VARIOUS FORMS OF EXPERIMENTAL ARTERIAL DISEASE IN THE RABBIT *

MINER C. HILL, M.D.
NEW YORK

Despite the fact that the experimental lesions in the rabbit's aorta occurring after the administration of adrenalin and other substances cannot be considered analogous to arteriosclerosis as seen in man, and despite also a reasonable doubt as to whether these lesions are really caused by such experimentation or are spontaneous in character, their study is of considerable importance in connection with the general problem of the degenerative and reparative processes in arteries. In the hope of adding somewhat to our knowledge of the subject, and also of clearing up, if possible, some of the doubtful points, I have studied during the past year the results of various forms of experimental injury. The methods employed include the administration of adrenalin and direct injury by crushing the vessel or applying an irritant, such as silver nitrate. The suspension experiments of Klotz¹ have been repeated and, following the suggestion of an editorial writer² in the *Journal of the American Medical Association*, based on recent investigations of loco-weed disease, the prolonged administration of barium by the mouth has been carried on, with the hope of thus explaining the spontaneous lesions. In all these experiments an equal number of animals have been used as controls and a careful study has been made of all animals used in other experiments in order to obtain information concerning the spontaneous occurrence of arterial disease.

As the general status of experimental arterial disease, including an excellent summary of the literature, has recently been presented by Adler,³ such will not here be considered.

---

* From the Pathological Laboratory New York University and Bellevue Hospital Medical College; aided by a grant from the Rockefeller Institute of Medical Research.

1. Klotz, O.: Arbeits-Arteriosclerose Experimentelle. *Centralbl. f. allg. Path. u. Anat.*, 1908, xix, 535.

2. Editorial: Barium, a Cause of Loco-weed Disease. *Jour. Am. Med. Assn.*, 1908, li, 1338.

3. Adler, I.: Present Status of Experimental Arterial Disease. *Am. Jour. Med., Sc.*, 1908, cxxxvi, 241.

The question of spontaneous lesions may naturally be considered first. The significance of spontaneous lesions in connection with those produced experimentally was first seriously raised by Miles,⁴ who reported spontaneous lesions in nearly 35 per cent. of presumably normal animals and who suggested that the lesion assumed to be due to adrenalin might be truly spontaneous. This view has been opposed by Miller,⁵ who summarizes the several large series of observations on normal animals without the discovery of spontaneous lesions, and by Pearce,⁶ who found such lesions in but 6 per cent. of the animals examined. It is of interest, in view of the possible influence of local conditions, that Miles' 35 per cent. represents western rabbits, while Pearce's 6 per cent. is based on New York and Massachusetts rabbits. Furthermore, it is of interest that, while Pearce found lesions in but 6 per cent. of normal animals, lesions occurred with greater frequency in animals which had been in the laboratory for long periods of time and had been used for inoculation and other general experimental work. This latter observation suggests that a variety of influences may operate to produce these lesions in the rabbit.

SPONTANEOUS LESIONS

The animals examined for spontaneous lesions were those used in the class work of the departments of physiology and pharmacology, and for which I desire here to thank Professors Lusk and Wallace. These were presumably normal animals, used shortly after their arrival, in short-period experiments. In addition are included animals used in the department of pathology in various experimental studies.

The series comprises 210 rabbits. The aorta was examined from the aortic valve to the iliac bifurcation, and the iliac, carotid and subclavian branches followed for some distance. Of these animals thirty-one, or 15 per cent., exhibited arterial disease. In only one was the lesion as diffuse as in the adrenalin lesion, and in this animal only were lesions found below the diaphragm. This was a young rabbit, weighing 940 grams, in which the lesion appeared as a diffuse roughening of the thoracic and upper abdominal aorta. Four showed changes in the descending portion of the thoracic aorta and one a small pin-head-sized patch in the first part of the innominate artery; in all others the lesions were confined to the ascending and transverse portion of the arch of the aorta. The usual

4. Miles, A. B.: Spontaneous Arterial Degeneration in Rabbits. *Jour. Am. Med. Assn.*, 1907, xlix, 1173.

5. Miller, J. L.: Spontaneous Arterial Degeneration in Rabbits. *Jour. Am. Med. Assn.*, 1907, xlix, 1489.

6. Pearce, R. M.: Occurrence of Spontaneous Arterial Degeneration in the Rabbit. *Jour. Am. Med. Assn.*, 1908, li, 1056.

lesion consisted of three or four irregular depressions just beyond the aortic valve.

Divided according to the purpose for which the animals were used, we have 117 rabbits utilized for class work in physiology and pharmacology with no previous treatment, and 83 for experiments in pathology. The latter experiments included inoculation of sheep's blood for the Wassermann test and various injections in connection with a study of experimental nephritis. Of the former, sixteen, or 13.5 per cent., and of the latter fifteen, or 18 per cent., showed lesions, a somewhat greater incidence in animals kept for some time under laboratory conditions than in those used immediately. This difference is too slight to be seriously considered, but is in keeping with Pearce's previous observation concerning the influence of experimental and laboratory conditions.

Of the above rabbits, fifty were bought in Chicago and are presumably representative of rabbits of the middle west; seventy-six purchased from one New York dealer and represent animals raised in New Jersey; concerning the source of the remainder, no definite information could be obtained. Of the fifty Chicago rabbits, six, or 12 per cent., presented spontaneous lesions; of the seventy-six eastern, eight, or 10.5 per cent. As far as these sources of supply are concerned, there is, therefore, little difference in the occurrence of spontaneous lesions.

These observations, however, in connection with the available statistics of other investigators, demonstrate conclusively that the spontaneous lesions occur not infrequently. That the age of the animal is a factor is doubtful, for five of the thirty-one animals in this series weighed less than 900 grams, two weighing 440 and 410 grams, respectively. Of these, the former presented the most extensive lesion found in the series. This is of interest in view of the fact that Pic and Bonnamour,⁷ as well as Pearce and Stanton,⁸ have called attention to the difficulty of producing adrenalin lesions in young (small) rabbits.

Histologically the spontaneous lesion cannot be distinguished from that due to adrenalin. The most prominent features are changes in the elastica and calcification. The elastic tissue loses its wavy appearance and the individual fibers become much thickened. Later these become calcified. Not infrequently a hyperplasia of the intima is seen—more frequently, indeed, than is the case in the adrenalin lesion. This may

---

7. Pic, A., and Bonnamour, S.: Contribution à l'étude du déterminisme de l'athérome aortique expérimental. *Compt. rend. Soc. de biol.*, 1905, lviii, 219.

8. Pearce, R. M., and Stanton, E. M.: Experimental Arteriosclerosis. *Jour. Exper. Med.*, 1906, viii, 74.

possibly be due to the greater age of the spontaneous lesion. Lesions of the type described by Ophüls⁹ have not been found.

In connection with the study of these spontaneous lesions I have investigated the effect of barium administered by the mouth, thus adopting the suggestion of the editorial writer in the *Journal of the American Medical Association*, previously mentioned.² This writer calls attention to Crawford's study of the barium content of certain plants and the explanation of "loco-weed disease" as a poisoning by barium. He then makes the suggestive observation, in view of the well-known effect of barium on the vascular system, that possibly the greater frequency of spontaneous arterial degeneration observed by Miles (35 per cent.), as contrasted with Pearce's 6 per cent. (New York), might be due to the forage plants of Colorado containing minute quantities of mineral poisons and, among others, possibly barium. As this theory appeared very plausible, especially so in view of Miller's experiments with barium, I have attempted to produce a chronic barium intoxication by giving rabbits small doses of the chlorid with their food. Four rabbits received barium chlorid by stomach-tube, daily, immediately after their feeding period. Three animals died after varying periods, but one received a total of eighty-six doses of half a milligram each during a period of fourteen weeks (February 1 to May 7) without the development of arterial lesions. Though small in number for definite conclusions, such experiments indicate that barium intoxication probably does not explain the spontaneous lesions.

The production of arterial lesions by single injections of adrenalin has been rendered very doubtful by the recent observations concerning the frequency of spontaneous lesions. Pearce and Baldauf,¹⁰ in 1906, reported such lesions in each of a series of six rabbits receiving one-half to one and a half minims of adrenalin. Unfortunately no control experiments were made. At Dr. Pearce's suggestion I have repeated these experiments. Two animals received, each, in divided doses, four minims of a 1 to 1,000 solution of adrenalin, and two were set aside as controls. One of the former died after ten, and one after forty-one days. No lesions were evident in either; the controls also showed no lesions. It is evident that the previous positive results described by Pearce and Baldauf are in all probability to be explained by the occurrence of spontaneous

9. Ophüls, W.: Spontaneous Arteriosclerosis of the Aorta (Atheroma) in a Rabbit. *Jour. Am. Med. Assn.*, 1907, xlviii, 326.

10. Pearce, R. M., and Baldauf, L. K.: A Note on the Production of Vascular Lesions in the Rabbit by Single Injections of Adrenalin. *Am. Jour. Med. Sc.*, 1906, cxxxii, 737.

lesions. Meyers,¹¹ working with a large number of animals, has reached a similar conclusion.

In other respects, unlooked-for results have been obtained with the use of adrenalin. Thus, in one group of four animals varying in weight from 1,500 to 2,000 grams and receiving, each, twenty-nine injections, over periods of fifty-two to one hundred and four days, the characteristic diffuse lesions of the experimental disease were found in only one. In an equal number of controls no lesions were found. As the total amount of adrenalin administered to these animals varied from 167 to 174 minims, one must agree with other investigators that constant results do not always follow the administration of this substance. During the early periods of this treatment the animals failed to show the distress so characteristic of the intravenous injection of adrenalin and a fresh supply was used in the latter half of the injection. The use of an adrenalin altered by age, or otherwise deteriorated, cannot, therefore, be invoked as an explanation of the small percentage of positive results.

It is evident from the preceding discussion that the question of spontaneous versus experimental arterial degeneration is not a simple one—indeed, is one beset with many difficulties of interpretation, and, as Adler wisely concludes, demands a suspension of judgment until further investigation has cleared up the doubtful points.

DIRECT CHEMICAL AND MECHANICAL INJURY

For the purpose of comparing the lesions of the spontaneous and adrenalin disease with those of degeneration and repair following direct injury, the vessels in two series of animals were injured by chemical and mechanical means, respectively. For chemical injury, a 3 per cent. solution of silver nitrate, as employed by Harvey¹² for the study of bone formation in arteries, was used. Laparotomy was done on three large rabbits and a silver solution was painted over a small portion of the abdominal aorta. The excess was removed after one minute by means of a sponge wet with salt solution. Of the three animals, two survived and were killed after forty-six days. In both the injured areas showed marked thickening of the adventitia. Histologically, the intima and inner portion of media were unaffected. In the outer portion of the media very definite calcification was present, and about this, involving especially the adventitia, was a great amount of fibrous thickening.

11. Meyers, M. K.: Die Wirkung von intravenösen Injektionen von Hypophysenextrakt und Brenzkatechin, sowie von einmaligen Adrenalininjektionen auf die Aorta von Kaninchen. *Centralbl. f. allg. Path. u. path. Anat.*, 1909, xx, 109.

12. Harvey, W. H.: Bone Formation in Rabbit's Aorta by Application of Irritant to Vessel Wall. *Jour. Med. Research*, 1907, xvii, 25.

In another series the same portion of the aorta was crushed with an artery clamp, the jaws of which were covered with thin rubber tubing to prevent tearing. The pressure was applied for ten seconds. On removing the clamp, interstitial hemorrhage in the vessel wall could be seen, but no external bleeding occurred. Six large rabbits were so treated and killed after periods varying from fourteen to forty-seven days.

Macroscopically, the aorta appeared more opaque at the point crushed, but there was no appreciable thickening and no thrombosis. Microscopically, an atrophy of the connective tissue and smooth muscle of the media was seen, with stretching, straightening and rupture of the elastic fibers and marked hypertrophy of the intima. Despite the thickening of the intima, however, the vessel-wall was thinner at this point than elsewhere, owing to atrophy of the media. No calcification was seen. Although there is an interesting contrast between the results following the chemical and mechanical injury, the two lesions being of entirely different type, neither closely resembles the common lesion of the spontaneous or the adrenalin disease, though the repair following mechanical injury is an exaggerated form of that type of adrenalin injury in which intimal proliferation (Pearce) is occasionally seen.

For the purpose of still further studying the influence of the mechanical factor without employing toxins or causing direct injury, the recently published experiments of Klotz¹ were repeated.

Klotz describes the production of arterial lesions in rabbits as the result of suspending the animals daily by the hind legs for three minutes. This treatment was carried on for periods varying from ninety-two to one hundred and thirty days. He had demonstrated previously, by manometric studies, that by this procedure the pressure in the carotid and subclavian vessels could be raised. Thus he was reproducing a factor considered of prime importance in the etiology of human arteriosclerosis. The five rabbits subjected to this procedure showed more or less marked changes in the aorta, and the carotid and subclavian arteries. In the animal subjected to daily suspension for one hundred and thirty days there was found at autopsy a spindle-shaped aneurism of the thoracic aorta extending from the sixth rib to the diaphragm, a thickening of the wall of the aorta as far down as the renal artery, and a marked thickening of the carotid and subclavian arteries. It is of interest that, while the lesions in the aorta were of the adrenalin type, with primary change in the media, the peripheral vessels showed a primary thickening of the intima. As the arteries of the extremities by which the animals were suspended were free of lesion, it seemed justifiable to consider the

mechanical factor responsible for the arterial disease, especially as the negative controls appeared to rule out spontaneous lesions.

I have performed this experiment with two groups of animals. The rabbits chosen were all full-grown and in good condition. In the first experiment six animals were used; three subjected to suspension, three kept as controls. They were treated exactly as described by Klotz for a period of one hundred and thirty days, after which the animals were killed. Careful post-mortem examination of the aorta and its branches to the second or third division revealed no gross changes. Numerous sections were cut from different levels of the aorta and its branches and examined microscopically with negative results. Of the control animals, one showed most diffuse lesions in the ascending arch of the aorta.

I then decided to repeat the experiment, modifying Klotz's method by giving 100 cubic centimeters of water per stomach-tube, thirty to forty-five minutes before suspension, in the hope of increasing the vascular tension by the production of artificial plethora. In preliminary experiments it was found that this amount of water is absorbed within thirty to forty-five minutes after ingestion, and, allowing for the regulatory mechanism of diuresis, it seemed that at this period we would have the maximum temporary filling of the blood-vessels. The period of suspension was increased also to five minutes. Three animals were so treated daily for a period of a hundred days, but in no animal of the series could lesions of the arteries be demonstrated. I have no explanation to offer for these results, which differ so greatly from those obtained by Klotz.

SUMMARY

The occurrence of spontaneous arterial disease in the rabbit is an important factor in the experimental study of vascular disease, and a factor the status of which must be definitely determined before the results of experimentation along this line can be definitely accepted. In the course of this investigation 210 presumably normal animals were examined and spontaneous lesions found in 15 per cent. A slight but apparently negligible difference has been found in the incidence of these lesions in eastern and western rabbits and also in rabbits used immediately after purchase as compared with those passing through a long laboratory existence. The lesions occur in young (small) rabbits almost as frequently as in old rabbits. Histologically, the common type of spontaneous lesion cannot be distinguished from that due to adrenalin. Macroscopically, however, it differs from the diffuse lesion of adrenalin by being usually limited to a few foci occurring at the origin of the aorta. The peculiar spontaneous lesion described by Ophüls has not been

seen. The prolonged administration of barium by the mouth does not cause, as has been suggested, the appearance of spontaneous lesions. These spontaneous lesions apparently explain the results previously ascribed to single injections of adrenalin, for a repetition of the "single injection" experiments have given negative results. Likewise, in some groups of experiments, the long-continued administration of adrenalin in fairly large doses has led to the production of the diffuse lesion, considered characteristic of adrenalin, in a comparatively small percentage of animals.

Direct chemical injury, as painting the vessel with silver nitrate, causes a fibrous thickening of the adventitia and outer portion of media with calcification of the latter. Direct mechanical injury, as crushing by measured pressure with forceps, leads to atrophy of the media without calcification and to definite proliferation of the intima. The latter type resembles somewhat certain late stages of the adrenalin lesion with intimal proliferation, but neither can be considered analogous to either the spontaneous or adrenalin lesions.

Attempts to produce vascular lesions through increasing the blood-pressure by mechanical means, as in the suspension experiments of Klotz, have given negative results.

St. Luke's Hospital.