sible for the activity in the lesioned rat. Resistance of ACTH and of vasopressin to pepsin digestion has been previously reported and is confirmed in the present study(8,9). Loss of CRF activity of the SME extracts following incubation with pepsin constitutes definitive evidence for a CRF distinct from vasopressin.

Pitressin (Exp. 3), purified natural vasopressin and synthetic vasopressin(7) have been demonstrated to deplete adrenal ascorbic acid in the hypophysectomized rat. The pepsin labile factor may also have an extrapituitary action. The fact that Pitressin and the pepsin labile factor induce a much greater degree of adrenal ascorbic acid depletion in the lesioned than in the hypophysectomized rat suggests that the major action of both agents is on the adenohypophysis.

A working concept for action of vasopressin in inducing adrenal ascorbic acid depletion has been presented(7). Vasopressin may release ACTH from the adenohypophysis, from the kidney, and possibly from other tissues by competing for polypeptide binding sites. The pepsin labile factor may act in a similar fashion. That the release of ACTH is relatively specific is indicated by failure of oxytocin(6), glucagon (unpublished observation) and polymyxin B to deplete adrenal ascorbic acid in the lesioned rat.

Both the pepsin labile factor and vasopressin satisfy the classical criteria for endocrine roles. They are present in an area whose ablation results in an endocrine deficiency (failure of ACTH release in response to acute stimuli) and administration of either agent to the lesioned rat corrects the deficiency (release of ACTH). Demonstration of the pepsin labile factor at site of origin of the portal venous system lends support to the concept that a substance elaborated in the hypothalamus is carried *via a* vascular link to the adenohypophysis where it initiates ACTH release.

Summary. Ability of acid extracts of calf hypothalamus (stalk-median eminence area) to induce adrenal ascorbic acid depletion in the median eminence lesioned rat is attributable in large measure to a pepsin labile factor distinct from ACTH and from vasopressin.

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Species Differences in Cholesterol Biosynthesis by Arterial Tissue.* (24150)

DANIEL L. AZARNOFF†‡ (Introduced by G. L. Curran)
Department of Medicine, University of Kansas Medical Center, Kansas City, Kan.

The role of the artery in production or maintenance of atherosclerosis is unknown. Since the artery itself is the primary target of the disease, its own metabolic activity may be of great importance in formation of atheroma. Until recently the evidence available(1,2,3) seemed to indicate that the lipids found in atheromata are deposited directly from the plasma. However, studies with P^{32} indicate that at least part of the phospholipid moiety

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† U. S. Public Health Service Research Fellow of Nat. Heart Inst.

‡ Present Address: Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis, Mo.

is actually synthesized by arterial tissue *in situ*(4) and that this synthetic rate can be influenced by naturally occurring substances such as epinephrine(5) and cortisone(6). Reports utilizing radioactive tracer technics have indicated that aortas of rabbits(7,8), fowl(7,9), swine(10), and calves(11) are capable of incorporating C^{14}-labeled acetate into cholesterol. In only the latter 2 species, however, was the purification of isolated cholesterol adequately performed to eliminate all radioactive contaminants. Since cholesterol is the major sterol in atheromata, the incorporation of C^{14}-labeled acetate into cholesterol by arterial tissue is of interest. This process was studied in several different species of animals for the following reasons: 1) There is a well known variation of arterial response in different species to feeding high cholesterol diets. 2) Differences have been reported for content of various enzymes in coronary artery as compared to aorta and in atheromatous as compared to non-atheromatous areas of the coronary artery(12). 3) To date adequate cholesterol purification procedures in studies of arterial cholesterol biosynthesis were performed in only 2 species of animals. 4) The possibility of inhibiting arterial cholesterol synthesis, if there be such, by vanadium salts or other inhibitors of cholesterol biosynthesis has attractive therapeutic implications.

Materials and methods. Human aorta was obtained at autopsy within 8 hours of death or during great vessel surgery. It was immediately placed in iced buffer and taken to the laboratory where adherent fat was stripped from the adventitial surface. Two 0.5-1.0 mm thick slices were made from the intimal side with a Stadie-Riggs microtome. Approximately 3 g of tissue were placed in 20 ml phosphate buffer(13) containing 10 mg sodium acetate-1-C^{14} (0.5 mc/mM) and incubated for 2 hours at 37°C in an atmosphere of 95% oxygen and 5% CO_2. At the end of incubation period carrier cholesterol was added as indicated (Tables I and II). The entire contents of the incubation flask were made to a concentration of 70% ethanol and 5% potassium hydroxide and saponified under reflux for 2 hours. The non-saponifiable material was extracted with petroleum ether, (B.P. 30-60°C). The combined petroleum ether extracts were evaporated to dryness under a stream of nitrogen and the residue taken up in 90% ethanol. Cholesterol was precipitated from this solution as the digitonide. Cholesterol digitonide was washed with 90% ethanol, 40% ethanol – 60% ether, and ether successively. Radioactivity of the digitonide was determined and the cholesterol then purified *via* dibromide(10). The dibromocholesterol was repeatedly recrystallized from methanol to constant radioactivity. Radioactivity was determined using a thin end-window Geiger-Mueller tube. A sufficient number of counts were obtained so that 2 counts above background were reliably higher than ambient ionization counts at a level of confidence less than 0.01. When coronary artery was obtained, the easily accessible portions (usually proximal portion of right and left coronary arteries) were rapidly dissected free from adherent fat, opened along their long axes and incubated without preparing slices. Groups of the various species of adult animals were sacrificed by a blow on the head and the aorta from the aortic ring to the level of renal artery removed and chilled in cold buffer. The adventitial surface was stripped free of adherent fat and a pool of arteries (Table II) from the same species and sex, was incubated in a manner similar to that described above for coronary artery. Because of the relative thickness of dog aorta, slices of this tissue were prepared for incubation.

Results. Although human aorta and coronary artery are capable of incorporating acetate into a digitonin-precipitable substance (Table I), this material is not cholesterol, inasmuch as all radioactivity disappears from the cholesterol fraction with preparation of the dibromide. Synthesis of a digitonin-precipitable substance which was not cholesterol was also found by Schwenk and Stevens (14) when they incubated Yoshida ascites tumor cells with radioactive acetate. Increasing the incubation time to 5 hours, or addition to the incubating medium of di and triphosphopyridine nucleotides, adenosine triphos-

TABLE I. Incorporation of Acetate-1-C^{14} into Cholesterol by Arterial Tissue (Humans).

Aorta	Age	Sex	Cholesterol carrier, mg	Radioactivity* Cholesterol carbon		Cause of death
				Digitonide	Dibromide	
J.H.	50	♀	0	608	0	Obtained during surgery for insertion of Hufnagel valve
W.D.	15	♂	4	1690	0	*Idem*
T.R. (area mostly atheromata)	48	♂	0	229	0	Trauma
T.R. (area relatively free of atheromata)			0	368	0	"
H.S.	54	♀	0	204	0	Acute leukemia
E.S.	34	♂	2	195	0	*Idem*
A.M.	78	♂	0	201	0	Carcinoma of rectum
J.S.	25	♂	0	233	0	Rheumatic heart disease
W.T.	58	♂	0	404	0	Carcinoma of prostate (rec'd estrogen therapy)
C.R.	6 mo	♀	4	1870	0	Suffocation
Coronary artery						
E.S.	34	♂	8	100	0	Acute leukemia
A.C.	51	♀	4	140	0	Chronic myelogenous leukemia

* Radioactivity = counts/min. at infinite thickness × mg cholesterol carrier.

phate, or glucose singly or in combination, also did not induce conversion to cholesterol. The possibility exists that conversion of acetate to cholesterol does not take place when autopsy material is used because of inactivation of a necessary enzyme following death. However, the fact that aorta obtained during surgery behaved in a similar manner mitigates against an enzyme inactivation as the explanation. Also it has been shown by Maier and Haimovici(15) that at least the oxidative capacity of dog and rabbit aorta does not change appreciably when tissue from freshly sacrificed animals is compared to that from animals kept under conditions simulating those of humans from the time of death until autopsies are performed. The apparent inability of human aorta and coronary artery to incorporate acetate into cholesterol adds further corroboratory evidence that cholesterol found in atheroma in humans is not synthesized *in situ* but rather is deposited from plasma cholesterol.

A similar inability to incorporate acetate into cholesterol is seen in the aortic tissue of dogs, cats, and rats (Table II). This is in agreement with studies of Gould(16) on dog aorta. Although the possibility exists that addition of carrier cholesterol could dilute out a minimal amount of radioactivity, it seems unlikely. The data in Table II, however, are in disagreement with the results of

TABLE II. Studies on Incorporation of Acetate-1-C^{14} into Cholesterol by Arterial Tissue (Experimental Animals).

Aorta	Sex	Carrier cholesterol, mg	No. of animals pooled	Radioactivity* Cholesterol carbon	
				Digitonide	Dibromide
Dog	♀	0	1	590	0
	♀	2	1	620	0
	♂	2	1	216	0
Chicken	♂	4	4	632	440
	♀	4	4	1840	829
Rabbit	♂	4	3	1150	576
	♀	4	3	1400	936
Guinea pig	♂	8	7	600	257
	♂	4	7	450	226
	♀	8	7	2280	514
	♀	4	7	926	732
Rat	♂	8	10	1480	0
	♀	8	10	1970	0
Cat	♀	4	4	316	0

* Radioactivity = counts/min. at infinite thickness × mg cholesterol carrier.

Ranney and Weiss(17), who report increase in rate of "cholesterol synthesis" of rat aorta following administration of α-p-biphenylyl butyric acid. Cholesterol in the investigations of Ranney and Weiss was assayed for radioactivity as the total non-saponifiable fraction. This fraction contains several other sterols in addition to cholesterol(10). The importance of employing cholesterol purification procedures when one wishes to measure "cholesterol synthesis" is further borne out by our unpublished findings that even plasma itself is capable of converting C^{14}-labeled acetate to a digitonin-precipitable substance which is not cholesterol. Further, experiments reported here were carried out *in vitro* to eliminate the possibility of extra-arterial cholesterol synthesis in the intact animal providing preformed labeled cholesterol which could then be deposited in the aorta.

From the literature and data in Tables I and II, various species of animals can be divided into 2 groups based upon aortic cholesterol metabolism. Group I comprises those animals whose aortas *can* incorporate acetate into cholesterol and includes rabbits, chickens, guinea pigs, swine(10), and calves(11). Group II comprises those animals whose aortas *cannot* incorporate acetate into cholesterol and would include humans, dogs, rats, and cats. Two other characteristics of the grouping of these species appear evident. Animals in Group I consume primarily a herbivorous type diet and can be made atherosclerotic with relative ease by feeding a high cholesterol diet while animals in Group II are carnivores and omnivores and it is difficult, if not impossible, to produce atherosclerosis in these animals by high cholesterol intake alone. At present the significance of this difference is not evident and no satisfactory explanation is available for the observed differences. Preliminary attempts to characterize the radioactive digitonin-precipitable substance synthesized by aortas of Group II animals have been unsuccessful.

Summary. Coronary arteries and aortas of humans and aortas of dogs, cats, and rats cannot incorporate C^{14}-labeled acetate into cholesterol *in vitro*. Aortas of guinea pigs, rabbits, and chickens can accomplish this conversion.

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