assaying spasmolytic and antihistaminic drugs in isolated strips of surviving gut; laxatives in terms of stool softening in rhesus monkeys; marihuana-active substances in terms of ataxia in dogs; and sympathomimetic agents in terms of ability to elevate the cat's blood pressure. The task of assaying biological activity by intraindividual comparison is not limited to drugs, but arises in many cases in which the potency of commensurable stimuli—agents, factors, etc.—has to be correlated numerically on the basis of a common response. It is believed that the principles of this procedure may be serviceable in various disciplines other than pharmacology.

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Identification and Chromatography of Androgens as Their 2,4-Dinitrophenylhydrazones

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Frequent use is made of 2,4-dinitrophenylhydrazine (DNPH) for the characterization of aldehydes and ketones, and the chromatographic adsorption of a number of the hydrazones of DNPH is described by Strain (*2*). However, the reagent does not appear to have been widely used in the study of steroid ketones. Veitch and Milone (*3*) have published a method for the estimation of estrone as the 2,4-dinitrophenylhydrazone, but their procedures seem not to have been applied to androgens. These studies suggested the possibility of using DNPH for the isolation and characterization of the constituents of commercial androgen preparations. In 1941 Coffman (*1*) reported the preparation of several androgen esters of p-phenylazobenzoic acid and was able to resolve mixtures of several of these colored esters chromatographically. In dealing with commercial products, however, this type of derivative could not be expected to distinguish between free and esterified androgenic steroids.

This paper constitutes a preliminary report of the preparation and properties of the DNP-hydrazones of testosterone, testosterone propionate, methyl testosterone, androsterone, and dehydroisoandrosterone. First attempts to prepare the hydrazone of one of these androgens (testosterone propionate) directly in an oil solution indicate that such a procedure is possible and may be developed into a useful analytical method. Over-all yields of about 60 per cent have been obtained to date. Details will be published as soon as the techniques are perfected.

The chromatographic separation of mixtures of some of these hydrazones has been accomplished, which indicates the possible application of the reagent to urinary extracts of ketosteroids for investigations in androgen metabolism.

[1] Thanks are due Mrs. Doris C. Chambers, of the Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, for carrying out the nitrogen analyses reported.

A 10- to 15-mg. sample of each androgen, dissolved in 1–2 cc. of ethanol, was allowed to react with a 10 per cent excess of reagent (prepared by adding 2 or 3 drops of concentrated hydrochloric acid to the calculated amount of the free base dissolved in a minimum of hot alcohol). and the mixture boiled for about 2 minutes. Frequently the hydrazone precipitated immediately from the hot solution, but if it did not, water was added to the point of cloudiness. On cooling, precipitation occurred. The precipitate was filtered off, washed with water to remove hydrochloric acid, and dried *in vacuo* over phosphorus pentoxide. After solution in benzene, it was adsorbed on activated alumina (Aluminum Ore Company, Grade F 20, minus 80 mesh), and the chromatogram developed with 10–15 per cent chloroform in benzene. On this adsorbent the bands formed may be brown, orange, or yellow, depending upon the individual compound and to some extent on the developing mixture. Following development, either the column was extruded and cut or, if extrusion proved difficult, the colored zones were dug out with a spatula. The hydrazones of the androgens prepared to date are all readily soluble in benzene and chloroform, moderately so in hot alcohol, but only slightly soluble in ligroin.

After elution with chloroform and filtration, the solvent was removed by distillation under reduced pressure and the hydrazone recrystallized from hot aqueous ethanol to constant melting point. Crystallization from benzene-ligroin pairs produced no change in melting point, but the color of the crystals was usually different from that seen when alcohol was employed. Yields of 95–100 per cent of theory are readily obtained.

TABLE 1

Androgen	M.P.*	Crystalline form and color	N found (%)	N calculated (%)
Testosterone	202.5°–203.5°	Red needles	11.62	11.96
Testosterone propionate	209.9°–210.5°	" "	10.67	10.68
Methyl testosterone	221.5°–223.5°	Orange "	11.41	11.86
Androsterone	232.5°–234°	Yellow "	11.68	11.91
Dehydroisoandrosterone	241°–242°	Yellow micro needles in thick mat	—	—

* Melting points were determined with an aluminum block apparatus calibrated by means of the U.S.P. melting-point reference standards, but are not otherwise corrected.

Table 1 lists the data for the androgen hydrazones prepared to date.

When a mixture of the hydrazones of testosterone and testosterone propionate was subjected to chromatographic adsorption, as described above, the constituents were readily separated and recovered in yields of 97 per cent and 91 per cent respectively, the fractions being identified by melting-point determination after recrystallization. The derivative of testosterone was adsorbed at the top of the column and that of the propionate below.

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