* and in incubations with subcellular fractions of liver. Very little, if any, estrone-16-^{14}C administered to rats appeared in the urine as the sulfate or glucosiduronate and although the liver microsomes from this species were shown to be able to conjugate estrogens with glucuronic acid, no such reaction occurred if the soluble fraction of the cell was also present. In contrast, under identical conditions, large amounts of the normal estrogen conjugates were formed by the guinea pig.

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Hadacidin Potentiation* of Lethal Action of Ionizing Irradiation. I. Effect of Mammalian Tumor Cells in Culture.† (29895)

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The antibiotic hadacidin (N-formylhydroxyamino acetic acid), isolated from culture filtrates of *Penicillium frequentans* Westling(1) has been reported to be an inhibitor of tumor growth(2,3) and to potentiate the cytotoxicity of phenethylbiguanide for KB cells in culture(4). Since phenethylbiguanide has been reported to stimulate glycolysis in mammalian cells(5) and the radiation response of mammalian and other cells varies with the oxidation state(6) it was of interest to ex-

* Potentiation — a degree of synergism that is greater than additive.

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