counting techniques but uses sample sizes that are smaller by two orders of magnitude. The sample sizes used in the present work (1 g or less) can almost certainly be further reduced by a few orders of magnitude. The method will shortly be applied to the dating of carbon samples whose dates are well established by conventional methods.

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References and Notes

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10. We thank R. M. DeVries, R. Middleton, A. B. McDonald, and M. Rubin for their advice and for valuable discussions, and T. S. Lund and the tandem operations group at the University of Rochester Nuclear Structure Research Laboratory for their assistance in carrying out these experiments. The laboratory is supported by a grant from the National Science Foundation. The participants from the University of Toronto are supported in part by a grant from the National Research Council of Canada.

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Carbon-14–Labeled Diethylstilbestrol Synthesis by the McMurry Method: Concurrent Formation of Hexestrol

Abstract. *Isotopically labeled diethylstilbestrol prepared from p-methoxypropiophenone by coupling with $TiCl_3$-$LiAlH_4$ contained approximately 50 percent hexestrol. Nonlabeled preparations and a subsequent labeled preparation had less than 2 percent hexestrol in them. Initial examinations for purity by gas-liquid chromatography, thin-layer chromatography, and isotope dilution did not show the presence of hexestrol. Specific ion monitoring of a gas chromatography–mass spectrometry determination revealed the presence of the diethylstilbestrol-hexestrol mixture.*

Uniformly labeled [^{14}C-diphenyl]diethylstilbestrol ([^{14}C]DES) was prepared by the method of McMurry (*1*). [^{14}C-phenyl]*p*-Methoxypropiophenone was reacted with a reagent prepared from one equivalent of $LiAlH_4$ and two equivalents of $TiCl_3$. The resulting methoxy derivative was demethylated with sodium hydroxide in ethylene glycol, and the product was purified by preparative thin-layer chromatography. Thin-layer chromatography on silica gel with a petroleum ether–ether system (4 : 6 by volume) indicated only *cis*-and *trans*-DES. The ratio of the *cis* to *trans* form was lower than expected for an equilibrium mixture; however, the rate of equilibration has been shown to vary with type of solvent, temperature, and the presence of other chemicals (*2*). Gas-liquid chromatography (GLC) of the trimethylsilyl derivative (4 percent OV-101; 80 to 100 mesh Gas-Chrom Q; column, 1.8 m by 4 mm; 150° to 250°C at 5° per minute) showed only *cis*- and *trans*-DES.

Our first hint of a purity problem came during isolation of the products after a degradation experiment in which DES (one part of the ^{14}C-labeled product under study with 173 parts of unlabeled DES) was allowed to stand for 3 weeks in 0.01*N* NaOH. A radioactive peak with relatively little mass was observed with a gas chromatrographic system that simultaneously monitored mass (by flame ionization) and radioactivity. Because of this observation, we analyzed trimethylsilyl derivatives of the undiluted ^{14}C-labeled material by gas chromatography–mass spectrometry (GC-MS). As in earlier GLC analyses, two GLC peaks were obtained. The first (small) peak had the retention time of *cis*-DES and the second peak had the retention time of *trans*-DES. A plot of ion abundances for *m/e* (ratio of mass to charge) 412 (M^+ for DES) and *m/e* 207—one of the abundant fragment ions for pseudo DES (*3*)—showed that the first part of the second GLC peak resulted from a compound that yielded an ion of high abundance at *m/e* 207 and an ion of low abundance at *m/e* 412, whereas the last part of GLC peak resulted from a compound that yielded an ion of low abundance at *m/e* 207 and an ion of high abundance at *m/e* 412. A mass spectrum taken during the first part of the second GLC peak was nearly identical to that of the trimethylsilyl derivative of hexestrol. Because of the uniform labeling of benzene rings, the increase in mass due to ^{14}C is distributed over a wide range of masses; therefore the (M + 2) peaks (414 for DES and 416 for hexestrol) and the (P + 2) peak for the 207 cleavage peak of hexestrol were not greatly increased.

With the evidence that the [^{14}C]DES preparation contained ^{14}C-labeled hexestrol and that a partial separation could be effected by GLC, we searched for better systems. The best GLC system found (3.5 percent OV-101; 100 to 120 mesh Gas-Chrom Q; column, 1.8 m by 2 mm; 180° to 230°C at 1° per minute) gave two partially resolved peaks with retention times of 15.7 and 16.2 minutes for hexestrol and DES, respectively. Areas under the curves suggested that approximately 60 percent of the material was hexestrol. Liquid chromatography [4.5 mm by 30 cm μPorasil column, 5 to 10 μm (Waters Associates, Milford, Mass.), 5.0 percent tetrahydrofuran (THF) linearly programmed to 12.5 percent THF in hexane at a flow rate of 1.5 ml per minute over 15 minutes] gave complete separation of hexestrol from *trans*-DES (liquid scintillation counting of trapped peaks showed that 40 percent of the material was hexestrol).

An isotopic dilution determination made by recrystallization from benzene (10 μg of ^{14}C-labeled material and 400 mg of ^{3}H-labeled DES) gave a ratio of ^{14}C to ^{3}H that increased slightly with recrystallization, suggesting that the [^{14}C]DES was of higher purity than the ^{3}H-labeled DES. Recrystallization of 400 mg of hexestrol from benzene to which 5 μg of pure DES labeled with ^{14}C in the side chain had been added gave a nearly constant specific activity. Thus, during recrystallization from benzene, not only does DES serve as a carrier for hexestrol, but hexestrol also serves as a carrier

for DES. With methanol-water as the solvent system, isotopic dilution (10 μg of ^{14}C-labeled material and 400 mg of ^{3}H-labeled DES) showed after two recrystallizations that nearly 60 percent of the labeled material was not [^{14}C]DES.

GC-MS analysis of material obtained from an unlabeled reaction conducted on a larger scale, showed the formation of approximately 2 percent hexestrol, suggesting that a stoichiometry problem, caused by the highly reactive nature of $TiCl_3$, may have caused the concurrent formation of hexestrol. A repetition of the labeled reactions yielded DES with little hexestrol formation. This problem emphasizes the need for caution in the use of isotope dilution as a means of determining purity and emphasizes the need for intensive and varied purity determinations on labeled chemicals prior to their use in studies involving residue determinations. Attention has been drawn to other purity problems involving labeled DES (*3*); however, hexestrol was probably not a problem in previous studies in that we analyzed by GC-MS the ethyl-labeled DES used in a study by Aschbacher *et al.* (*4*) and found no hexestrol.

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Unilateral Nephrectomy: Effect on Survival in NZB/NZW Mice

Abstract. *Male F_1 New Zealand Black × New Zealand White mice, which spontaneously develop immune complex renal disease, underwent unilateral nephrectomy at 3 months of age and were compared with sham-operated controls. At 12 months of age only 24 percent of mice with a single kidney were alive, while 85 percent of sham-operated controls survived to the same age. Unilaterally nephrectomized mice had more severe renal histologic changes, as shown by light and immunofluorescence microscopy.*

Helyer and Howie (*1*) reported a spontaneously occurring glomerulonephritis which progressed to renal failure in the F_1 generation of New Zealand Black and New Zealand White (NZB/NZW) mice. Burnet and Holmes (*2*), Hicks and Burnet (*3*), and McGiven and Lynraven (*4*) subsequently described the anticipated life expectancy and the gross and microscopic changes in NZB/NZW kidneys, which are now accepted as an animal analog of systemic lupus erythematosus. There is a marked sex-related difference in survival of NZB/NZW mice. Although close to 100 percent of these mice die in uremia, females develop renal failure sooner and die months earlier than males. All female animals are dead by about 1 year of age, whereas 60 to 70 percent of males live longer than 1 year.

The pathologic picture in the kidney is one of severe glomerulonephritis, thought to be secondary to immune complex deposition. Renal function begins to deteriorate at approximately 5 months of age in female and 7 months in male NZB/NZW mice. This is reflected in elevations of blood urea nitrogen (BUN) and creatinine concentration. Proteinuria may or may not be present early in the disease, but is usually present in the two months preceding death (*5*).

Fifty male NZB/NZW mice from colonies maintained at the National Institutes of Health (produced by NZB female × NZW male matings) were randomized at 10 weeks of age. Mice were housed five to a cage and were given standard mouse chow (Ralston Purina Co.) and water without restriction. After allowing 2 weeks for adjustment to their new environment, all mice were weighed and bled for baseline renal function values. Weights (mean, 41 ± 2 g) and BUN (27 ± 2.3 mg/100 ml) were comparable in all animals and well within normal limits for that age.

Table 1. Antibodies to DNA and mean BUN in unilaterally nephrectomized and sham-operated male NZB/NZW mice, at 8 and 10 months of age. Abbreviations S.E.M., standard error of the mean: *P*, probability.

Age of mice (mo.)	Mice with unilateral nephrectomy	Sham-operated controls	*P*
Mean percent of DNA bound (± S.E.M.)			
8	28.8 ± 1.2	36.7 ± 2.0	< .005
10	32.5 ± 2.0	43.8 ± 2.4	< .005
Mean BUN (± S.E.M.)			
8	26.3 ± 1.9	19.9 ± 0.8	< .05
10	47.3 ± 11.4	22.6 ± 1.1	< .05

All BUN determinations were done with a Beckman BUN analyzer in duplicate on blood from the tail vein.

Mice were randomly assigned to one of two groups: animals in group 1 underwent unilateral nephrectomy and those in group 2 were used as sham controls. The operative procedure was similar in each group. Animals were anesthetized with ether and their ventral surfaces were shaved. After the abdominal skin was cleaned with Betadine, a midline incision was made and the left kidney freed from its perinephric fat. All unilaterally nephrectomized animals then had the renal artery, renal vein, and ureter ligated with 3-0 silk suture and, when hemostasis was complete, the abdomen was closed with interrupted 4-0 silk sutures. Those animals serving as sham controls underwent the same operative procedure, but after mobilization of the renal pedicle, the incision was closed as above. After intraoperative mortality, in each case secondary to anesthesia, there were 21 mice in the experimental and 19 mice in the sham control groups.

Animals were bled every other week from the retro-orbital fossa. Blood was allowed to clot at 0° for 1 hour, and serum was obtained by centrifugation to remove cellular debris. The BUN determinations were made immediately; the remaining serums were frozen for future serological study. Antibodies to native DNA were measured by an ammonium sulfate precipitation assay as previously described (*6*), in which 10 μl of serum and 50 ng of ^{14}C-labeled native human KB DNA were used.

Mice in either group were counted as renal deaths if they had concentrations of BUN exceeding 100 mg/100 ml when they were killed or if found dead with a previous BUN concentration of more than 80 mg/100 ml. Renal tissue was removed when the animals were killed or at the time of spontaneous death and was fixed in 10 percent formalin for histologic sectioning. Additional kidney tissue was frozen for study by immunofluorescence microscopy.

Sections for light microscopy were cut and stained according to standard tech-