bers of animals, the dose of nitrogen mustard was smaller, having been predicated on that used in humans. While the results obtained were identical with those in the present study, they were open to question because no significant leukopenia could be demonstrated with this dose.

In the present study the dose of HN_2 was calculated to cause a significant drop of leukocytes without causing death. The difference in dosage which will accomplish this effect was found to be very critical, since the animals remained well and healthy on one dose and were killed quickly when the dose was increased very slightly. It is believed, therefore, that HN_2 was given in an amount which would affect the animals since leukopenia occurred regularly in our test group.

In Groups I and II the animals were made resistant to subsequent challenge with virulent tubercle bacilli by prior inoculation with BCG. Reference to Table I indicates that virulent organisms were confined to the injection site and adjacent nodes, a situation analogous to that of patients with quiescent tuberculosis. Treatment with HN_2 appears to have had no effect on animals thus treated, since the amount and distribution of the infection were essentially the same.

In Groups III and V the animals were also inoculated with virulent organisms but did not have the protection of BCG. The guinea pigs receiving HN_2 appeared to have less dissemination of the disease than those not receiving it. A statistical analysis of the data‡ in these groups reveals the difference between the mean amount of tuberculosis present in treated and untreated animals to have no significance.

No effect was evident when animals of Group IV, which were given BCG alone, were subjected to treatment with nitrogen mustard.

Summary. Nitrogen mustard administered intraperitoneally to guinea pigs in a dose of 0.3 mg/kilo for 6 days was sufficient to produce significant leukopenia but had no appreciable effect on amount or spread of tuberculous infection nor did it reduce the protection produced by prior vaccination with BCG.

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Method for Simultaneous Estimation of Pregnane-3α, 17α, 20α-Triol and Pregnane-3α, 17α, 20α-Triol-11-One in Urine. (23199)

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Cases of adrenogenital syndrome due to adrenal hyperplasia excrete large amounts of C-20-methyl steroids such as pregnane-3α, 17α, 20α, triol(1), and pregnane-3α, 17α, 20α, triol-11-one(2), in the urine. Although milligram amounts of pregnane-3α, 17α, 20α-triol are also excreted in cases of adrenal virilizing tumor(3) and small quantities of this steroid are present in the urine of normal persons(4), the presence of pregnane-3α, 17α, 20α-triol-11-one has been established so far only in cases of adrenal hyperplasia(2). In all the above publications the presence of both compounds was established by isolation and identification. However, until recently

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no accurate analytical method has been available which would enable an easy screening of urines for presence of pregnane-3α, 17α, 20α-triol and pregnane-3α, 17α, 20α-triol-11-one. Recently, Finkelstein and Goldberg(5,6) described a test for qualitative and quantitative estimation of pregnane-3α, 17α, 20α-triol-11-one. It was shown that pregnane-3α, 17α, 20α-triol-11-one could be visualized on filter paper by moistening the paper with 70% phosphoric acid, heating it at 87°C for 10 minutes, quickly cooling to room temperature and irradiating with U.V. light with a maximal emission at 3660Å. Under these conditions pregnane-3α, 17α, 20α-triol-11-one produces a brilliant blue fluorescence which is clearly visible with 1-2 μg of the compound sq. cm. Combined with paper chromatography, this reaction was found suitable for detection of pregnane-3α, 17α, 20α-triol-11-one in urinary extracts.

Results. With this method pregnane-3α, 17α, 20α-triol-11-one was detected in quantities above 40 μg/24 hr urine (which is the lowest limit of the test) only in urine of patients classified as female pseudohermaphrodites and macro-genitosomia praecox due to adrenal hyperplasia. On the other hand, pregnane-3α, 17α, 20α-triol-11-one was not detected in the urine of normal persons, or of patients with apparently "normal" endocrine function but receiving large amounts of ACTH and cortisone, or of patients with virilizing and non-virilizing adrenal tumors.

This method suffered, however, from the drawback that hot acid treatment was used for hydrolysis of urinary steroid conjugates, under which conditions pregnane-3α, 17α, 20α-triol is very rapidly destroyed(7). This steroid, of interest because of its occurrence in relatively high concentration in urine of patients with adrenal hyperplasia and also in cases with virilizing adrenal tumors, is thus destroyed in the test of Finkelstein and Goldberg(6).

Since pregnane-3α, 17α, 20α-triol is readily liberated from urinary conjugates with β-glucuronidase preparations(4,8), we investigated the possibility of developing a method, using enzymic hydrolysis and suitable for simultaneous determination of pregnane-3α, 17α, 20α-triol and pregnane-3α, 17α, 20α-triol-11-one. Three conditions had to be fulfilled in order to make such a single method for both compounds workable, (1), that conjugated urinary pregnane-3α, 17α, 20α-triol-11-one could be hydrolysed with β-glucuronidase under conditions employed for the hydrolysis of bound pregnane-3α, 17α, 20α-triol; (2), that both compounds could be identified under identical conditions, and (3), that pregnane-3α, 17α, 20α-triol could be easily separated from pregnane-3α, 17α, 20α-triol-11-one.

These requirements are satisfactorily met under the following conditions: (1) Enzymic hydrolysis of urine of cases of adrenal hyperplasia under conditions suitable for liberation of pregnane-3α, 17α, 20α-triol(8) has yielded amounts of pregnane-3α, 17α, 20α-triol-11-one similar to those obtained after hot acid hydrolysis. Both a molluscan preparation of β-glucuronidase(9) and Viobin Spleen glucuronidase have been used. (2) Detection of pregnane-3α, 17α, 20α-triol may be carried out under the following conditions, identical to those used for pregnane-3α, 17α, 20α-triol-11-one(6):—5 μg of pregnane-3α, 17α, 20α-triol in methanolic solution are applied on a Whatman No. 1 (for chromatography) filter paper on an area of approx. 1 cm^2. The paper is dried on air (evaporation of the solvent may be speeded up by warming with an ordinary electric lamp) then very briefly dipped in 70% phosphoric acid, blotted between 2 sheets of filter paper to remove excess reagent, and warmed for 10 minutes in an electric oven at 87°C on a preheated enamel tray. After rapid cooling a pink fluorescence is observed with pregnane-3α, 17α, 20α-triol under U.V. light of wavelength 3660 Å, while in daylight a violet color is seen. The fluorescence is clearly visible with 3-6 μg pregnane-3α, 17α, 20α-triol/sq cm. As mentioned previously, under the same conditions pregnane-3α, 17α, 20α-triol-11-one produces a brilliant blue fluorescence visible with 1-2 μg/sq cm. (3) The separation of the 2 compounds is easily achieved using paper chromatography in solvent systems described by Zaffaroni (10), or systems similar to those. For instance, chromatography of combined pregnanetriol and pregnanetriolone with benzene

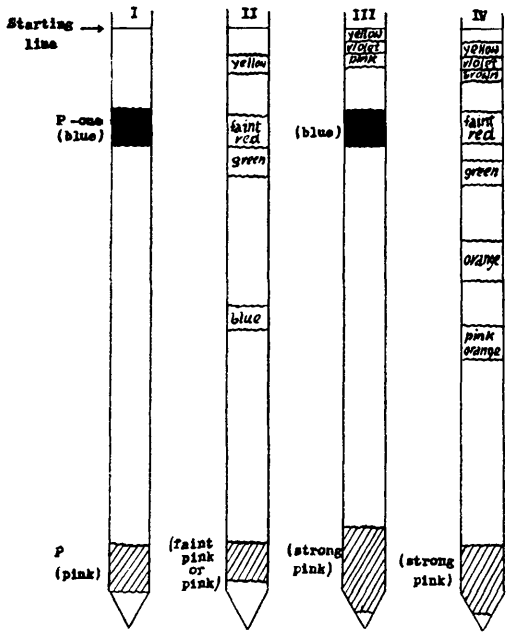

FIG. 1. Diagram of chromatograms of crystalline pregnane-3α,17α,20α-triol, pregnane-3α,17α,20α-triol-11-one and of urinary extracts. System: benzene/formamide:methanol (1:1). Running time 20 hr. Temp. 27°C. Strip I: pregnanetriol-10 μg (P) + pregnanetriolone-5 μg (P-one). Strip II: normal urine. Strip III: adrenogenital syndrome. Strip IV: adrenal adenoma.

on paper impregnated with formamide:methanol 1:1, at 27° for 20 hrs produces a separation of the compounds, as shown in Fig. 1, strip I.

Conclusions. An analytical procedure has been developed for simultaneous determination of pregnane-3α, 17α, 20α-triol and pregnane-3α, 17α, 20α-triol-11-one in urine. This procedure consists of enzymic hydrolysis, extraction of the urine with an organic solvent such as chloroform or ether, washing the extract with N/1 NaOH and water till neutral, and evaporating the extract *in vacuo* until dry. The dry residue is then dissolved in a small volume of methanol or ethanol and applied to a filter paper for chromatography for detection of both compounds through their characteristic fluorescence and estimation by comparison with standards. The significance of a method for simultaneous estimation of pregnane-3α, 17α, 20α-triol and of pregnane-3α, 17α, 20α-triol-11-one as an aid in differential diagnosis between adrenal tumor and adrenal hyperplasia is obvious, since, as pointed out above, pregnane-3α, 17α, 20α-triol is excreted in excess in cases of adrenal tumor as well as in adrenal hyperplasia, whereas pregnane-3α, 17α, 20α-triol-11-one is apparently excreted in large quantities only in cases of adrenal hyperplasia.

Typical results obtained with the method described are given in Fig. 1. Strip I shows the separation of the crystalline compounds. Strip II shows the fluorescence colors obtained with most samples of urine of normal persons. Significant is the absence of the blue spot given by pregnane-3α, 17α, 20α-triol-11-one and the presence of the faint pink or pink in position characteristic for pregnane-3α, 17α, 20α-triol, which indicates small quantities of this compound. Strip III illustrates fluorescence colors given by urinary extracts of a case of adrenogenital syndrome, due to adrenal hyperplasia (female pseudohermaphrodite). Here a distinct blue spot is visible at the same distance from the starting line as crystalline pregnane-3α, 17α, 20α-triol-11-one, and aslo a strong pink in a parallel position to crystalline pregnane-3α, 17α, 20α-triol. Strip IV is a chromatogram of a case of adrenal adenoma which shows absence of pregnane-3α, 17α, 20α-triol-11-one, but a high concentration of pregnane-3α, 17α, 20α-triol (strong pink fluorescence). A full report will be published.

Summary. A test has been described for simultaneous estimation of urinary pregnane-3α, 17α, 20α-triol and pregnane-3α, 17α, 20α-triol-11-one. After liberation from their conjugates by β-glucuronidase hydrolysis and extraction of the free compounds from urine, they are distinguished and estimated by paper chromatography and by the specific fluorescence colors which they develop on heating the paper strips, moistened with 70% phosphoric acid, for 10 minutes at 87°. Increased excretion of pregnane-3α, 17α, 20α-triol has been observed both in patients with adrenal hyperplasia and with adrenal tumors. Pregnane-3α, 17α, 20α-triol-11-one has been detected so far only in urine of patients with adrenal hyperplasia. Based on this differential excretion distinction be-

tween adrenal tumors and adrenal hyperplasia seems to be possible.

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Progesterone a Precursor of Testicular Androgens in Sheep.* (23200)

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Recent studies have provided evidence for a possible relation between fecal androgen and progesterone metabolism in the bovine (1). Furthermore, several androgenic compounds have been identified in the feces of a pregnant cow following the administration of progesterone(2). While feces from normal bulls and steers are inactive(3,4) it is interesting to note that progesterone administration to a bull produced statistically significant increases in the androgen content of the feces (1). *In vitro* formation of testosterone and Δ^4-androstene-3, 17-dione from progesterone has been demonstrated for rat testis(5) and human testicular tissue(6).

In view of this finding, the results of an experiment in which progesterone was given to a ram and a castrate male sheep are presented as indicating possible conversion of progesterone to androgens by the ovine testis.

Materials and methods. Collections of feces were made daily from 2 male sheep, one of which had been castrated as a lamb. The ram was a 2-year-old New Zealand Romney x Lincoln and weighed approximately 140 lb during the period of experimentation. The second animal was a New Zealand Romney wether, 14 months of age, weighing 80 lb. After preliminary collection period of one week each animal received daily during the next week a subcutaneous injection of 0.5 g and 0.25 g respectively, of progesterone in arachis oil. Total feces were dried daily for 48 hours in an oven at 45°C and ground to a fine powder in a Wiley mill. White Leghorn cockerels from the College Farm were fed the dried feces as 10% of the control ration for 21 days, starting when chicks were 4 days old. The increase in comb weight was used to assess androgen content of the fecal samples when compared with the comb response to methyl-testosterone incorporated in the feed. In a preliminary trial a sample of dried feces from a ewe in the last month of pregnancy was tested against 3 levels of methyltestosterone and one level of Δ^4-androstene-3,17-dione in the chick feed. The latter fecal sample had been stored in a dry condition for 2 years.

Results. The chick comb response revealed some androgenic activity in the feces of a pregnant ewe (Table I), whereas the response to the feeding of feces from both the untreated ram and wether was slight (Table II). When progesterone was administered, androgen excretion in the feces of the ram was very markedly increased. This finding was in con-

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