graphs of specimens with greater "thickness" will be of special interest.

Work is in progress to accomplish electron radiography by transmission on a quantitative basis. The uniform intensity of the sources was measured by the methods of absolute β-counting. These values can be compared with the electron intensities in the betagraph, determined with a microdensitometer. The results represent a measure for the absorption of electrons of a given mean velocity in small areas (limit, 30 μ diameter) which is related to the "thickness" of the structure of the specimen.

The application of α-emitting monolayers (radium, polonium, plutonium) opens up new possibilities for studies of specimens in α light. Pictures of butterfly wings depicted in the radiation from a radium stearate source show about the same shades as the betagraph in Fig. 2.

Descriptions of other applications of radioactive monomolecular films will appear elsewhere.

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Dicarboxylic Acid Bis-(β-Tertiaryaminoalkyl) Amides as Curare Substitutes

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A series of bis-(β-dimethylaminoethyl) esters of dicarboxylic acids was recently reported (*1*) to possess powerful curarelike activity. Concurrently with the esters, a group of bis-(β-tertiaryaminoalkyl) amides of the dicarboxylic acids was prepared and examined for curariform action.

The bis-amides were obtained in excellent yields by brief refluxing of a slight excess of the unsymmetrical disubstituted ethylene diamines with the dimethyl or diethyl esters of the dicarboxylic acids. In most cases the bis-amides were crystalline solids readily recrystallized from organic solvents. The bis-tertiary amino amides were transformed into bis-quaternary ammonium salts by refluxing in methanol solution with an excess of the appropriate alkyl halide, most commonly methyl or ethyl iodide.

Examples of the types of products made are: bis-dimethylaminoethyl oxamide (mp, 123°–124°; calcd.: C, 52.1; H, 9.6. Found: C, 52.1; H, 9.6); its bis-methiodide (mp, 288°–290°; calcd.: C, 28.0; H, 5.5. Found: C, 28.2; H, 5.5); bis-dimethylaminoethyl succinamide (mp, 134°–135°; calcd.: C, 55.8; H, 10.1. Found: C, 55.6; H, 9.9); its bis-methiodide (mp, 251°–252°; calcd.: C, 31.0; H, 5.9. Found: C, 31.1; H, 5.9); its bis-ethiodide (mp, 189°–190°; calcd.: C, 33.7; H, 6.4. Found: C, 33.7; H, 6.1); bis-diethylaminoethyl adipamide bis-methiodide (mp, 134°–135°; calcd.: C, 38.4; H, 7.1. Found: C, 38.9; H, 6.8); bis-morpholinoethyl malonamide (mp, 127°–128°; calcd.: C, 54.8; H, 8.6. Found: C, 54.8; H, 8.4); and its bis-methiodide (mp, 153°–154°; calcd.: C, 33.3; H, 5.6. Found: C, 33.7; H, 5.4).

Interestingly, both the bis-tertiary amino amides and the derived quaternary salts were relatively devoid of curarelike activity, requiring huge doses to produce any perceptible block of neuromuscular transmission in the cat. However, both in the tertiary and quaternary form these compounds have been found to possess the remarkable ability, anticholinesteraselike, to prolong the duration of the block produced in the cat by the bis-esters (*1*) as much as four or five times. Details of the pharmacological results will be published elsewhere by E. J. de Beer and J. C. Castillo of these laboratories. Further work along these lines will be reported later.

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The Enzymatic Dehydrogenation of Estradiol to Estrone[1]

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An enzyme system that catalyzes the conversion of α-estradiol to estrone, *in vitro*, was demonstrated by using a purified protein fraction of beef liver and rat tissue homogenates as enzyme sources.

A 2-mg sample of a crudely purified preparation from the acetone-insoluble, water-soluble fraction of beef liver was tested for ketosteroid and found to be negative (*1*). A 5-mg sample of crystalline α-estradiol[3] was likewise negative.

Five mg of the estradiol was added to 0.2 mg of the purified beef liver preparation in 20 ml of water and incubated for 12 hr under conditions previously found to be within the range of maximal enzymatic activity. Twenty ml of a 0.1% solution of 2-hyrdoxy-3-naphthoic acid hydrazide, containing 50% ethyl alcohol and 5% acetic acid, was stirred into the mixture, brought to boiling, and cooled to room temperature. The yellow precipitate formed was separated from both the excess hydrazide and any estradiol present by centrifugation, repeatedly washed with 50% ethyl alcohol, and refluxed with pyruvic acid for 30 min. Distilled water was added to saturation, and a white crystalline precipitate was formed on cooling (*3*). The crystals were purified by repeated washings with warm 5% sodium bicarbonate solution and warm distilled water. After drying in air, the crystals were examined microscopically and found to be colorless plates similar in form to estrone. The mp was 252°–254° C. When some of the crystals were mixed with a known sample of crystalline estrone[3] (mp 253°–

[1] Aided in part by a grant from the Maryland Division, American Cancer Society, to R. W. TeLinde and Howard W. Jones, Jr.

[2] Leslie Hellerman gave valued assistance in this work.

[3] Supplied by courtesy of Schering Corporation.

255° C), the mp rose to 253°–254° C. The ketosteroid test was positive. Positive rat tests for estrogenic activity, as estimated by increases in uterine weight (*2*), were obtained by as little as 32 μg, which resulted in uterine weight increases from an average of 25 mg to 82.5 mg for rats of 36.3 gm average weight. The yield estimated on the basis of solubility in ethyl alcohol was 2.2 mg.

To establish the activity of the liver preparation as enzymatic the following tests were performed:

1. Inactivation by boiling: Fifteen test tubes, each containing 0.1 mg of the liver preparation in 20 ml of distilled water, were used. Tubes 1–5 were boiled 15 min and cooled; ketosteroid tests were negative. Two mg of crystalline α-estradiol was added to tube 6–10 and incubated 6 hr at 37° C; all contents were strongly positive for ketosteroid. Tubes 11–15 were boiled 15 min, 2 mg of crystalline α-estradiol was added, and the tubes were incubated 6 hr at 37° C; all ketosteroid tests were negative. Five tubes containing 2 mg crystalline α-estradiol only in 20 ml of distilled water were incubated 6 hr at 37° C; all ketosteroid tests were negative.

2. Inactivation by silver nitrate or bichloride of mercury: Nine tubes, each containing 0.1 mg of the liver preparation in 20 ml of distilled water, were used. To tubes 1–3, 0.034 g $AgNO_3$ was added. To tubes 4–6, 0.0544 g $HgCl_2$ was added. Tubes 7–9 were used as controls. To all tubes 2 mg of crystalline α-estradiol was added, and, after 6 hr incubation at 37° C, each was tested for ketosteroid; tubes 1–3 and 4–6 were negative, and 7–9 were strongly positive.

3. Rate of production of ketosteroid, when concentration of enzyme was small as contrasted to that of the substrate, was shown to be constant (Fig. 1).

4. Variation of activity with temperature: Two mg α-estradiol and 0.1 mg liver preparation in 20 ml distilled water were incubated 6 hr at 2-degree intervals from 29°–45° C. Maximal ketosteroid production, estimated colorimetrically, occurred between 33°–35° C, approximately 50% at 31° and 37°, 25% at 29° and 39°, and 10% at 45° C.

5. Influence of pH on activity: Two mg α-estradiol and 0.1 mg liver preparation were incubated at 35° C with 20 ml of 0.1 *N* isoelectric buffer solutions from pH 4.8 to 8.8. Maximal ketosteroid production occurred between pH 6.8 and 8.0, approximately 50% at 6.2 and 8.8, 15% at 4.8.

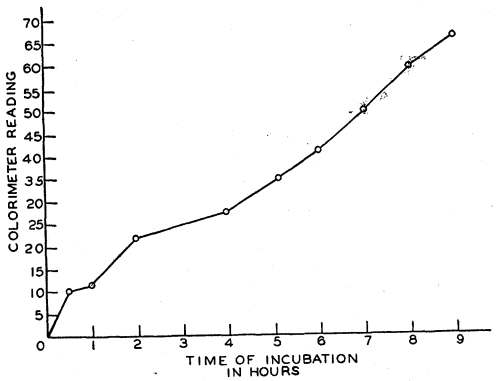

FIG. 1. Two mg crystalline α-estradiol, 0.02 mg liver preparation, 20 ml distilled water/tube were incubated at 37° C; 1 tube/hr was quantitatively tested for ketosteroid, using a Klett photoelectric colorimeter and #54 filter. Time of incubation in hr is plotted against colorimeter readings.

The enzyme was shown to be a dehydrogenase by the following test: Six small flasks, each containing 2 mg crystalline α-estradiol and 0.2 mg liver preparation in 10 ml 0.1 *N* citrate-phosphate buffer pH 7, were used. To flasks 1–3 was added 0.5 ml 1% methylene blue. Flasks 4–6 were controls. Anaerobic incubation under nitrogen was performed for 3 days at 37° C, then each was tested for ketosteroid; 1–3 were positive and 4–6 negative. Methylene blue was completely decolorized in flasks 1–3.

Inhibition experiments showed the enzyme system was not inhibited by cyanide *M*/500, or epinephrine 1: 1000, and only partially (approximately 2/3) inhibited by ethyl alcohol 70%–80%.

Crystalline α-estradiol was incubated with rat tissue homogenates, and tests for ketosteroid were performed. Positive results were obtained with liver, kidney, breasts, and testis, but those with uterus, ovary, and adrenal were negative, as were the controls.

Attempts at further purification will be made to demonstrate possible substrate specificity of the enzyme.

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