Age Changes in Composition of Aorta of the Rabbit.* (22706)

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(Introduced by G. L. Duff)
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The important disabling and lethal vascular changes of ageing in man include those of atherosclerosis, calcification and hypertension. There has been considerable research, in man and in animals, on dietary induction of these conditions, and on alterations in blood chemistry that accompany their development (1). There are also reports on chemical alterations in vessels affected by atherosclerosis (2), calcification(3) and hypertension(4). However, there has been relatively little reference to age changes in chemical composition of vessels *not* affected by one or more of these conditions. Study of this aspect of vascular disease seems important, since the vessels of the young are relatively free of these damaging conditions. The present study was undertaken to determine some of the chemical phenomena of vascular ageing in the hope that the results might indicate some local factors that might render the vessels of the old more susceptible, if indeed they are, than those of the young. In view also of the rarity in ageing rabbits of spontaneous vascular disease equivalent to that in man, it was hoped that comparison of the findings in these two species might yield information bearing on the pathogenesis of the diseases noted above. It has been reported that in man the water content of the aortic wall either remains constant(3,5) or rises slightly(6) with increasing age. This is accompanied by progressive elevation of the total lipid(3,5) and of cholesterol(2,7). Calcium(3,5,8,9) and phosphorus (3) have also been reported as reaching higher concentrations in the human aorta with advancing age. Data on the sodium and potassium content of the human aorta have not come to our notice.

There appear to be no comparable data on age changes in the chemical composition of the rabbit aorta. Our preliminary studies showed that in ageing rabbits there is a significant and progressive rise in the water content of the aorta, associated with an even more striking fall in the quantity of combined aqueous and fat-soluble extractibles, (unpublished data). The present study was undertaken to confirm and amplify this observation.

Materials and methods. Forty-four female and 7 male New Zealand white rabbits were used. Because an adequate number of old males could not be obtained, the results tabulated and tested statistically are for the females only. There were 27 young animals, aged 2.5 to 3.5 months, and 24 old ones of 37 to 55 months. All were apparently healthy. Samples of blood were collected from a few animals (via the ear artery) several weeks before killing, and also from some immediately before killing. Some were subjected to blood pressure determinations by the non-traumatic method of Grant and Rothschild (10). All were fed Ogilvie Miracle Rabbit Pellets® and tap water *ad libitum*. The average period of acclimatization in our animal quarters after delivery from the dealer was one month. The environment was at normal indoor or "room temperature." The animals were killed by a blow on the back of the neck, and autopsies done immediately. The aorta from arch to renal arteries was removed, opened longitudinally, blotted free of gross blood with filter paper, and the adventitial fat dissected off as completely as possible. The specimen was transferred to a stoppered weighing bottle, placed in a desiccator, and weighed to 0.1 mg. The tissue was then lyophilized and the dry weight obtained. In transfer procedures the aorta was handled with forceps only. Sodium, potassium and calcium concentrations were determined using aortas from 18 young rabbits (all female) and 16 old ones (12 females, 4 males). Samples were pooled in 3's from young, and in pairs

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TABLE I. Age Changes in Aortic Water of Rabbit.

No. of animals	Avg age, mo	Water, %
25	3	62.9 ± .4
19	46	65.4 ± .3

from old animals, to provide quantities large enough for accurate measurement. The pooled samples were ashed with nitric and perchloric acids. Sodium and potassium were determined by flame photometry, and calcium by the microadaptation of the Clark-Collip method(11). Data from female samples are designated in Table II as Group 1-a and 2-a. The aortas of the remaining 9 young (7 females, 2 males) and 8 old (7 female, 1 male) rabbits were used for determinations of total lipid, cholesterol and non-lipid phosphorus. These vessels were also tested for carbohydrates, reducing sugars and sulfur. Data from females are listed as groups 1-b and 2-b, Table III. After freeze-drying, the specimens were extracted in a Soxhlet apparatus, using 15 cc ethanol for a period of 24 hours, followed by cold ethyl ether overnight. The fat-free weight of the sample was recorded and the aorta was extracted in 15 cc double distilled water for an additional 24 hours. Following this the sample was desiccator-dried and weighed. The alcohol-ether extracts were evaporated and the residue taken up in petroleum ether. Total cholesterol content of this sample was then estimated by the Liebermann-Burchard reaction (12). The water extract was combined with the residue remaining after purification of the alcohol-ether extract and was tested for traces of carbohydrate, reducing sugars and sulfur. Molisch's reagent was used for carbohydrate, Benedict's quantitative reagent for reducing sugars and barium chloride solution for sulfur(13). The phosphorus content of the aqueous extract was estimated on duplicate aliquots using the Fiske-Subbarow technic (14).

Results. The qualitative tests for carbohydrate, reducing sugars and sulfur yielded negative results. Table I shows the mean values for water content of the aortas of 25 young and 19 older female animals. The aortas of the older animals contain an average of 2.5% more water than those of young animals, a small but highly significant difference ($p < .001$). Because of this change in water concentration, other values are reported per unit of dry weight.

In Table II are shown the aortic sodium, potassium and calcium concentrations. The potassium content remains unchanged, while there is a moderate but significant increment of sodium, and the calcium concentration is approximately doubled in the older animals ($p < .001$ for both Na and Ca). These changes are more marked than the increase in water concentration, and are significant even when related to the wet weight.

Table III compares the aortic content of lipid, cholesterol and phosphorus in the young and old animals. The results indicate a slightly but not significantly lower total lipid, and moderately higher levels of cholesterol ($p < .05$) and of phosphorus ($p < .02$) in the aortas of the older animals.

Discussion. These results confirm our pre-

TABLE II. Age Changes in Sodium, Potassium and Calcium of Aorta of Rabbit.

Group	No. of pooled samples	Age, mo	Na	K	Ca
			(mEq/100 g dry wt)		
1-a	6 (18)*	3	22.4 ± .4	11.3 ± .4	98 ± 9
2-a	6 (12)	46	28.6 ± 1.2	11.5 ± .3	219 ± 15

* Figures in parentheses show No. of aortas used.

TABLE III. Age Changes in Lipid, Cholesterol and Non-Lipid Phosphorus of Aorta of Rabbit.

Groups	No. of animals	Age, mo	Lipid, % dry wt	Cholesterol	Non-lipid phosphorus
				mg/100 g dry wt	
1-b	7	3	16.3 ± 1.7	463 ± 37	158 ± 12
2-b	7	45	13.7 ± 1.3	558 ± 22	210 ± 15

liminary finding of a higher aortic water concentration in old than in young rabbits although the increase found in this study, using freeze-drying, is less marked than that found previously by oven-drying at 110°C. Comparable studies in man have shown that aortic water either remains constant(3,5) with increasing age, or increases(6).

The rise in water content of the aorta associated with a rise in the sodium but constant potassium level might indicate that all or most of the added water was extracellular, *i.e.* that the aged aorta had become relatively oedematous. While this may well be so, there is some evidence to indicate that the change may be more complex. Other studies(15) have indicated age changes in the concentrations of sodium and potassium in rabbit erythrocytes, characterized by progressive replacement of intracellular potassium by sodium. If the same should be true of the cells of the aortic wall, then the extra water and sodium in the aortas of old animals could be both intra- and extracellular. Elevation of aortic water, sodium and potassium was found by Tobian and Binion(16) in hypertensive rats. The relation of the sodium increase to blood pressure in our animals is not clear. In general, adult rabbits have shown higher pressures than animals comparable in age to those of the present young group. However, other rabbits with a marked elevation of blood pressure due to bilateral renal compression did not have significantly altered aortic water, sodium or potassium concentration. A similar discrepancy was found for red cell electrolytes(15).

In the case of the other mineral constituents studied, calcium and phosphorus, our findings are in general agreement with those reported by others in man(3,5,8,9). In spite of the fact that in none of the aortas examined was there the slightest evidence of gross calcification, analysis showed a 100% increase of calcium in the older aortas. Phosphorus was increased by slightly more than 20%. The increment of phosphorus in the older animals is in about the same ratio to that of calcium as was found in man by Buck (3), *i.e.* 0.36:1. The calcium and phosphorus concentrations in the rabbit aorta were both considerably lower than Buck's lowest human values. This is curious because serum calcium levels in the rabbit are normally nearly double those in man(17).

A further difference between age changes in chemical constituents in the rabbit aorta and those in man is seen in the concentrations of total lipid and of cholesterol. For both these constituents the concentrations in the rabbit aorta are much lower than those reported in man(3,5,7). In addition, while the concentration of total lipid rises progressively with advancing age in man, our findings in the rabbit indicate no such increase, though there is an increase of doubtful significance ($p<.05$) in aortic cholesterol. It is worth noting that the only old male whose aortic cholesterol and total lipid were determined had values for both which were lower than those of any of the 7 old females studied.

In the interpretation of any age alterations in the rabbit one is handicapped by the lack of precise data on the natural longevity of rabbits as noted by Leary(18). On the basis of available information we have chosen to consider the two groups of animals in the present study as representing approximately childhood and middle-age.

Summary. A study of the aortas of 44 female rabbits in 2 age groups (3 mos. and 46 mos.) corresponding approximately to human childhood and middleage, indicates elevation of the water, sodium, calcium, non-lipid phosphorus, and possibly of the cholesterol content of the vessel wall in the older group. The potassium content remains relatively constant. The generally lower levels of total lipid and of cholesterol in the rabbit aorta compared with that of man probably account at least in part for the freedom of the former from spontaneous atherosclerosis.

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Serial Propagation of 3 Strains of Rabbit Fibroblasts; Their Susceptibility to Infection with Vaccinia Virus.* (22707)

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Tissue culture technics have been employed frequently for the propagation of viruses *in vitro*(1,2). There have been, however relatively few studies that attempt to elucidate in a simplified system which is potentially provided by tissue culture many fundamental problems regarding virus multiplication. To approach problems of this type in a definitive manner, it is necessary to have a continuous supply of uniform experimental material such as is provided by cultures of mammalian cells which can be propagated serially under controlled conditions. Cultures derived from different sources may vary considerably in their viral range and in their response to viral infection in a fashion analogous to laboratory animals. This indicates the need for cultures of more than one cell type as well as cells derived from a variety of animals. These studies were undertaken to investigate the possibility of establishing *in vitro*, strains of fibroblasts derived from rabbit tissues and to compare their relative susceptibilities to infection with vaccinia virus.

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TABLE I. Composition of Cell Culture Media.

Medium	Composition* (V/V) %	%	%
CF	5 CEE	10 NHS	85 V-614
F7		10	90
F15	5 BEE	10	85
F17	10 CEE	20	70
F21	5	20	75
705	5	20	75 703

* The following abbreviations are employed: CEE = chick embryo extract; BEE = beef embryo extract; NHS = normal horse serum; V-614 = solution of Fischer *et al.*(5); 703 = solution 703 of Healy *et al.*(6).

Materials and methods. Media. The composition of the media employed for propagating cells is indicated in Table I. All media were sterilized by filtration through Selas filters of 02 porosity. Chick embryo extract (CEE) and beef embryo extract (BEE) were prepared as described previously(3). *Establishing cell cultures on glass.* Cell cultures were initiated in roller tubes according to the method of Swim and Parker(4). When growth in the roller tubes was well established, the cells were dislodged from the glass by treating with 0.02% trypsin (Nutritional Biochemicals Corp., 2 x crystallized—50% Mg SO_4) in V-614 solution(5) or in solution 703 (6), pH 7.9-8.3, for 10 minutes at 37°C. The