EXPERIMENTAL MYOCARDITIS; A STUDY OF THE HISTOLOGICAL CHANGES FOLLOWING INTRAVENOUS INJECTIONS OF ADRENALIN.*

By RICHARD M. PEARCE, M. D.

(*From the Bender Laboratory, Albany, N. Y.*)

PLATE XX.

During a recent investigation[1] by Dr. Stanton and myself of the lesions occurring in the aorta of the rabbit as the result of repeated injections of adrenalin, the heart muscle, in the few instances in which it was examined, was seen not infrequently to be the seat of very definite degenerative lesions and occasionally also of proliferative changes. The association of such conditions with an experimental vascular lesion suggested the possibility of producing experimentally a chronic myocarditis analogous to that seen not infrequently in man in connection with grave arterial disease. As the material at hand was insufficient for a comprehensive study a second series of investigations was inaugurated. In these an attempt has been made to study the sequence of the changes and to determine to what extent they are due to the direct action of adrenalin and to what extent secondary to the arterial lesions. Few of the investigators who have produced vascular lesions with adrenalin have described the associated myocardial changes. Fischer[2] mentions the occurrence of necrotic and fibrous changes and, rarely, calcification. K. Ziegler,[3] in a series of eight animals, found changes very similar to those hereinafter detailed.

Methods.—These were practically the same as in the earlier experiments by Dr. Stanton and myself, except that the initial dose was placed at one-tenth cubic centimetre, and rapidly raised to five-tenths and occasionally higher. All injections

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were made into the ear vein, and usually on alternate days. As previous experiments had shown that ten to twelve injections of adrenalin were sufficient to produce well marked arterial lesions, the injections in this series with a few exceptions were thus limited and the animals were then allowed to remain without further treatment for periods varying from one to three weeks. Thus the animals which survived the treatment represent an average injection period of twenty-four days, and a total elapse of from twenty-seven to forty-five days, a sufficient time, as indicated by the few preliminary observations, for the development of proliferative lesions in the myocardium. The animals dying during the course of the injections furnished sufficient material for the study of early degenerative changes.

The histological methods were limited to the use of Scharlach R for frozen sections, and of hæmatoxylin and eosin and Mallory's connective tissue stain for material hardened in Zenker's fluid and imbedded in paraffin.

Results.—Thirty-six rabbits were injected. These may be divided into two groups: the first group including fourteen animals which died during the period of injection, and the second group, twenty-two animals, which received from seven to fifteen injections and were killed at various intervals thereafter. The first group represents animals receiving from one to five injections, of which none died later than the tenth day. All animals dying in this stage succumb apparently to circulatory and respiratory disturbance and present at autopsy acute dilatation of the heart, œdema and congestion of the lungs, and occasionally hæmorrhages from the serous and mucous membranes or in the interior of organs. No macroscopic lesions of the aorta are demonstrable. When death follows the injection within a few minutes the cardial dilatation, especially after the fourth or fifth injection, is extreme. Under such circumstances the heart loses much of its conical shape; the apex is more or less rounded and the overdistended auricles project laterally and greatly broaden the base. The left ventricle is very anæmic and its pale almost bloodless substance is in sharp contrast to the blue-black color of the thin walled auricles.

Degenerative Changes.—The heart muscle of two of three rabbits dying within a few minutes after a single injection shows a well marked œdema causing more or less separation of the muscle fibres: in the third animal the œdema is slight. In all three the muscle fibres show irregular swellings, and the striations are somewhat indistinct. In one, multiple fractures of more or less homogeneous fibres are frequently seen, but as these conditions could not be found in either of the other hearts, they were considered as probably artifacts.

But one animal died after two injections. This death occurred on the fourth day, thirty-six hours after the second injection. The œdema of the myocardium is so great as to cause wide separation of the muscle fibres in many areas. In some of these areas the fibres have lost their striations and are very granular. A few small areas of intense œdema take a diffuse blue stain similar to myxomatous tissue.

Three animals died after three injections. The notes on one of these follow:

RABBIT No. 25.—Male, weight 1670 grammes. The initial dose was one-tenth cubic centimetre of a one to one thousand solution of adrenalin injected into the ear vein. On the following day the dose was raised to two-tenths and after an interval of two days it was repeated. The animal was found dead seventeen hours after the last injection. At autopsy œdema and congestion of the lungs and extreme dilatation of the heart are present. Histologically the heart reveals focal areas of most intense œdema. These occur not only in the wall of the ventricle but also in the papillary muscles. Crossing these areas are strands of connective tissue in the midst of which fragments of heart muscle may occasionally be seen. Some of these fragments stain well while others are merely shadows of fibres in the midst of the finely granular coagulated serum. Some have ragged irregular ends pointing definitely to displacement by fracture. Elsewhere the fibres are widely separated by a serous fluid, are very granular, have swollen nuclei, stain lightly with eosin, and have but indistinct striations. On cross section many of the fibres show a clear zone about the nucleus, evidently a perinuclear œdema. Occasionally, bundles of fibres appear as if torn across, the space between being filled with serum. The branching of the fibres is brought out well by the œdema. Swollen hyaline fibres are not infrequent. The larger blood vessels are widely distended; the capillaries on the other hand are not prominent. No lesions are seen in the walls of the blood vessels.

In a second animal of this period which received three injections of one-tenth cubic centimetre the œdema was not so prominent, but on the other hand more extensive granular and hyaline

degeneration of the fibres was present. After the fourth injection one animal died and after the fifth, six. These last represent the period of greatest mortality as well as the gravest degenerative lesions. All animals dying at this time showed extreme dilatation of the heart and histologically, in addition to the changes just described, a severe granular and hyaline transformation with more or less necrosis and disorganization. This condition is illustrated by the following protocol:

RABBIT No. 6.—Male, weight 1720 grammes. On September 26, 29, and 30 one-tenth cubic centimetre of adrenalin solution was injected into the ear vein; on October 2 and 3, two tenths. The animal died during the night of October 3. Upon post-mortem examination the heart was found to be dilated, the left ventricle pale and bloodless, and the lungs œdematous; the other organs were normal. Sections from three levels of the heart show general œdema and extreme disintegration of the muscle fibres. On both cross and longitudinal section the latter are seen to be irregularly swollen, granular, without striation, and not infrequently hyaline. The nuclei are swollen and frequently surrounded by a zone of œdema. In many fibres with extensive vacuolization of the protoplasm the nucleus does not stain. About such fibres small accumulations of mononuclear leucocytes are occasionally present and in some instances foci of epithelioid cells. In areas of extreme degeneration the outlines of the muscle fibres on cross section are indistinct and the picture is that of a uniformly granular mass with regularly placed lightly staining chromatic rings. These various changes affect the papillary muscles also. No changes in the vessels of the heart are demonstrable. Histological examination of the aorta is negative.

In other animals of this period the degenerative changes in the heart were of the same general character though varying more or less in degree. In two, a slight diffuse growth of connective tissue between the muscle fibres occurred.

Attempts to demonstrate fat in frozen sections of formalin hardened material treated with Scharlach R and counterstained by hæmatoxylin were uniformly negative. This method however revealed, in a few hearts, isolated fibres or small groups of fibres taking a diffuse yellow stain. These were more or less hyaline, and without nuclei; they however occurred in close relation to other similar fibres which did not thus react. This reaction has not as far as I am aware been previously described and its significance is not apparent.

The lesions in these hearts are due, manifestly, either to the

direct toxic action of adrenalin upon the heart-muscle or to mechanical disturbances of the circulation caused by the effect of adrenalin on the vascular mechanism. They cannot be secondary to alterations in the walls of the large vessels, for in this series none of the animals exhibited macroscopic lesions of the aorta and in but three were degenerative changes evident microscopically.

Proliferative Changes.—Of the twenty-two animals survivng seven to fifteen injections, all but three showed degenerative changes in the myocardium, though seldom of the severe grade observed in the above descriptions. In twelve, more or less definite new connective tissue formation was evident. This new tissue was present, in some hearts, as focal accumulations of fine connective tissue about individual hyaline or granular fibres or about small groups of such fibres. In others a diffuse arrangement of new tissue was demonstrable in considerable areas of the myocardium and in two cases a very severe and diffuse fibrous myocarditis existed. The new tissue as a rule was most abundant about the blood vessels but had no relation to the sub-epicardial tissue or to the endocardium. It was very prominent in the papillary muscles. All parts of the ventricle suffered, though it any difference existed, the lesions were more severe at the apex. Focal accumulations of leucocytes were not prominent in the more chronic lesions, though occasionally present in the earlier conditions. When present these were usually of the lymphoid cell type. No suppuration or other evidence of infection was seen at any time; the lesion apparently was a pure connective tissue proliferation following degeneration of muscle fibres. The following protocol is that of the most advanced lesion observed:

RABBIT No. 22.—Male, weight 1685 grammes. During a period of eleven days seven injections of adrenalin varying in amount from one-tenth to eight-tenths cubic centimetre were given (total amount five and two-tenths cubic centimetres). The animal was killed by chloroform five days after the last injection.

Autopsy.—Weight 1650 grammes. A small amount of clear serous fluid is found in the peritoneal and pleural cavities. The heart is large and firm; the walls of the left ventricle are distinctly thickened; the auricles are dilated. The entire circumference of the aorta at its origin is the seat of closely massed, depressed, round or oval, calcified areas which form a complete ring about the

vessel and extend irregularly into the sinuses of Valsalva and along the ascending portion of the aorta for a distance of one and two-tenths centimetres. The orifices of the coronary arteries, in the natural position of the aorta, are entirely obscured but by pressure on the heart substance their presence is indicated by the escape of minute points of blood. These lesions cause considerable deformity of the vessel. A few isolated plaques of similar appearance are scattered elsewhere in the aorta and a considerable number are grouped about the origin of the cœliac axis.

Histology.—Sections through the entire ventricle from base to apex show extreme degeneration of muscle fibres and a new formation of connective tissue diffusely arranged, for the most part, but with here and there focal areas in which the muscle fibres are entirely absent. The muscle fibres as a rule are swollen, vacuolated, and exceedingly granular, though some are atrophied. Fibres with irregular constriction and swelling and more or less hyaline transformation are not uncommon. The nuclei are distorted, stain poorly, and frequently are entirely absent. In some fibres the nucleus is contracted and surrounded by a clear zone; in other fibres the nucleus is greatly swollen. The connective tissue is more abundant in the mid and inner portions of the ventricle and but slight beneath the pericardium and in the papillary muscles. The focal areas are more numerous at the apex of the ventricle and towards the endocardial surface. The connective tissue is very loose and œdematous Between the elongated wavy vesicular nuclei is considerable intercellular substance practically free of lymphoid cells. While the arrangement of the new tissue is in general diffuse it is frequently more abundant about the blood vessels. In the focal areas the fibrillated character of the new tissue is very evident. The walls of the blood vessels show no change. Sections stained by Mallory's connective tissue method show everywhere between the muscle fibres a more or less well marked blue reticulum, while the focal areas take a deep stain relieved only by the deep red of fragments of hyaline fibres. The methods for demonstrating elastic tissue show a diffuse increase of fine elastic fibrils in the denser areas of connective tissue; they reveal no changes in the blood vessels.

Control Observations.—Although the literature treating of various experimental lesions of the myocardium of the rabbit contains no references to the occurrence of degenerative and proliferative changes in the hearts of apparently normal rabbits, it seemed advisable to determine definitely this point. As the sacrifice of a series of normal rabbits appeared undesirable the sections of heart muscle from a large number of animals injected with various organs and fluids of the dog during the past few years, in the course of several investigations of cytolysins, were utilized. A careful study of this material failed to reveal fibrous changes and showed few degenerative lesions. The latter were of slight

degree and never of the type occurring after the administration of adrenalin.

Mode of Action of Adrenalin.—The association of a severe fibrous myocarditis with vascular lesions involving the orifices of the coronary arteries, as in the experiment given above in detail, suggests that the latter condition must have some causal relation to the myocardial changes. Indeed in the early part of the investigation it was thought that such a relation would eventually be demonstrated. Such, however, does not appear to be the case, for in one instance a myocarditis of equally severe type developed in the entire absence of lesions in the aorta. Moreover in six of the twelve animals exhibiting changes in the myocardium no lesions of the aorta were present, and in three of the remainder the lesions were but slight. The fibrous changes in the myocardium would appear therefore to be independent of the vascular lesions and not analogous to the myocarditis associated with arterial diseases in man.

Other possible explanations are based on a direct toxic action of adrenalin on the heart muscle, or an indirect action through the mechanical disturbances of the circulation which it causes. Although the physiological action of adrenalin on smooth muscle is well known we have no knowledge of its toxic action on the various types of muscle cells. Degenerative lesions in the parenchymatous cells and in muscle cells other than those of the heart and aorta are rarely seen, and it seems improbable that the toxic principle of adrenalin, if it exists, should be so selective as to affect only the muscle cells of the heart and the smooth muscle of the aorta and its larger branches. Indeed the limitation of degenerative lesions to the heart and larger blood vessels, and their practical absence in all other tissues, speak against any toxic action and point to an influence of a mechanical nature affecting these structures alone.

The action of adrenalin in constricting the arterioles with consequent increase in blood pressure is well known. This increase of blood pressure is alone not sufficient to explain the lesions, for if it were, we would expect the vascular changes due to adrenalin to be more general. But it is admissible to conceive of a temporary

ischæmia of terminal vascular territories, due to the direct action of the drug upon the smooth muscle cells of the coronary arterioles, which lasts a sufficient length of time to cause some interference with the nutrition of the affected areas. From physiological experiments this period would appear to continue about ten to fifteen minutes. If to this we add the increased contractile effort of the heart necessary to overcome the greatly augmented intravascular tension, we have factors sufficient to explain the degenerative lesions of the early experiments. The heart muscle under conditions of poor nutrition is pushed to its extreme power and dilatation results with injury of single fibres or groups of fibres. This injury is due in part to nutritive and in part to mechanical disturbance. That the dilatation is extreme, that the muscle is anæmic, and that serious alterations of the fibres are associated with this condition after but a single injection of adrenalin has been repeatedly demonstrated during the course of this investigation.

Practically the same explanation was advanced by Dr. Stanton and myself to explain the necrosis in the lesions of the aorta due to adrenalin. K. Ziegler's explanation of the vascular lesions is also very similar. He points out that the inner and middle portions of the media of the aorta, the usual seat of necrosis, is the part of the vessel most scantily supplied by the vaso-vasorum and therefore most susceptible to necrosis at the time of vascular spasm and local anæmia.

Comparison with Other Experimental Lesions of the Myocardium.—The degenerative changes due to adrenalin do not conform in all respects to those caused by other agents used experimentally. The hyaline and granular changes in the muscle fibres, the alterations in striation, and the slight accumulations of lymphoid cells are similar to those found by various investigators (Comba,[4] Welch and Flexner,[5] and Mollard and Regaud[6]) who have studied the effect of diphtheria toxin on the heart muscle. On the other hand they differ in the absence of fatty changes, in the extreme œdema of the interstitial tissue, and in the disorganization of the fibres. In the same way also they differ from the lesions produced by Welch[7] in his experimental study of the effect of high

temperature and from those produced by Ribbert[8] with Staphylococcus pyogenes aureus, by Charrin[9] with Bacillus pyocyaneus, by Tallquist[10] with Streptococcus pyogenes, by Björksten[11] with various bacteria or their toxins, and by Flexner[12] with ricin and abrin.

With the exception of the slight focal increase of connective tissue described by Charrin, Comba, and Mollard and Regaud, as a rare result of repeated injections of bacterial toxins, the only form of fibrous myocarditis mentioned in the literature of experimental lesions of the heart is that following ligation of the coronary arteries. The histological changes following such ligation have been described by Kolster, Porter, and Baumgarten[13]. The last, in his very detailed description, states that by the fourteenth day the new connective tissue had largely replaced the widespread areas of anæmic necrosis. This is of interest in connection with the lesions above described, in that the most marked connective tissue proliferation occurred in a rabbit killed sixteen days after the first injection.

Relation to Lesions in Man.—No definite comparisons can be made. The animals with acute degeneration of the myocardium and extreme dilatation of the heart which die suddenly after the fourth or fifth injection represent a condition apparently similar to that occasionally seen in man, where unusual strain causes sudden death due to an acute dilatation of a heart previously injured by some toxic agent or disturbance of nutrition. The later fibrous lesions have no analogy to the commoner chronic forms of myocarditis in man associated with arteriosclerosis or following known toxic conditions. That some of the more unusual forms of fibrous myocarditis in man which are difficult of explanation may be due to circulatory disturbances of the same general nature as those caused by adrenalin injections in the rabbit cannot be denied. It is to these only that the results of this experimental investigation appear to have any relation.

EXPLANATION OF PLATE XX.

Fig. 1.—Fibrous myocarditis with more or less focal arrangement of the new tissue. Rabbit No. 22; see description in text.

Fig. 2.—Higher magnification of a different portion of the same section show-

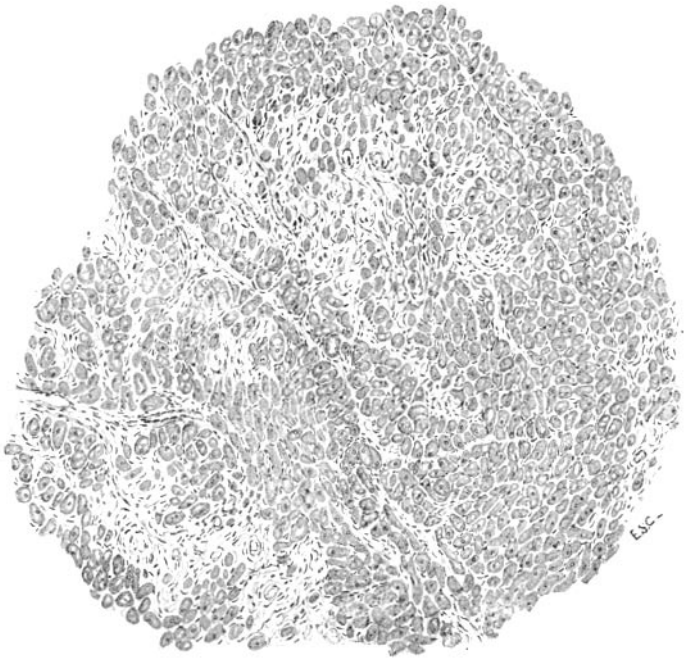

Fig. 1.

Fig. 2.

ing the character of the muscle degeneration and also a moderately diffuse increase of connective tissue.

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