than of females, a finding comparable to the sex difference in the rate of phosphopyruvic acid formation(1) in rat tissues. It must be noted, however, that this sex difference in the rate of phosphopyruvic acid formation was not found in mice, indicating the influence of species on the regulation of enzyme reactions. Pertinent to the experiments reported is the effect of diethyl stilbestrol and castration on the metabolism of prostatic tissue, described by Barron and Huggins(5). The regulatory effect of hormones may partly be due to their effect on the synthesis of enzymes and partly to their interaction on the catalytic protein. This question is being further studied by comparison of *in vivo* and *in vitro* effects of pure hormones.

Summary. Castration of male white rats resulted in a decreased rate of phosphopyruvic acid formation from 3-phosphoglyceric acid, by dilute tissue homogenates. Ovariectomy caused an elevation of enzyme activity in the tissues of females. Enzyme activity of hypophysectomized rats decreased only 14 days after the operation. The effect of age on this enzyme reaction was also demonstrated. Tissues of young females had a higher enzyme activity than those of mature females, except the ovaries which were considerably lower than in the mature animals. The effect of age was not noticeable in young males. Injection of diethyl stilbestrol in young males or testosterone in young females caused a decrease in phosphopyruvic acid formation. Cortisone and ACTH decreased the rate of this enzyme reaction in both sexes.

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Identification of Sterols on Filter Paper and Their Separation by Paper Partition Chromatography.* (18347)

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Partition paper chromatography, in which the partition is between the water of the hydrated cellulose of the filter paper and the organic mobile solvent, has been applied to a wide variety of chemical compounds(1,2). However, the separation and identification of steroids by this method is difficult, due to extremely low solubilities in H_2O and to a lack of good color reactions capable of detecting these compounds on filter paper. Recently, paper partition chromatography has been successfully applied to *keto steroids* by the formation of hydrazones with Gerard's Reagent T prior to chromatography(3) and by the use of non-aqueous solvent systems (4). This communication describes methods and conditions for the identification and separation of several non-keto steroids on a micro scale on filter paper.

Identification. Two satisfactory methods were developed for the identification of cholesterol, ergosterol, 7-dehydrocholesterol, vit. D_2 and vit. D_3—using Whatman's No. 1

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filter paper. In the first, the paper containing the compound spots was suspended 5 cm above a bromine solution (0.25% Br_2 in pyridinesulfate dibromide, or 2% Br_2 in $CHCl_3$ or CCl_4, at 40°C) for 30 seconds. The paper was next sprayed with a saturated solution of KI in methyl alcohol and then with a 20% starch solution. The compound spots become dark blue while the surrounding area remains colorless or sometimes becomes light blue. The color fades with time and drying to a reddish brown. Ten gamma cholesterol spots can be detected; the method is even more sensitive for ergosterol. A more sensitive method involves spraying the sterol spots with a 20% solution of antimony pentachloride in chloroform. This color reaction was discovered and utilized by Steinle and Kahlenberg for the colorimetric determination of cholesterol(5). Immediately after spraying with the $SbCl_5$ solution, cholesterol develops a brown color, which darkens somewhat on standing, while ergosterol and 7-dehydrocholesterol give a purplish-red color which changes to a dark blue on standing; vit. D_2 and D_3 develop a pink color which changes to brown with standing. With 5 microliter spots, cholesterol can be detected in 5 γ concentrations, vit. D_3 in 3 γ, vit. D_2 in 2 γ, 7-dehydrocholesterol in 1 γ, and ergosterol in concentrations as low as 0.5 γ.

Separation. In attempting to determine a method for the separation of the above sterols, ascending(6), descending(7), and circular chromatograms(8) were run for any one solvent combination. Over 100 solvent combinations of the following solvents with one another were investigated: phenol, methanol, ethanol, n-propanol, n-butanol, t-amyl alcohol, benzene, xylene, toluene, acetone, carbon tetrachloride, cyclohexanone, chloroform, ether, petroleum ether (Skellysolve B), p-cresol, o-nitrotoluene and pyridine. The chief difficulty encountered was that most of the solvent combinations tried gave either no migration of the sterols or migration to the solvent front with no separation. The difficulty in separating the sterols is, at least in part, due to the great similarity of the 5 compounds studied. The only group here which tends to affect the R_f value is the hydroxyl at carbon atom 3, and this group is common to all the sterols studied. Furthermore, hydroxyl groups have a comparatively small effect in comparison to other substituent groups; alkane groups exert a very small effect in altering the R_f value(3). Water seems to have a striking effect on the migration, in that solvent combinations containing large proportions of water gave no migration, and as the water/organic ratio of the solvent combination grew smaller, migration occurred.

Two solvent combinations tried gave definitive separations; one was: phenol, 13.5 parts by volume; methanol, 30; water, 56.5. It was necessary to measure these components quantitatively, for varying the parts of phenol by one gave markedly different results. Using ascending chromatograms, this combination separated 7-dehydrocholesterol from cholesterol and ergosterol, using 10 μg quantities of each. The 7-dehydrocholesterol advanced slightly behind the solvent front, while the other 2 did not migrate at all. With phenol 14.0, methanol 30, and water 56 parts, only ergosterol remained behind, while 7-dehydrocholesterol and cholesterol were slightly behind the solvent front.

Using a solution of 80 parts methanol, 10 parts benzene and 10 parts petroleum ether (acid washed Skellysolve B, b.p. 60-70), 10 μg each of vit. D_3, cholesterol and 7-dehydrocholesterol were separated and distributed in 3 bands—from the solvent front on backward—on a circular chromatogram. The circular filter paper (12.5 cm) was placed over an evaporating dish (containing the solvent) which in turn was placed in a similar evaporating dish containing ice water. A 5-liter beaker was inverted over the whole set-up (Fig. 1). Antimony pentachloride in $CHCl_3$ was used to identify the various bands. Cholesterol could be separated from ergosterol

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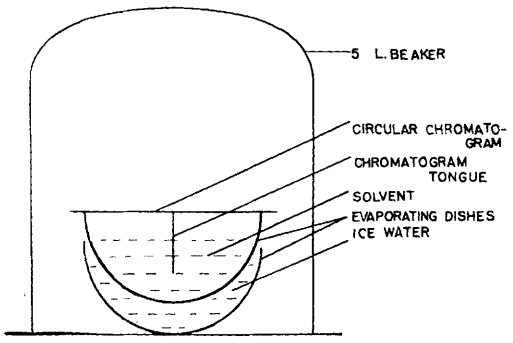

FIG. 1.
Apparatus for chromatographic separation of steroids.

using the same solvent and conditions. The importance of water-vapor and/or temperature was demonstrated, since no separation of cholesterol, ergosterol and vit. D_3 could be obtained on a circular chromatogram at 2°C in a cold room, nor at room temperature (approx. 30°C with the icebath eliminated).

Recently, Kostir and Slavik(9) devised a method for paper chromatography in which the partition is between 2 organic phases—one stable (acetylated filter paper), and the other mobile—in distinction to the ordinary partition between the water of the hydrated cellulose of the filter paper and the organic mobile solvent. They state that this method should enable one to separate compounds having low water solubility such as the fatty acids, steroids, etc., which are not resolved in the water-organic partition. We could not effect separation of sterols using this method. We think it advisable, however, to investigate this aspect of chromatographic separation of sterols further.

Summary. Methods for identification and separation of several non-keto steroids in 10 μg quantities on paper chromatograms are described.

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Effect of Cortisone Acetate upon Plasma Amino Acids in the Eviscerate Rat. (18348)

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The level of blood amino acids in the eviscerate-adrenalectomized rat is lower than that of non-adrenalectomized, eviscerate rats (1,2). The administration of adrenal cortex extract to eviscerate and to adrenalectomized-eviscerate rats causes a rise in plasma amino acids(2). In the present study, it was shown that the administration of cortisone acetate to eviscerate and to adrenalectomized-eviscerate rats caused a rise in plasma amino acids.

Methods. Male rats of the Sprague-Dawley strain were eviscerated at 250 g weight. Adrenalectomy was performed at the same operation. Immediately following operation, all of the animals were given continuous intravenous infusions of 0.9% sodium chloride with glucose and insulin. The volume was 20 cc per 24 hours per rat. The glucose load was 40 mg per 100 g of rat per hour, and the insulin dose (Regular Insulin, Lilly) was 4 units per 24 hours per rat. The details of the methods have been described(3,4). Cortisone acetate (Merck) in saline suspension was administered by subcutaneous injection at the beginning of the experiment and 6 hours later and 24 and 30 hours later in those animals which were studied for 48 hours. Eviscerate rats with and without cortisone acetate were studied simultaneously. Samples of blood

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