the results obtained. That at least the first possibility is involved can be inferred from the fact that, at high thrombin concentrations, the clotting delay due to radiation is no longer evident. At high thrombin concentrations there is an increase in the rate at which activated fibrinogen appears in solution(4). Apparently, at a high concentration of thrombin, the elaboration of normally activated fibrinogen is sufficiently rapid to overcome the radiation-induced reduction of polymerization. The increase in the difference in clotting time between the irradiated and the control solutions as the thrombin concentration is decreased is then a reflection of the decreased amounts of normally activated fibrinogen formed, the latter being replaced by increasing amounts of radiation-altered material. This modification of the fibrin strands produces an altered clot structure, since these strands comprise the network of a clot which is more highly accessible to the action of urea. At a relatively high thrombin concentration, this effect is also abolished, and this favors the interpretation that the primary modifications produced by radiation become manifest in the early stages of the clotting process.

The effect of low doses of radiation on fibrinogen is apparently not due to an oxidation of sulfhydryl groups, for as pointed out previously, sulfhydryl reagents failed to abolish the radiation-induced clotting delay. Nevertheless, these findings do not exclude the possibility that the polymerization phases of the clotting process are sulfhydryl dependent, since cysteine, glutathione, and thiourea protected radiation-sensitive sites in the fibrinogen molecule at doses ranging from 100 to 600 kiloroentgens(3). The persistence of clotting delay in the presence of similar concentrations of some of the same reagents indicates, however, that sulfhydryl reagents and x-rays act on separate sites in the fibrinogen molecule; both of these sites are essential for clotting to take place.

Summary. Purified bovine fibrinogen was exposed to single x-ray doses in the ranges of 25-100 r and 100-500 r. In each case the logarithm of the thrombin clotting time increased linearly with increasing dosage. The clotting delay due to irradiation was dependent on the thrombin concentration but independent of fibrinogen concentration. Addition, prior to irradiation, of sulfhydryl reagents failed to abolish the radiation-induced clotting defect. Irradiation of fibrinogen increased the rate of solution in concentrated urea of the fibrin clots obtained after addition of thrombin.

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Steroid Secretion by the Isolated Rat Adrenal.* (22363)

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Adrenal vein blood obtained from the rat has been demonstrated to contain several steroid hormones. Corticosterone is present in largest amount but hydrocortisone and aldosterone can also be detected(1,2). Bush found that the ratio of corticosterone to hydrocor-

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tisone secretion by the rat adrenal was approximately 20:1, whereas, in most other animals the secretion of hydrocortisone is greater than that of corticosterone(1). It has been demonstrated also that the rat adrenal gland secretes steroid hormones under *in vitro* conditions when stimulated by ACTH(3). Heard found that the rat adrenal incubated in the presence of ACTH secretes four steroids(4). On the basis of chromatographic mobility, ultraviolet light absorption, and staining reactions, these have been identified as corticosterone, cortisone, hydrocortisone, and an unknown substance.

The purpose of the present investigation was to determine the ratio of hydrocortisone and cortisone secretion to total steroid hormone production of the rat adrenal under *in vitro* conditions. To accomplish this, a method has been developed which permits quantitative determination of steroids containing an alpha-beta, unsaturated ketone structure in ring A and, also, steroids possessing a 17, 21 dihydroxy-20-ketone side chain. Thus, it is possible to determine the total steroid production of the isolated adrenal as well as the secretion of steroids such as hydrocortisone and cortisone. The results demonstrate that the secretion of hydrocortisone, cortisone, and other steroids possessing a dihydroxyketone side chain amounts to less than one-half of the total *in vitro* steroid production of the rat adrenal gland.

Procedure. Male Sprague-Dawley rats weighing 150-175 g were used in these studies. Animals were sacrificed by decapitation. The adrenals were quickly removed, dissected free of surrounding fat, weighed, and bisected. Each adrenal was placed in a beaker containing 2 ml of Krebs-Ringer-phosphate-glucose solution at pH 7.4. The glucose concentration in the medium was 200 mg%. One unit of ACTH was added to the contents of the beaker and the mixture incubated aerobically for 2 hours at 37°C in a Dubnoff metabolic incubator. On completion of incubation, the medium was decanted into a stoppered centrifuge tube leaving the adrenal slices in the beaker. The beaker was washed twice with one ml portions of distilled water and the rinsing water combined with the incubation medium in the centrifuge tube. The aqueous solution was extracted with 5 ml of ethylene dichloride and the mixture centrifuged at 1500 r.p.m. for 5 minutes. Following centrifugation, the aqueous phase was removed by aspiration and 4 ml of the ethylene dichloride extract was pipetted into a clean tube. The latter solution was taken to dryness under nitrogen at 50°C and the residue containing the steroids dissolved in 3 ml of methyl alcohol. Aliquots of the methyl alcohol solution were utilized for determination of the steroid concentration by a spectrophotometric technic and by the Porter-Silber method(5). Spectrophotometric measurement was accomplished by determining the absorption spectrum of the methanol solution from 220 to 260 mμ on a Beckman Model DU spectrophotometer. Steroids which possess an alpha-beta, unsaturated ketone structure in ring A demonstrate maximum absorption at 240 mμ. Steroid concentration was calculated from the absorption spectrum by the method of Allen (6). The Porter-Silber procedure measures only those steroids which have a 17,21 dihydroxy-20-ketone side chain such as cortisone, hydrocortisone, and Compound S.

Paper chromatographic separation of the steroid compounds secreted was achieved by combining the methyl alcohol solution obtained from 20 adrenal incubations. The toluene-propylene glycol chromatographic system of Zaffaroni was used(7). Twenty micrograms of compounds F, E and B were also placed on the chromatogram in order to compare the chromatographic mobility of the steroids secreted by the rat adrenal with that of known compounds. Steroids present on the chromatograms were detected by visualization under ultraviolet light and by staining with alkaline silver nitrate(7). Identification of the steroid secreted in largest quantity (steroid 4) was accomplished after elution of this substance from the chromatogram by use of the sulfuric acid chromogen technic(8).

Results. Recovery of steroids utilizing the method described was determined by adding known amounts of cortisone (free alcohol) to the incubation mixture. In these studies of

TABLE I. Recovery of Cortisone from Incubation Medium.

	Steroid recovery	
	Spectrophotometric method	Porter-Silber method
10.3 γ cortisone added	10.78 ± .19 γ* (104.7%) 11†	9.6 ± .11 γ* (93.2%) 12†

* Mean ± S.E. of mean.
† No. of determinations.

steroid recovery the adrenal slices were boiled to inactivate enzyme systems before being placed in the incubation medium. Table I demonstrates that recovery of cortisone was practically complete as measured by the spectrophotometric and Porter-Silber methods.

Table II compares the steroid secretion by isolated adrenals as measured by the spectrophotometric and phenylhydrazine - sulfuric acid methods. It is apparent that the spectrophotometric assay detects more than twice the quantity of steroid material determined by the Porter-Silber reaction.

TABLE II. Steroid Secretion by the Rat Adrenal Gland *In Vitro*; 19 Determinations in Each Series.

	Spectrophotometric method	Porter-Silber method
Actual steroid secretion/adrenal	11.4 ± 0.77 γ*	4.86 ± 0.24 γ
Steroid secretion per 100 mg adrenal wt	80.1 ± 5.28 γ	34.1 ± 4.58 γ

* Mean ± S.E. of mean.

The results of paper chromatographic separation of steroids secreted by rat adrenals are presented in Fig. 1. Four steroids are detected when the chromatogram is visualized under ultraviolet light and these are labelled in order of increasing mobility in the toluene-propylene glycol system. Steroid 1 has the same chromatographic mobility as does hydrocortisone. The mobility of steroid 2 is equal to that of cortisone. The third steroid band moves more rapidly than cortisone but slower than corticosterone. Steroid 4 moves at the same rate as does corticosterone in the toluene-propylene glycol system. Steroids 1, 2 and 4 reduce alkaline silver nitrate and it is, therefore, concluded that these steroids possess an alpha-ketol side chain.

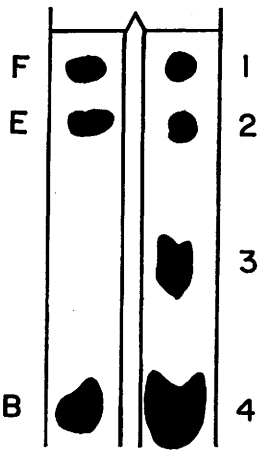

FIG. 1. Paper chromatographic separation of steroids secreted by isolated rat adrenal gland. Dark areas represent steroids which absorb ultraviolet light. Compounds F, E, and B were placed on left limb of chromatogram. The methanol solution containing the steroids secreted by 20 rat adrenals incubated with ACTH was placed on right limb of chromatogram.

Absorption spectra of the sulfuric acid chromogens of steroid 4 and that of corticosterone are presented in Fig. 2. It is apparent that the two spectral curves do not coincide. That of steroid 4 is, however, identical to the spectrum that has been reported for 11-epi-corticosterone (Δ 4-pregnene-11 α, 21 diol-3, 20 dione)(9).

Discussion. The investigations of Bush(1)

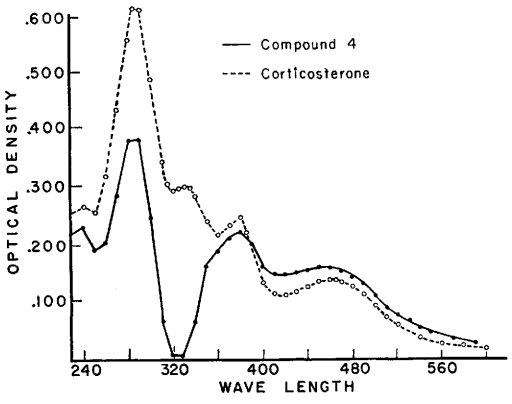

FIG. 2. Sulfuric acid chromogen absorption spectra of compound 4 and corticosterone. These steroids were eluted from the paper chromatogram, taken to dryness, and dissolved in concentrated sulfuric acid. Absorption maxima of compound 4 agree closely with those reported for 11-epi-corticosterone(9).

and Singer(2) have shown that adrenal vein blood obtained from the rat contains several steroid hormones including corticosterone, aldosterone, hydrocortisone, 11-dehydrocorticosterone, and 11-hydroxyandrostenedione. The steroid secreted in greatest quantity is corticosterone and the other hormones are produced in small amounts. Heard(4) has stated that the isolated rat adrenal cortex secretes both hydrocortisone and cortisone as well as corticosterone and an unknown substance. The results obtained in the studies reported herein also show that four steroids containing the alpha-beta, unsaturated ketone structure are secreted by the isolated rat adrenal. Quantitative measurement of steroid secretion demonstrates that almost one-half of the total adrenal hormones secreted under *in vitro* conditions consists of steroids which possess the 17, 21 dihydroxyketone side chain. The secretion of hydrocortisone, however, must account for only a small portion of the Porter-Silber reactive material since the area of the chromatogram containing this steroid was relatively small. If compound 2 is cortisone, as suggested by Heard, this would account for an additional quantity of steroid containing the dihydroxyketone side chain. The secretion of cortisone, however, was also small as judged by the area of the chromatogram containing this steroid. It is, therefore, possible that at least a portion of the steroid material present in area 3 contains a 17, 21 dihydroxy-20-ketone side chain. Heard(4) has obtained evidence which indicates that there are at least 2 steroids present in area 3 and believes that one of these is Compound S, a steroid which has a dihydroxyketone side chain and would, therefore, be measured by the Porter-Silber technic.

It has been demonstrated that corticosterone is the major hormone secreted by the rat adrenal *in vivo*(1). The steroid secreted in greatest quantity in these experiments possessed a chromatographic mobility equal to that of corticosterone. In addition, this compound, like corticosterone, contains an alpha-beta, unsaturated ketone in ring A and has an alpha-ketol side chain. However, the product formed by addition of sulfuric acid to this substance demonstrated an absorption spectrum different from corticosterone but identical to that reported for 11-epi-corticosterone. It is, therefore, concluded that under *in vitro* conditions the chief hormone secreted by the rat adrenal is 11-epi-corticosterone.

The explanation of the finding that the isolated rat adrenal secretes 11-epi-corticosterone, while under *in vivo* conditions corticosterone is produced, is not apparent. Previous investigations, however, have shown that there is a discrepancy between *in vivo* and *in vitro* enzymatic reactions concerned with steroid hormone metabolism. Dorfman (10) has demonstrated that if C_{21}-Δ^4-3 ketosteroids are incubated with liver or adrenal tissue, the reduction products formed are in the 5-alpha series. When these compounds are administered to the living animal, the reduction products obtained have the 5-beta configuration. Since in these investigations it has been demonstrated that an epimer of corticosterone is secreted by the isolated rat adrenal cortex, it is suggested that some of the other hormones produced *in vitro* may also be stereoisomers of the compounds secreted *in vivo*.

Summary. (1) A method to determine the *in vitro* steroid secretion by the rat adrenal is described. This procedure measures total steroid hormone production as well as secretion of steroids which have a 17, 21 dihydroxy-20-ketone side chain. (2) It has been demonstrated that the *in vitro* secretion of adrenal hormones which possess a dihydroxyketone side chain amounts to almost one-half of the total steroid secretion. (3) The isolated rat adrenal secretes four steroid hormones in response to ACTH. The compound secreted in largest amount has been shown to be 11-epi-corticosterone.

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Iodine Metabolism in a Region of Endemic Goiter. (22364)

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The great avidity for iodine exhibited by the thyroid gland of subjects living in certain regions where endemic goiter is prevalent has been demonstrated by Stanbury *et al.*(1,2) and by Roche *et al.*(3). It appeared to us of interest to carry out further measurements on different aspects of iodine metabolism in the regions previously studied by us. Results of thyroid and renal radioiodine clearance, conversion ratios, protein-bound I^{131}, stable iodine urinary excretion, rate of hormonal iodine formation and salivary radioactivity are reported in the present article.

Material and methods. All subjects were apparently healthy and euthyroid adults. One group (cases 1 to 14) came from the region of Bailadores, where previous studies had been carried out(3), and the other group (cases 15 to 21) from the region of Tabay, near the city of Mérida. Both regions lie within the Venezuelan Andes, at an altitude above 4,500 feet. For the study of thyroid I^{131} uptake, subjects were chosen whose thyroid was either barely palpable, or palpable but not readily visible. Radioiodine was supplied by Abbott Laboratories at Oak Ridge. External thyroid measurements were carried out with a Nuclear (Model DS-1) scintillation counter, placed 40 cm away from the thyroid cartilage. The resulting activity was multiplied by 0.85(1), and the activity measured 40 cm from the thigh was subtracted from the resulting value. The result was expressed as % of the administered dose, as measured 40 cm away from a 250 cc beaker (when the dose was given orally) or in a 20 cc syringe (when the dose was given intravenously). The beaker was thoroughly washed and the washings given to the patients. The syringe was counted before and after intravenous administration of the dose, and the second count was subtracted from the first. Measurements of plasma, saliva and urine radioactivity were performed in a well-type scintillation counter (Nuclear, Model 3037 B) and compared with the activity of suitably prepared standards.

Measurements of thyroid and renal clearances were carried out as follows: 50 μc of I^{131} in 10 cc of saline solution were injected intravenously. The thyroid was counted at 2 and 29 minutes during 2 consecutive minutes. The results, properly corrected, were taken to represent neck activity at 3 and 30 minutes respectively, and were expressed in terms of % of the administered dose. Blood was obtained at 5, 10 and 30 minutes and radioactivity of 3 cc of plasma was counted, compared with a standard and expressed in terms of % of the administered dose per liter of plasma. The patient was asked to urinate at 35 minutes and urine radioactivity was measured and compared with the standard. Thyroid clearance was calculated from a formula given by Kretchmar(4), modified from the method of Berson *et al.*(5):