

## CARDIAC CHANGES IN DOGS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CRUZI*

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Since the discovery of Chagas' disease in Panama in 1931 we have had the opportunity of studying the pathological changes which occur in the acute fatal cases, DeCoursey (1) and Johnson and de Rivas (2). Our survey work indicates, however, that a very large percentage of the persons who become infected with this parasite do not succumb to the infection, but become what has been termed by Chagas and others as chronic cases. The extent of the damage to the heart musculature in the non-fatal cases is not definitely known and the conditions which exist in the so-called chronic cases is equally obscure. For a general review of the subject the reader is referred to a recent article by Yorke (3). Chagas (4) believed that a spontaneous cure does not occur, and that those who escape death in the acute stage all pass into a chronic stage. E. Chagas (5) on the other hand, believes that as time goes on the infection becomes attenuated owing to the defensive reaction of the host and consequently if re-infection does not take place the infection may come to an end. There is sufficient evidence, however, to show that the opinion held by E. Chagas is not the rule. Dias (6) reports a case in which the infection was present in the host for a period of 12 years and in which he was able to rule out the possibility of re-infection. In our own experience we have obtained positive complement-fixation reactions with serum of individuals which were positive by blood examination three and four years ago, Johnson and Kelser (7). Since it has not been possible to carry out a

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study of the lesions which occur in chronic Chagas' disease in the human host we have followed the infection in dogs which were experimentally infected with the parasite. Although we cannot state definitely that the changes which we have observed in dogs during the chronic period are necessarily the same as those which occur in human beings, we do believe that, because of the similarity of the lesions in the two hosts during the acute stage, there is very likely a definite parallelism in the chronic stage. It is for this reason that the following observations are recorded.

#### METHODS

The parasite *Trypanosoma cruzi* which was used in these experiments was obtained from three different sources, and are designated in the text as strain "O," strain "D" and strain "M." Strain "O" was isolated from an opossum on June 23, 1931 and has been carried since that time in guinea pigs. The "D" and "M" strains were recovered from human hosts. The "D" strain was isolated from an acute fatal case of the disease and "M" strain from an acute case which recovered. These last two strains were inoculated directly into dogs.

On the basis of the lesions produced it seemed that there was a difference in the virulence between the animal strain and the two human strains. With the animal strain the intensity of the infection was much less than the other two and there was therefore far less myocardial damage.

The animals were infected by intraperitoneal inoculation of whole defibrinated infective blood. On the following day, examination of blood smears was started and this procedure was continued until the animals died or were sacrificed. Data concerning strains of parasites used, prepatent period, patent period, date of autopsy and etc. are given in table 1.

The employment of the blood smear examination for checking the presence of the parasites was of no value during the chronic periods of the disease, for the reason that during this period no blood forms are present. This was shown by animal inoculation. Various methods were tried and the complement fixation test was finally considered the most valuable for detecting the pres-

ence of the parasites in the tissues. The procedure was that devised by Kelsler (8) in which an artificial culture antigen is used.

A total of 19 dogs have been used in this study; 10 were inoculated with strain "O" and nine with the human strains "D" and "M." All animals sacrificed during the period when parasites were present in the blood were considered as acute cases and those sacrificed after the disappearance of the trypanosomes

TABLE 1

DOG NUMBER	STRAIN USED	DATE INOCULATED	DATE POSITIVE	DATE LAST POSITIVE	DATE AUTOPSIED	CHRONIC PERIOD†
						days
19	"O"	4/18/34	5/24/34	P.W.K.*	8/ 2/34	
20	"O"	4/18/34	5/14/34	8/15/34	10/18/34	63
21	"O"	4/18/34	5/25/34	P.W.K.	7/ 2/34	
23	"O"	4/18/34	5/31/34	8/24/34	10/30/34	66
24	"O"	4/18/34	5/31/34	8/22/34	8/25/34	3
25	"O"	8/16/34	10/ 3/34	P.W.K.	10/30/34	
26	"O"	8/16/34	10/ 3/34	4/19/35	6/ 2/36	408
27	"O"	8/16/34	10/ 2/34	P.W.K.	10/18/34	
28	"O"	8/16/34	10/ 2/34	12/19/34	7/25/36	583
29	"O"	8/16/34	10/ 3/34	P.W.K.	10/30/34	
31	"M"	8/19/35	9/ 9/35	12/14/35	6/ 2/36	70
32	"D"	4/ 3/35	4/24/35	P.W.K.	5/ 9/35	
33	"D"	5/ 9/35	6/17/35	11/19/35	7/25/36	248
34	"D"	2/26/35	2/28/35	Died	4/ 3/35	
41	"D"	7/16/35	8/14/35	12/18/35	5/12/37	510
43	"D"	7/16/35	8/14/35	Died	9/21/35	
50	"M"	12/ 6/35	12/13/35	2/20/36	5/ 4/37	438
51	"M"	12/ 6/35	12/19/35	2/15/36	5/12/37	451
53	"M"	12/ 6/35	12/14/35	1/29/36	5/12/37	468

\* Blood stream positive when killed.

† Period elapsing between last appearance of parasites in blood and death of animal.

from the circulation were considered chronic cases. Two of the dogs which were inoculated with the human strains died of the infection; one had been inoculated with the "D" strain and the other with the "M" strain. The number of acute cases totalled 9 and the chronic cases 11.

The hearts were removed immediately after the death of the animal and pieces were fixed in several different fixatives. Zen-

ker's solution was found to be most suitable; with this fixative the ordinary hematoxylin-eosin staining procedure stained both cells and parasites in a very satisfactory manner. Neutral 10 per cent formalin, 95 per cent alcohol and Schaudinn's solution were also used. Hematoxylin-eosin was the stain of choice. Various others were used but the results were in no sense superior to those obtained by a properly performed hematoxylin-eosin stain.

#### OBSERVATIONS

##### *Description of lesions produced by strain "O"*

Ten dogs comprised the group inoculated with this strain. Five of these were sacrificed during the period when trypanosomes were present in the peripheral circulation (D-19, D-21, D-25, D-27 and D-29). The blood stream infection in these animals was very light and never more than 40 parasites were counted in any one thick film preparation.

The microscopical examination of the heart tissues showed very little in the way of pathological changes. The muscle fibers showed practically no alterations; even those which contained parasites were without demonstrable structural changes. The parasites usually occupied the central portion of the fiber, in many cases displacing the nucleus laterally or toward one or the other of the poles. In no instance were parasites found in any structure except the cardiac fiber itself. The number of parasitized cells were few and in D-19 and D-27 an extended search was necessary before any were found.

Scattered throughout the myocardium were small accumulations of round cells. These accumulations in some instances were composed entirely of lymphocytes and mononuclear wandering cells and in other instances fibroblasts seemed to be the predominating type. The large mononuclear cells were actively engaged in the process of phagocytosis and fragments of nuclei, red cells and muscle were seen in their cytoplasm. Although these inflammatory foci were seen in all parts of the myocardium the greatest number was usually to be found near the auriculoventricular junction.

The remaining dogs in this group, five in number, were not sacrificed until the trypanosomes had disappeared from the blood stream, that is they could no longer be found by microscopic examination of blood smears. By referring to table 1 the interval between the last appearance of the parasites in the circulation and the date that the animals were sacrificed can be determined. It will be seen that one, D-28, was not killed until a period of 583 days had elapsed. The others varied from three days up to 408 days. The probable presence of parasites in the tissues during the negative blood phase was shown in two dogs by the application of the complement-fixation reaction. In both animals, tested at various intervals, the reactions were strongly positive and at the time that they were killed the tests were 4 plus.

In these dogs lesions were extremely few. D-24 killed three days after the disappearance of the parasites, showed the greatest number. Focal inflammatory lesions of the same character as has already been described were again the only changes noted. Parasites could not be found in D-20, D-23, D-26 and D-28 although their presence was indicated by the areas of infiltration and in addition in D-26 and D-28 by the positive complement fixation reactions.

In general the pathological lesions seen in this group of dogs consisted of a low grade focal inflammation occasioned by the degeneration of cardiac cells. The picture of the chronic form differed from the acute in that the reaction was less intense and no parasites could be found in the tissues.

*Description of lesions produced by strains "D" and "M"*

Nine dogs were inoculated with the human strains, five with strain "D," and four with strain "M." Two, D-34 and D-43, died as a result of the infection; one, D-32, was sacrificed shortly after the parasites first appeared in the blood and the remaining six, D-31, D-33, D-41, D-50, D-51, and D-53, were allowed to live for varying periods after the disappearance of the parasites from the circulation. In seven of these animals, on several occasions, the number of parasites appearing in the blood stream

was over 300 per thick film preparation. In two, however, the highest number found was 32.

The pathological lesions which were found on examination of the two fatal cases were typical of those described for man. There was in D-34 a considerable degree of myocardial degeneration with an inflammatory reaction which was more or less generalized. The muscle fibers were thin and stringy and widely separated from one another, the spaces between being filled with round cells of various types. Some of the cardiac fibers showed loss of striations and an increased affinity for eosin stain, appearing more or less like the cardiac cells undergoing degeneration in such toxic infections as diphtheria. In D-43 on the other hand, although the myocardial damage was considerable the majority of the cells were of normal size and no serious alterations in their structure was noticeable. In both of the dogs there were a great number of parasitized fibers. The presence of the parasites in these elements did not appear to injure them in any respect and until the muscle cells actually bursted from the increase in the number of parasites contained in them they were unaltered structurally. As long as the cardiac fibers containing the parasites remained intact there was no cellular reaction around them. The rupture of the cells with liberation of the parasites, however, called forth an inflammatory reaction. The cells usually involved were lymphocytes and several types of mononuclear wandering cells. These last mentioned types were seen with their cytoplasm loaded with nuclear fragments and broken down muscle fibers and other débris. It was a rare occurrence when parasites were found phagocytized and to find them in elements other than muscle fibers was most unusual. Although the general pathological picture seemed to be at first glance a diffuse one, closer examination showed the underlying process to be focal in nature.

The myocardial damage and consequent interstitial reaction was evident in all portions of the myocardium examined; however, there were areas which were more seriously involved than others. The myocardial layers adjacent to the epicardium and endocardium always showed greater pathological changes than the more

central portions. These outer and innermost layers became progressively more involved the nearer to the base of the heart they were located.

D-32, killed shortly after the appearance of trypanosomes in the blood stream, showed very little myocardial damage. The parasitism of the muscle fibers was slight and the focal inflammatory lesions were not numerous. Here again the seeming predilection of the parasites for the outer and innermost layers of the myocardium was apparent.

D-31, D-33, D-41, D-50, D-51 and D-53 were sacrificed at different periods during the chronic stage. The serum of each animal was examined by the complement fixation test and each gave positive reactions. It will be seen from table 1 that the periods of chronicity varied from 70 to 510 days. In each of these dogs on microscopical examination focal areas of inflammation were present. These were composed of the usual collection of round cells and cellular débris. In what appeared to be older lesions numerous fibroblasts were present and many were undergoing mitosis. Scattered throughout the myocardium were numerous small scarred areas representing healed focal inflammatory lesions. This seemed to be the only method of repair of the affected areas as there was no evidence of actual regeneration of the cardiac fibers. In none of these animals could parasites be found, even after long searches.

#### DISCUSSION

*Trypanosoma cruzi*, the causative agent of Chagas' disease, when inoculated into dogs gives rise to a parenchymatous myocarditis with an accompanying interstitial reaction. These changes may be of an acute character as seen in D-34 and D-43 where the pathological changes were of a serious type or they may be of a sub-acute or chronic type in which the lesions are distinctly focal in nature and less destructive in character. The lesions are initiated by the invasion and subsequent rupture of the cardiac fiber by the parasite, but until actual rupture of the fiber takes place no alteration in its structure occurs and no surrounding cellular infiltration is occasioned. Upon rupture of the

cardiac cells and liberation of the parasites the focal lesion so characteristic of this infection is formed. It has been stated by Crowell (9) and others that toxins produced by the parasites are responsible for some of the damage to the myocardium. Niimi (10) published an article in which he states that he was able to isolate a toxin from cultures of *T. cruzi* which inoculated into experimental animals caused blood changes. In our series of dogs no evidence of a substantial nature was obtained which would indicate the presence of a toxin. The damage which was seen could be readily accounted for on the basis of the mechanical action of the parasites. In this infection as well as in other trypanosome infections, in our opinion, sufficient evidence has not been presented to show the actual existence of a true toxin.

We have found that the lesions which occurred in our series of hearts were present in greater numbers in some areas than in others. This observation applies to the acute phase as well as to the sub-acute and chronic phases. In all the hearts the focal lesions were most numerous in the layers adjacent to the epicardium and endocardium near the auriculo-ventricular junction. The central and apical portions showed much less involvement. An adequate explanation for this could not be found. It might possibly be associated with the blood supply. Crowell (11) describes this as occurring in an armadillo heart which he had the opportunity of examining. In acute cases this finding, although masked by the more or less diffuse character of the interstitial reaction, is nevertheless present.

Definite pathological changes were demonstrated in animals in which the trypanosomes had been absent from the circulation for periods as long as 538 days. These changes differed from those of the acute cases only in extent and intensity. The characteristic focal round cell inflammatory lesions were present, although few in number. Scarring to a greater or lesser degree is also seen in the chronic form. Parasites are extremely rare and in most cases are not found in microscopical examination of the tissues. However, the presence of the focal lesions and the fact that complement fixation tests are positive leave little doubt as



to their presence even though they cannot be demonstrated microscopically.

Our results indicate that there was a difference in virulence between the strains of parasites used in these studies. This difference was seen in the intensity of the blood infection and microscopic pathology. Animals inoculated with a strain of parasites isolated from an opossum gave a much less marked reaction than the two strains recovered from human hosts. This animal strain had been isolated in 1931 and had been passed through many guinea-pigs before it was used in dogs, while the human strains were of more recent origin. The passage through guinea-pigs might conceivably have been responsible for the attenuation of the opossum strain. Chagas (12) reports the attenuation of a strain *T. cruzi* after passage through human hosts.

#### CONCLUSIONS

1. The cardiac lesions of acute fatal Chagas' disease in experimentally infected dogs appear to be the same as those of the fatal infection in humans.

2. The existence of a chronic stage of this disease in dogs has been demonstrated. The lesions differ from the acute lesions only in intensity and destructive character.

3. The lesions of experimental Chagas' disease in dogs are seen to occur more frequently in the outer and innermost layers of the myocardium; the central portions are less involved.

4. The possible attenuation of a strain of *T. cruzi* by passage through guinea pigs is suggested.

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