rise in serum inorganic phosphate and magnesium. On cessation of therapy the serum calcium level declines again to normal levels.

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Changes in Patterns of Secretion of Corticosteroids in Rabbits after Prolonged Treatment with ACTH.* (20959)

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(Introduced by M. Finland.)

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The nature of the adrenocortical secretion in diverse species has now been evaluated *in vivo* and *in vitro*. It was first shown that the isolated bovine gland, perfused with ACTH, synthesizes a mixture of corticosteroids, the major components of which are 17-hydroxycorticosterone (hydrocortisone) and corticosterone(1). Subsequent examination of the steroids present in adrenal vein blood has revealed that either hydrocortisone or corticosterone (or a mixture of both) is uniformly present in highest concentration in all species studied(2). The relative amounts of hydrocortisone and corticosterone, however, vary widely among species. It has been reported that the adrenal glands of sheep and monkeys secrete primarily hydrocortisone, those of rabbits and rats produce predominately corticosterone, while cows, ferrets, cats and dogs secrete mixtures of both(2,3). Recent studies indicate that human adrenal vein blood contains primarily hydrocortisone, although lesser amounts of corticosterone have been detected(4). These differences between species have been reported by Bush(2.3) to be constant and unaltered by ACTH; the inference has been drawn that the species differences may be fixed genetically.

The view that the nature of the steroids released in response to ACTH is fixed in unalterable fashion seemed at variance with certain observations on the comparative effects of ACTH and corticosteroids on lymphoid tissues of the rabbit. Thus, ACTH, cortisone and hydrocortisone induced significant changes in the weight and concentrations of ribonucleic acid in lymph nodes. Corticosterone, on the other hand, the presumed product of adrenal secretion in rabbits, had no significant effect at the same dosage levels as the other 2 steroids(5,6). The response of the rabbits to ACTH was not explicable on the assumption that rabbits secrete corticosterone exclusively. Inasmuch as the effects of ACTH on lymph nodes were observed after relatively prolonged administration, the possibility was considered that prolonging the treatment with ACTH might alter the pattern of secretion of corticosteroids in this species. Accordingly, experiments were undertaken to test this view. The results to be reported demonstrate that the prolonged administration of ACTH to the rabbit leads to an alteration in the pattern of secretion of corticosteroids so that hydrocortisone becomes the predominant steroid component of adrenal vein blood.

Methods. White male rabbits weighing between 2.5 and 3.5 kg, with no apparent dis-

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ease, were used in these experiments. To obtain sufficient corticosteroid for analysis, adrenal vein blood from identically treated rabbits was pooled and analyzed as a single sample. Four groups of rabbits were studied. Group I represented untreated rabbits; the other groups received porcine ACTH as follows: Group II received 15 I.U. of ACTH in gelatin† intramuscularly 2-3 hours before collection of the adrenal vein blood was begun. Groups III and IV received ACTH daily for 7 days and 21-28 days, respectively. For the latter groups, a highly purified ACTH preparation (25-50 I.U. per mg as evaluated by bioassay in mice(7)) was suspended as a dry powder in sterile peanut oil containing 5% beeswax. This suspension contained one mg per ml of highly purified ACTH, and was given intramuscularly daily in 0.5 ml doses for the indicated period of time. The prolonged treatment with this preparation of ACTH produced significant loss of body weight and marked hyperplasia of the adrenal glands. The adrenal weights were approximately doubled after 7 days of treatment and approximately tripled after 21-28 days of treatment. The adrenal vein blood was collected from a segment of the vena cava so isolated that only blood from the left adrenal vein could enter it. The left adrenal vein could not be cannulated directly inasmuch as it runs its course almost entirely within the adrenal cortex, entering the renal vein almost at the junction of the left renal vein and inferior vena cava. The right adrenal gland was technically more difficult to isolate in this manner, hence the left gland was used exclusively in these studies. To achieve the vena caval pouch for the collection of adrenal vein blood, the animals were anesthetized with intravenous sodium pentobarbital. A longitudinal midline incision was made over the abdomen, and the inferior vena cava and peritoneal surfaces of the left kidney and left adrenal gland were exposed. Double ligatures were placed around the renal vein distal to the adrenal gland and around the vena cava above and below the left renal vein. All tributaries entering the pouch were ligated. Ligation of the vena cava was the last procedure immediately preceding the opening of the pouch and onset of collection of blood through a glass cannula using mild suction. An average of 4-6 ml of blood per hour was obtained in this manner; the oozing of blood from the adrenal vein was visualized directly in all cases. Five percent glucose in physiological saline was administered through the femoral vein at a rate of 1-2 ml per minute in order to retard the development of circulatory collapse. Thrombosis in the adrenal vein was minimized by the intravenous administration of 10 mg of heparin, and sufficient additional heparin was added to the glucose and saline to deliver about 10 mg per hour. Collection periods extended for 2-3 hours. The samples of adrenal vein blood were pooled upon collection and stored at —20°C. The corticosteroids were extracted from the thawed blood with ethyl acetate, using the method of Meyer(8). The residues obtained from the ethyl acetate extracts were fractionated between 80% ethanol and hexane. The hexane was discarded and the corticosteroids were obtained from the aqueous ethanolic solution. The ethanol was removed *in vacuo* under nitrogen and the corticosteroids were extracted from the aqueous fraction with methylene chloride. The residues obtained after evaporation were fractionated by paper chromatography using the chloroform-formamide and toluene-propylene glycol systems of Zaffaroni(9), using 2 cm wide paper strips for each sample. The corticosteroids present on papergrams were detected by ultraviolet scanning and by reaction of narrow strips to a variety of spot test reagents which included blue tetrazolium and concentrated sulfuric acid. For quantitative estimation of the steroid content, the steroid zone was eluted from paper with methanol, and the ketol content of aliquots of the residue was determined using the procedure of Chen and Tewell(10), using both hydrocortisone and corticosterone as standards. In all cases an area of paper derived from the same papergram, not containing steroid, was eluted and analyzed to obtain a "paper blank." Recovery by this method of corticosteroids

† All the ACTH was kindly supplied by Armour Laboratories.

TABLE I. Effect of Administration of ACTH on Concentrations of Corticosterone and Hydrocortisone on Adrenal Vein Blood of 4 Groups of Rabbits.

No. rabbits	Treatment	Vol. sample (ml)	H†	C†	Ratio H/C
			μg/100 ml		
6	0 (control)	69*	<6–8	155	<.05
	ACTH				
5	Single dose	65	<6–8	140	<.05
4	7 days	50	62	126	.5
5	21–28 days	60	307	75	4.

* Estimated value: Pool of 45 ml of adrenal vein blood plus 75 ml from 1 rabbit consisting mainly of extra-adrenal blood.

† H = hydrocortisone; C = corticosterone.

added to blood is 80-100%. Corticosterone and hydrocortisone were identified on the basis of comparison with reference standards, mobility in paper chromatographic systems, the green-yellow fluorescence on paper strips with concentrated sulfuric acid, mixed chromatograms and by the sulfuric acid chromogen technic of Zaffaroni(9).

Results. The results obtained are in Table I. The adrenal vein blood from untreated rabbits contained corticosterone but no detectable hydrocortisone. Since the method employed would have detected 6-8 μg of steroid per 100 ml of blood with ease, the ratio of hydrocortisone to corticosterone is <0.05, in agreement with the findings of Bush(3). When ACTH was given once as 15 I.U. in a gelatin menstruum, the results were similar. This suggests that the considerable trauma associated with the collection procedure may have stimulated the release of endogenous ACTH at rates sufficient to stimulate biosynthesis of corticosteroids at or near maximal capacity. Similar observations following brief treatment with ACTH have been reported(3).

When daily treatment with ACTH was given for one week or more, hydrocortisone appeared in the adrenocortical secretion. After 7 days of treatment, the ratio of hydrocortisone to corticosterone was about 0.5; after 21-28 days hydrocortisone became the predominant steroid and its concentration was 4 times that of corticosterone. There is an indication that the total corticosteroid output was increased in this circumstance, perhaps as a reflection of the increase in adrenal weight. Evaluation of the absolute rate of output of corticosteroid could not be attained precisely due to variations in rates of blood flow, adrenal weight, collection periods, etc. However, a general figure of 40-75 μg of steroid per g of gland per hour represents the approximate magnitude of the rate of production. No clear effect of ACTH, regardless of the manner of administration, on the rate of production of steroid per g of adrenal tissue per unit time could be ascertained.

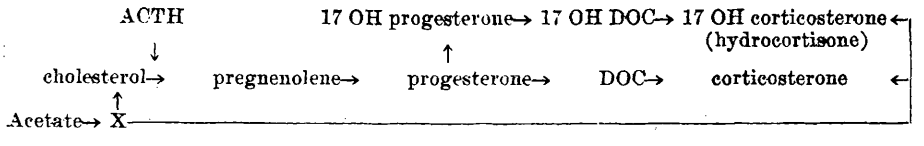
All of the papergrams of the adrenal vein samples contained, in addition to the 2 identified components, zones of unidentifiable material giving ultraviolet reactions typical for Δ^4-3 ketones of varying polarity. No 17-ketosteroid reaction zones were detected. Those ultraviolet reactive zones that did not react with blue tetrazolium may have represented adrenal products since they were not present in detectable amounts in extracts of equivalent volumes of peripheral rabbit blood. In general, all samples studied, with the exception of those obtained after ACTH had been given for 3-4 weeks, showed the presence of 2 such zones, one more polar than hydrocortisone and the other of polarity similar to those of cortisone and electrocortin. After 3-4 weeks of administration of ACTH, 3 ultraviolet positive zones more polar than hydrocortisone and 3 zones of polarity intermediate between hydrocortisone and corticosterone were observed. Whatever the nature of these unidentified substances, the suggestion from these studies is that the administration of ACTH for 3-4 weeks may also influence the number or concentration of these unidentified trace components. Although present in minor concentration, these trace products are not necessarily without physiological interest; a trace component of the adrenocortical secretion, electrocortin, has recently been described and may be primarily responsible for the adrenal control of electrolyte metabolism (11,12).

Discussion. These data may serve to explain why ACTH and hydrocortisone exerted similar effects on the lymph nodes of rabbits when corticosterone in large doses was without significant effect. The treatment with ACTH

in the lymph node studies was administered for 5 days or more(5,6). Our present results indicate that significant amounts of hydrocortisone were probably produced by the stimulated adrenal glands. There are indications that certain effects of ACTH are different in different animal species, presumably through variable effects on adrenal steroidogenesis. These data have been reviewed elsewhere(7,13).

Although genetic factors are undoubtedly operative in the control of adrenal secretions, the results herein reported demonstrate that the nature of the adrenal secretion within a given species is not unalterably fixed. Whether factors other than exogenous ACTH have a similar effect on the adrenal secretion remains for future investigation. It has been demonstrated, *in vitro,* that exogenous ACTH rapidly increases the rate of production and release of corticosteroids without significant alteration of the nature of the steroids produced(3,14,15). The effect of ACTH described in these studies is dissimilar in that it requires days or weeks to achieve the profound alteration in the pattern of adrenal secretion without striking increase in the rate of production of corticosteroid. The rapid action of ACTH has been extensively studied *in vitro;* this action appears to be involved in the conversion of cholesterol to progesterone from which both hydrocortisone and corticosterone are derived(15,16). In confirmation of these *in vitro* observations Bush has found that ACTH increases the total steroid output without altering the relative ratios of hydrocortisone to corticosterone under certain conditions(3).

The action of ACTH which is demonstrable after prolonged treatment must operate differently in that the pattern of steroids but not necessarily the absolute amount is altered. The following sequence in the biosynthesis of corticosteroids from cholesterol *in vitro* has been proposed(16), and the locus of action of ACTH is indicated:



From this sequence it is apparent that the rate of hydroxylation of progesterone at C-17 and C-21 is a determining factor in the synthesis of hydrocortisone and corticosterone and that synthesis of corticosterone is the major pathway of conversion of progesterone if C-17 hydroxylating activity is low or absent. If this hypothesis is correct, the absence of secretion of hydrocortisone in untreated rabbits would be due to a deficiency or inhibition of the C-17 hydroxylating system involved in biosynthesis of hydrocortisone. Conversely, according to this view, the appearance of hydrocortisone in the adrenal secretion requires that prolonged treatment with ACTH selectively increase the activity of the C-17 hydroxylating system. Further work is clearly necessary to test this hypothesis.

Summary. The adrenal vein blood of untreated rabbits contains corticosterone as the predominant steroid component. A single dose of ACTH did not alter significantly the nature or amount of steroid output of the adrenal gland. When ACTH of porcine origin was given for one week, hydrocortisone as well as corticosterone was found in the adrenal vein blood in a ratio of 1:2. The administration of ACTH for 21-28 days led to the predominance of hydrocortisone in the adrenal secretion, and the ratio of hydrocortisone to corticosterone was 4:1.

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Changes in Clot Retraction Time and Platelet Adhesiveness Following Whole Body Irradiation.* (20960)

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The exact nature of the clotting defect in the hemorrhagic state following whole body radiation is unclear. It is apparent that the concentrations of fibrinogen(1), calcium, prothrombin(1), ac-globulin(2), antihemophilic globulin(3) and the plasma precursor of serum prothrombin conversion accelerator(4) are normal in the irradiated animal. The most significant concomitant of the prolonged clotting time and the hemorrhagic syndrome is the marked thrombocytopenia. Emphasizing the significance of the thrombocytopenia have been the studies demonstrating temporary reversal of the clotting defect following transfusion into these animals of normal platelets (5,6). It is not known whether there are qualitative changes in platelets of irradiated animals or whether these changes may precede onset of thrombocytopenia. Cohn has shown in his charts that there is a delay in initiation of clot retraction by the third post radiation day prior to onset of thrombocytopenia but no reference is made to this observation in the text(7). There is a very close relation between the rate of initiation of clot retraction as measured by clot retraction time and level of platelet adhesiveness(8,9).

In this study evidence is presented of changes in platelet adhesiveness and clot retraction time preceding onset of thrombocytopenia in the irradiated dog. There is also present in the irradiated animal a circulating plasma factor which inhibits platelet adhesiveness and prolongs the initiation of clot retraction.

Methods. Citrated venous blood was placed in a lusteroid tube and immediately after drawing blood a sample was drawn in a red-cell diluting pipette. The diluting fluid was 3.8% sodium citrate and 0.2% formalin. The pipette was placed in shaker for 10 minutes and the fluid then examined directly on a counting chamber. Platelet adhesive index was determined by the glass-wool method of Moolten and Vroman(10). The normal index in the dog varied from 1.15 to 1.35 and is reproducible with less than 10% error. Clot retraction time was determined by the method of recalcified venous blood in castor oil(8). This was modified to account for the more rapid clotting of recalcified dog's blood. 4.5 ml of venous blood was drawn into a syringe containing 0.5 ml of 3.8% sodium citrate. One ml of this blood was placed in each of 2 glass tubes in a water bath at 18°C and .06 ml of a 5% $CaCl_2 \cdot 2H_2O$ solution was added to each tube. Immediately following addition of calcium, blood was drawn up in a siliconized capillary pipette and deposited as a single drop in castor oil. The tubes of castor oil were kept at 18°C and the test done in quadruplicate. The clot retraction time was

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