In 1944, a correct account of the reactions of diazonium salts with alcohols was published in *Organic Reactions* (*7*), but it has apparently been overlooked by the majority of those who have since discussed this reaction.

In conclusion, two points should be emphasized: (1) ethyl alcohol is not, in general, dependable for replacing diazonium groups by hydrogen (*7*); (2) reduction with hypophosphorous acid is a reliable way to replace diazonium groups by hydrogen (*7, 8*). A second general method for replacing diazonium groups by hydrogen has just been described by Roe and Graham (*9*).

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Biosynthesis of Estrone and β-Estradiol in the Perfused Ovary[1]

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Zaffaroni, Hechter, and Pincus (*1*) found that the adrenal gland converts C^{14}-labeled acetate into adrenal hormones when it is perfused with a solution of this salt. Brady (*2*) showed that testis slices are capable of synthesizing testosterone from C^{14}-acetate, and Savard, Dorfman, and Poutasse (*3*) demonstrated conversion of labeled acetate to testosterone and Δ^4-androstene-3,17-dione in the perfusion of human testis. It therefore seemed interesting to investigate whether the ovary may synthesize estrone or β-estradiol from the same starting material.

The experiment was carried out by perfusing sow ovaries with sodium acetate labeled with C^{14} in the carboxyl group. The technique, including radioactivity counts, and the apparatus were the same as described in an earlier investigation (*4*).

Two perfusions of long duration were carried out with ovaries obtained from one pregnant and one nonpregnant sow. The animals each weighed about 200 lb. Two thousand units of heparin were injected intravenously before beginning the operation to obtain the ovaries. The large and small intestines were removed, and the aorta was litigated just below the origin of the ovarian arteries. The ovaries were freed of all adnexa. The aorta was then cut just above the origin of the ovarian arteries, and the entire specimen mounted in the perfusion apparatus. (As to the apparatus and procedure, *cf.* [*4*].)

The first experiment was carried on for 44 hr. During this time 1000 RU of gonadotrophin and 0.1125 mc of acetate labeled in the carboxyl group were added at 20 min, 9 hr, and 25 hr after circulation in the perfusion apparatus started. At the 9- and 25-hr intervals the perfusing medium was changed. The organ was without circulation for 30 min prior to beginning the perfusion.

In the second experiment, which lasted 13 hr, one injection of 1200 RU of gonadotrophin and 0.225 mc of labeled acetate was made 7 min after circulation was reinstated; the interruption of the circulation lasted 21 min.

The perfusion liquid in both experiments was a mixture of pig's blood and White's solution as described before (*4*). In each experiment the approximate volume of perfusate charging the system was 500 ml.

The weight of the ovaries in the first experiment was 6.1 g, in the second, 3 g. In both instances the organ appeared to have been well circulated by the perfusion liquid. No gross response to the gonadotrophin was evident, but there was also no significant degeneration discernible.

Extraction of the perfused ovaries. Immediately after the perfusion was terminated the mixture of ovaries and perfusion liquid was converted into a mash in a Waring Blendor. One third of the total volume of chloroform was added and again blended for about 10 min. The homogenous mixture was centrifuged at about 2000 rpm in the cold room. Three easily separable layers were formed; the lowest was a clear chloroform solution. The middle layer could be easily separated and consisted mostly of protein. This was stirred with acetone, and the acetone solution was filtered from the insoluble residue, which was again treated with acetone. The combined acetone extracts were evaporated to dryness and extracted with pentane. This solution was added to the chloroform layer. The uppermost layer from the centrifugation was a water solution which was extracted with chloroform. This extract was also added to the main choloroform portion. The combined extracts were then taken to dryness. The fatlike residue was boiled with methanol, and after cooling the clear methanol solution was decanted from the solid residue. This operation was repeated three times. The methanol extracts were evaporated to dryness and divided into two equal portions.

a) Isolation of estrone. The first part of the methanol extract was dissolved in about 10 ml methanol, and 200 mg of pure estrone was added and dissolved by warming. In the cold the estrone crystallized out

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and was filtered. It was recrystallized from methanol, and then showed a mp of 264°–266° C (Stage) which was not changed by another crystallization from methanol. This fraction had a specific count of 5 cpm/mg.[3] All mother liquor material was dissolved in ether, and this solution was extracted with 5% alkali. The alkali extract was acidified and extracted with ether. The residue from the ether solution was distilled in high vacuum at a bath temperature of 120°–160° C, and the substance thus obtained was recrystallized twice from acetone. The melting points of the crystals were the same as above. The specific count for this fraction was 3 cpm/mg.

b) Isolation of β-estradiol. The second portion of the methanol extract was dissolved in about 10 ml methanol, and 200 mg of β-estradiol (mp 172°–174°) was added. The material which crystallized out on cooling was crystalline but impure and could not be purified by further crystallization. It was recombined with the mother liquor and this mixture dissolved in ether, which was extracted with 5% KOH. The alkaline extract was acidified and extracted with ether. The residue from this ether solution was distilled in high vacuum at 120° bath temperature, and the material obtained was recrystallized from acetone, the crystals washed with cold ether and then recrystallized from acetone-hexane. The mother liquor was decanted from the crystals and these again crystallized from aqueous acetone. The crystals thus obtained sintered at 173° and melted at 177°–181° C (Stage). A further recrystallization from methanol gave a mp of 176°–177° C. This fraction gave a specific count of 3 cpm/mg.

c) Isolation of cholesterol. All mother liquor material in the second experiment was combined and boiled for 5 hr with 15 ml of a 10% solution of KOH in 95% ethanol. This reaction mixture was extracted with pentane, which after evaporation gave a crystalline residue of yellow color. It was treated with digitonin in the usual fashion, and the cholesterol was isolated from the digitonin complex. The specific count was 434 . Dibromination and reconversion gave a pure cholesterol which had a specific count of 75, thus showing the presence of higher counting companions (HCC) in the crude cholesterol (*5*).

The experiments here reported show that isolated surviving sow ovaries when perfused with sodium acetate labeled in the carboxyl group produce labeled estrone, β-estradiol, and cholesterol. Westerfeld *et al.* (*6*) many years ago isolated β-estradiol in substance from ovaries, and MacCorquodale *et al.* have secured the simultaneous presence of estrone by unmistakable reactions (*7*). Beall also observed the presence of both estrogens together in horse testes (*8*).

Like the testis in the earlier investigation, the ovary produces C^{14}-cholesterol simultaneously with the hormones. The experiments as described do not allow any conclusion as to whether the hormones are derived from this cholesterol, or whether cholesterol and the hormones are produced from a common precursor.

[3] All counts in this investigation were made in a gas flow counter. For the preparation of the samples for counting compare (*4*).

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Structural and Energy Relationships in the Formation of Iron and Aluminum Oxides, Hydroxides, and Silicates[1]

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Iron and aluminum oxides, hydroxides, and silicates are of great importance in the mineral content and formation of soils and sediments. Their structural relationships, known from x-ray diffraction methods and bonding considerations, are shown herein to help explain their occurrence. Recently, Ervin (*1*) considered the diaspore-corundum and boehmite-Al_2O_3 systems, and he has shown that they are structurally related. Structural interrelationships with genesis can be shown for iron and aluminum silicates, as well as for their oxides and hydroxides. These considerations, together with energy changes, are summarized in Fig. 1. (Downward slope of arrows is indicative of ease of occurrence.)

In the aluminum system, dehydration of $Al(OH)_3$ (gibbsite) results in the formation of AlOOH (boehmite, reaction *f*, Fig. 1). Dehydration may be accomplished by increase of temperature or decrease of suspension pH. Milligan (*2*) has reported boehmite to

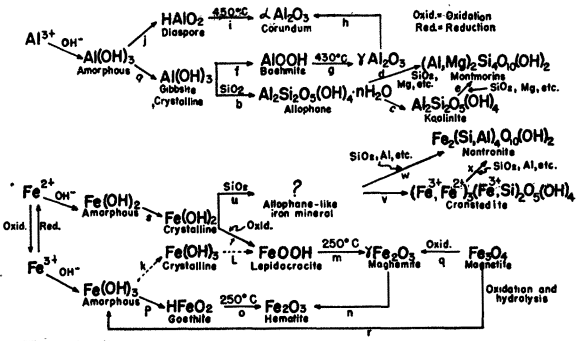

FIG. 1. Structural and energy relationships of iron and aluminum oxides, hydroxides, and silicates. (Downward slope of arrows indicative of ease of reaction occurrence.)

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