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Experimental heart lesions

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EXPERIMENTAL HEART LESIONS

BY

MILDRED ESTHER SCHEETZ, M. D.

A THESIS

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STATE UNIVERSITY OF IOWA.

1913.
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INTRODUCTION.

By the term "heart lesions" it is inferred that any lesion of the heart is meant, and it is true that the lesion may be of the pericardium, the myocardium or the endocardium, but I propose to limit myself in the main to the lesions of the endocardium, and especially in its reference to the valves of the heart.

That endocarditis is due to an infectious agent is now a fact that is no longer disputed. But the factors that determine the infection of the endocardium is quite obscure, no satisfactory explanation based on experimental knowledge has been offered as to why the valves are involved so much more frequently than the mural endocardium, nor as to how the bacteria to which the infection is due, find their avenue of entrance into the tissue infected.

Now it has been generally assumed that the bacteria localize by implantation, more upon the valves of the heart than the mural endocardium, because of the irritation due to the friction of the ever passing blood which with the lessened vitality of the tissue just at the closing points of the valve cusps, furnish a suitable site for implantation of the bacteria contained in the blood stream. But this has never been
quite satisfactory, opinions have differed and there are many now that take the view that the localization of infection upon the heart valves is due to infectious embolism, due to the fact that the small tortuous capillaries in the base of the heart valves provide a site for bacterial emboli safe from the phagocytic power of the endothelium.

For a thorough understanding of how this may occur some knowledge of the embryology and histology of the heart valves is essential.

**EMBRYOLOGY.**

In the early stages of the embryo's development the heart appears as a tube. The veins of the body unite into a common cavity - the sinus venosus - at the posterior end of this tube. In the course of development this tube becomes bent upon itself and from it, later, pouches develop, the atrium and the ventricle, the original tube still persisting and connecting them. As development proceeds, the sinus venosus loses its distinctive feature as a separate structure to become incorporated in the termination of the superior and inferior vena cava and a strip of the right auricle between the orifices of these two vessels and the coronary sinus; the original tube
ceases to exist as tube, but persists as a band of peculiar fibers, the auriculo-ventricular bundle.

Tandler\(^2\) in the development of the heart shows that the atrial and ventricular musculatures are continuous around the atrial canal, until their continuity is broken by a wedgeshaped aggregation of embryonic connective tissue, along the atrio-ventricular groove, the thin edge of the wedge being directed inward. In later stages, this wedge gradually becomes prolonged toward the cavity of the heart and cuts so deeply in between the atrial and ventricular musculature throughout the entire circumference of the atrio-ventricular groove that the direct continuity these parts is interrupted, but the trabecular portions are still in continuity at the anlagen of the valves—
the valve apparatus then undergoes further elaboration in the differentiation of valve cusps, chordae, tendonae, musculi, pappillares, etc. While in the earlier period the endocardial portion greatly surpassed the muscular, the latter increases greatly on the atrial surface until it actually seems as if they were exclusively musculature, covered with endocardium. But later the musculature degenerates and connective tissue takes its place, standing in intimate connection with the
connective tissue wedge in the earlier stage. The free margins of the valves at certain points retain for a long time the characteristic structure of the endocardial thickenings, while in others points they are converted into typical connective tissue - these thickenings represent the "Noduli Albini". The semilunar valves being first plump folds of the distal swelling of the bulb, become thinner, and pouch-like cavities are formed which are the anlagen of the Sinus Valsalva. The connective tissue of the semilunar cusps loses its succulent character becoming fibrous and tendonous, with a simple covering of endocardium. So it is seen in the beginning of valve development that there was but an outpushing of the endocardium carrying with it a bit of the connective tissue beneath, which soon becomes fibrous in character, and much muscle which soon atrophies and occupies but a small bit of the valves length - wherever connective tissue and muscle was found, blood-vessels also could be noted.

Histology of the Valves.

Standard works give very meagre descriptions of the histology of heart valves. Kollicker describes the auriculo-ventricular valves as composed of a strong layer of fibrous tissue with abundant elastic fibers
running out from the fibrous ring about the valve orifice, each surface being covered with a layer of endocardium that is thicker on the auricular side, he says the semilunar valves have the same structure, but are thinner. Luschke was the first to describe the blood vessels in the valves, a little later he declared the vascularity of the semilunars. There was much controversy over this. Coen found the mitral valves vascular, but the aortic not-vascular. Darrier in a careful work in 1895, found that semilunar valves never contained blood vessels, that a normal tricuspid never contained them, that the mitral curtain, where its base is applied to the aortic valve, contained numerous vessels as far out toward its edges as the muscular layer extended, which is 1/4 of the way in an infant and 1/6 of the way in adults; that in pathologic conditions the whole valve might be valvular. Seipp in 1895 in a work on elastic tissue of the heart, goes into the subject at length, summing up all the literature that can be found on the subject. His observations were very accurate and detailed and much like those made in later times. There are
but few that give a detailed description of the tissue entering into the various layers of the valves. Curtis\textsuperscript{7} enumerates four distinct layers of the semilunar valves, but does not give detail. Veraguth\textsuperscript{8} describes various layers of the auriculo-ventricular and semilunar valves according to the appearance of the cells and intercellular substance and according to the embryology of the structures as given by Hertwig. Veraguth gave five layers to the valve -

1- Endothelial covering, a thin tissue rich in cells with spindle shaped nuclei and some larger nuclei.

2- A distinct zone of much intercellular substance and a few large cells.

3- Center, layer of fibers running parallel to rim of valve and very marked where chordae tendonaee are attached - also a few large cells.

4 and 5, Like 1 and 2, on the other side of the valve.

In the mitral valve he found the central layer quite pronounced, in the semilunars it is totally wanting. Veraguth maintained that this arrangement is borne out by the developmental appearances
of the valves in the embryo; according to Hertwig the auriculo-ventricular valves are formed by a fibrous degeneration of muscle tissue, which originally surrounded a small auriculo-ventricular opening; this fibrous tissue is covered on both sides by a layer of "gelatinous" tissue lining the heart. The semilunar valves, being composed only of an outfolding of this "gelatinous" tissue, have not the same fibrous center. Veraguth concludes that the two outer layers he describes are remains of the embryologic "gelatinous" tissue, and his central layer in the auriculo-ventricular valves is the fibrous degenerated muscle layer. The valves, according to him are symmetrical on their two faces and if slit down the middle between them would show similar halves - This assertion is more nearly true on the aortic valves than of the mitral, but even in the latter the distribution of elastic tissue on the two faces is not at all the same and the layer of muscle so often seen is always near the auricular surface.

Homans and Burrage gave observations made in the laboratories with Dr. Councilman, viz.,
Aortic valves - from birth to 5 years of age -
the valve is composed of fibrous and elastic tissue:

The fibrous tissue is densest on the aortic side
of the valve toward the tip, and at the base. The
fibers of the aortic side are for the most part cut
in cross section, i.e., their direction is circular.
Some however are longitudinal, or nearly so. Cells
are not numerous. In the middle of the valve, the
fibrous tissue is not dense, the fibers are in spiral
bundles and run in the long axis of the valve es-
pecially near the ventricular side. At the base of
the valve, the "hinge" area, the middle layer of loose
fibrous tissue is so broad as to occupy nearly the
whole width of the valve; toward the end of the valve
it is obliterated by fibers coming in from the ven-
tricular side. On the aortic side of the valve there
is a thin layer of cross cut elastic fibers, immediately
beneath the endothelium - this layer keeps its same
character throughout the length of the valve. In the
adult valves, the bundles of fibrous tissue are thicker,
less wavy, the valve less cellular, and less loose tissue
in the middle layer, than it is in the infantile heart -
there is a progressive change toward an adult type; the
valves thins in proportion to its length, the densest
portion of the fibrous tissue, instead of lying near the
aortic surface comes to occupy the greater proportion of
the width of the valve while the loose layer in the middle becomes very thick. In the infant the tip of the valves shows a sort of knob connected with the rest by a very thin portion; this is never seen in adult valves.

Mitral Valves - Infantile.

The mitral valve is longest, thicker and more flexible than the aortic. It is supplied at its base and fora variable part of its length with cardiac muscle. It possesses a thick covering of elastic tissues on both its surfaces, but the layer on the ventricular surface thins out rapidly as it passes out of the valve. The fibrous tissue is densest on the ventricular side, but occupies the greater portion of the valve. As the region of the Chordea tendonae is reached the fibrous bundles stream out to them from the ventricular surface.

The elastic tissue resembles that of the aortic valve, but differs in the thickness at the base. The muscle of the mitral curtain is a strong wedgeshaped area of the usual cardiac variety, found at the base of the valve with the fibers circular in direction. The amount of muscle and the extent to which it runs along the valve varies very much; in some cases it extends to nearly half the length of the valve, in other
cases it ends abruptly at the base. It is always found immediately beneath the auricular elastic layer and in all cases blood vessels are seen. The adult valve bears the same relation to the infantile as does the aortic, viz.; the elastic tissue is more fully developed and the fibrous tissue is more dense. The valve becomes longer and thinner. The blood vessels are always with the muscle on the auricular side. Schöffer finds a mass of nonstriated muscle at the base of the valve, acting as constrictor of the orifice, and in this run capillaries, which are also found in the wide strands of connective tissue in the "hinge area" coming from the ostia venosa; he also finds that the semilunar valves contain at their base bundles of muscle continuous with the myocardium of the auricles, and are supplied with blood vessels as far as the strands of muscle extend.

Degenerative lesions - There are thickenings of the heart valves whose pathologic character is in doubt and but very little can be found upon the subject.

Veraguth describes certain thickenings of the valves which apparently are not pathologic in character - they were found at any age and are apt to appear at the points where the valve surfaces come in contact with each other in closing and apparently were developed from his "second" layer.
On microscopic sectioning he found them to consist of a very dense connective tissue formation sometimes almost homogeneous in appearance and containing a few drawn-out shrunken cells; he considered them to be a development from embryologic remains which take on growth because of the irritation from the constantly coming together of the valve surfaces.

He points out that they are not hypertrophy resulting from overwork, as they have been found in the very young, nor can they be from acute infections as the layer between them and the surface is unchanged. Homans¹⁰ frequently found such thickenings accompanied on unaccompanied by definite pathologic changes. In aortic valves they occurred on the ventricular surface, where the valves met in closing. They were either conical or rounded in shape consisting, on section, of dense connective tissue with many fine elastic fibers, seemingly growing out from the ventricular elastic membrane at their base; in later life they were usually accompanied by certain degenerations. He suggests that they are an adaptation of the valve to correct a faulty closing as they are best seen on the aortic valves, and often on one or two cusps only. Adami and Nicholls¹³ speak of small bodies the color of raspberry jelly and of pinhead
size, seen frequently along the closing edge of the mitral segments, more rarely near the edge of aortic cusps, they are seen only in infants, disappearing usually in childhood. According to Kaufmann they represent unused remains of the (vascular) nodes or eminences from which the valves are developed. Wegelin in quite a recent publication points out that in the course of development small pockets lined with endothelium are formed on the ventricular aspect of the auriculo-ventricular valves and on the distal sides of the semilunar valves. These cavities eventually become covered in by the proliferation of the endothelium to form closed sacs. Valvular hematomas are always found to be covered by endothelium, and Wegelin holds that they are formed by the extravasation of blood into the pockets. It is suggested that the nodules are remains of these extravasations become organized.

Mucoid degeneration in the aortic valves - In the normal histology of the valves an area of loose cellular tissue was mentioned, very wide at the "hinge area" of the valves and running from here toward the free edge, between the ventricular elastic layer and the dense aortic fibrous layer. In young valves this was
found at times to take a deep blue stain with hematoxylin thus resembling myxomatous or mucoid tissue. In older valves, tissue having this same appearance is seen as a degenerative lesion more commonly than any other. It occurs toward the tip of the valve beneath the ventricular elastic layer and encroaching considerably on the dense fibrous tissue of the opposite side. Such areas show an increased number of cells with round or oval nuclei and a few connective tissue fibers, many of which appear fragmented. Stains for elastic tissue show a few elastic fibers. This condition accompanies and frequently occurs in the midst of large thickenings of the valve surface.

A natural explanation of this condition is that the thickening by interfering with the nutrition of the valve caused the degeneration for such myxomatous or mucoid tissue is often seen in the midst of large thickenings and also, it is curious to note that it is in the layer where a mucoid stain is taken in the very young valves.

Mitral valves - like the aortic, are apt to show thickening, these being on the auricular side where the valves meet, but the thickening is apt to include the whole width of the valve, and is not noticed
in young valves but particularly in advanced life and the condition is often associated with fatty degeneration.

Fatty Degeneration - This appears upon the valves and mass frequently upon the mural endocardium in the form of slightly elevated patches of yellowish white color, that are due to the transformation of the protoplasm of the connective tissue and endothelial cells into fat; in advanced cases, fat droplets may be seen in the spaces between connective tissue cells. In aortic valves this occurs most often midway from base to tip, almost never near the base and frequently throughout the outer half. It occupies the middle plane of the valve encroaching much more on the ventricular elastic layer than on the dense fibrous tissue. This is found in the majority of valves in individuals over 30 years of age, and is the beginning of atheromatous change. It is usually accompanied when extensive, by a generally diffused thickening of the ventricular surface, analogous to the sclerotic changes in arteries. The fat cells are often very large and are apt to lie in groups, often pushing the elastic fibers aside. In the mitral these changes take place in the auricular elastic layer and just beneath it. They are more often seen near the base of the valves, but in arrangement and distribution
Acute Infections in relation to degenerative lesions - In autopsies noted, all degenerations found were chronic, at least none recognized as acute or directly connected with pneumonia, diphtheria, or tuberculosis, etc. Veraguth gives a good account of the pathologic anatomy of valve infections. One instance is quoted by Homan of a mitral valve previously thickened and then followed by acute infection. In this case there was a destruction of tissue on the auricular side of the valve near the tip, extensive fibrin formation in the necrotic tissue and beneath this, in the main substance of the valve a considerable proliferation of large connective tissue cells. Large masses of streptococci were seen in the necrotic area. Veraguth reasoned that the round cell infiltration of fibroblasts took the place of polymorphonuclear leucocytes which were not present to any extent. No blood vessels were seen new or old. Adami says that when suppurative microbes are deposited on non-vascular areas, as for instance the outer third of heart valves, that there is a migration of leucocytes toward the infected spot, showing that these leucocytes came from the nearby vessels and in the heart valves.
are further complicated by being exposed to the irritating influence of the blood stream, which deposit upon the roughened area fibrin, forming "vegetations". The causative agent may grow in these vegetations if small they may be completely absorbed by the leukocytes, if not absorbed, the vascularity of the inflamed cusps extends into them, and their fibrin becomes replaced by granulation tissue, they then become organized and fibroid. In some cases the relative age of the different depths of thickening can be told, furthest from the auricular surface the tissues and blood vessels were richly supplied with elastic fibers, nearer the surface the tissue becomes less elastic and more like ordinary sująar tissue, while the vessels were smaller and younger. On the surface, when the repair from the last attack was still going on, the young connective tissue cells were large and abundant, and the vessels thinwalled and capillary. Such conditions would seem to show that the thickening was due to repeated attacks of acute infection; at other times it was impossible to tell whether the thickening was of the benign type described earlier, or of the type just described; the vascularity of the tissue, it is true, tends to show the difference.

In the mitral valves, there was no such extensive
distraction of large thickened flaps, as in aortic cases; one large thickened flap was practically eaten away from the ventricular side; it seems evident that a valve that has no internal blood supply cannot repair itself as well as one in which the blood supply is abundant. This valve, in which the destruction of tissue was extreme, possessed only a number of capillary vessels with no elastic tissue in their walls, vessels which were connected apparently with the surface of the valve only and were little more than mere blood spaces.

There was little attempt at repair, and it would seem as if an aortic valve stood a poorer chance of repair without deformity than a mitral under similar circumstances - and when it is generally assumed that bacteria localize by implantation, the great frequency of mitral and tricuspid over aortic and pulmonic remains to be explained.

Infections - in relation to infectious emboli of valves - That the infection takes place through infective emboli in the blood vessels is now being accepted, though Orth, in a wide experience, claims he has never met a case of it, although allowing it to be possible, in relapsing cases where new vessels have been formed in the tissues of the cusps, for this process to take place.
It is true that overstrain may be a cause and is to some extent supported by experimental evidence.

Repeated observations have shown that nearly all cases of valve lesions are due to microorganisms. So the old division of endocarditis into "simple" and "ulcerative" no longer holds good — for etiologically, the condition cannot be regarded as a distinct entity, a great variety of microorganisms enter into its causation, so it is more correct to speak of a streptococcus or pneumococcus infection, than endocarditis.

The bacteria at work are numerous and the infection may be mixed. The chief organisms are — the Diplococcus pneumoniae, the Streptococcus parous; the Staphylococcus pyogenes aureus; but the B. doli, B. diphtheria, B. influenza, the B. pyocyaneous, B. tuberculosis; gonococcus; micrococcus endocarditis rugatus; micrococcus endocardidis encapsulatis and B. endocarditis griseus have been met with. With regard to the frequency with which the various valves are involved, Washbourne refers to 309 cases of infective endocarditis, the mitral was alone affected in 115, the aortic 69, the aortic and mitral together in 73, the tricuspid in 28, the pulmonary in 19 of the cases.
Henderson gives an account of 8 cases, 7 of which were diagnosed in life, of infectious or malignant endocarditis, called by him "acute septicaemia", with localization on the endocardium. It has been generally accepted that cardiac rheumatism predisposes to infectious endocarditis by damaging the valves rendering them more liable to secondary infections. Payton and Paine investigated extensively the pathogenesis of rheumatic fever, and hold that rheumatic endocarditis is itself primary, and does not require an added infection to produce a progressive lesion.

Koster demonstrated that extension in an established human endocarditis may be due to embolism, and suggested that endocarditis may begin as an embolic process. No experimental proof of this mode of origin, however, was found.

Lissauer after reviewing the literature upon the subject extensively concludes that while endocarditis may occur exceptionally as an embolic process, no one has been able to show experimentally that this mode of origin really plays a role in the localization of bacteria in the heart valves - there is great difficulty in producing endocarditis experimentally without previous injury to the valves. Some previous lack of functioning from stress
or low vitality, seems essential. Ribbert produced endocarditis in rabbits by intravenous injections of emulsions of staphylococci and potato emulsions, the potato particles filling the small vessels and lowering vitality, making a fit place for bacterial growth, and showing that endocarditis was due in some cases at least to embolism.

Orth and Wyssowitsch had similar results from injections of suspensions of staphylococci and streptococci with finely pulverized charcoal. Fulci also had similar results from similar injections, but found that they were unable to produce endocarditis by intravenous injections of virulent cocci without the foreign particles, deducing from this that a previous lesion or lowered vitality must be present to prepare a reception for the action of the circulating bacteria. Recently Lissauer obtained endocarditis in two out of twenty rabbits after repeated injections of a non-virulent white staphylococcus without any foreign particles being present. The injections were repeated at frequent intervals, the doses being moderate. One rabbit died in two months after six injections and the other in six months after 10 injections both showing small vegetations on the mitral and tricuspid valves. No explanation was
given as to why repeated injections over a long period should produce endocarditis. We are led to infer that the frequent irritation and blocking of the small capillaries leads to a repeated sclerosis.

Horder has shown that cocci are cultivated from the blood in cases of chronic infectious endocarditis in 90% of the cases and that from these, as well as from allied streptococci cultivated from the throat or feces, well developed cases of endocarditis can be obtained by injections into animals.

Rosenau in 1909 sought to prove that the production of endocarditis by intravenous injections of organisms isolated from cases of endocarditis is due to peculiar qualities of the bacteria, he noticed that a chronic or subacute form which begins insidiously on a previously diseased valve, often with no demonstrable source of infection, and which nearly always runs a fatal course, is due to bacteria of a very low virulence, and that various strains differ quite markedly in certain details, but at certain stages they are all freely susceptible to phagocytosis, adhere more or less markedly to the surface of solid media, grow in clumps in broth, and produce endocarditis quite regularly when injected intravenously into rabbits. If injected into other regions they produce only slight illness and rapidly are destroyed.
by phagocytosis.

Davis in his examinations of animal reactions to streptococci injected similar various strains being of the type described by Davis and Rosenau in the recent milk epidemic of sore throat in Chicago— with the exception of two—a streptococcus vividans and a hemolytic streptococcus.

On examination the heart was found affected in a greater proportion of cases.

Jackson in her work on the myocardium, investigated the cause of "rheumatic myocarditis" made use of the same strains, found in succeeding experiments beginning with animals killed within 10 hrs after injection first large collections of bacteria in the minute blood vessels of the heart, with no necrosis or any cellular infiltration about the collections; in longer periods of life, 24 hours to 3 days, before being killed, the heart showed clumps of bacterial masses, with muscle cells immediately around them being necrotic, no cellular infiltration. In rabbits dying spontaneously at intervals of 5 to 7 days—areas of necrosis with cellular infiltration were found in the heart.

Bracht and Wachter by using different strains of streptococci produced changes in rabbit hearts which
are different from those caused by the ordinary
streptococcus pyogenes (hemolyticus) but not identical
with those in the human myocardium.

Cupps and Miller found that certain strains of
bacteria caused lesions in heart muscle. Geipel found
it difficult to account for the presence of nodules
in the heart muscle of a child dead of nephritis and
mitral endocarditis as no specific organism could be
found; it is now attributed to a certain infectious
agent known to produce such lesions in other hearts
when patient was suffering from acute articular
rheumatism; Rosenau in later experiments with his
modified pneumococci demonstrated that the hemorrhage
and vegetations appeared upon valves because of emboli
within the fine capillaries in the musculature at
the base of the valve, believing that endocarditis
was more common in childhood because of the greater
abundance of the muscle tissue of the valves.

Washbourne made various observations and experi-
mentations as to the phagocytic power of that the endo-
theelial lining of the heart has upon the bacteria of
the blood - he states that at points of narrowing,
where the blood stream impinges upon the endocardium,
this phagocytic action is most likely to occur and so
the auricular aspect of the auriculo-ventricular valve and
the ventricular aspect of the semilunar valves are favorable
areas for this process - but at the same time, these are
the regions which from their movements and functionings are
subject to greatest stress and in consequence when weakened
from work and low vitality, these cells, instead of de­
stroying the bacteria taken up, permit the intracellular
multiplication and they themselves become destroyed, the
bacteria multiplying and involving the other cells in the
immediate neighborhood, destroying them and thus making
the beginnings of the vegetations seen upon the leaves of
the valves.

The following experiments were made in the bac­
teriological laboratory with a view to ascertaining just
what action, and what alteration in structure took place
in cardiac tissue upon the entrance of certain material
and microorganisms into the general circulation - exper­
imentation and observation was made especially with regard
to the action upon valvular tissue of-

1- Common irritants and foreign bodies in the
circulation.

2- Irritants or foreign bodies, plus a microorganism.

3- Simple infections-and modified throat bacteria.

Investigation was then made as to-

1- The lesion, if any, by an irritant, and the extent
of the weakening and the invasion of virulent organisms upon
these lesions after a longstanding irritation; also

2. To determine if simple infections localized upon vascular tissues because of implantation, or of infectious emboli.

Technique - Practically all injections were made into the posterior auricular vein of half grown rabbits. Half-grown rabbits were used because they yield the best results, it being difficult to produce endocarditis at all in old rabbits, while in the very young, healing is apt to occur. In a few cases dogs were used, but they are not so susceptible to bacterial influence as rabbits, and are harder to handle in the laboratory. Of irritants used, alcohol proved the best, very small doses 0.5 cc of 34% alcohol given daily over a long period of time when the stomach of the animal was empty, reproducing as near as possible the normal manner of the heavy drinkers alcoholism. For foreign bodies, tobacco seed was used in the dog and finely ground charcoal in the rabbit. These were injected alone in a saline solution or mixed with a low degree-virulence staphylococci to produce if possible, infectious embolism of the small capillaries. For simple infection, Staphylococci from throat grown on blood agar or nutrient broth for twenty-four hours was used—or the saliva from many mouths collected and a small portion injected to test the conclusion of
of Rosenau that "saprophytic streptococci" so named by Horder, or "Streptococcus vividans" by Schoenmuller, are in reality pneumococci that have become attenuated and peculiarly modified as the result of passing through the circulation and environmental conditions. These may be strains taken from the throat and gain entrance through the tonsils in inflammatory conditions, localizing upon the valve tissue and causing endocarditis. The dose given was large enough usually to kill in 24 to 48 hours.

**Gross Observations**

With alcohol alone, the thickening of the valve in small nodule-like vegetations, whitish and hard-like fibrous tissue was the only result noted, the rest of the valve appeared hard and shrunken, no erosions were visible. In the case of long standing irritation from alcohol where B. coli were injected in small repeated doses, nothing different was to be detected grossly, except a slight roughening of the nodular-like elevations.

In the case of the tobacco seed, small hemorrhages were seen in the mural endocardium and one large (comparatively) hemorrhage at the base of the mitral valve, showing the effect of blocking by an emboli. The same result was obtained when the charcola and cocci were injected together showing that the smallness and tortuosity of the capillaries
in the valve made them especially liable to the blocking by emboli and that if an infectious agent be such as to form clumps, they alone could lodge in the capillaries, produce hemorrhages and probably because of the relatively slight vascularity in the valves could gather a mass of fibrin, etc., about them before the leucocytes could gather in such numbers as to cause their destruction. Hemorrhages and areas of degeneration were found near the apexes of the papillary muscles, presumably caused in the same way.

No hemorrhages or lesions were found on the semilunar cusps - though Rosenau found them in a few cases in his experimental work on this subject.

The injection of the twenty-four hour culture of staphylococci produced hemorrhages and small scars on the valves, the capillaries were markedly dilated, at the base of the valve, and in a few cases hemorrhages at the apexes of the papillary bundles were found. These hemorrhages were accompanied by similar hemorrhages in the kidney and the lungs - although the valvular hemorrhages occurred without any found elsewhere when the dose was small, when the dose was so large that the animal died within twenty-four hours, cardiac renal and pulmonary hemorrhages would be found without hemorrhages upon the valves. It seems that a certain
time must elapse before valvular hemorrhage takes place. When the cocci injected were not clumping well, the cardiac muscle seemed to be the one principally affected, large doses causing marked abscesses, and areas of necrosis. The rabbits when injected with saliva from the mouths of the student class, died within 48 hours, all showed valvular hemorrhages, beginning vegetations and hemorrhages of the papillary bundles; cultures from the blood showed modified pneumococci, and lesions in the glomeruli of the kidney.

Microscopic sections were made by the paraffin method and stained by hematoxylin and eosin and also by Gram's method. The sections through the valves and papillary bundles, the site of hemorrhages and dilated blood vessels, show much extravasation of blood, both subendothelial and muscular, with desquamation of endothelial cells and blood vessels packed with blood. What appears to the eye as a subendothelial hemorrhage always proves to be such under the microscope - Bacteria were never found in the areas of hemorrhage, especially when the animal died within 24 hours - nor when the lesion was of long standing, as then areas of necrosis or fibrosis were alone to be found. But when death occurred at the end of the second or third day, bacteria were found,
usually adjacent to an area of hemorrhage. At this time there is scarcely any leucocytic infiltration about the bacteria which is in marked contrast to the lesions in the glomeruli of the kidney, where leucocytic infiltration is marked at the end of 48 hours. In no instance was there any evidence of thrombosis at this stage, nor evidence that leucocytes were the carriers of the bacteria.

The lesions in the kidney were very much like those described by Aschoff and Gaskell and more recently by Baehr, in ulcerative or chronic endocarditis in man; there was sclerosis following areas of degeneration and hemorrhages due no doubt, as claimed by those authors, to bacteria emboli, followed by leucocytic infiltration and then sclerosis. The hemorrhages occur almost exclusively in the fine capillaries of the glomeruli, and if the animal does not die before the end of 48 hours, there may be leucocytic infiltration as far as Bowman's capsule—At the time of death from endocarditis fresh renal hemorrhages are rare, but healing or fibrous scarring in the glomeruli frequently observed.
Experiment No. I.

Rabbit inoculated by injection in posterior vein of ear with 1 cc of 24 hour stock culture of staphylococcus pyogenes aureus. Rabbit appeared quite sick for three days, then recovered. In 10 days reinoculated with same dose of same organism just obtained from pus that came from an abscess in vicinity of man's hip; animal became very sick, temperature somewhat raised; was killed with chloroform.

Post-mortem examination - Heart wall abscessed, mitral and tricuspid showing nodules and hemorrhages at base of abscesses on kidney, superior surface of diaphragm in subcutaneous tissues and in knee joints.

Bacteria recovered from serous exudate.

Experiment No. 32

Guinea pig inoculated with 1 cc of 24 hour stock culture of B. coli, by intraperitoneal injection. No symptoms developed, the dosage was repeated in 10 days and again in 10 days. No change was noted. In three months the pig was again inoculated with 0.5 cc
of same organism, no symptoms noticed. Animal killed with chloroform.

Post-mortem examination - Tricuspid valve showed slight hemorrhage. The leaves of the mitral were contracted and open, appearing to be adherent to each other and to the heart wall, which appeared thickened. The liver was pale in color.

Experiment No. 8.

A rabbit fed daily on 15 cc of alcohol (34%) for a period of 6 months, animal killed with chloroform.

Post-mortem Examination - Small white nodules like fibrous contractions were seen on both mitral and tricuspid, endocardium lighter in color than normal; muscle wall appeared thick and tough. Liver markings were exceedingly prominent and organ was lighter in color and firmer.

Experiment No. 9.

Rabbit fed daily on 15 cc of alcohol (34%) for a period of three months. At the end of this time 0.5 cc of twenty-four hour bouillon culture of attenuated bouillon culture was injected into the posterior auricular
vein. Rabbit showed slight symptoms of weakness for a few days, then became better; was killed one month later with chloroform.

Post-mortem examination - a few white nodules on mitral and tricuspid valves, no hemorrhages or dilated capillaries seen, muscle wall thick and fibrous; no hemorrhages or degenerations noted. The liver showed prominent markings but there was little else of note.

Experiment No. 10.

This animal, a rabbit, died spontaneously and post-mortem examination showed a large retroperitoneal abscess on left side, which by bacteriological findings was proved to be due to the Staphylococcus pyogenes aureus. The heart wall was thickened and flabby; there was marked dilatation of the capillaries at the base of the mitral valve, a few encrustations on the tricuspid leaves were found. The liver showed signs of degeneration, the kidney and spleen had foci of degenerations. No blood cultures obtained.

Experiment No. 11

This rabbit suddenly died while being etherized for experimental work of a different sort. Heart walls were soft and flabby but nothing abnormal seen on auriculo-ventricular valves. The animal was evidently suffering
from some severe intoxication as in the kidney and liver were found foci of degeneration and some slight hemorrhages - no bacteria from blood cultures were to be obtained.

Experiment No. 4.

Rabbit inoculated with 2.5 cc of virulent staphylococcus pyocyaneus aureus, intravenously in posterior vein of ear, rabbit appeared sick for two days, ear somewhat inflamed; on third day rabbit appeared as well as ever. At intervals of five days the rabbit was reinoculated, becoming gradually very weak, emaciated, listless, joints still, acting very rheumatic, finally attacked with dyspnoea convulsive movements and death.

Postmortem examination - abscesses at base of mitral valve and on apices of papillary muscles in left ventricle, also at base of tricuspid, none seen on semilunars. Many abscesses in muscle wall of heart - about 100 to 200 abscesses scattered over body, in the voluntary muscle joints, kidney, etc., although pancreas and lungs apparently not affected. Pure cultures of the organism injected were found in all the abscesses.

Experiment No. 18.

Rabbit inoculated intraperitoneally with sputum gathered from class, 2.5 cc being injected. Symptoms began within 24 hours, with temperature, a depressed and
listless attitude, loss of appetite, became gradually weaker dying in convulsions.

Postmortem examination - hemorrhages in the base of the mitral valve, heart wall soft and soggy, pneumococci found in hearts blood. Liver showed white patches and many granulating masses on the peritoneum. Intestines swollen and inflamed. Pneumococcus found in the inflammatory exudate which was fibrous and thick, these cocci also found in kidney, spleen and liver.

Experiment No. 3

Rabbit - Sputum from class injected subcutaneously into abdominal wall. In three days an intense suppuration occurred, walled off. Pneumococcus recovered from pus. In three days the animal was reinoculated with sputum from same class, 2.5 cc being given intraperitoneally, animal showed signs of sickness within 15 minutes, died within 12 hours.

Post-mortem findings - Heart valves (mitral and tricuspid) show capillaries markedly swollen, no distinct hemorrhages, walls of heart soft and distended. Pneumococcus recovered from blood of heart, also from inflammatory exudate; kidney, lungs, spleen and liver. Marked congestion and solidification of peritoneum around coelic axis; hyperemia of all organs.
Experiment No. 15

Rabbit inoculated with 2.5 cc virulent staphylococcus albus. Inoculation was intraperitoneal, but resulted in large subcutaneous abscess, animal kept one week, killed with chloroform.

Postmortem examination - Small capillaries in papillary bundle of left ventricles - heart wall flabby, hemorrhagic conditions of spleen, liver and kidneys. Cultures yielded the organisms injected.

Experiment No. 21.

Rabbit injected intravenously with 3 drops of adrenalin; injections were made daily for one week, increasing the dose by one drop each day; animal allowed to live for 2 weeks, killed with chloroform.

Postmortem examination - Heart walls thickened and fibrous areas of degeneration noted in myocardium; valves white, somewhat shrunken at base, areas of degeneration in spleen, liver, kidneys and lungs.

Experiment No. 6.

Rabbit injected intravenously with 2 cc of olive oil into the posterior auricular vein. Symptoms of dyspnoea with convulsions took place within the hour.

Postmortem examination - Heart apparently normal, no lesions were seen on valves or heart wall. Sections
showed many emboli in lung, a few isolated spots in kidney, none in brain, and a few in heart muscle. Sections stained with Sharla\textsuperscript{ahh} R for the oil.

Experiment No. 2.

Rabbit - subcutaneous injections of canthardin - dissolved in acetic ether - dose 0.001 gram - were made into the abdominal wall of a guinea pig daily for three successive days-animal killed with chloroform.

Postmortem examination - heart walls flabby, no hemorrhages seen on auriculo-ventricular valves, but endocardium appeared slightly roughened, intense hyperemia of liver and kidneys, a few foci of degeneration in the spleen.

Experiment No. 40.

Staphylococcus aureus 2.5 cc injected subcutaneously into posterior auricular vein, three successive injections made - at 7 day intervals, no symptoms arising, animal killed with chloroform.

Postmortem examination - a few whitenodules on mitral valve, none noticeable on semilunars or tricuspid, a few hemorrhagic areas in mural endocardium of left ventricle, small areas of hemorrhage in kidneys, no change noticed in lungs or spleen, liver showed areas of degeneration.
Experiment No. 37.

Inoculation of rabbit with 2.5 cc of Staphylococcus aureus, from serum culture tubes growth from throat swabbings - the culture was mixed with finely ground charcoal and injected into posterior auricular vein - three successive injections were made one week apart; rabbit killed with chloroform.

Postmortem examination - Mitral and tricuspic markedly congested, line of hemorrhage above at base of valve. Heart walls apparently normal. Small areas of degenerations seen in lung and kidney - cultures yielded organism injected.

Experiment No. 15

Received each day 1.5 cc plain alcohol (34%) per oram, in two months after colon bacilli were injected intravenously, doses repeated every fifth day - animal showed symptoms of acute infection, which then subsided and animal was killed with chloroform.

Postmortem examination - the heart was small and firm, walls rather lighter in color. The endocardium of the valves was roughened and small bits of blood and fibrin seemed to adhere closely. The liver showed slight cirrhosis, in the kidney there were areas of degeneration.
Experiment No. 12

Guinea pig that died spontaneously - no foci of infection noted, but the liver was found nearly destroyed by fatty degeneration - Heart showed areas of degeneration in the left wall, valves were clear; hemorrhage in apices of papillary bundles of left ventricle. On section heart showed fragmentation of muscle tissue and extravasation of blood.

Experiment No. 17

Guinea pig that died of puerperal infection.

Postmortem examination showed areas of degeneration in liver and kidney but nothing of note found in heart either macroscopically or microscopically.

Experiment No. 18

Rabbit injected with 1 cc of normal saline with which tobacco seed was mixed. Injection made into posterior auricular vein. At the end of one week autopsy was done. Small hemorrhages were found in lung with hemorrhagic infarcts in kidney and spleen. The heart showed one large hemorrhage on tricuspid valve, a few dilated capillaries on the mitral, with nothing of note in the heart walls.
Experiment No. 27.

Dog - small, healthy and active - no lesions shown by cardiogram - was injected with 2 cc of virulent staphylococcus aureus from throat cultures, mixed with finely crushed charcoal particles. These injections were made every 10 days for three weeks. The heart record remaining apparently normal, 20 cc of virulent staphylococci was given, being injected into the saphenous vein. Dog died within 24 hours.

Postmortem examination of heart - Heart walls rather soft and flabby, the mitral valve showed areas of hemorrhage and was markedly thickened, having a spongy feel to the touch. The tricuspid seemed glued and adherent to the heart wall. No areas of hemorrhage seen. On sectioning it was found that the vessels were filled with staphylococci in clumps and that the endothelial cells had exerted their phagocytic activity and were filled with the cocci - no leucocytes were in evidence the time being too short for them to make their appearance.
Fig. 1- Drawing of tricuspid valve from heart of rabbit No. 18 that had been injected with tobacco seed - capillaries at base of valve was enlarged and one large hemorrhage appeared at about midway the length of valve - hemorrhage was cut out and sectioned in paraffin - See PLATE 3, Fig. 1.

Fig. 2- Section through left ventricle showing hemorrhage of papillary bundle. Taken from heart of rabbit No. 4 that died from septicemia, having been injected with saliva gathered from student class. Pneumococcus found in blood and cultures. Sections made in paraffin, See PLATE 5, Figs. 1 and 2.

Fig. 3- Heart from rabbit No. 3, showing dilated capillaries and area of degeneration on papillary bundle and endothelial hemorrhages. See PLATE 3, Fig. 2.
PLATE II.

Fig. 1- Aortic valve - normal - from infant - showing fibrous layer on aortic side and the elastic layer on ventricular side, with the widely separated fibers of the loose connective tissue of the hinge area - no muscle apparent in valve.

Fig. 2- Mitral valve - normal- from infant, showing muscle extending one-third way down the length of valve, nearer the auricular side, the fiber being in cross section, showing their direction to be circular, elastic tissue for a considerable distance along ventricular side.

Fig. 3- Aortic valve - normal - hinge area showing loose connective tissue in central portion, appearing much like mucoid tissue, with dense fibrous tissue on aortic side.
PLATE III.

Fig. 1- Hemorrhage from tricuspid valve, from heart of rabbit injected with tobacco seed - note marked extravasation of blood and intact endothelium on both surfaces - Hematoxylin and eosin - X250

Fig. 2- Subendothelial hemorrhage from base of tricuspid valve, from heart of rabbit No. 15. Note the intact endothelium and the thickened endocardium - Hematoxylin and eosin X250.
PLATE IV.

Fig.1- Mitral valve from heart of rabbit having been injected with mixture of crushed charcoal and Staphylococcus aureus - Note enlarged nodules of endothelium with areas of necrosis in centers of thickening. X 150.

Fig.2- Degeneration of muscle fibers from base of mitral valve from heart of rabbit dying from slow injections of charcoal and Staphylococcus aureus. Hematoxylin and eosin. X 150
Fig. 1

Fig. 2

PLATE IV.
PLATE V.

Fig.1- Cross section of papillary bundle from heart of rabbit No. 4, having been injected with saliva and dying from septicemia showing large mononuclear cells surrounding blood vessel - the embryonal cells of Coombs, as seen in myocarditis.

Fig.2- Section from above - large mononuclear cells surrounding blood vessels and infiltrating tissues.
CONCLUSIONS.

As a result of the foregoing experiments, I believe that the endocarditis in the rabbit which followed injections of microorganism was due to an embolic process; that the emboli form in the small and tortuous capillaries in the valve, blocking the vessel, and hemorrhages result; that the cocci may form emboli it is necessary that there be some irregularity of the vessel wall, through some firmer irritant or that the infectious agent be capable of clumping and blocking the capillaries. The action on the endocardium of cocci from cultures and the throat is then due in part to the presence of fine capillaries in the valves, and in part to the peculiar mode of growth of these cocci which tend to grow in clusters and embolic-like clumps. These clusters become wedged in the fine capillaries of the valves and the relatively avascular structure of the valve serves to protect the cocci from the leucocytes long enough to allow them to develop so as to produce masses around which fibrin or other material is precipitated which for mechanical reasons again serves to protect the organisms - the production of valvular hemorrhages and endocarditis by simple intravenous injections of these cocci is almost a constant result as long as some foreign materialis injected with them, or the cocci
are of the kind that tend to form in clumps and adhere to surfaces as do staphylococci, streptococci and the cocci of the throat.

The injection of mixed cultures from saliva and the tonsils is followed by valvular hemorrhages and endocarditis, hence, there must be present some cocci, (as pneumococci) similar to those found in chronic infectious endocarditis, which when carried into new surroundings become modified and take on pathogenic properties much like the typical "endocardococcus" and experimental results tend to show that these organisms may cause various forms of endocarditis, that designated as "simple infective endocarditis" as well as the malignant form of chronic infectious endocarditis.
SUMMARY

Inflammatory changes in the endocardium may affect any portion of it, the valves, the tendonous cords, the papillary muscles or the mural lining. The valvular form is by far the most common, the mural endocardium is rarely affected alone.

Primary, or simple endocarditis is said to occur but it is now known to be but a secondary to some other condition, such as an intoxication or infection. Endocarditis is more common in the young than in adults; the greater susceptibility of the endocardium in children may be explained as due to the greater number of capillaries in the valves in youth.

I believe that when some microorganism, especially those that inhabit the throat, find their way by the tonsil into the blood stream, their virulence becomes greater and as they tend to cluster and fill the small capillaries, the heart suffers, especially the valves, which although they have better blood supply in youth than in old age, still have very small and tortuous vessels, allow the bacteria to gather in small clumps, that may be easily destroyed, and recovery ensues, but leaves the valve damaged. The repeated hemorrhages in the valves leads to sclerosis and this in turn to
to valvular disease (usually mitral regurgitation with more or less stenosis).

It is a well known fact that chronic malignant endocarditis under special consideration can be and is engrafted on a previously diseased and sclerosed valve - the results obtained here go to show that both simple endocarditis and the malignant fatal form can result from the same organism; in the simple, the mild infection caused first a vascularization of the valve, with perhaps hemorrhage - scar tissue results, the tissue becomes avascular, there may be many very fine capillaries form and this condition predisposes to infection.

On account of the sclerosed condition of the valve, repair becomes more difficult, the overgrowth becomes larger and larger, and it becomes the malignant type with ulcerating vegetations, which may either break off, be carried through the circulation and cause infarction in the brain, spleen or kidneys, and if they still contain bacteria, form miliary metastatic abscesses - or, in the milder forms, healing may take place with fibroblastic change, calcification and deformity of the valve occurs, but the condition leads sooner or later to death.
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