

The Antiatherogenic Effect of Iridium¹⁹² upon the Cholesterol-fed Rabbit *

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Most of the methods employed to assess the severity of an atherosclerotic process influence the investigator to regard the lesion primarily in terms of lipid or sterol derangement. The grossly observed yellowish-white color, the microscopically observed vivid scarlet after Sudan staining, and the easily analyzable triglyceride and cholesterol of a plaque emphasize the lipid phase of the lesion.

The experimental plaque certainly and the human plaque probably, particularly in its beginnings, are living tissues composed of rapidly growing cells (1). These cells furnish the site for the continuous deposition of those lipids that have attracted so much biochemical investigation. If the atherosclerotic process is compared with that of amyloidosis, we see that accumulation of extravascular material occurs in both, but in the latter, no significant cellular hyperplasia follows, hence no lumen-obstructing lesion.

Our own attention was brought to the original significance of the term "atheroma" when we discovered (2) that cortisone administration greatly diminishes the severity of atherosclerosis in the cholesterol-fed rabbit by interfering with the cellular hyperplastic phase of this process. We then reasoned that the atherosclerotic process could also be prevented or seriously curtailed by any other substance or agent that would, like cortisone, interfere with the cellular phase of atherogenesis. We decided therefore to subject a large section of the rabbit aorta to direct radiation before the induction of hypercholesteremia to de-

termine whether interference with cellular hyperplasia could be accomplished, and if so, whether such interference modified the atherogenic process. The present report describes these procedures and their results.

Methods

Exposure of thoracic and upper abdominal segments of rabbit aorta to iridium¹⁹². We determined in preliminary studies that a vinyl tubing catheter (i.d., 0.020 inches; wall, 0.008 inches) approximately 50 cm long inserted into the right femoral artery could be pushed into the aorta and to within 1 to 2 cm of the aortic semilunar valves of a mature rabbit. After the necessary length of catheter was determined, approximately 20 cm of radioactive iridium (iridium¹⁹²) wire (Squibb Iridi-tope)¹ was inserted into one of its ends. The catheter containing the wire was introduced into the aorta by pressure at the femoral arterial site of insertion. This procedure exposed the first 20 cm or more of the aorta directly to the gamma radiation emitted by the iridium¹⁹² contained within the vinyl tubing. The beta radiation emitted by the radioactive element probably was totally absorbed by the walls of the vinyl tubing. The catheter containing the radioactive iridium wire was removed after 48 hours in two series of studies. For control purposes, catheters containing a similar length of iridium wire without radioactivity were inserted and allowed to remain for the same time.

Since the half life of iridium¹⁹² is 74.4 days and the radioactive iridium wires were inserted at various times after their receipt from the manufacturer, the aortas of the two series of rabbits were exposed to slightly different amounts of radiation. The total average radiation emitted by the wires in each of the two series of experiments was estimated to be approximately 870 and 1,130 mg hours equivalent of radium, respectively. If we assume that the aortic wall was about 0.75 mm away from the central axis of the radioactive iridium wire, then the aortas of the rabbits of the two series received approximately 1,300 and 1,700 roentgens, respectively.

Five similar groups of rabbits were studied in each of the two series.

¹ E. R. Squibb & Sons, New York, N. Y.

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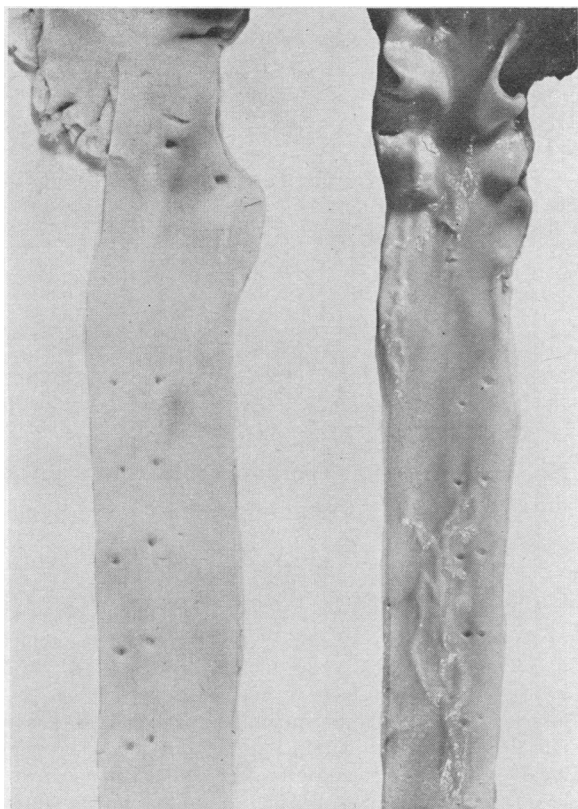


FIG. 1. AORTA OF A RABBIT FED A CHOLESTEROL-ENRICHED DIET FOR 3 WEEKS BEGINNING 2 WEEKS AFTER 48-HOUR INTRA-AORTIC EXPOSURE TO IRIIDIUM¹⁹² (LEFT) AND OF A SIMILARLY FED RABBIT WHOSE AORTA HAD BEEN EXPOSED TO THE CONTROL NONRADIOACTIVE WIRE (RIGHT). Note the beginning atherosclerosis in the latter.

Group 1. Twenty-one rabbits were exposed to radioactive iridium wire for 48 hours. Fourteen days after the removal of the wire, these rabbits were fed a measured amount (100 g of food per rabbit per day) of a cholesterol (2%)- and cottonseed oil (2%)-enriched diet.

Group 2. Twenty-one rabbits were exposed to a control nonradioactive iridium wire for the same time as the rabbits in group 1 were exposed to radioactive iridium. Then 14 days after the removal of the wire, they were fed exactly as the rabbits of group 1.

Group 3. Six rabbits were exposed to radioactive iridium wire for 48 hours and then fed the stock diet without any enrichment.

Group 4. Six rabbits were exposed to nonradioactive wire for 48 hours and then fed the stock diet without any enrichment.

Group 5. Eight unexposed rabbits were fed the same limited amount of the cholesterol- and cottonseed oil-enriched diet at the time it was given to the rabbits of groups 1 and 2.

Three weeks after they had first received the cholesterol-enriched diet 5 rabbits of groups 1 and 2 were

killed; the remaining rabbits were killed after 6 weeks. Two rabbits of groups 3 and 4 were killed at 3 and the remaining 6 weeks after the removal of their respective wires. The rabbits of group 5 were killed 6 weeks after receiving the cholesterol- and cottonseed oil-enriched diet.

Blood samples for cholesterol analysis were obtained from all rabbits every 3 weeks. At sacrifice, the aorta of each animal was inspected and graded (3) for atherosclerosis. Then sections of the aorta were obtained, fixed in formalin, and stained with Sudan IV. In addition, the first 10 cm of the aortas of all animals killed at the end of 6 weeks were removed and analyzed for cholesterol content. Sections of the hearts, livers, and lungs of these animals also were obtained, fixed, and stained with Sudan IV.

Results

The rabbits exposed for 48 hours to the radioactive iridium wire and later fed excess cholesterol and oil exhibited strikingly less atherosclerosis than the various control groups. Four of the five aortas of group 1 rabbits, which were examined 3 weeks after the cholesterol-enriched diet, exhibited no gross evidence of atherosclerosis

TABLE I

The effect of iridium¹⁹² upon the development of aortic atherosclerosis in the cholesterol-fed rabbit

No. of rabbits	Average serum cholesterol	Aorta	
		Atherosclerosis (0-4)	Cholesterol
	<i>mg/100 ml</i>		<i>mg/100 g</i>
Group 1: Rabbits exposed to iridium ¹⁹² and fed cholesterol-cottonseed oil diet			
14	1,271	0.6	2,711
Range:	856-1,813	0.0-2.0	1,800-3,500
SEM:*	±38	±0.1	±212
Group 2: Rabbits exposed to nonradioactive wire and fed cholesterol-cottonseed oil diet			
15	1,206	3.2	5,718
Range:	881-1,575	2.0-4.0	4,600-6,600
SEM:	±41	±0.2	±101
Group 3: Rabbits exposed to iridium ¹⁹² and fed stock diet			
4	41	0	475
Range:	27-60		400-500
Group 4: Rabbits exposed to nonradioactive wire and fed stock diet			
4	61	0	450
Range:	49-74		400-600
Group 5: Rabbits fed cholesterol-cottonseed oil diet			
6	1,249	2.2	4,638
Range:	967-1,536	2.0-2.5	3,550-5,860
SEM:	±77	±0.1	±405

* Standard error of the mean.

(Figure 1), whereas each of the five aortas of group 2 rabbits, which were examined at the same time, exhibited beginning streaks or areas of atherosclerosis. No significant gross pathology was observed in the aortas of those group 3 and group 4 rabbits that were also examined at this time.

On histological examination of the aortas exposed to the radioactive iridium wire, usually a

thin, poorly staining, superficial membrane consisting of a few, pleomorphic cells with dimly outlined, irregular, often misshapen nuclei could be seen (Plate 1A). Occasionally a macrophage containing Sudan-stained granules could be discerned. This straggly, scanty cellular growth was in striking contrast to the already luxuriant, sudanophilic, richly cellular masses observed (Plate 1 B) in the aortas of rabbits exposed to the non-



FIG. 2. AORTAS OF TWO RABBITS (775, 776) FED A CHOLESTEROL-ENRICHED DIET FOR 6 WEEKS BEGINNING 2 WEEKS AFTER 48-HOUR EXPOSURE OF AORTAS TO IRIIDIUM¹⁹² AND AORTAS (781, 784) OF TWO RABBITS SIMILARLY FED AFTER 48-HOUR EXPOSURE OF AORTAS TO NONRADIOACTIVE WIRE. The intense atherosclerotic processes present in the latter rabbits contrast strongly with the minimal degrees of atherosclerosis present in the aortas that had been exposed to iridium¹⁹².

radioactive wire and then fed the cholesterol-enriched diet.

The aortas of rabbits exposed to either type of wire, but fed only the stock diet, revealed no gross pathology at the 3-week period. On histological examination, however, each of them exhibited a thin, superficial membrane lying over the internal

elastic laminae. This membrane in the stock-fed rabbits earlier exposed to the radioactive iridium wire resembled that seen in the cholesterol-fed animals described above. The membrane in the stock-fed rabbits exposed earlier to the nonradioactive wire was far more cellular. These cells, moreover, appeared uniform in size and structure

PLATE 1A. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 400$) OF AORTA OF RABBIT FED EXCESS CHOLESTEROL FOR 3 WEEKS BEGINNING 2 WEEKS AFTER INTRA-AORTIC EXPOSURE OF RABBIT TO IRIIDIUM¹⁹² FOR 48 HOURS. There is a poorly defined, relatively acellular membrane covering the internal elastic lamina. Note the irregularity, paucity, and poor staining of the nuclei of this tissue compared with the richly sudanophilic hyperplastic tissue shown in Plate 1B. An occasional macrophage with slight sudanophilia can be seen near the internal elastic lamina.

PLATE 1B. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 400$) OF AORTA OF CONTROL RABBIT FED LIKE RABBIT OF PLATE 1A AFTER INTRA-AORTIC EXPOSURE TO NONRADIOACTIVE WIRE FOR 48 HOURS. The profusion and width of the surface growth contrasts sharply with that seen in Plate 1A. A number of Sudan-containing macrophages are present in the deeper portions of the new atherosclerotic mass.

PLATE 2A. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 100$) OF AORTA OF RABBIT FED EXCESS CHOLESTEROL FOR 6 WEEKS BEGINNING 2 WEEKS AFTER INTRA-AORTIC EXPOSURE OF RABBIT TO IRIIDIUM¹⁹² FOR 48 HOURS. A thin, glassy, superficial membrane showing sparsely distributed sudanophilic aggregations lies over the internal elastic membrane.

PLATE 2B. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 100$) OF AORTA OF CONTROL RABBIT FED LIKE RABBIT OF PLATE 2A AFTER INTRA-AORTIC EXPOSURE OF RABBIT TO THE NONRADIOACTIVE WIRE FOR 48 HOURS. Here the florid, richly sudanophilic, hyperplastic layer is many times the thickness of the one seen in the aorta of Plate 2A.

PLATE 3A. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 400$) OF THE AORTA OF ANOTHER RABBIT FED AND TREATED LIKE RABBIT OF PLATE 2A. The irregularity and poor staining of the nuclei in the thin, new, superficial lining seen in the aorta in Plate 1A are also seen in this section. The thickness of the surface layer of this aorta is about the same as that seen in Plate 1A. Note the occasional presence of Sudan-containing macrophages.

PLATE 3B. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 400$) OF THE AORTA OF A CONTROL RABBIT FED LIKE THAT OF PLATE 3A AFTER INTRA-AORTIC EXPOSURE TO THE NONRADIOACTIVE WIRE. The layer with its richness in cells, sudanophilia, and greater width contrasts strongly with the section shown in Plate 3A.

PLATE 4. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 100$) OF AORTA OF RABBIT FED EXCESS CHOLESTEROL FOR 6 WEEKS. There is typical atherosclerosis with sudanophilia present in the inner strata of the media.

PLATE 5A. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 100$) OF AORTA OF RABBIT FED STOCK DIET FOR 8 WEEKS AFTER INTERNAL EXPOSURE OF THE AORTA TO IRIIDIUM¹⁹² FOR 48 HOURS. The thin, glassy, almost colorless tissue lying upon the internal elastic membrane appears relatively acellular.

PLATE 5B. MICROPHOTOGRAPH (SUDAN IV STAIN $\times 100$) OF AORTA OF CONTROL RABBIT FED LIKE RABBIT OF PLATE 5A AFTER INTERNAL EXPOSURE OF THE AORTA TO NONRADIOACTIVE WIRE FOR 48 HOURS. Surface tissue lying upon the internal elastic membrane can be seen. It appears to be more cellular and greater in width than the comparable tissue shown in Plate 5A.

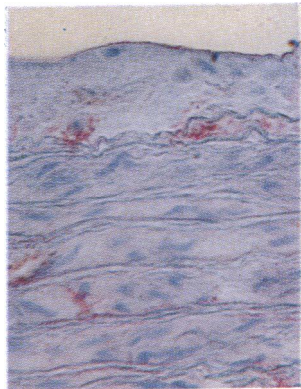


Plate 1A

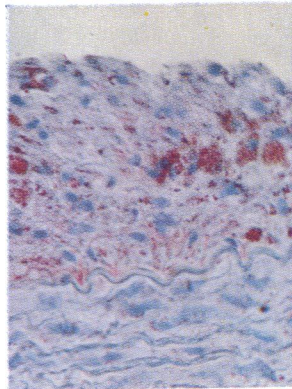


Plate 1B

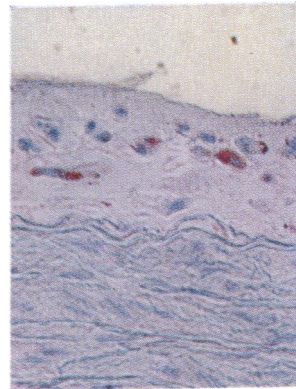


Plate 3A

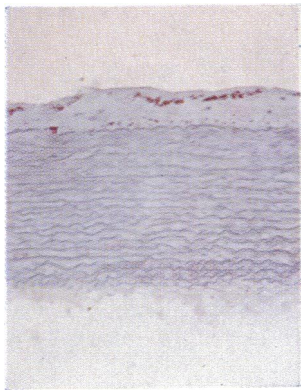


Plate 2A

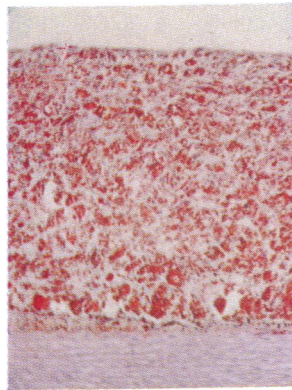


Plate 2B

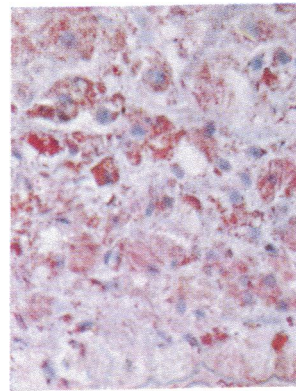


Plate 3B

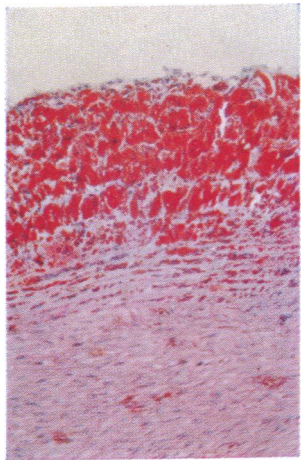


Plate 4

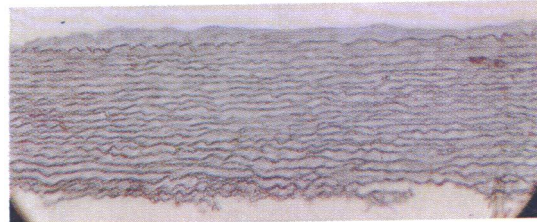


Plate 5A

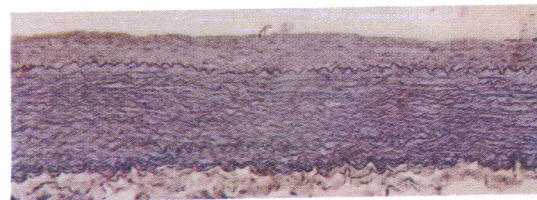


Plate 5B

and contained relatively round vesicular well-stained nuclei.

The effects of aortic exposure to iridium¹⁹² were even more strikingly evident when the remaining rabbits of all groups were killed at the end of the 6 weeks of excess cholesterol feeding. Eleven of the 14 aortas of the group 1 rabbits revealed little or no atherosclerosis (Figure 2; Table I), whereas the atherosclerosis was quite severe in 13 of the 15 aortas of the group 2 rabbits (Figure 2; Table I) and present to a lesser degree in the remaining 2 rabbits. There was no evidence of either atherosclerosis or fibrosis in any of the aortas of the remaining rabbits of groups 3 and 4 killed at this time (Figure 3). Significant atherosclerosis was observed in all of the rabbits of group 5 that were fed only the cholesterol-enriched diet.

These gross observations were confirmed by the histological studies. The aortas of the group 1 rabbits allowed to survive for the 6 weeks were found to have the earlier described, thin, superficial membrane (Plates 2A and 3A) containing sparsely distributed, pleomorphic cells with an occasional Sudan-containing macrophage. On the other hand, the aortas of group 2 rabbits (Plates 2B and 3B) exhibited a richly hyperplastic, sudanophilic growth. A similar, hyperplastic, sudanophilic process was observed in the aortic sections of group 5 rabbits (Plate 4). The aortic sections of the group 3 rabbits that had been exposed to the radioactive iridium wire but fed the stock diet usually exhibited the thin superficial membrane (Plate 5A) earlier noted, but again it appeared relatively acellular. The aortic sections of the group 4 rabbits exhibited a similar type of membrane, but usually it was considerably thicker and still primarily cellular (Plate 5B).

The chemical analyses of the aortic strips confirmed the gross findings (Table I), for there was about half the concentration of cholesterol present in the aortic segments of the cholesterol-fed rabbits previously exposed to radioactive iridium wire as there was in the aortic segments of the rabbits exposed to the nonradioactive wire or of the rabbits simply fed the excess cholesterol. The aortic segments of groups 3 and 4 rabbits contained about the same concentration of cholesterol (Table I), an amount that we have usually observed in the normal aorta (2).



FIG. 3. AORTA OF A RABBIT FED A STOCK DIET FOR 6 WEEKS AFTER 48-HOUR EXPOSURE OF AORTA TO IRIIDIUM¹⁹² (LEFT) AND OF A RABBIT SIMILARLY FED AFTER 48-HOUR EXPOSURE OF AORTA TO NONRADIOACTIVE WIRE (RIGHT). No gross lesions can be discerned in either aorta.

These gross, microscopic, and chemical observations indicate quite clearly that prior exposure to iridium¹⁹² had conferred a marked immunity against atherogenesis upon the aortic area that had received its radiation. That this effect was due to the iridium¹⁹² appeared certain when the coronary vasculature was examined microscopically. Despite the scanty atherosclerosis present in the aortas of group 1 rabbits, their coronary vasculature showed about the same degree and intensity of sudanophilic hyperplasia as the vasculature of rabbits in groups 2 and 5.

Histological examination of the livers and kidneys of group 1 rabbits killed at 6 weeks revealed no discernible abnormalities. The lungs from 2 of these 14 rabbits, however, revealed large areas of scar tissue that apparently had replaced normal lung tissue.

Discussion

The marked protection against subsequent atherosclerosis afforded the aorta of the cholesterol-fed rabbit by prior exposure to iridium¹⁹² appeared to be due primarily to the failure of this segment of the vascular tissue to react with its accustomed hyperplasia to the presence of excess cholesterol. Certainly the exposure to radiation did not alter the hypercholesteremic response of the rabbits. Nor did it prevent the entrance of excess cholesterol into their aortas, for both by histological and chemical studies an excess was present. This excess was not so great as that found in the rabbits exposed to nonradioactive wire or in the control rabbits fed only excess cholesterol. But these latter groups of rabbits promptly reacted to cholesterol feeding with a profuse aortic hyperplasia, and this latter process in itself markedly promotes the entrance of excess cholesterol into the aortic wall (4). Finally, the marked coronary atherosclerosis present in the iridium¹⁹²-exposed rabbits strongly suggests that plasma cholesterol had not been changed in any way that would afford protection against atherosclerosis.

Actually, the present findings in the rabbits exposed to radiation are very similar to those we observed earlier (2) in rabbits fed cholesterol and given cortisone. In both types of rabbits, failure to respond to cholesterol feeding with profuse aortic atherosclerosis apparently stemmed from the inability of the aortic tissue to effect its accustomed florid hyperplastic reaction when confronted with excess cholesterol in the blood.

The rabbits showed no clinically obvious harmful effects from radiation, and, other than the pulmonary findings, no histological evidence of damage was found. The thin, superficial, membranous covering of the aorta, moreover, was found as frequently in the rabbits exposed to the nonradioactive wire as in those exposed to the radioactive iridium wire. We believe, therefore, that this ribbon of tissue resulted merely from the 48-hour aortic lodgment of the vinyl tubing and its continuous impingement upon the aortic lining.

Neither the results of the present study nor those of the earlier study in which cortisone was employed (2) indicate that either iridium¹⁹² or

cortisone will ever find a place in the armamentarium against clinical atherosclerosis. We do believe, however, that the results of both studies strongly suggest that atherosclerosis is more than a matter of simple lipid and sterol accumulation, and therefore attempted antiatherogenic prophylaxis or therapy may include more possibilities than removal or alteration of just blood lipids and sterols. One such process may be that of influencing the cellular component of the atherogenic reactions, a component without which significant arterial obstruction may not evolve, regardless of the quantity or quality of the blood lipids and sterols.

Summary

A 20-cm length of iridium¹⁹² wire (Squibb Iriditope) was placed for 48 hours in the proximal portion of the aortas of a series of rabbits. These rabbits, when subsequently fed a diet containing excess cholesterol, failed to exhibit significant atherosclerosis, as compared to the control animals, in that portion of the aorta which had received the irradiation. The histological and chemical studies performed upon these rabbits suggest that the antiatherogenic effect of the irradiation was due primarily to the suppression of the intimal hyperplastic response to a persistent hypercholesteremia usually observed in the rabbit's aorta.

Acknowledgment

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References

1. Friedman, M. Pathogenesis of the spontaneous atherosclerotic plaque. A study on the cholesterol-fed rabbit. *Arch. Path.* 1963, **76**, 318.
2. Friedman, M., S. O. Byers, and S. St. George. Effects of cortisone administration upon the pathogenesis of experimental atherosclerosis. *Arch. Path.* In press.
3. Friedman, M., R. Homer, and S. O. Byers. An evaluation of potassium iodide as a therapeutic agent in the treatment of experimental hypercholesteremia and atherosclerosis. *J. clin. Invest.* 1956, **35**, 1015.
4. Friedman, M., and S. O. Byers. Endothelial permeability in atherosclerosis. *Arch. Path.* 1963, **76**, 99.