THE EFFECT OF ARTERIAL OCCLUSION IN ATHEROMA—AN EXPERIMENTAL STUDY

C. J. MIENY, M.B., CH.B. (PRET.), F.C.S. (S.A.), F.R.C.S. (ENG. AND EDIN.), *Department of Surgery, University of the Witwatersrand*

Arterial reconstruction for atherosclerosis obliterans has gained general acceptance in the last 15 years. Because of the segmental nature of the disease in the arteries to the extremities, high hopes were entertained earlier that disobliteration, replacement-grafting or bypass-grafting procedures would restore bloodflow to ischaemic limbs. Unfortunately this has not been borne out by experience in a significant number of cases.

The late patency rates after reconstructive surgery followed up for at least 1 year, differed strikingly in the various clinics. In aorto-iliac artery reconstruction the failure rate at 1 year was fairly constant at about 35%.[1,2] In ilio-femoral disease, the closure rate at 1 year varies between 40% and 70%.[3-7]

Causes of Late Failure

Attention was urgently turned to a study of the possible causes of late failure. These can be divided into 2 broad sub-groups:

(*a*) Those failures which can be blamed on technical errors at operation or shortcomings in the materials used for grafting.

(*b*) Factors inherent in the diseased vascular system of the patient. These may be of haemodynamic origin, viz. deficient head of pressure or deficient blood flow-off, or progression of the latent disease process in the arterial walls.

Crawford *et al.*[4] considered that 57% of their failures after operation on femoro-popliteal occlusion could be blamed on technical errors, and 43% on some aspect of the underlying disease. They report that 66% of re-occlusions following surgery occurred in the arterial segment distal to the reconstruction.

Warren *et al.*[3] found that re-occlusions or progression of the disease after reconstruction in ilio-femoral atheroma in 29 limbs occurred in 10 cases followed-up for at least 2 years. Nine of these 10 re-occlusions were distal to the reconstructed segment, while in 13 limbs with femoral artery blocks observed arteriographically without surgery for at least 1 year, no lesions were seen to develop in the popliteal artery or its branches below the block.

In a further communication Warren *et al.*[8] reported a study which comprised 23 limbs followed up arteriographically over a mean span of 4·5 years, without using surgery. In only 2 cases was there evidence of progression in the segment below the block, despite advanced disease of the popliteal branches in half the cases at the start of the survey, whereas 18 different episodes of progression were noted in the upper segment proximal to the block. The theory of a protective effect of a block on the distal segment has been entertained by some authors.[3, 8-10]

It is generally accepted that hypertension in conjunction with probably a number of other metabolic factors contributes to the deposition of atheroma in man and the experimental animal. Is it possible that the converse, i.e. a lower than normal pressure, protects the arterial wall against the disease?

MATERIALS AND METHODS

40 New Zealand white rabbits of both sexes, weighing between 2½ lb. and 7 lb. were used in the experiment. The animals were fed on a diet to which 1 G of cholesterol per rabbit per day had been added. This was done by mixing chow with cholesterol dissolved in ether. The ether was allowed to evaporate leaving a thin layer of cholesterol, coating the rabbit chow. With very few exceptions the animals took readily to this diet.

Immediately after the diet was started, the right common iliac artery was ligated. This was done through a transperitoneal approach under intravenous 'sagatol' anaesthesia.

In 12 rabbits blood-cholesterol estimations were done at monthly intervals (Table IV). The animals either died or were sacrificed at various time intervals from 15 days to 170 days.

In 18 rabbits intra-arterial blood pressures were recorded at the time of sacrifice (Table III): Catheters were placed in the

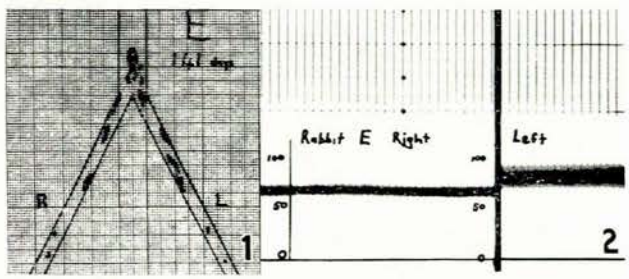

Fig. 1. Semi-schematic drawing showing the macroscopical extent of the disease on the normal left side, and on the right side distal to an occlusion.
Fig. 2. The systolic blood pressure distal to the occlusion is equal to the diastolic pressure on the normal side. The pulse pressure on the normal side is almost twice that on the occluded side.

patent left femoral artery as well as in the right femoral artery distal to the occlusion. The pressures were measured by means of strain-gauge manometers (Fig. 2).

At postmortem examination the aorta and ilio-femoral arterial tree was removed and pinned to a board, after the arteries had been laid open. A macroscopical assessment of the disease was then made in the normal left artery and distal to the occlusion on the right side. Semi-schematic drawings were made on graph paper to assess the quantitative difference in the extent of the disease on the 2 sides (Fig. 1).

The arteries were then studied histologically. In the early lesions, frozen sections were cut and stained with oil Red O to detect early deposits of lipids in the intima. With this method lipids stain a bright red.

In the well-established lesions the arteries were rolled lengthwise as described by Stein.[11] Sections were then cut transversely and stained with haematoxylin and eosin. In this way the whole length of the artery could be scanned in a few sections cut at different levels.

The degree of disease present histologically on the 2 sides was then compared and finally coordinated with the macroscopical assessment (Figs. 3 and 4).

RESULTS

The results are presented in Tables I to IV.

The earliest lesions were seen at 45 days in the aorta and at 64 days in the iliac vessels. The lesions were graded according to their macroscopical extent: *Grade 1*, when only a single plaque was present in the vessel; *Grade 2*, when more than one plaque was present; *Grade 3*, when there were multiple plaques present; and *Grade 4*, when the plaques were coalescing and covering considerable areas of intima.

There was disease present in the ilio-femoral arteries in 5 of the 16 rabbits sacrificed within 3 months. In 2 rabbits the

disease was present on the left side only and in 3 rabbits there was disease present on both sides. It would thus seem that an occlusion of the artery did not significantly delay the onset of the disease distal to the occlusion.

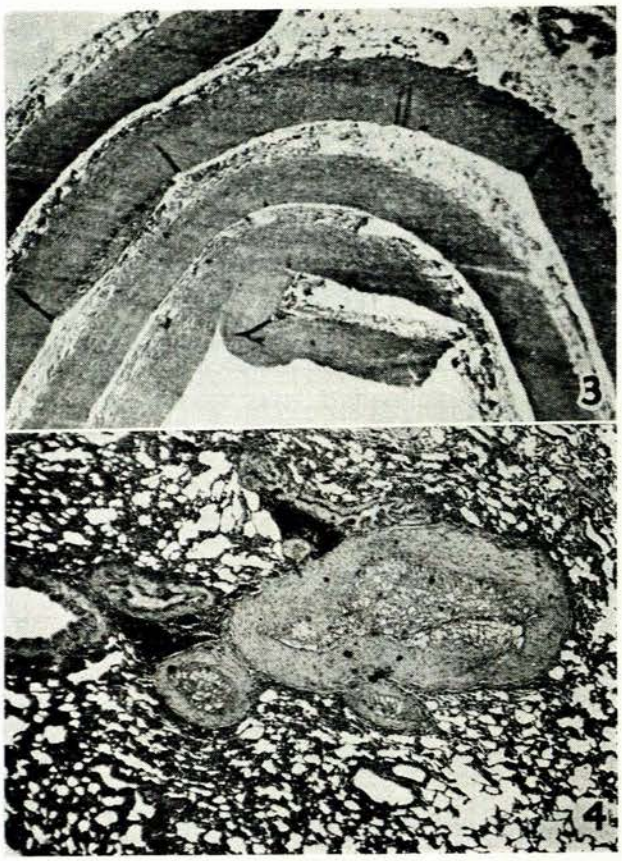

Fig. 3. An example of a histological section of a rolled ilio-femoral artery. Note the plaque of atheroma between the 2 vertical lines.
Fig. 4. Section of a pulmonary blood vessel shows the lumen almost completely obliterated by atheroma.

TABLE I. GRADE OF DISEASE

Rabbit	*Time/days*	*Aorta*	*Left*	*Right*
1	64	2	—	—
2	64	2	1	—
3	24	—	—	—
4	35	—	—	—
5	15	—	—	—
6	15	—	—	—
7	78	2	1	—
9	45	1	—	—
10	52	2	—	—
13	Died at op.	—	—	—
16	68	2	1	1
18	62	2	—	—
20	Died at op.	—	—	—
21	70	2	1	1
22	58	1	—	—
28	83	3	2	2

In Table II are presented those rabbits with well-established disease in the distal vessels. In the last column of the Table a combined assessment of the macroscopical lesions and the microscopical appearances is given. The disease predominated on the right or 'protected' side on 8 occasions and on the left or 'unprotected' side on 10 occasions. No discernible difference in the extent of the disease on the 2 sides could be noted in 5 rabbits.

TABLE II. GRADE OF DISEASE

Rabbit	*Time/days*	*Aorta*	*Left*	*Right*	*Final assessment*
8	108	3	2	2	L
11	95	3	2	2	R
12	150	4	2	2	R
14	170	4	3	3	R
15	110	4	2	2	L
17	110	4	2	2	L
19	91	4	2	2	L
23	94	4	2	2	E
24	153	4	3	3	R
25	153	4	3	3	R
26	150	3	2	2	L
27	121	4	2	2	L
A	100	3	2	2	E
B	100	3	2	2	R
C	101	3	2	2	L
D	140	3	3	3	L
E	141	3	2	2	E
F	141	—	—	—	—
G	133	3	2	2	E
H	133	3	2	2	L
I	133	3	2	2	R
J	143	3	2	2	E
K	143	3	2	2	R
L	143	3	3	3	L

TABLE III. INTRA-ARTERIAL BLOOD PRESSURES

Rabbit	*Time since occlusion*	*Left*		*Right*	
		Systolic mm.Hg	*Diastolic mm.Hg*	*Systolic mm.Hg*	*Diastolic mm.Hg*
14	170	96	74	70	58
23	94	88	70	60	52
24	153	94	72	58	50
25	153	86	64	62	46
26	150	86	70	60	54
28	83	82	58	64	50
A	100	110	80	70	60
B	100	100	80	76	66
C	101	110	74	70	56
D	140	106	86	88	76
E	141	96	84	84	78
F	141	80	56	40	34
G	133	100	74	80	70
H	133	76	62	66	56
I	133	120	80	88	70
J	143	86	70	66	50
K	143	90	72	74	54
L	143	92	74	70	52

TABLE IV. BLOOD CHOLESTEROL LEVELS

Rabbit	*Time since occlusion, days*	*1st month pre-diet*	*2nd month*	*3rd month*
A	100	70	960	1000
B	100	80	1260	800
C	101	84	1190	1240
D	140	105	1310	1480
E	141	68	600	520
F	141	53	130	270
G	133	28	500	650
H	133	32	350	900
I	133	41	800	1200
J	143	25	450	700
K	143	36	1100	800
L	143	22	850	1300

An occlusion in the artery did not protect the distal arterial tree from progression of the disease.

In Table III the BP in the artery distal to the occlusion remained persistently lower than the systemic blood pressure.

The blood cholesterol rose sharply on the diet, to more than 1,000 mg./100 ml. in many instances, as depicted in Table IV. Rabbit F failed to take the diet adequately and her blood

cholesterol remained below 300 mg./100 ml. even in the third month. This rabbit did not develop the disease.

DISCUSSION

Since the pioneering experiments of Anitchow[12] in 1913 in which he created atheroma in rabbit arteries by feeding them a diet with a high cholesterol content, this animal has been used extensively in the study of atheroma. Some authors[13, 14] have pointed out the difference between the lesions as seen in the rabbit and in man. The most important difference is the site of occurrence of the plaques, which form mainly in the abdominal aorta in man and in the thoracic aorta in the rabbit. The complications of ulceration and thrombosis are not seen in the rabbit. The individual lesions, however, present striking similarities both macroscopically and microscopically.[15, 16] It is probable that if the experimental conditions that elicit the lesions in the rabbit were less forced, these lesions would resemble the human disease even more closely.

Blood Pressure and Atheroma

There is clinical evidence indicating that hypertension accelerates the onset and accentuates the progress of atherosclerosis in man.[17-20] Conversely it is interesting to note that individuals with hypotension are much less apt to develop atherosclerosis than those with average normal levels of BP. The study of Hunter[21] in 1939 showed that with a BP 20 points less than average, the life expectancy is decidedly better than that of the average population, the actual mortality in this group being only 71% of the expected, with many fewer deaths resulting from cardiovascular disease.

It has also been shown in the experimental animal that hypertension accentuates atheroma induced by cholesterol feeding.[22-24]

There are 2 possible ways in which BP may play a role in the pathogenesis of atheroma.

1. *Intravascular Filtration Pressure*

Whereas the filtration pressure in all other tissues in the body is the relatively low capillary BP, in the arteries the arterial BP, which is of course many times the magnitude of the capillary pressure, determines in large part the movements of fluid across the endothelial intimal membrane into the subendothelial tissue spaces.[18, 25, 26] The penetration of substances such as cholesterol may be increased by virtue of such high filtering pressure. Indeed, it has been shown that in the presence of hypertension the cholesterol content of the wall of the aorta is significantly increased although the serum-cholesterol concentration is not elevated.[19, 20]

Wilens[27] has shown experimentally in an arterial preparation that the rate of filtration of serum cholesterol through the intima was directly related to the pressure exerted on the column of blood in the arterial lumen. At 20 mm.Hg no filtration occurred. At 30 mm.Hg slow filtration commenced. At increasing pressures the rate of filtration was correspondingly accelerated.

Further evidence to support the filtration theory has been forthcoming from the experiment of Dayton[28] in which he has shown, using labelled carbon atoms, $4C^{14}$, that although the arterial wall can synthesize cholesterol, the major part in the lesions is derived from plasma cholesterol.

2. *Metabolic Changes in the Arterial Wall*

There is a lower respiration rate in the wall of the thoracic aorta compared with the abdominal aorta in the rat[29] and the rabbit.[30] Hypertension results in significant elevation of the oxygen consumption of all segments. These findings indicate that different levels of blood pressures may profoundly alter the metabolic activity of the aorta.

Blood Pressure Distal to an Arterial Occlusion

There is normally very little pressure gradient in a main limb artery. After occlusion of a vessel there is a drop of BP distal to the block which creates a pressure gradient in the small muscle vessels of the mid-zone. These vessels connect the branches of the main artery and normally carry little blood. As these channels dilate the pressure gradient is reduced by an increasing pressure below the block which continues to rise until a level is reached which is approximately that of the previous diastolic pressure.[31-33] Under resting conditions the blood-flow at this pressure level is equal to the flow in the normal leg.

The blood pressure distal to a block is consistently lower than in the normal limb even after many weeks.[34, 35]

CONCLUSIONS

Although it is generally accepted from clinical evidence in man, and from experimental findings in animals, that hypertension accelerates and accentuates atheroma,[22, 23] this is not invariably the case. Neither could a direct relationship between the level of blood pressure and the degree of atheroma be shown.[24]

Katz and Stamler[36] found that hypertension did not significantly augment the lesions of spontaneous atheroma in chickens.

Wakerlin *et al.*[24] reported that in thiouracil-cholesterol fed dogs made hypertensive by partially clamping the renal artery, there were atheromatous lesions present distal to the clamp in the renal artery in spite of the fact that the BP at this site was 20 - 70 mm.Hg lower than the systemic BP.

The relative lack of atheromatous lesions in the pulmonary vessels are often remarked upon and the low BP in the lesser circulation is regarded as a protecting factor.

However, Miller[37] stated that lesions are present in the pulmonary vessels in 6 - 8% of cases of atheroma, while Brenner[38] has placed the incidence as high as 65 - 70%. One-fifth of cases with pulmonary atherosclerosis have no demonstrable pulmonary hypertension. Atheroma of the pulmonary arteries in rabbits fed on a cholesterol diet is a common lesion. There is also no significant difference in the extent of the lesions in the pulmonary circulations in normotensive and hypertensive rabbits.[39]

In the present experiment, although there was a persistent hypotension in the artery distal to the block relative to the pressure on the normal side, there was no significant evidence that the arteries distal to the block were protected from atheroma.

SUMMARY

The effect of arterial occlusion on the development of atheroma in rabbits fed on a cholesterol diet is studied.

Intra-arterial BP's are recorded distal to the occlusion, and in the normal femoral artery.

It is concluded that in spite of persistent hypotension in the artery distal to the occlusion, there is no delay in onset, nor protection from progression of the disease.

I am indebted to Prof. D. J. du Plessis, who encouraged me to embark on this investigation and who placed the facilities of the Department of Surgery, University of the Witwatersrand, at my disposal, and to Mr. A. Veenstra for taking the photographs.

REFERENCES

1. Shepherd, R. C. and Warren, R. (1960): Surg. Gynec. Obstet., **110**, 346.
2. Cocket, F. B. and Norman, A. G. (1958): Brit. Med. J., **1**, 726.
3. Warren, R., John, H. T., Shepherd, R. C. and Villavicencio, J. L. (1961): Surgery, **49**, 1.
4. Crawford, E. S., De Bakey, M. E., Morris, G. C. and Garret, E. (1960): *Ibid.*, **47**, 79.
5. Hoye, S. J. and Warren, R. (1956): New Engl. J. Med., **254**, 102.
6. Warren, R. and Villavicencio, J. L. (1959): *Ibid.*, **260**, 255.
7. Cannon, J. A. and Barker, W. F. (1955): Surgery, **38**, 48.
8. Warren, R., Gomoz, R. L., Marston, J. A. P. and Cox, J. S. T. (1964): *Ibid.*, **55**, 135.
9. Du Plessis, D. J., Gaylis, H. and Stein, L.: Personal communication.
10. Dill, L. V. and Isenhour, C. E. (1941): Surg. Gynec. Obstet., **72**, 38.
11. Stein, H. B. (1937): S. Afr. J. Med. Sci., **2**, 117.
12. Anitschow, N. C. (1933): *Atherosclerosis*, p. 271. New York: Macmillan.
13. Prior, J. P., Katz, D. M. and Ziegler, D. P. (1961): Arch. Path., **71**, 672.
14. Duff, G. L. (1935): *Ibid.*, **20**, 81 and 259.
15. Duff, G. L. and McMillan, G. C. (1951): Amer. J. Med., **11**, 92.
16. Leary, T. (1934): Arch. Path., **17**, 453.
17. Corcoran, A. C., Lewis, L. A., Dustan, H. P. and Page, J. H. (1956): Ann. N.Y. Acad. Sci., **64**, 620.
18. Gubner, R. and Ungerlieder, H. E. (1949): Amer. J. Med., **6**, 60.
19. Rosenthal, S. R. (1934): Arch. Path., **18**, 473 and 660.
20. Faber, M. (1946): Acta med. scand., **125**, 418.
21. Hunter, A. (1939): Journal of the Institute of Actuaries, **70**, 60.
22. Bronte-Stewart, B. and Hepinstal, R. H. (1954): J. Path. Bact., **68**, 407.
23. Hepinstal, R. H. and Parker, K. A. (1957): Brit. J. Exp. Path., **38**, 55.
24. Wakerlin, G. E., Moss, W. G. and Kiely, J. P. (1957): Circulation Res., **5**, 426.
25. Petrof, J. R. (1922 - 23): Beitr. path. Anat., **71**, 115.
26. Lange, F. (1924): Virchows Arch. path. Anat., **248**, 463.
27. Wilens, S. (1951): Science, **114**, 389.
28. Dayton, S. (1959): Circulat. Res., **7**, 468.
29. Christie, R. W. and Dahl, L. K. (1957): J. Exp. Med., **106**, 357.
30. Fisher, E. R. and Geller, J. H. (1960): Circulat. Res., **8**, 820.
31. John, H. T. and Warren, R. (1961): Surgery, **49**, 14.
32. Winblad, J. N., Reemtsma, K., Vernatt, J. L., Laville, L. P. and Creach, O. (1959): Surgery, **45**, 105.
33. Edholm, O. G., Howarth, G. and Sharpey-Shafer, E. P. (1951): Clin. Sci., **10**, 361.
34. Theis, F. (1933): Surg. Gynec. Obstet., **57**, 737.
35. Eckstein, R. W., Gregg, D. E. and Pritchard, W. H. (1941): Amer. J. Physiol., **132**, 35.
36. Katz, L. N. and Stamler, J. (1953): *Experimental Atherosclerosis*, p. 224. Springfield, Ill.: C. C. Thomas.
37. Miller, H. P. (1925): Med. Clin. N. Amer., **9**, 673.
38. Brenner, O. (1935): Arch. Intern. Med., **56**, 211, 457 and 724.
39. Hepinstal, R. H. and Bronte-Stewart, B. (1954): J. Path. Bact., **68**, 395.