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EXPERIMENTAL CHAGAS DISEASE IN PUREBRED BEAGLE DOGS ACUTELY INFECTED WITH *TRYPANOSOMA CRUZI* (B STRAIN)

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SUMMARY

The pathogenesis of Chagas' disease was studied in purebred beagle puppies that received a subcutaneous inoculation of approximately 500,000 *Trypanosoma cruzi* (B strain) per kg of body weight. All the dogs developed acute Chagas' disease. Clinical signs were observed and peripheral blood parasitemias were periodically quantified. Tissue concentrations of amastigotes and the severity of lesions were determined. A prepatent period of 4-7 days post-inoculation and a period of peak parasitemia from 13-24 days were observed. A positive statistical correlation between terminal parasitemia counts and tissue amastigote counts was found. By 13 days post-inoculation all the primary body tissues were subject to parasitic infection. In addition, early morphologic changes associated with acute Chagas' disease were seen as regional lymphadenitis and hyperplasia. The highest tissue lesion counts were between 18-31 days. A progressive myopathy, cardiopathy and neuropathy were reflected in the clinical findings and confirmed by histologic examination.

INTRODUCTION

American trypanosomiasis has received critical attention since this disease was discovered by Chagas in 1909 [2]. The significance of Chagas' disease as a dangerous plague of the South American continent has generated numerous research efforts to reveal its pathogenesis [9]. Experimental studies in dogs have furnished information as to the transmission of the disease [4], cardiac changes in experimentally infected animals [8] and testing programs for the detection of useful therapeutic agents [5, 6]. GOBLE [5] has studied variations in the incubation period, potency, and pathogenesis of experimental Chagas' disease in dogs as influenced by age and sex of host, route of inoculation and strain of parasite. Other studies cited by GOBLE [5] furnish data on complement fixation, neurotropism, diagnostic isolation, pathology, hematology, electrocardiography and intradermal reaction.

The purpose of this study is to document clinical and morphologic data from purebred beagle puppies acutely infected with B strain *Trypanosoma cruzi*.

MATERIAL AND METHODS

The B strain of *T. cruzi* was originally isolated from a Brazilian patient in 1942 and has been used extensively for experimental studies on Chagas' disease [7].

In this investigation B strain *T. cruzi* was obtained from the heart blood of female donor mice of the Manor-Swiss strain, 10-14 days after infection. Four male and eight female purebred beagle puppies, ranging from 2-4 months of age received a subcutaneous inoculation in the middorsal lumbar region of approximately 500,000 parasites per kilogram

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of body weight. These puppies served as untreated infected controls during the course of chemotherapeutic investigations.

Daily observations for clinical signs were made beginning on the day after inoculation. Blood was withdrawn from ear veins periodically for microscopic quantification of *T. cruzi* in the peripheral blood. One microliter of undiluted blood was placed under an 11.0 mm square cover slip and the total number of parasites in the sample was counted.

The dogs were sacrificed over a period of 7 to 31 days post-inoculation by chloroform inhalation. At necropsy all tissues and organs were examined grossly. Selected organs were sampled and placed in Susa and Carnoys' fixatives. Paraffin sections were cut at 5 microns and routinely stained with hematoxylin and eosin. In addition, brains of eight dogs were placed in 10% formalin and were stained with luxol fast blue-cresyl violet.

An estimated concentration of amastigote stage pseudocysts in the tissues was determined by counting the cysts per tissue section and assigning the following designations: +, 1 to 3 cysts; ++, 4 to 6; +++, 7 or more. Tissue amastigote grades were obtained by adding the + signs for individual dogs.

The degree of lesion formation in the tissues was graded as slight, moderate or marked. Lesion grade averages were obtained by adding the + signs and dividing the sum by the number of organs examined for each dog.

Statistical correlation coefficients and statistically significant probability levels for terminal parasitemia counts versus tissue amastigote grades and average lesion grades, and amastigote grades versus average lesion grades were calculated using the rank correlation method of LITCHFIELD & WILCOXON [10].

RESULTS

Based on previously published data on the characteristics of B strain *T. cruzi* infections in dogs [5], erratic prepatent periods of at least 1 or 2 weeks were anticipated. Consequently, microscopic examination of the peripheral blood of the first four dogs inoculated was not initiated until day 8 post-inoculation. The remaining eight dogs were examined earlier and showed a prepatent period ranging from 4 to 7 days. Thereafter, parasites were detected in greatly varied numbers on the days indicated in Table I. Between 18 and 31 days post-inoculation, a trend toward a decrease in parasitemia prior to sacrifice was observed.

The first clinical sign appeared on day 3 post-inoculation as a bilateral conjunctivitis with copious lacrimation in dog No. 1. On the day of sacrifice, the other signs observed initially on day 18 were progressively more pronounced. These signs included 6 instances of muscular weakness and 3 of paraplegia. A moribund condition was preceded by prostration in four dogs. Other signs listed in Table II include anorexia, labored respiration, hypersalivation and tactile hypersensitivity.

Table II provides a summary of gross findings at necropsy. One of the two dogs sacrificed 7 days post-inoculation showed hyperemia and enlargement of regional lymph nodes. At 13 days a lymphoid organ hyperplasia was well established and was consistently observed until 31 days when the last dog was sacrificed.

Additional gross findings were subcutaneous gelatinous edema (13 and 18 days), 3 instances of "chagomata", and general muscular atrophy in four dogs.

Table III presents the estimated concentration of amastigote stage cysts in various organs. Thirteen days after infection, amastigotes were frequently found in lymphoid organs, connective tissue, skeletal and cardiac muscles including the heart valves (Figs. 1A and 1B) and in peripheral nerves (Figs. 1C and 1D). They were found less frequently in thyroid, adrenal and salivary glands, tongue, esophagus, ileum, aorta, extrinsic eye muscles and kidney; none were found in lungs, pancreas, pituitary gland or spinal fluid. Dog Nos. 10 and 11 had high concentrations of amastigotes in brain tissue at days 28 and 29. Gonads and bone marrow samples of dogs sacrificed after 24 days post-inoculation were not examined.

Table IV presents a summary of the histopathologic changes. At 7 days post-inoculation lymphoid organ hyperplasia, skeletal muscle myositis, hepatitis and focal interstitial nephritis were observed. After 13 days post-inoculation, infectious granulomas, necrosis and inflammatory cellular infiltrations were

TABLE I

Parasite Counts of Peripheral Blood from Beagle Puppies Infected with a Subcutaneous Inoculation of Approximately 500,000 *T. cruzi* (B Strain) per kg of Body Weight

Dog Identification Number	1	2	3	4	5	6	7	8	9	10	11	12
Sex	F	F	F	F	F	F	M	M	F	M	F	M
Day Sacrificed Post-Inoculation	7	7	13	13	18	18	24	26	26	28	29	31
Days Post-Inoculation	Parasites Per 1 Microliter of Peripheral Blood											
1	0	0	0	0	0	0	0	**	**	0	**	**
3	0	0	0	0	0	0	0	**	**	0	**	**
4	*	*	*	*	*	*	1	**	**	< 1	**	**
5	< 1	0	< 1	< 1	< 1	0	*	**	**	*	**	**
7	< 2	1	9	3	5	1	15	**	**	7	**	**
8			*	*	*	*	33	+	+	40	+	+
9			132	28	16	8	91	*	*	38	*	*
11			441	91	16	12	123	+	+	94	+	+
12			*	*	*	*	129	*	*	155	*	*
13			> 5000	216	9	9	*	+	+	*	+	+
14					*	*	199	+	+	457	+	+
15					35	30	*	+	+	*	+	+
16					*	*	532	+	+	571	+	+
17					22	10	*	+	+	*	+	+
18					16	7	535	80	43	836	111	25
19							*	120	50	*	173	32
21							478	98	53	392	123	33
23							768	*	*	476	*	*
24							1838	*	*	911	*	*
25								*	2	*	166	37
26								308	3	488	*	*
27										*	92	37
28										214	*	*
29											69	25
31												8

< = less than
> = greater than
* = no sample taken
** = not examined

F = female
M = male
\+ = parasites present but not counted until day 18

TABLE II

Summary of Clinical and Gross Morphological Findings at Autopsy for Beagle Puppies infected with a Subcutaneous Inoculation of Approximately 500,000 *T. cruzi* (B Strain) per kg of Body Weight

Dog Identification Number		1	2	3	4	5	6	7	8	9	10	11	12
Sex		F	F	F	F	F	F	M	M	F	M	F	M
Age of Dog at Inoculation (Months)		3	3	3	3	3	3	2	3	3	2	4	3
Day Sacrificed Post-Inoculation		7	7	13	13	18	18	24	26	26	28	29	31
Prepatent Period, Days Post-Inoculation		5	7	5	5	5	7	4	8*	8*	4	8*	8*
First Appearance of Clinical Sign (Days Post-Inoculation)		3						21	21	26	21	18	30
Clinical Findings	Paraplegia										+	+	+
	Anorexia								+			+	
	Weak and Phlegmatic							+	+	+	+	+	+
	Prostrate and Moribund							+	+		+		+
	Keratoconjunctivitis	+						+		+	+	+	
	Epiphora	+									+		
	Labored Respiration							+	+	+	+		+
	Sialorrhea								+	+			
	Hyperaphia												+
Gross Findings	General Subcutaneous Gelatinous Edema				+	+							
	"Chagoma" at Injection Site						+		+		+		
	General Muscular Atrophy							+			+	+	+
	Focal Pleural Surface Edema			+									
	Kidneys: Cortico-Medullary Focal Hyperemia							+					
	Slight Hydrocephalus			+		+		NE	NE	NE		+	NE
	Dark Red and Watery Bone Marrow				+	+	+	NE	NE	NE	NE	NE	NE
	Lymph Nodes: Enlarged		+	+	+	+	+	+	+	+	+	+	+
	Lymph Nodes: Marked Hyperemia		+	+		+	+			+			
	Lymph Nodes: Edematous					+							
	Granular Spleen Surface				+			+	+	+		+	+

F = female
M = male
* = first examination

\+ = positive finding
NE = not examined

TABLE III

Summary of the Estimated Concentration of Amastigote Stage Cysts of *T. cruzi* (B Strain) in Various Organs of Beagle Puppies, After a Subcutaneous Inoculation of Approximately 500,000 Organisms per kg of Body Weight

Dog Identification Number	1	2	3	4	5	6	7	8	9	10	11	12
Sex	F	F	F	F	F	F	M	M	F	M	F	M
Age of Dog at Inoculation (Months)	3	3	3	3	3	3	2	3	3	2	4	3
Day Sacrificed Post-Inoculation	7	7	13	13	18	18	24	26	26	28	29	31
Skin at Injection Site	+	+	+++	++		++	+			+		
Thyroid Gland			++									
Thymus Gland			++				+++	+++	+++	+++	+++	+
Regional Lymph Nodes		+++	+++	+++	++	++		+	+++	+	+++	+
Spleen			+++	+					+	+++	+	
Tongue			++	+								
Esophagus				+						+		
Salivary Glands			+									
Skeletal Muscles			+++	++	+		+++	+++	+	++	+	+
Lung												
Heart			+++	++		+	+++	+++	++	+++	+	++
Aorta									+			
Liver			+							+		+
Pancreas												
Ileum			++									
Kidney										++		
Brain							NE	NE	NE	+++	+++	NE
Spinal Fluid							NE	NE	NE			NE
Pituitary Gland							NE	NE	NE			NE
Peripheral Nerve			++	+++		+		+	+			
Eyes			+	++			NE	NE	+			NE
Adrenal Gland			+	+			NE	NE	NE		NE	NE
Gonads							NE	NE	NE	NE	NE	NE
Bone Marrow		+	+++				NE	NE	NE	NE	NE	NE
Connective Tissues			+++	+++		++	+	+	+	++		
Tissue Amastigote Grade	1	5	35	21	3	8	11	12	14	22	12	6

\+ = 1-3 cysts per section
++ = 4-6 cysts per section
+++ = 7 or more cysts per section

NE = not examined
Blank = not remarkable

TABLE IV

Summary of Histopathologic Findings in Beagle Puppies Infected with a Subcutaneous Inoculation of Approximately 500,000 *T. cruzi* per kg of Body Weight

Dog Number		1	2	3	4	5	6	7	8	9	10	11	12
Sex		F	F	F	F	F	F	M	M	F	M	F	M
Age at Inoculation (Months)		3	3	3	3	3	3	2	3	3	2	4	3
Day Sacrificed Post-Inoculation		7	7	13	13	18	18	24	26	26	28	29	31
Lymphoid Hyperplasia	Regional Lymph Nodes	+++	+++	+++	+++	+++	+++	++	+++	+++	++	+++	+++
	Spleen			++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	Thymus Gland				++	+++		+++	+++	++	+++	+++	+++
Infectious granuloma formation associated with necrosis and inflammatory cellular infiltration	Injection Site Subcutaneous		+	+++	+	+++	+++	++	+++	+++	+++	+++	+++
	Skeletal Muscles	+++	+	+++	+++	+++	+++	+++	+++	++	+++	+++	+++
	Diaphragm Muscle			+++	+++	+++	+++	++	++	+	+++	+++	+
	Myocardium & Pericardium			++	++	+++	++	+++	+++	+++	+++	+++	+++
	Gastrointestinal Muscularis Mucosa			++	++	++	++						+
	Tongue					++	+	+	++	+	++	++	
	Esophagus				++	++	+	+++	+	+	+	+++	+
	Submaxillary Gland			++	+	+++	+++	+		+	++	++	++
	Liver	++	++	++	+++	+++	+++	+++	+++	++	+++	+++	+++
	Pancreas					+	+		+			+	
	Kidneys	++			++	+++	++	+++	++	++	+++	++	
	Adrenal Glands				++	+++	+	NE	NE	NE	+	NE	NE
	Brain & Meninges			+		+++	+++	NE	NE	NE	+++	+++	NE
	Vagus & Sciatic Nerves					++	++	+		++	+++	++	
	Extrinsic Ocular Muscles			++	+++	++	+++	NE	NE	+++	++	+++	NE
	Spleen			+++	+++	+++	+++	+++	+++	++	+++	+	+++
	Regional Lymph Nodes		+			+++	+++	+++			++	+	+
	Thymus Gland											+++	+++
	Femur Marrow					+++	+++	NE	NE	NE	NE	NE	NE
	Pulmonary Alveolar parenchyma			+++		+++	+++				+		
	Uterus					+		NE	NE	NE	NE	NE	NE
	Pituitary Gland						+	NE	NE	NE			NE
Inflammation	Pyelonephritis									+			+
	Optic Nerve Perineuritis						++	NE	NE	++	++		NE
	Lacrimal Glands & Conjunctiva	+				+++	+++	NE	NE	+		+	NE
Average Lesion Grade (Total No. Lesions/Tissues Examined)		0.4	0.3	1.1	1.3	2.3	2.0	1.8	1.6	1.5	1.8	1.8	1.7

F = Female M = Male + = Slight ++ = Moderate +++ = Marked NE = Not examined

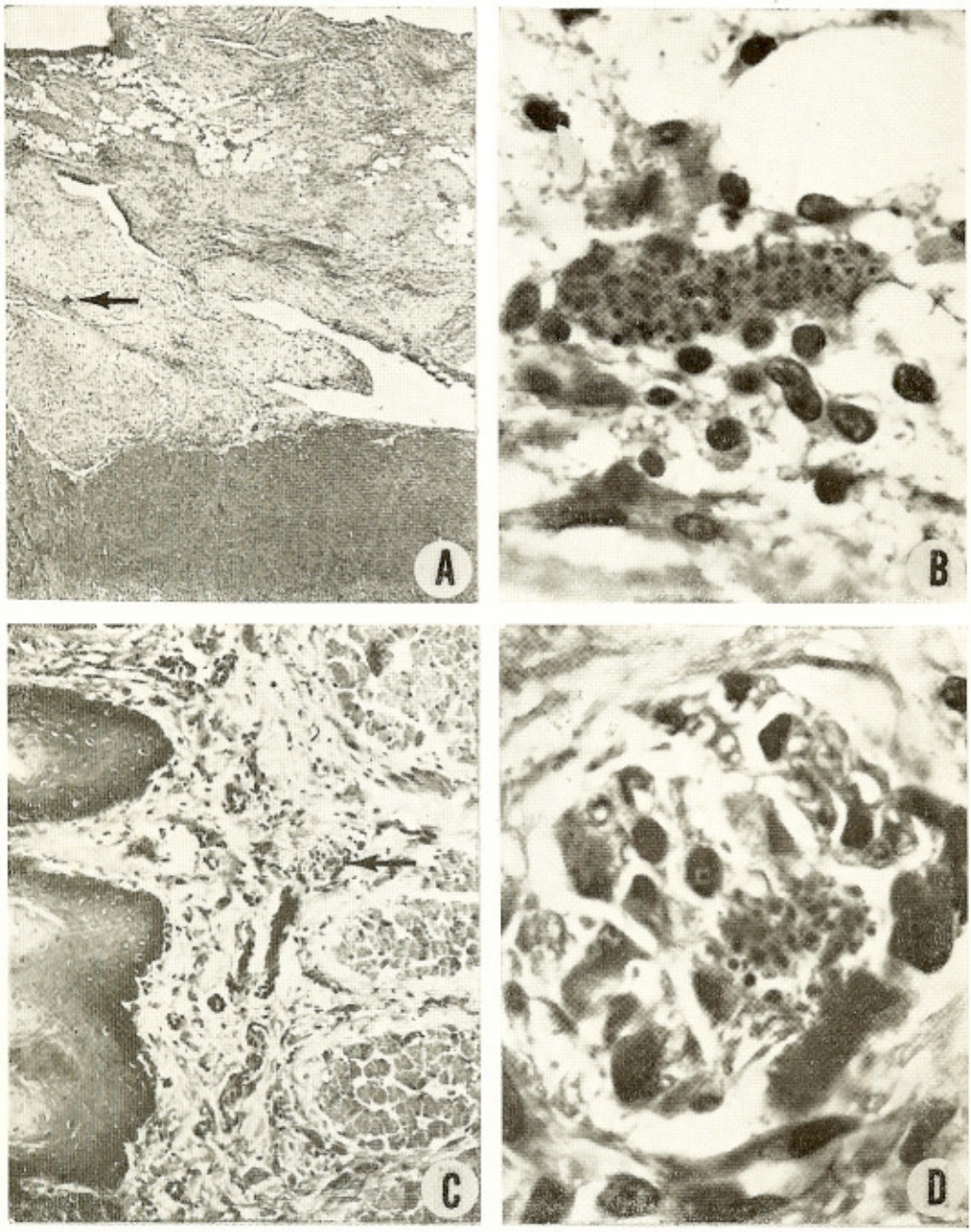

Fig. 1 — A) Section through mitral valve of heart of Dog No. 9. Arrow points to focal granuloma in the valve cusp (H. & E., 35 ×). B) Higher magnification of area indicated by an arrow in Fig. 1A shows a cyst with amastigote stages of *T. cruzi* (H. & E., 1300 ×). C) Section of tongue from Dog No. 8. Arrow indicates a cross-section of a peripheral nerve in the submucosa (H. & E., 140 ×). D) Higher magnification of nerve shown in Fig. 1C. Amastigote stages of *T. cruzi* are present in debris of axis cylinders. (H. & E., 1300 ×)

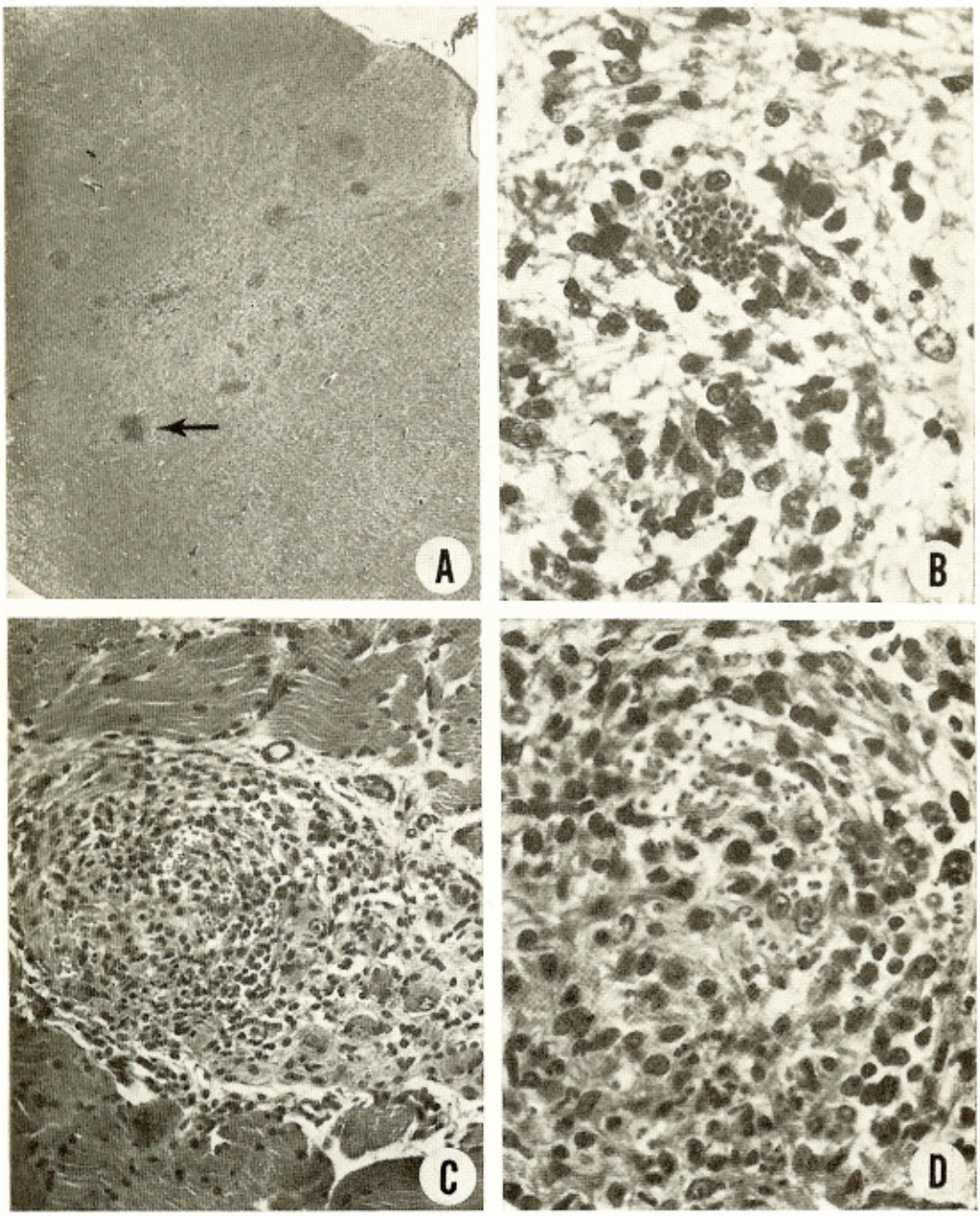

Fig. 2 — A) Arrow indicates one of many disseminated focal encephalitis in medulla oblongata of Dog No. 11 (H. & E., 15 ×). B) Higher magnification of focal lesion indicated by an arrow in Fig. 2A shows simultaneous occurrence of infectious granuloma and amastigote stages of *T. cruzi* (H. & E., 530 ×). C) Infectious granuloma in a ganglion in the muscularis of the esophagus of Dog No. 4. Note partial loss of nerve cell bodies (H. & E., 210 ×). D) Higher magnification of the central area of the granuloma shown in Fig. 2C. There are groups of amastigotes scattered among the cellular debris (H. & E., 530 ×)

consistently found in most of the tissue examined. A few organs such as pancreas, pulmonary alveolar parenchyma, pituitary gland and muscularis mucosa of intestine, showed an irregular occurrence of these lesions. Thymus gland granulomas were found at 29 and 31 days only.

There was a positive statistical correlation coefficient [10] ($r = + 0.68$) which is statistically significant at probability ($p = 0.05$) between the terminal parasitemia counts and tissue amastigote grades. Between 18-31 days the lesion grade averages remained consistently high (Table IV). No significant statistical correlation was found between the terminal parasitemia and average lesion grades, or between amastigote grades and average lesion grades.

Blood trypanosomes (Table I) and tissue amastigote populations (Table III) showed similar peak occurrence ranges of 13-24 and 13-28 days, respectively. The greatest number of lesions (Table IV) occurred between 18-31 days.

DISCUSSION

Early manifestations of acute Chagas' disease in all the dogs were regional lymphadenitis and hyperplasia. Chagoma and conjunctivitis appeared early in several dogs. By 18 days post-inoculation the remaining dogs exhibited pronounced muscular weakness which later became associated with labored respiration, paraplegia and prostration. Between 24-31 days these findings indicated imminent death.

This investigation with purebred beagle dogs showed a prepatent period of 4-7 days with the highest parasitemias occurring between 13-24 days post-inoculation. At 7 days amastigote stage pseudocysts were observed microscopically in the cells of soft connective tissue, the site of inoculation, in regional lymph nodes, and bone marrow. By 13 days the distribution of amastigote stages in organs was variable. Because of the limitations involved in sampling tissues, the size of sections examined and the minute size of many amastigote foci, parasites may have been present in the tissue but not seen in the section examined. The amastigote stage cysts were not always associated with any adjacent tissue reaction and frequently the tissues had only infectious granulomas without parasites. Occasionally parasites were found in a granuloma. This coexistence was observed in nerve tissue (Fig. 2B).

The occurrence and distribution of lesions generally followed that of the amastigotes found. However, the number of lesions generally exceeded the number of amastigote cysts. If the escape of parasites from the pseudocysts initiate the host's immune response and lesion development then it may be assumed that parasites invaded these tissues to a greater extent than was detected by random histologic counting.

The positive statistical correlation coefficient ($r = + 0.68$) and statiscally significant probability ($p = 0.05$) between the terminal parasitemia counts and tissue amastigote grades may be dependent on the following events: The initiation of the parasites' intracellular reproductive cycle with simultaneous increases of blood trypanosomes and amastigote populations. Similar peak occurrence ranges of 13-24 and 13-28 days, respectively, were recorded. However, intracellular reproduction precedes lesion development and a greater variation in their peak occurrence ranges (13-28 days and 18-31 days) could be expected. An even greater difference was noted between the peak parasitemia time range (13-24 days) and greatest number of lesions (18-31 days). These observations were consistent with the known sequence of *T. cruzi* reproduction in mammals [3, 9, 11].

The number and distribution of amastigotes found in the brains of dogs (numbers 10 and 11) indicated that parasites could have been found earlier than 28 days. Sites of predilection in brain tissue were not observed. Amastigotes were found in gray and white matter, granular and molecular layers, dura and pia mater, cerebrum, cerebellum, olfactory lobes and optic chiasma. Their presence produced severe granulomatous encephalitis (Fig. 2A) and meningitis which were observed as early as 13 days post-inoculation. The basal area including the medulla oblongata, medulla spinalis, pons and tractus cerebrospinalis showed the greatest concentration. Although no special effort was made to isolate ganglia systematically, they were occasionally observed in sections (Figs. 2C and 2D) and contained either amastigotes or showed granulomatous changes as evidence of recent

parasitic invasion. Since the vagus, sciatic, optic and peripheral nerves were early sites of infection it seems reasonable to assume that the peripheral and then the central nervous system become major sites for cellular destruction.

The amastigote stage cysts, infectious granulomas, inflammation, myocarditis, encephalitis, neuritis and other pathologic changes were typical of those caused by Chagas' disease and have been described in the literature [1, 5, 8, 10]. The pleural surface focal edema, kidney focal hyperemia, and the slight hydrocephalus found are of doubtful significance since they are known to occur as spontaneous diseases in beagle dogs of the Lederle colony.

At 13 days post-inoculation all the primary body tissues were subject to parasitic infection. A progressive myopathy, cardiopathy and neuropathy were reflected in the clinical findings and confirmed by histologic examination. Inevitably all the dogs would have died of severe myocarditis and extensive infection of the central nervous system.

RESUMO

Doença de Chagas aguda, experimental, em cães puro-sangue "beagle" infetados pelo Trypanosoma cruzi *(cepa B)*

O Autor estudou a patologia da Doença de Chagas em filhotes de cães puro-sangue da raça "beagle", que receberam por inoculação subcutânea, cerca de 500.000 tripanosomas da cepa B, por quilo de peso. Observaram-se os sintomas clínicos, contou-se periòdicamente o número de parasitas no sangue periférico, determinaram-se a concentração de formas amastigotas nos tecidos e a gravidade das lesões. O período pré-patente foi estabelecido em 4 a 7 dias após a inoculação e o ponto máximo de parasitemia entre 13 a 24 dias. Constatou-se uma correlação, estatìsticamente significante, entre a intensidade da parasitemia terminal e as contagens das formas tissulares. Treze dias após a inoculação, em todos os tecidos primários identificava-se a infecção parasitária. As alterações morfológicas mais precoces associadas à tripanosomíase aguda foram linfadenite e hiperplasias regionais. O maior número de lesões teciduais ocorreu entre 18-31 dias. Miopatia, cardiopatia e neuropatia progressivas foram confirmadas pelo exame histológico.

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