Effect of "Short-term" Hypertension on the Uptake of C¹⁴-Cholesterol by the Aortic Wall of the Rat

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SUMMARY

To investigate the effect of blood pressure *itself* on lipid accumulation in the aortic wall, we have studied the effect of "short-term" (about 2 weeks) hypertension on the uptake of cholesterol-4- C¹⁴ by the aortic wall in normo-cholesterolemic female rats. Renal hypertension was produced by partial constriction of a left renal artery. The uptake of C¹⁴-cholesterol by the aortic wall of the rat was expressed in terms of the total and the specific activity of cholesterol and the ratio of the total activity of cholesterol in the aortic wall to the specific activity of cholesterol in the serum. The results indicate that cholesterol uptake by the aortic wall was not accelerated by the existence of hypertension. These results are discussed with respect to the effect of hypertension *itself* on atherogenesis.

Additional Indexing Words:

Filtration theory   Renal hypertension   Atherogenesis α -Lipoprotein
 β -Lipoprotein   Injurious effect on the arterial wall

MANY epidemiological as well as experimental studies have suggested that hypertension has an aggravating effect on the process of atherosclerosis.^{1), 2)} This effect of hypertension has been explained by and taken as the support of the "Filtration Theory,"^{2), 3)} which is widely accepted as the pathogenesis of atherosclerosis. Namely, it is believed that increased blood pressure acts on lipoproteins in the circulation in such a way that the amount of lipoprotein infiltrating the arterial wall increases. However, the real cause of the effect of hypertension on atherosclerosis is yet unsettled.¹⁾ Hypertension may cause first some damage to the intima which leads to accelerated lipid infiltration and lipid accumulation. To investigate the effect of blood pressure *itself* on lipid accumulation in the aortic wall, we have studied the effect of "short-term" hypertension on the uptake of C¹⁴-cholesterol by the aortic wall in normo-cholesterolemic rats.

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METHODS

Wistar female rats were used in this experiment. They were maintained in an air-conditioned animal room on Purina chow for a month before and during the experiment.

Renal hypertension was produced in 10 rats under Pentobarbital anesthesia by partial constriction of a left renal artery by the method of Wilson and Byrom.⁴⁾

Four weeks after the operation, arterial blood pressure was measured in unanesthetized rats by a modified Gallagher and Grimwood microphonic method described by Ito, Amano and Kasahara.⁵⁾

The cholesterol-4-C¹⁴ solution was made by a slight modification of Siperstein and Chaikoff's method.⁶⁾ C¹⁴-cholesterol was first dissolved in a small amount of warm absolute ethanol and to this solution distilled water containing a few drops of Tween 20 was added and made up to the desired volume. By this method, according to our experience, a clear aqueous C¹⁴-cholesterol solution could be made more easily and required less Tween 20.

Under light ether anesthesia, 5 control and 5 hypertensive rats were weighed and each injected with 0.5 ml. of cholesterol-4-C¹⁴ solution (ca. 5 μ C.) per 100 Gm. of body weight into a right jugular vein. Eight hours after the injection they were sacrificed. A blood sample was drawn by heart puncture from each rat under ether anesthesia and the heart, together with the thoracic and abdominal aorta, was removed. After opening heart and aorta, we cut the aorta free of the heart at the base. The aorta was rinsed rapidly in a 0.9% saline solution to remove apparent blood and blood clots and blotted lightly on a filter paper. It was weighed quickly on a torsion balance and homogenized in chloroform-methanol (2:1) using a Potter-Elvehjem glass homogenizer. The heart was weighed and the heart weight to body weight ratio was calculated.

Serum lipids were extracted with chloroform-methanol (2:1) at room temperature. The chloroform-methanol extract was evaporated in a water bath (40–50°C) under a N₂ atmosphere, and the lipids were re-extracted with chloroform. An aliquot of chloroform extract was applied to a planchet to make an infinitely thin sample.⁷⁾ Radioactivity was determined by a windowless 2 π gas flow counter.

Table I. Comparison of Body Weights, Heart-Body Weight Ratios, and C¹⁴-

	Body Weight (Gm.)	Heart-Body Weight Ratio (mg./Gm.)	Aorta	
			Cholesterol Concentration (%)	CPM/mg. of Aorta
Hypertensive Group ¹	97 $\pm$ 22	5.34 $\pm$ 0.958	0.35 $\pm$ 0.063	3.99 $\pm$ 0.563
Control Group ¹	133 $\pm$ 11	3.42 $\pm$ 0.321	0.38 $\pm$ 0.089	6.00 $\pm$ 0.936
Statistical Significance Test*	P=0.02	0.01>P>0.001	N.S.**	0.01>P>0.001

¹ Mean $\pm$ S.D.

² Significance of this ratio: See the text

* Student "t" test

** non-significant

An aliquot of the chloroform extract was evaporated to dryness and this sample was used for total cholesterol determination by a $\text{FeCl}_3\text{-H}_2\text{SO}_4$ method.⁸⁾

RESULTS AND DISCUSSION

The blood pressure of operated rats ranged from 63 to 259 (average: 155) mm.Hg. In our previous measurements of blood pressure in 195 normal rats, blood pressure ranged from 64 to 118 and never exceeded 120.⁹⁾ However, because of widely accepted values in the literature,^{4),10)} we chose to define a hypertensive rat as one that had a pressure greater than 140 mm.Hg. In these studies we have used 5 hypertensive rats paired with 5 control rats which is a sufficient number to give statistical validity to any differences observed.

The average blood pressure of hypertensive rats 2 days before sacrifice was 202 mm.Hg with a range of 149 to 259. The hypertensive rats gained significantly less weight after the operation than the control rats (Table I), although the initial body weights of the two groups were, on the average, the same (ca. 80 Gm.).

The heart weight to body weight ratio was significantly higher in the hypertensive rats, supporting the existence of hypertension¹⁰⁾ (Table I).

According to our previous experience, hypertension developed within 2 weeks after the operation. Therefore, our rats were probably hypertensive for about 2 weeks before these studies.

Serum cholesterol levels were slightly higher, though statistically not significant, in the hypertensive group, and this was reflected in the slightly but significantly lower average specific activity of serum cholesterol in that group (Table I). The total radioactivity (cpm./ml. serum) was identical between both groups (Table I).

Cholesterol in the Serum and Aortic Walls Between Hypertensive and Control Rats

CPM/ $\mu\text{g.}$ - Cholesterol	Serum			Ratio ²
	Cholesterol Concentration (mg./100 ml.)	CPM/ml. of Serum	CPM/ $\mu\text{g.}$ - Cholesterol	CPM/mg. of Aorta CPM/ $\mu\text{g.}$ -Chol. in Serum
1.18 $\pm$ 0.516	116 $\pm$ 20.1	10,388 $\pm$ 2,777	9.08 $\pm$ 2.174	0.47 $\pm$ 0.160
1.66 $\pm$ 0.403	94 $\pm$ 13.7	10,989 $\pm$ 953	11.88 $\pm$ 1.038	0.51 $\pm$ 0.087
N.S.	N.S.	N.S.	0.05>P>0.02	N.S.

Newman and Zilversmit used the average of 3 determinations (22, 29 and 48 hours) for calculating the specific activity of serum cholesterol after feeding of C^{14} -cholesterol in rabbits. Since our animals were injected with only a small amount of C^{14} , it was decided to determine the cholesterol specific activity at a single early time point; namely, 8 hours after injection. Multiple blood samples were not taken to avoid stressing the rats, which might influence blood pressure. Determination of serum cholesterol specific activity at a single time point would not cause a large error in the estimation of the influx of cholesterol into the aorta judging from the data of Newman and Zilversmit.¹¹⁾

The concentration and specific activity of cholesterol in the aortic wall were essentially the same between the hypertensive and control groups (Table I). However, the total activity of cholesterol in the aortic wall was significantly higher in the control group, which may be due to the higher specific activity of serum cholesterol in this group. The ratio of the total activity of cholesterol in the aortic wall (cpm./mg. aorta) to the specific activity of cholesterol in the serum (cpm./ μ g.-cholesterol) was identical between both groups. This ratio would express a real influx of cholesterol into the aortic wall from the circulation if one could assume no efflux from the wall. Efflux during an 8-hour experimental period would probably not be significant.¹²⁾ The release of cholesterol *in vivo* from the normal rat aortic wall has been estimated to be about 24% per 24 hours.¹³⁾

If hypertension itself acts on serum lipoproteins by accelerating their filtration into the aortic wall as is suggested by the "Filtration Theory",²⁾ one might expect an increase in the activity of C^{14} -cholesterol in the aortic wall (cpm./mg. aorta) or at least in the ratio of the total activity in the aortic wall (cpm./mg. aorta) to the specific activity in the serum (cpm./ μ g.-cholesterol). However, as mentioned above and shown in Table I, these relationships in the hypertensive group are identical to or rather lower than those in the control group. These results could not be explained by the prevailing "Filtration Theory". Several authors have shown an increase in atherogenicity by hypertension in cholesterol-fed experimental animals and birds.^{2), 14)–17)} However, their results were based on the effect of "long-term" hypertension in cholesterol-fed animals and birds. Moreover, the clinical and epidemiological studies on the effect of hypertension on atherosclerosis were all carried out on patients having chronic hypertension.^{18)–20)} Therefore, from these experimental and clinical observations, it would be very difficult to get information about the effect of hypertension *itself* on the filtration of lipid into the aortic wall from the circulation separate from the other effects of hypertension such as the injurious effect on the aortic wall.

In the human it is well known that cholesterol is carried mainly by the

β -lipoproteins; however, in normal rats most of the cholesterol is carried in the α -lipoprotein fraction. Therefore, in our experiments intravenously injected C^{14} -cholesterol might have been carried mostly by α -lipoprotein. In fact, in the serum one hour after intravenous injection of cholesterol-4- C^{14} , the ratio of the specific activity (cpm./ μ g.-cholesterol) in α - to β -lipoprotein was 1.56. From the viewpoint of atherogenesis, α -lipoproteins have not been regarded as being atherogenic, although there seems to be no definite proof of this point. However, in terms of "filtration" into aortic walls, the "Filtration Theory" does not distinguish between these lipoprotein classes. The theory states that the β -lipoprotein is atherogenic because it is probably the chief carrier in plasma of unstably held lipid which would be shed within the vessel as the filtered lipoprotein breaks down;³⁾ α -lipoprotein is thought to pass more easily through the vessel wall due to its smaller size.²⁾ Therefore, regardless which lipoprotein was predominantly labeled in our experiments, this experimental approach may be valid in testing the effect of hypertension on lipid filtration into the aortic wall.

The exact mechanism of cholesterol uptake is still unknown. The observed uptake of C^{14} -cholesterol could perhaps represent an exchange process. If this is the case in our experiments, it may be not surprising if hypertension did not affect the uptake of C^{14} -cholesterol by the aortic wall. It would be very difficult to distinguish between an exchange process and a filtration process in this kind of experiment.

According to Newman and Zilvermit, influx of cholesterol into atheroma *in vitro* under conditions where no filtration pressure is exerted is almost the same as is found *in vivo*.²¹⁾ Their observations would coincide very well with our results. Therefore, it would appear that hypertension aggravates the atherogenic process through its injurious effect on the arterial wall and not through an increased pressure on lipid particles in the circulation.

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