ous solutions tend to spread excessively on the paper. Solutions containing even weak hydrochloric or sulfuric acid char a variety of the papers tried, even at moderately elevated temperatures. The method may prove useful in other applications of partition chromatography where the concentration of the substance to be identified is low in relation to the sensitivity of the color or other indicator reactions.

A Simple Device For Exposure of Groups of Mice to Uniform X-Ray Doses[1]

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In the course of experiments involving exposure of groups of mice to doses of X-rays[2] it was necessary to utilize a holder which assured uniformity of exposure. Based on a metal "pie plate" device of Snyder (*1, 2*), this device was constructed to minimize the possible errors in dosage due to reflection of X-rays and interference in passage through the metal screen cover.

Circles of ¼″-thick plywood, 5½″ and 7½″ in diameter, were cut and enclosed in a 2″-wide strip of ¼″-mesh wire hardware cloth, so that the wooden plaque was mounted in the middle of the wire cloth band. A cover of plastic screen mesh, ordinarily used in window screening, was made by stretching a piece of the plastic mesh across an embroidery hoop of the proper diameter. (See Fig. 1.)

FIG. 1.

In such a device the X-rays do not pass through any metal before reaching the animals, thereby avoiding adulteration of the beam with secondary radiations. The plywood "floor" of the device is elevated 1″ above the table; thus there is a minimum of back scatter. Twenty-four mice can be simultaneously irradiated in the 7½″ tray and ten to fourteen mice in the 5½″ tray.

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[1] Designed in connection with radiation study under Atomic Energy Commission and Office of Naval Research contract.

[2] By Milton Friedman, radiologist, New York University.

Infrared Spectrometry in Metabolic Studies With Deuterium-labeled Steroids[1]

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Infrared spectrometry, which has been of the greatest utility for the detection, identification, and isolation of steroid metabolities excreted in urine (*2, 3, 6*), has been extended to the identification of steroids with deuterium in the molecule. Changes in the infrared absorption spectra as a result of the replacement of hydrogen by deuterium were noted first by Hardy, Barker, and Dennison (*4*) in 1932. The analysis of deuterium-containing organic compounds of biological interest by means of infrared spectrometry has since been described by Herget and Hardy (*5*). Our investigations have been concerned with the detection of a deuterium steroid in an extract containing a number of closely related compounds. One marked advantage of the spectroscopic technique, especially in physiological studies, is that the material to be examined can be recovered without loss or alteration. It is thus possible to employ deuterium as a tracer where a pure compound can be obtained only in limited amount.

In Fig. 1 the spectrum of pregnanol-3(α)-one-20 is compared with the spectrum of the same compound where a hydrogen atom at C-11 and at C-12 has been replaced by deuterium. Two absorption bands attributed to the C-D stretching vibrations appear at 2,165 and 2,145 cm^{-1} in the deuterium-containing compound. In the neighborhood of 1,200 cm^{-1} pronounced differences are apparent in the two spectra. These latter differences can be ascribed to the perturbing effect of the increased mass of the deuterium atoms on the molecular vibrations. In general, with an increase of the number of deuterium atoms in a steroid there is a progressively greater change in the spectrum between 700 and 1,300 cm^{-1}, as well as an increase in intensity of the bands at 2,165 and 2,145 cm^{-1}. These observations are expected since it has been shown that the spectrum in the region 700–1,300 cm^{-1} is extremely sensitive to any change in structure (*3*).

The C-D absorption bands in the neighborhood of 2,150 cm^{-1} are particularly useful for the identification of a deuterium-containing molecule because, in the concentrations used, this region is transparent in the absence of

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deuterium. For this reason increased amounts of the nonisotopic steroid in the sample do not interfere with the detection of deuterated molecules. Absorption in this region not only affords a positive indication of the presence of deuterium in the molecule but forms the basis for quantitative determination of the percentage excess of isotope in the sample. The only effect of dilution with a nonisotopic compound is to increase the total weight of the steroid which must be examined. As an illustration of the sensitivity of infrared analysis, the presence of deuterium could be established in as little as 25 μg of pregnan-11,12-d_2-ol-3(α)-one-20 containing five atoms percent excess of the istotope. This was accomplished with a microcell (*1*) of thickness of 3 mm and a volume of 0.04 ml of carbon tetrachloride as solvent. Changes in the spectrum were measured in both the 2,150 cm^{-1} and 700–1,300 cm^{-1} regions. The presence of a C-D bond in a series of different steroids has been established and using thicker microcells, even smaller quantities of substance suffice for estimation.

The usefulness of this analytical method has been demonstrated in the following metabolic experiment. Allopregnan-5,6-d_2-ol-3(β)-one-20-acetate was injected into a normal woman. After collecting the urine for 20 days, fractionation was effected by the procedures which have been described (*2, 3, 6*). Feces collected during the experimental period were studied in analogous fashion. An examination of the region of the spectrum in which the C-D stretching bands occur demonstrated that the isotope was present in both the crude α- and β-hydroxy ketonic fractions as well as in the α- and β-hydroxy non-ketonic fractions from both urine and feces. No such bands were detected in the neutral nonketonic-nonalcoholic fraction. Chromatographic separation of these crude fractions resulted in the separation of several crystalline compounds together with several amorphous fractions or mixtures. These are being investigated further, and the results will be reported in detail at another time. Nevertheless, since certain fractions exhibited strong C-D bands near 2,150 cm^{-1} while others either lacked these bands or had bands of a very low order of intensity, it was possible to locate rapidly the metabolites containing deuterium among the many substances excreted by a normal individual. It was further possible to establish the approximate deuterium content of these metabolites.

It is anticipated that metabolites of the steroid hormones will frequently be obtained in amounts so small that, without some prior knowledge of the approximate isotopic content, analysis by means of the mass spectrometer would be either difficult or impossible. It appears from these results that infrared spectrometry especially in physiological investigations will be a valuable supplement in the detection, analysis, and identification of deuterium compounds.

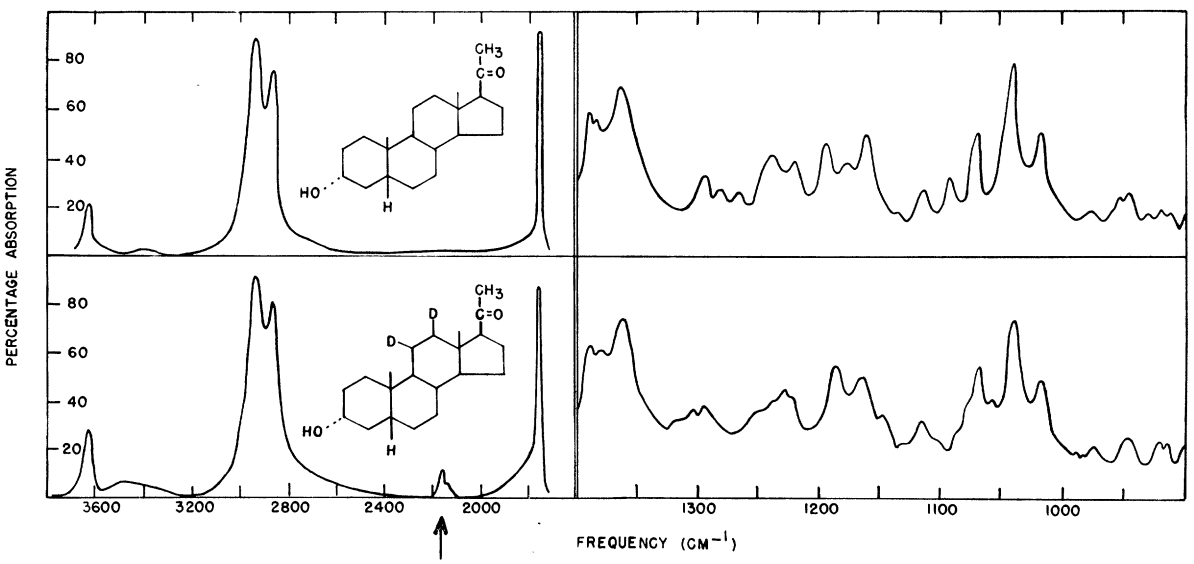

FIG. 1. Infrared absorption spectra of normal and deuterium steroids in CS_2 solution; upper curve—pregnanolone, m.p. 150–151°/C; $[\alpha]_D^{25} = +109.0°$ in $CHCl_3$; lower curve—pregnan-11,12-d_2-ol-3(α)-one-20, m.p. 150–151°/C; $[\alpha]_D^{25} = +108.6°$ in $CHCl_3$. (1 mm cell: concentration 10 mg/ml.) Arrow indicates C–D stretching band.

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