hypoxia and intercoronary arterial pressure gradients may exist to provide more favorable conditions for collateral growth.

Summary. The effect of pregnancy and administered estrogen on the coronary collateral circulation of otherwise normal dogs was investigated. The data show that neither pregnancy nor estrogen given for 21 weeks resulted in significant alteration from controls in peripheral coronary occlusion, or in the roentgenographic appearance of the coronary arterial vasculature following injection with a barium gelatin mixture.

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Adrenal Steroid Secretion in Rabbits Following Prolonged ACTH Administration.* (29812)

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The principal secretory products of the adrenal cortex of adult mammals are corticosterone and cortisol. Bush has shown that the relative quantity of these compounds varies from species to species(1). He and other workers have demonstrated that the rabbit is predominantly a corticosterone producer, the corticosterone/cortisol ratio (B_K/F_K) being approximately 20:1(1,2,3).

Based on the observation that administration of corticosterone to rabbits had no effect on the lymphoid tissue whereas administration of ACTH had a pronounced effect, Kass *et al* investigated the secretion of the rabbit adrenal cortex following a 7-28-day-period of porcine ACTH administration(2). This treatment converted the rabbit into a predominantly cortisol producer.

In a recent review by Saba *et al*, ACTH is said to have two effects on adrenal secretion (4). The first effect is seen as an immediate rise in the output of the adrenal steroids without a change in their ratios. The second effect, after prolonged ACTH administration manifests itself as an alteration in the ratio of the adrenal products. To our knowledge this long term effect of ACTH administration is attributed primarily to the work of Kass and coworkers and has never been confirmed. The concept that prolonged ACTH administration changes the ratio of steroid output by the adrenal cortex is of sufficient importance that a repetition of the experiments seemed warranted.

Methods. Male rabbits weighing 2.7-3.5 kg were maintained on Purina rabbit pellets and had water *ad libitum*. The animals were divided into 3 groups. Porcine ACTH was given to Group I for 15 days and to Group II for 30 days. The porcine ACTH† was suspended in sesame oil and was administered twice daily subcutaneously to both

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TABLE I. Adrenal Weights and Steroid Secretions.

	Control (5)*	ACTH	
		Group I 15 days (5)	Group II 30 days (6)
Total adrenal wt, mg	327.8 ± 18.3†	429.6 ± 33.5 P <.05‡	495.8 ± 53.8 P <.05
$\frac{\text{Adrenal wt, mg}}{\text{Rabbit wt, g}} \times 100$	9.84 ± .55	14.00 ± 1.33 P <.02	16.73 ± 1.75 P <.01
μB_K/min/g of left adrenal	13.10 ± 2.45	4.92 ± 2.22 P <.05	3.15 ± 1.14 P <.01
$\mu g F_K$/min/g of left adrenal	.86 ± .33	2.04 ± .82 n.s.	2.88 ± .95 n.s.
B/F ratio	23.66 ± 6.52	2.74 ± .79 P <.02	1.23 ± .19 P <.01
Total μg/min for left adrenal	2.11 ± .39	1.40 ± .53 n.s.	1.54 ± .58 n.s.

* No. of animals/group.
† Mean ± standard error of mean.
‡ Compares experimental groups to control group.
n.s. = not significant.

groups in 1/10 ml doses so that each rabbit received 25 units of ACTH per day. Control animals, Group III, were injected with similar quantities of sesame oil. At the end of the treatment period the animals were anesthetized with sodium pentobarbital, and a midline abdominal incision was made. Blood from the left adrenal gland was collected by cannulating the left renal vein. The renal vein was tied both distally and proximally to the adrenal gland and all tributaries entering this pouch were also ligated. At times the adrenal gland lay too close to the vena cava to place a ligature around the renal vein. In these cases ligatures were put around the vena cava above and below the adrenal gland. Immediately following the cannulation, 1000 units of heparin were administered intravenously. Blood was collected for periods ranging from 60 to 105 minutes and averaged 8 to 10 ml per hour. The whole blood was immediately frozen and stored at minus 15°C.

Before extraction was undertaken 4-C^{14} corticosterone and 7α-H^3-cortisol were added to each sample to serve as recovery standards and to enable us to calculate the secretion rates. The solvents used for extraction were redistilled or of spectroquality, and all evaporations were carried out in an atmosphere of nitrogen below 40°C. Extraction procedures on whole blood were identical to those reported previously(5). Cortisol and corticosterone were isolated by paper chromatography using Zaffaroni systems(6). Regions which had Rf's similar to those of corticosterone and cortisol were eluted from the paper, acetylated, and rerun in another Zaffaroni system. The acetate derivatives of cortisol and corticosterone from each animal as well as chromatography paper blanks were eluted, and 1 ml from each sample was counted in a Packard Tri-Carb scintillation counter for use in recovery and secretion rate calculations. Another aliquot was taken for quantitation. The Porter-Silber reaction(7) was used for cortisol and the blue tetrazolium reaction for corticosterone(8). A radioactive strip counter was used in locating the radioactive tracers added. As further proof of the identity of the isolated compounds sulfuric acid spectra were run and proved to be identical with that of authentic cortisol and corticosterone(6).

Statistical analyses were performed according to methods described by Edwards (9).

Results. Table I illustrates that adrenal weights increased significantly in the ACTH treated animals in absolute values as well as when expressed as the function body weight. At the start of experiment the body weights of all groups were virtually identical. Animals receiving ACTH failed to gain weight whereas control animals continued to grow.

All steroid secretion values in Table I have been corrected for losses occurring during their isolation and characterization. The

control rabbits produced 13.1 μg of B_K/min/g adrenal while F_K was secreted at an average rate of 0.86 μg/min/g adrenal. The individual B_K/F_K ratios averaged 23.7 in the control group. The apparent discrepancy between the above ratio and that obtained when one divides the average B_K output by the average F_K secretion is due to the large variation in steroid production in the individual animals. Administration of ACTH produced a 63% decrease in B_K output after 15 days of administration and 75% after 30 days administration. These reductions were both significant when compared to the control. The apparent doubling and tripling of F_K output in the 15- and 30-day injected groups were not statistically significant. The ratios of B_K/F_K in Group I and II were significantly reduced when compared to the control animals. The last line in Table I compares the total steroid output of the left adrenal gland on a per minute basis, and it is clear that ACTH did not produce any significant change in total amount of steroid secreted per minute. It is apparent that when one adds the output of B_K and F_K per minute per gram of gland the secretion is diminished in the ACTH treated rabbits; however, if the glandular hypertrophy is taken into account, outputs of control and experimental animals are similar.

Discussion. Although the B_K/F_K ratios reported by Kass and coworkers in their control animals are similar to those reported here, several differences are apparent. In both our 15- and 30-day ACTH treated animals we observed an alteration in B_K/F_K ratios although not to the extent previously reported by the above workers. Kass *et al* reported a 50% reduction in B_K output and an approximate 50-fold increase in F_K production resulting in a B_K/F_K ratio of 1:4 following 12-25 units of ACTH daily. In this report F_K production tripled while the B_K output was reduced 75% and this resulted in a reduction of the B_K/F_K ratio from >20 to approximately unity. In addition Kass and coworkers reported an increase in total steroid output after 21-28-day treatments with ACTH. We have been unable to detect an increase in total B_K and F_K production on a per minute basis although the adrenal glands were significantly hypertrophied. We interpret this to mean that under the conditions of anesthesia and surgical stress the hypertrophied glands of the ACTH animals were not secreting larger amounts of steroids than the control animals. It should also be noted that the steroid secretions of our animals are in ranges previously reported by others(3,10) and are 11 to 21 times higher than those reported by Kass *et al.*

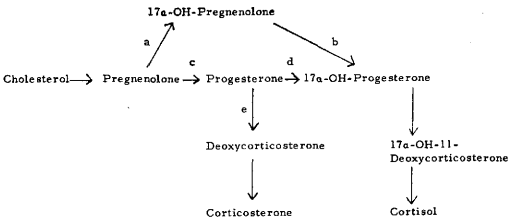

FIG. 1. Adrenal steriod biogenetic pathway.

Fig. 1 illustrates the biosynthetic scheme for adrenal steroid production. It is generally thought that the immediate effect of ACTH administration is an enhancement of the conversion of cholesterol to pregnenolone. If both B_K and F_K originate from a common precursor, then the chronic administration of ACTH must act later in the biosynthetic scheme to account for the alteration in the relative quantities of these two steroids. We were unable to detect any intermediate compounds such as 17α-OH-pregnenolone, deoxycorticosterone, progesterone, 17α-OH-progesterone, or 17α-OH-11-deoxycorticosterone in our animals which might have aided in determining the site or sites of action of chronically administered ACTH. Kass and coworkers attributed the change in the B_K/F_K ratio in ACTH treated rabbits to an increase in activity of step *d* and perhaps a reduction in activity of the enzyme systems at step *e*. Recently Lipsett and Hökfelt have demonstrated that 17α-OH-pregnenolone can serve as a precursor for cortisol in normal adrenal tissue(11). This suggests the possibility of cortisol synthesis proceeding from pregnenolone thru steps *a* and *b*. Thus, the alteration observed in the B_K/F_K ratios after prolonged ACTH administration might well be the result of an enhanced activity of step *a* and/or a reduced activity of step *c*.

Summary. Porcine ACTH was administered to rabbits for periods of 15 and 30 days. At the end of this time the quantity of corticosterone isolated from adrenal venous blood was significantly reduced, while the secretion of cortisol tripled. This resulted in a drop in the corticosterone/cortisol ratio from a control value of 20:1 towards unity. The hypertrophied adrenals of the ACTH treated rabbits secreted the same quantity of steroids per unit time as the control animals. A possible explanation of these changes is discussed.

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Metabolism of Arterial Tissue With Special Reference to Esterase and Lipase.* (29813)

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In previous work on the fate of fresh aortic homografts in dogs fed an atherogenic diet, it was found that thoracic aorta(1) usually refractory to atherosclerosis remains so even when implanted into the abdominal aorta, site of maximum involvement in the dog, whereas abdominal implants remain susceptible(2). Similar results were obtained with aortic homografts implanted into the thoracic aorta(3). These findings suggest a biologic dissimilarity between thoracic and abdominal aorta in the dog.

In another series of experiments, a species difference was noted in the oxidative capacity of the succinic oxidase and cytochrome oxidase systems in aortic tissue of man, rabbit and dog(4,5), species presenting various degrees of susceptibility to atherosclerosis.

The present investigation deals with esterase and lipase activities of normal aortic tissue of dog, rabbit and man.

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Material and methods. Tissues used in this investigation were aortas of man, rabbit and dog. Liver and serum were used for comparison. Animal specimens were secured from male mongrel dogs 2-3 years old and male chinchilla rabbits 6-12 months old. Rabbits were sacrificed by exsanguination and dogs by light anesthesia and exsanguination, and tissues removed immediately. Human specimens free of atherosclerosis were secured from autopsies within 5 hours after death. Age of subjects ranged from 13 to 63 years. Preliminary experiments with animal tissues demonstrated that esterase activity remained constant during this lapse of time, even at room temperature.

After removal from the host, tissues were placed in ice-cooled containers with tight fitting tops. Aortas were flushed with ice-cold saline solution, freed of adherent fat and connective tissue, rinsed with saline solution, blotted, minced, introduced into chilled ho-