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-

mogenizing tubes and homogenized in iced water, 1:9 w/v. Liver was subjected to the same procedure, except for mincing. The homogenates maintained their activity for 16 hours if kept on ice. They were diluted according to needs, immediately prior to use. Study of the aorta was performed on its 3 segments, *i.e.*, ascending thoracic and arch (hereafter referred to as arch), descending thoracic and abdominal.

Esterase and lipase activities were determined by the β-naphthyl laurate method (6,7). Its main steps were those originally described by the authors. Serious difficulties, however, were encountered due to spontaneous hydrolysis of the substrate during the last steps of the procedure, namely coupling reaction and subsequent acidification and extraction of the azo dye. These were therefore improved by rigorous standardization as to time and temperature, so that no spontaneous hydrolysis occurred, as attested by a colorless blank. The latter consisted of all the components, except that the homogenate was inactivated by boiling. The final steps were conducted as follows: At end of incubation period (2 hours at 37.4°C) experimental tubes were placed in an ice-cold water bath for 7 minutes, chilled diazo-blue solution was then added and coupling allowed to proceed for 4 minutes. This was followed by addition of 2 ml ice-cold trichloracetic acid solution (40%), mixing, addition of 10 ml ice-cold ethyl acetate and vigorous shaking. During above procedures the experimental tubes were kept in the ice-cold bath. Separation of the 2 phases was effectuated by centrifugation for one minute at 2500 rpm. Each determination was done at 3 levels of tissue concentration. Total incubation volume was 7.5 ml, containing (final concentration) : β-naphthyl laurate 4×10^{-4} M and veronal buffer pH 7.4, 1.35×10^{-2} M. For lipase determination it contained in addition, sodium taurocholate and calcium chloride of final concentrations 1.07×10^{-2} M and 3×10^{-5} M respectively. For esterase determination, amounts of fresh tissue per experiment were: 7-10 mg for aorta; 0.3-0.4 mg for human and dog liver and 0.1-0.2 mg for rabbit liver; 0.03-0.05 ml for human and dog serum and 0.01-0.02 ml for rabbit serum. Identical results were obtained with plasma. With the exception of dog serum, lipase could not be detected in any tissue studied. Amounts of dog serum per lipase determination were 0.004-0.007 ml. Enzyme activity was expressed in micrograms of β-naphthol liberated per milligram tissue nitrogen or per ml serum per hour. Nitrogen was obtained by micro-Kjeldahl determination(8).

TABLE I. Esterase Activity* of Aortic Tissue (entire wall).

	Arch	Desc thoracic	Abdominal
Dog	86.1 ± 3.22†	62.5 ± 3.35	41.1 ± 2.38
Determinations‡	13	13	13
Rabbit	78.9 ± 3.63	49.2 ± 2.40	35.5 ± 1.68
Determinations	11	11	11

* Activity is expressed in μg of β-naphthol liberated per mg tissue nitrogen per hour. Lipase could not be detected in any tissue.

† Mean ± S.E.M.

‡ The 3 aortic segments were derived from same aorta.

Probability of differences between aortic segments in same species: Dog $p < .001$; Rabbit $p < .001$.

Probability of differences between species: Arch $p > .10$; Desc Thor $p < .01$; Abd $p > .05$.

Results and discussion. Table I summarizes results obtained with dog and rabbit entire aortic wall. It reveals decreasing esterase activity from arch to abdominal aorta and a significant difference ($P < .001$) between aortic segments.

Previous attempts at detecting esterase in normal human and mammalian arterial tissue were carried out mostly with histochemical techniques and yielded conflicting results(9-14). Zemplenyi *et al*(13) reported the presence of esterase in aortic tissue of rat, rabbit, guinea pig and hamster as well as a species difference in its localization and degree of activity in various aortic layers. Other investigators using the entire wall of rabbit thoracic aorta have demonstrated by means of biochemical techniques the presence of esterase and absence of lipase(15).

The results obtained with the entire aortic wall (Table I) reflect the overall activity of its 3 major layers, namely intima, media and adventitia. The results with aortic layers are summarized in Table II. Separation of pure

TABLE II. Esterase Activity* of Aortic Layers.

	Arch		Desc. thoracic		Abdominal		
	Intima-media	Media	Intima-media	Media	Intima-media	Media	Adventitia
Dog Determinations‡	65.6 ± 3.46† (9)	77.7 ± 2.86 (9)	66.4 ± 2.44 (9)	68.7 ± 2.52 (9)	67.8 ± 2.11 (9)		12.0 ± 2.03 (7)
Rabbit Determinations	80.1 ± 3.63 (9)		59.3 ± 3.83 (9)		61.7 ± 5.47 (9)		10.1 ± 1.59 (7)
Man Determinations			41.8 ± 2.25 (7)	40.3 ± 2.26 (7)	44.1 ± 4.50 (7)	37.6 ± 1.35 (7)	

* Activity is expressed in μg of β-naphthol per mg tissue nitrogen per hour. Lipase could not be detected in any tissue.

† Mean ± S.E.M.

‡ The 3 aortic segments were derived from same aorta.

Probability of differences between same layer in various segments or various layers in same segment:

Dog Int-Med: P >.5; Med P <.05; Int-Med vs Med: Arch P <.02; Thor P >.5; Int-Med *vs* Adv: Abd P <.001.

Rabbit Int-Med: Arch *vs* Thor and Abd P <.02; Thor *vs* Abd P >.5; Int-Med *vs* Adv: Abd P <.001.

Man Int-Med P >.5; Med P >.10; Int-Med *vs* Med: Thor P >.5; Abd P >.10.

Probability of differences between species:

Dog vs Rabbit Int-Med: Arch P <.02; Thor P >.10; Abd P >.10; Adv: Abd P >.10.

Dog vs Man Int-Med: Thor P <.001; Abd P <.001; Media: Thor P <.001.

Rabbit vs Man Int-Med: Thor P <.01; Abd P <.05.

intima not being possible due to its extreme thinness, the innermost layer isolated also contained media; it will be referred to hereafter as intima-media. Esterase activity of the latter showed no significant difference between the 3 aortic segments in dog, as well as between thoracic and abdominal segments in rabbit and man, whereas in rabbit's aortic arch it was significantly higher (P<.02).

Since the innermost intima-media is primarily involved in the atherosclerotic process, its higher activity in rabbit's aortic arch which parallels the greater susceptibility of that segment to atherosclerosis may appear of interest. In man and dog, however, a similar parallelism does not seem to exist between esterase activity and greater susceptibility to atherosclerosis of the abdominal aorta as compared to the thoracic.

Media from human thoracic and abdominal aorta displayed similar values (P>.10). In the dog it showed significantly higher values in the arch as compared to descending thoracic (P<.05). Due to difficulty in obtaining media free of adventitia in dog's abdominal aorta and rabbit's 3 aortic segments, results were erratic and therefore discarded.

Similar difficulty was encountered in obtaining adventitia free of medial fibers in dog's and rabbit's aortic arch and descending thoracic. Adventitia seemed to possess much lower activity than that of the other 2 aortic layers. Abdominal adventitia, free of media, showed about 1/6th their activity in both rabbit and dog.

Esterase activity, as determined in the entire wall (Table I) displayed a decreasing gradient from arch to abdominal aorta whereas no difference was noted when determined in intima-media of the 3 aortic segments in dog or descending thoracic and abdominal in rabbit. Since intima *per se* contributes very little to the mass of the entire wall, it would appear that the decreasing gradient observed may bear a direct relationship to relative proportions of media and adventitia in a given segment.

Table III summarizes results obtained with serum and liver. It reveals that human and dog serum possess similar esterase activity (P>.10), whereas in rabbit it is about 13 times higher (P<.001). It also reveals high esterase activity in the liver and a significant difference between species (P<.001).

Our findings indicate that aortic innermost intima-media and media possess relatively low esterase activity, about 1/25th, 1/100th and 1/65th that of liver, in dog, rabbit and man respectively.

It appears that a species specificity exists

TABLE III. Esterase and Lipase Activity* of Serum and Esterase Activity of Liver.

	Serum		Liver
	Esterase	Lipase	esterase
Dog Determinations	63.2 ± 2.35† 24	2763 ± 250 14	1675 ± 94.7 21
Rabbit Determinations	895 ± 84.4 32	0 10	6117 ± 448 15
Man Determinations	68.1 ± 2.82 19	0 10	2706 ± 262 9

* Activity is expressed in μg of β-naphthol liberated per mg tissue nitrogen or per ml serum per hour.

† Mean ± S.E.M.

Probability of differences between species.—Esterase: *Serum* Dog *vs* Rabbit P <.001; Dog *vs* Man P >.10; Rabbit *vs* Man P <.001; Liver P <.001.

for esterase activity of the innermost intima-media, man exhibiting the lowest values and rabbit's aortic arch the highest. A species specificity also exists with regard to liver and serum, the former showing the highest activity in rabbit and lowest in dog, whereas serum shows similar activity in man and dog and much higher in rabbit. The enzymatic differences observed may prove interesting when correlated to susceptibility to atherosclerosis. Thus, the rabbit, most susceptible of the 3 species studied, displayed the greatest esterase activity for all 3 tissues studied.

Summary. Esterase was demonstrated in normal aortic tissue of dog, rabbit and man. Its activity was determined in various layers of the 3 aortic segments (arch, descending thoracic, abdominal) as well as in liver and serum. Absence of lipase, except for dog serum, was constantly demonstrated. Of the 3 aortic layers studied (innermost intima-media, media, adventitia), the adventitia displayed the lowest activity, about 1/6th that of the other two. Intima-media showed no difference in activity between the 3 aortic segments in dog, or between descending thoracic and abdominal in rabbit or man, whereas a higher activity was noted in rabbit's arch. A species difference seems to exist, aortic intima-media showing the lowest values in man, and highest in rabbit's arch. A species difference was also noted with regard to liver and serum, the former showing the highest activity in rabbit and lowest in dog, whereas serum showed similar activity in dog and man, and much greater in rabbit.

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