SUSCEPTIBILITY OF THE *CEBUS APELLA* MONKEY TO DIFFERENT STRAINS OF *T. CRUZI* AFTER SINGLE OR REPEATED INOCULATIONS[1]

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INTRODUCTION

Since 1909, when the findings of Carlos Chagas (a physician of the Brazilian Health Service and an investigator at the Oswaldo Cruz Institute) became known *(1)*, Chagas' disease has been reported in every country of Central and South America.

In Argentina, two physicians (Maggio and Rosenbusch—*1a*) reported in 1914 that although numerous *Trypanosoma cruzi* could be observed in the vector *Triatoma infestans* existing in 10 Argentine provinces, no associated disease could be detected in humans. Later another Argentine (Mazza—*2*) continued Chagas' work and confirmed American trypanosomiasis to be a serious endemic pathology. He worked at the Misión de Estudios de Patología Regional in Jujuy from 1929 to 1946, where he recorded some 1,400 cases and performed about a hundred necropsies of acute and chronic cases.

Chagas' disease is now widely recognized as one of Latin America's most important public health problems. It is estimated that at present over 65 million people live in endemic areas where the risk of infection exists, and that at least 20 million people living in both rural and urban parts of Latin America are infected *(2a)*. The number of infected people in Argentina has been estimated at about three million *(3)*.

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The acute phase of the infection occurs most often in childhood and is generally mild, passing unnoticed in about 85% of the cases *(4)*. It is estimated that only some 5% of all the acute infections are actually diagnosed; of the resulting acute disease cases, past work has classified 75% as mild, 18% as moderately severe, and 6% as severe *(3)*. As a consequence, the disease poses a far greater threat to adults, as may be inferred from the high morbidity and mortality rates found among chronically infected patients *(5)*. Furthermore, chemotherapeutic treatments now available for combating the disease, especially in the chronic phase, are far from satisfactory.

PAST ANIMAL EXPERIMENTS

Most of the animals infected experimentally to date have been mice *(6)*, rabbits *(7)*, or dogs *(8)*, all of which are very susceptible to *T. cruzi*. However, observations about the infections in these hosts are generally limited to the acute phase of the disease, because of the high mortality produced by the parasite. In general, what chronic infection has been observed in these animals has shown an erratic course involving slight disturbances.

In addition, other authors' findings have indicated that autoimmunity probably plays an important role in the pathogenesis of the human cardiac and gastrointestinal lesions associated with chronic Chagas' disease. These findings suggest that development of an immunoprophylactic method, if it were possible, would require further investigation, using an experimental animal model capable of developing clinical manifestations of the chronic human disease *(9)*.

Dorland *(10)* studied the virulence of *T. cruzi* strains isolated in Texas and California for monkeys by inoculating the feces of naturally infected bugs through the animals' ocular conjunctiva. All of the monkeys developed the infection and some showed Romaña's sign. Histopathologic studies demonstrated a slight diffuse chronic myocarditis.

Torres and Tavares *(11)* studied the course of the infection in *Cebus* monkeys inoculated several times, using different routes and inocula. The animals, which died between 95 and 243 days after infection, presented a slight diffuse chronic myocarditis with marked individual variations. This made it difficult to evaluate the results, even though more pronounced lesions were observed in the animals that had been reinoculated over short periods of time.

Other researchers *(12, 13)* have infected rhesus monkeys with *T. cruzi* and have followed the infections for long periods. Megaesophagus has been reported at least twice in these primates. However, this species is not native to the areas endemic for Chagas' disease, and it is very expensive and difficult to obtain.

In sum, past research progress on Chagas' disease has been slow, due partly to a lack of a suitable animal model capable of producing lesions resembling those found in humans—notably those arising from an inflammatory process producing fibrosis in the heart that invariably leads to myocardial damage and may cause heart failure, and effects involving the autonomic nervous system that result in disperistalsis and megaviscera. Hence, a principal objective of the WHO Tropical Disease Research

Program has been the development and evaluation of such animal models for reproducible experiments. This work is essential for the improvement of our knowledge about Chagas' disease pathogenesis and immunopathology, especially for discerning the mechanism responsible for chronic disease manifestations, evaluating the chemotherapeutic effects of drugs, and developing a safe and effective vaccine.

MATERIALS AND METHODS

The work being reported here was directed primarily at making the New World nonhuman primate *Cebus apella* serve as an experimental model for chronic chagasic pathology in man. Our aim was to come as close as possible to establishing an ideal animal model—an ideal model being defined as one that would do the following:

1) support a long-lasting subclinical parasitemia detectable by xenodiagnosis and/or hemoculture as well as by conventional serology;

2) present cellular and/or humoral immune reactions;

3) develop the cardiac and digestive forms of the disease with typical histopathologic lesions;

4) survive the acute phase of the infection;

5) display lesions in a relatively short period of time;

6) develop the disease in a manner pretty much independent of the age and sex of the particular infected animal involved;

7) utilize animals native to the endemic area and easy to obtain; and

8) be available at a reasonable cost.

Specifically, we attempted to induce experimental chagasic infections in *Cebus apella* monkeys by means of single or repeated inoculations with different strains of *T. cruzi* (using two different inoculation routes and different numbers of parasites in the inocula) in order to determine the susceptibility of this species to infection with *T. cruzi*.

Forty-eight *Cebus apella* monkeys (40 adult males, six mostly juvenile males, and two juvenile females) that had normal hematologic and serologic enzyme parameters *(14)*, were free of chagasic infection (as determined by serology and xenodiagnosis), and were also free of ECG pathology, were selected from an outdoor colony. (This colony, situated in Escobar about 30 kilometers from Buenos Aires and supported by the UNDP/World Bank/WHO tropical disease research program, maintains a breeding population of about 200 *Cebus* monkeys.) The 48 animals were placed in an indoor colony in individual cages, with water *ad libitum* and food provided according to a standard pellet diet (25% protein, 290 calories/100 g) prepared by Cargill (Buenos Aires, Argentina) and supplemented with fresh fruit.

Temperature, humidity, and light conditions were provided that were adequate for the needs of the experiment. Periodic and orderly control of the monkeys' behavior, body weight, and intestinal parasites found in feces was carried out in the cages.

At the time of the first inoculation the "juveniles" ranged in age from one to five years, and their weights ranged from 940 to 1,950 g. The estimated ages of the adults at the time of the first inoculation ranged from five to

10 years (the average estimated age being eight years), and their weights ranged from 2,110 to 3,320 g.

Parasite Strains and Inoculations

The animals were divided into four different groups—one control group and three groups inoculated with three different *T. cruzi* strains (Table 1). The control group consisted of 30 adults; another group, which received *T. cruzi* strain "CA1," was composed of 10 adults; the third group, which received the "Colombian" strain until May 1984, included four juveniles (two males and two females); and the fourth group, which received the "Tulahuen" strain, was made up of four young males. All of these *T. cruzi* strains had been maintained in the laboratory by periodic passage through Swiss mice.

The 10 adult monkeys receiving the CA1 strain were inoculated with the parasite's metacyclic forms by the conjunctival route. Three received one inoculation of 4×10^4 parasites; four received one inoculation of 1×10^6 parasites; and the last three received two inoculations, the first of 4×10^4 parasites and the second of 1×10^6 parasites one year later.

The two other inoculated groups (receiving the Colombian and Tulahuen strains) were repeatedly inoculated with 3×10^6 blood forms of the parasite by the intraperitoneal route—a route intended to produce better absorption of the parasite. The four young males receiving the Tulahuen strain were inoculated 10 or 11 times at intervals ranging from a few days to 30 weeks (see Figure 3), and the four juveniles inoculated with the Colombian strain were inoculated 18 or 19 times at intervals ranging from three to 24 weeks (see Figure 2). The periodic reinoculations were performed in order to approximate conditions found by people living in endemic areas, where the periods of natural reinfection vary.

TABLE 1. Basic characteristics of the *Cebus apella* monkeys inoculated with the CA1, Colombian, and Tulahuen *T. cruzi* strains and of the uninoculated controls. Data on the inoculations administered are shown below.

	T. cruzi strains inoculated			Controls (no inoculations)
	CA1	Colombian	Tulahuen	
Number of animals	10	4	4	30
Sex	male	2 male, 2 female	male	male
Estimated age at first inoculation (years)	6–10	3,3 1,2	4–5	5–9
Weight (g)	2,110–3,320	940–1,800	1,660–1,950	2,250–2,570
Date of first inoculation	6/80 to 7/81	9/82 to 10/82	11/82 to 12/82	—
Number of inoculations as of 6/84	1 to 2	18 to 19	10 to 11	—
Number of *T. cruzi* administered (per inoculation)	4×10^4 to 1×10^6	3×10^6	3×10^6	—
Route of inoculation	conjunctival	i.p.	i.p.	—

Subsequent Monitoring and Testing

Patent parasitemia, serologic changes, electrocardiographic and echocardiographic alterations, and cardiac or gastrointestinal disorders detectable by radiology were monitored as follows:

Parasitology. The parasitemia was monitored in each monkey, initially by direct observation of the *T. cruzi* parasite via the fresh-drop or Strout test *(15)*. This was to be done three times a week after the first inoculation, before each reinoculation, and at the end of the first and each subsequent month. When the direct testing yielded negative results, each negative animal was tested by xenodiagnosis using 40 third-instar nymphs of *Triatoma infestans*. In this case, each animal was exposed to the bugs for 30 minutes every three months. A pool of feces from each group of bugs was then examined for *T. cruzi* at 30 and 60 days after the bugs' exposure.

Serology. Sera were collected from all of the monkeys (including the controls) at regular intervals. Each animal was bled every seven days for the first month after inoculation or initiation of the experiment. Thereafter, each animal was bled twice a month.

The collected blood was allowed to clot at room temperature, serum was withdrawn, and each collected serum was divided into small samples and stored at −70°C. All of the collected sera were tested for antibodies against *T. cruzi* by indirect hemagglutination (Cellognost-Chagas, Behringwerke) and ELISA *(16)*. These tests were performed weekly during the first month and monthly thereafter.

Hematology. Each monkey's hematocrit, hemoglobin level, white blood cell count, and red blood cell count were determined monthly with a Clay Adams Hematology Analyzer HA-5 Counter.

Serum enzymes and proteins. The activity in plasma of various enzymes (GOT, GPT, LDH, alpha HBDH, and gamma GT) was assessed by means of Boehringer kinetic tests. This was done once a week during the acute and subacute phases of the infection, and twice a month thereafter during the first year. The level of plasma proteins was assessed monthly.

Electrocardiography and echocardiography. ECGs were recorded every week for the three first months after inoculation and twice a month thereafter. For this purpose the animals were given 10 mg/kg of ketamine hydrochloride anesthesia (Ketalar®, Parke Davis, Buenos Aires, Argentina). They were then placed in a dorsal decubitus position on an adequate stretcher; and, after five minutes of rest, an ECG was recorded with a Fukuda FJC-7100 monitor at a speed of 25 mm/s.

Needle electrodes were placed in the subcutaneous cellular tissue in order to reduce dermic impedance. Limb electrodes were placed on the anterior portion of the forearms and on the inner portion of both legs. The precordial leads used were V1-V2-V4 and V6, and occasionally V3R—the latter in order to obtain a better evaluation of the right cavities.

The possible existence of subclinical conduction disturbances was investigated through intravenous injection of 1.0 mg/kg of Ajmalin aspartate [(17 R) Ajmalan-17, 21-diol aspartate; Craveri, Buenos Aires, Argentina], a *Rauwolfia* derivative that depresses intraventricular conduction.

Developments related to cardiac pathology were also studied by echocardiography; animals with alterations detected by this method or by ECG were grouped together; and animals to be sacrificed for anatomopathologic studies were selected at random from this group. A Berger C117 echocardiograph (M mode), made in Argentina with a 5MHZ transducer 0.5 cm in diameter, was used for this purpose.

The monkeys were prepared for this examination in the same way they were prepared for ECGs. Each animal was then placed in a dorsal and left lateral decubitus position during the examination.

For purposes of comparison, we considered as normal a collection of electrocardiograph and echocardiograph patterns obtained from the control group of 30 monkeys in our indoor colony (Tables 2 and 7).

Radiology. In order to evaluate the cardiac and gastrointestinal alterations produced by the infection, we performed radiologic examinations of the control and infected groups one and three years after inoculation by means of: (1) uncontrasted radiographs of the thorax (front view) and (2) contrast radiographs (video images) of the esophagus-stomach transit area, and also of the large intestine.

The first type of radiograph was obtained by placing each monkey in a vertical position at a focal distance of 1.80 m. An approximate indication of heart size relative to chest size (the cardiothoracic index) was derived by applying the equation

$$CTI = \frac{RD + LD}{TD}$$

where CTI is the cardiothoracic index; TD (thorax diameter) is a line drawn parallel to the transverse diameter of the heart that connects the most lateral extremities of the thorax; RD is the maximum transverse diameter of the right half of the heart; and LD is the maximum transverse diameter of the left half of the heart. A diagram of these three lines, drawn over an appropriate radiograph, is shown in Figure 1.

The second category of radiographs provided contrast-images of the upper and lower gastrointestinal tract (by means of CGR 1,000 mAmp equipment that was also employed for the thorax and esophagus-stomach X-rays), using serioscopy and image intensification with closed-circuit television. No antispasmodic drugs were used; the barium perfusion was controlled by pressure.

Anatomopathology. Animals to be sacrificed were selected at random from the inoculated monkeys that showed echocardiographic or ECG disturbances. Two controls were also sacrificed.

TABLE 2. ECG parameters found for the 30 control group monkeys, showing the average value ($\bar{X}$) of each together with the standard error (SE) and one standard deviation (SD).

	Heart rate (beats/min)	QRSA degrees	P(D2) sec.	PR(D2) sec.	QT(D2) sec.	Intrinsecoid deflection (V4) sec.	T(VI) sec.	T(VI) Pos. (80%) mm	T(VI) Neg. (20%) mm
$\bar{X}$	270.7	14.5	0.04	0.08	0.18	0.037	0.07	4.5	4.2
SD	29.9	49.3	0.00	0.01	0.02	0.004	0.01	0.7	1.4
SE	9.5	15.6	0.00	0.00	0.01	0.001	0.00	0.2	0.8

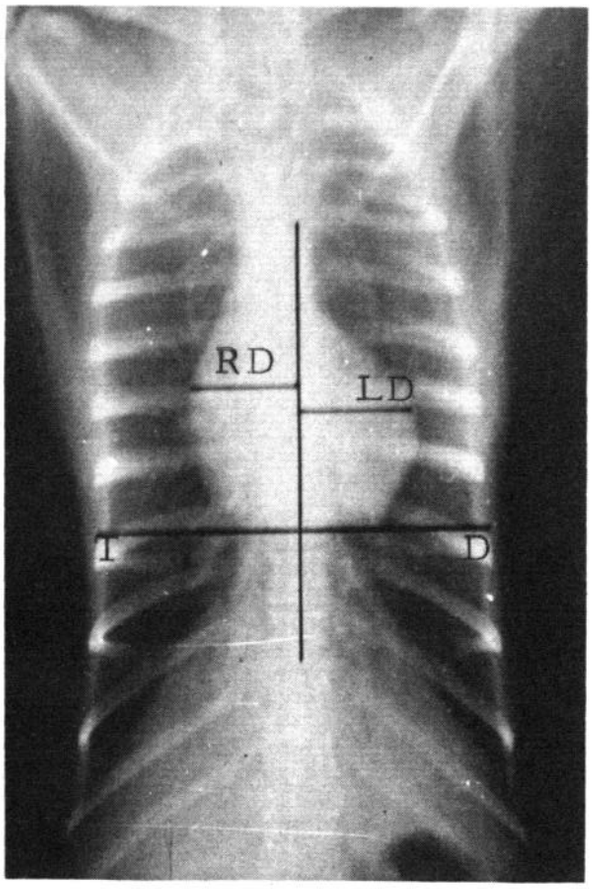


FIGURE 1. An X-ray showing the measurements used to derive the cardiothoracic index. RD is the maximum transverse diameter of the right half of the heart; LD is the maximum transverse diameter of the left half of the heart; and TD is the thoracic diameter.

A complete autopsy was performed on each animal. All organs and tissues were examined for gross lesions and were then fixed in a conventional formaldehyde buffer solution and Zamboni's fixative *(16a)* for application of hematoxylin-eosin and Masson's trichromic stain.

After fixation, three sections were taken from the walls of each chamber of the heart. Special attention was devoted to the septum, which was sectioned serially in the area located near the septal portion of the tricuspid valve; and a similar series of sections were also made in the portion of the auricular wall near the sinoatrial node.

In order to facilitate examination of the esophagus and colon, these organs were rolled along their longitudinal axes before being sectioned. Routine single sections were also taken from each monkey's brain, lungs, kidneys, adrenals, spleen, lymph nodes, and psoas muscles.

Results

Control Group

None of the aforementioned examinations revealed any alterations outside established parameters in the control group during the period of the experiment.

Parasitology

Seven of the 10 adult animals inoculated with the CA1 *T. cruzi* strain showed positive parasitemia by xenodiagnosis—one doing so at week five, five at week nine, and one at week 60. None of the 10 yielded positive fresh-drop or Strout test results during the acute phase (Table 3).

Similarly, none of the four juvenile monkeys inoculated with the Colombian *T. cruzi* strain yielded positive fresh-drop or Strout test results; but all four demonstrated parasitemia by xenodiagnosis—one at weeks 15 and 18; one at weeks 18, 21, and 39; one at weeks 35 and 54, and one at week 39 (Table 4 and Figure 2).

In contrast, all of the four juvenile monkeys inoculated with the Tulahuen *T. cruzi* strain showed positive parasitemia by the fresh-drop method during the first week after the first inoculation. Also, the Strout test yielded positive results with all of these monkeys for periods following the initial inoculation

TABLE 3. Summary of test results obtained with the 10 adult *Cebus apella* monkeys inoculated with the CA1 strain of *T. cruzi*.

	Designator assigned to each inoculated monkey									
	I	J	K	L	M	N	O	P	Q	R
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
Weight at first inoculation (g)	2,660	2,110	2,345	2,680	2,730	2,800	2,030	3,320	2,570	2,370
Estimated age at first inoculation	8 years	6 years	9 years	6 years	9 years	10 years	6 years	9 years	10 years	9 years
Date of first inoculation	6/2/80	6/2/80	6/2/80	7/3/81	7/3/81	7/3/81	7/3/81	6/2/80	6/2/80	6/2/80
No. of inoculations	1	1	1	1	1	1	1	2	2	2
Approx. no. of *T. cruzi* inoculated by the conjunctival route	4×10^4	4×10^4	4×10^4	1×10^6	1×10^6	1×10^6	1×10^6	4×10^4 1×10^6	4×10^4 1×10^6	4×10^4 1×10^6
Xenodiagnosis positive until	week 9	—	week 9	week 9	week 9	—	week 5	week 9	—	week 60
Maximum IHA titer	1/32	1/128	1/64	1/32	1/64	1/32	1/64	1/32	1/128	1/64
Time elapsed before detection of ECG changes	41 months	47 months	48 months	32 months	27 months	27 months	—	47 months	47 months	47 months
ECG compatible with	RBBB[a]	LVO[a]	RD[a]	IRBBB[a]	RBBB+LAH[a]	RBBB	Normal	IRBBB	RBBB	RD
Echocardiography	Pathology	Pathology	—	Pathology	Pathology	Pathology	—	Normal	Normal	—
Cardiothoracic index	0.57	0.56	0.54	0.55	0.61	0.58	0.58	0.66	0.55	0.56
Radiology of colon	Colon dilatation	Normal	Normal	Colon dilatation	Normal	Colon dilatation	Normal	Normal	Normal	Normal
Date of autopsy	5/29/84	—	—	—	5/28/84	5/29/84	—	—	—	—

[a] RBBB=right bundle branch block; LVO=left ventricle overload; RD=repolarization disturbance; IRBBB=intermittent right bundle branch block, LAH=left anterior hemiblock.

TABLE 4. Summary of test results obtained with the four juvenile *Cebus apella* monkeys inoculated with the Colombian strain of *T. cruzi*.

	Designator assigned to each inoculated monkey			
	E	F	G	H
Sex	Male	Male	Female	Female
Weight at first inoculation (g)	1,750	1,800	940	940
Estimated age at first inoculation	3 years	3 years	1 or 2 years	1 or 2 years
Date of first inoculation	9/10/82	9/10/82	10/4/82	10/4/82
No. of inoculations	19	19	18	18
Approx. no. of *T. cruzi* inoculated by the i.p. route	3×10^6	3×10^6	3×10^6	3×10^6
Xenodiagnosis positive until	week 39	week 39	week 54	week 18
Maximum IHA titer	1/128	1/128	1/128	1/128
Time elapsed before detection of ECG changes	20 months	—	13 months	18 months
ECG compatible with	RD[a]	—	LAH[a]	LVO[a]
Echocardiography	Pathology	Pathology	Pathology	Pathology
Cardiothoracic index	0.51	0.59	0.64	0.56
Radiology of colon	Normal	Normal	Normal	Normal
Date of autopsy	6/23/84	—	6/23/84	—

[a] RD=repolarization disturbance; LAH=left anterior hemiblock; LVO=left ventricle overload.

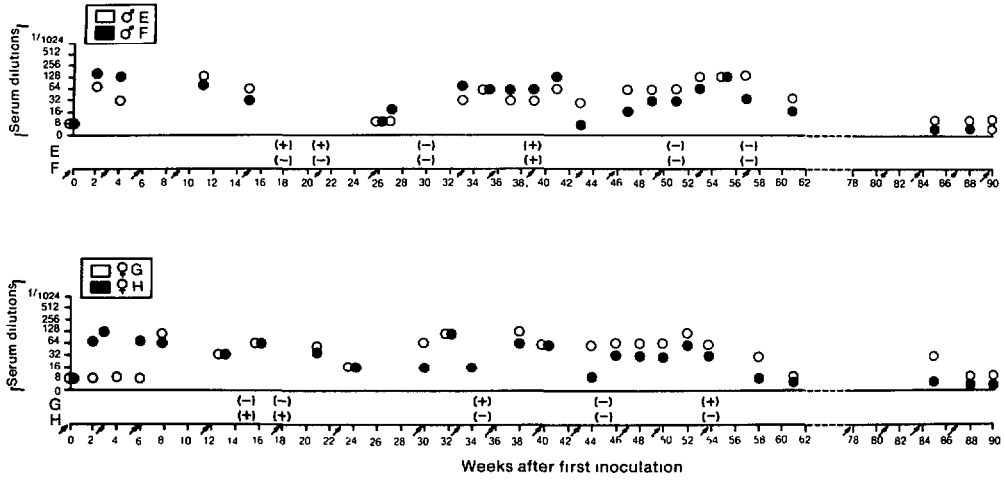


FIGURE 2. Results obtained from xenodiagnosis and IHA tests performed on the four juvenile *Cebus apella* monkeys inoculated repeatedly with the Colombian strain of *T. cruzi*. The boxes at upper left show each monkey's sex and letter designator, together with the shade (black or white) used to display data for that animal. The small circles show the IHA titers obtained at varying numbers of weeks after the initial inoculation. The plus and minus signs in parentheses show whether xenodiagnostic tests conducted in particular weeks yielded positive or negative results; and the small arrows show when reinoculations were administered.

that varied from eight to 30 weeks (Figure 3). Later, as the figure shows, xenodiagnosis yielded positive results through week 46 for one pair of monkeys and through week 49 for the other pair; and the Strout test yielded positive results again for five to seven weeks in three of the four monkeys following reinoculations that began on weeks 75 and 78 (Table 5).

Serology

IHA testing indicated that the 10 adult monkeys inoculated with the CA1 strain developed fairly high levels of serum antibodies against the parasite within 20 days of the first inoculation. These titers ranged from 1:32 to 1:128 (see Table 3). In general, the levels of these antibodies remained high for about three months and then tended to decline. The 10 monkeys also had elevated IgG levels, as determined by ELISA testing, that remained high over the course of the infection, ranging from 1.97 to 2.77. This indicates that low serum titers persisted throughout the chronic phase of the infection.

IHA testing revealed a somewhat different response in the four juveniles receiving the Colombian strain. Specifically, these monkeys appeared to develop high levels of serum antibodies between the second and the eighth week after the first inoculation, and these levels tended to stay fairly high over the course of subsequent reinoculations for 54 to 60 weeks before tending to decline. As in the previous case, these IHA titers ranged from 1:32 to 1:128 (see Figure 2). Also, the ELISA test revealed rising IgG levels over the course of the infection (Figure 4).

Similarly, the four juveniles receiving the Tulahuen strain showed high levels of IHA serum antibodies in response to multiple inoculations (see Fig-

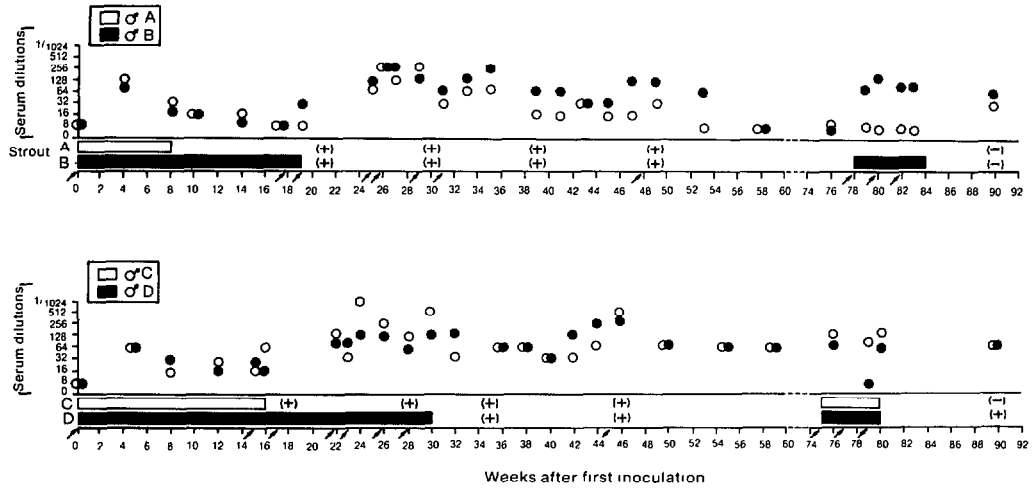


FIGURE 3. Results obtained from xenodiagnosis, IHA tests, and Strout tests performed on the four *Cebus apella* monkeys inoculated repeatedly with the Tulahuen strain of *T. cruzi*. The boxes at upper left show each monkey's sex and letter designator, together with the shade (black or white) used to display data for that animal. The small circles show the IHA titers obtained at varying numbers of weeks after the initial inoculation. The bars at bottom indicate weeks when the Strout test yielded positive results. The plus and minus signs in parentheses show whether xenodiagnostic tests conducted in particular weeks yielded positive or negative results; and the small arrows show when reinoculations were administered.

TABLE 5. Summary of test results obtained with the four *Cebus apella* monkeys inoculated with the Tulahuen strain of *T. cruzi*.

	Designator assigned to each inoculated monkey			
	A	B	C	D
Sex	Male	Male	Male	Male
Weight at first inoculation (g)	1,900	1,950	1,670	1,660
Estimated age at first inoculation	4 years	4½ years	4 years	4½ years
Date of first inoculation	11/8/82	11/8/82	12/2/82	12/2/82
No. of inoculations	11	11	10	10
Approx. no. of *T. cruzi* inoculated by the i.p. route	3×10^6	3×10^6	3×10^6	3×10^6
Strout test positive until	week 79	week 79	week 76	week 76
Xenodiagnosis positive until	week 49	week 49	week 46	week 46
Maximum IHA titer	1/256	1/256	1/1,024	1/256
Time elapsed before detection of ECG changes	18 months	—	18 months	11 months
ECG compatible with	RD[a]	Normal	RD[a]	LAH[a]
Echocardiography	Normal	Normal	Pathology	Normal
Cardiothoracic index	0.61	0.61	0.59	0.56
Radiology of colon	Normal	Normal	Normal	Dolichocolon
Date of autopsy	—	—	—	—

[a] RD=repolarization disturbance, LAH=left anterior hemiblock.

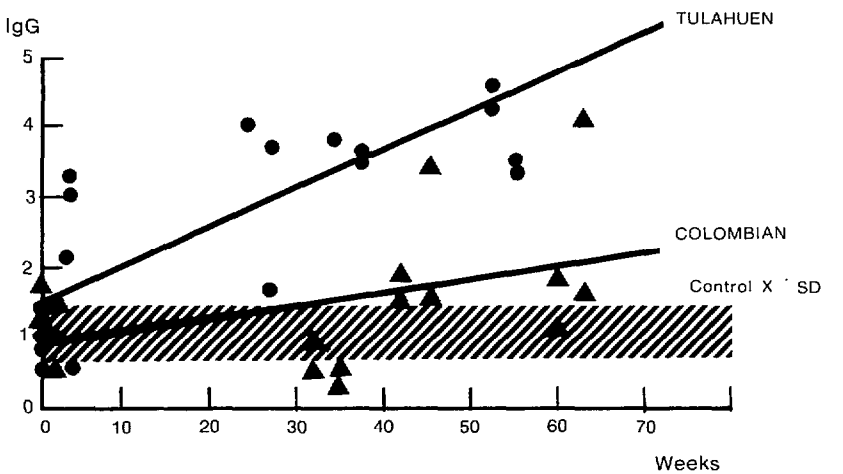


FIGURE 4. Positive IgG results obtained by ELISA testing of the eight *Cebus apella* monkeys inoculated repeatedly with the Colombian and Tulahuen strains of *T. cruzi* (triangles and circles, respectively), as compared to values obtained by testing the 30 controls (shaded band). Negative results obtained from the test monkeys are not shown.

ure 3); IgG levels (see Figure 4) were also elevated over the course of the infection.

Hematology, Serum Enzymes, and Serum Proteins

No significant hematologic or serum enzyme changes were detected in the inoculated monkeys. Likewise, the levels and electrophoretic fractions of plasma proteins determined immediately before sacrifice did not differ significantly in the inoculated monkeys as compared to the controls.

Radiology

No alterations were observed in the control group, regarding either the cardiothoracic index (CTI) obtained from the chest X-ray or the diameter and motility of the esophagus and colon as revealed by contrast radiography of the gastrointestinal tract.

Only two of the inoculated animals yielded notably altered CTIs. One with a CTI of 0.66, inoculated with the CA1 strain, had an intermittent right bundle branch block (see Photo A). The other, inoculated with the Colombian strain, had a CTI of 0.64 and a left anterior hemiblock (Photo B). Regarding human subjects, who are not strictly comparable, Maresh-Washburn (cited by Mosca et al.—*17*) states that a maximum CTI in the range of 0.53 to 0.58 can be found among infants weighing up to 11 kilograms.

The test of esophagus transit time with a barium meal showed no evidence of alterations, and none of the inoculated animals showed any noteworthy increase in esophagal diameters.

Of five inoculated animals that had ECG alterations and were sacrificed, two showed an enlarged colon diameter in the contrast study of that organ (Photos C and D).

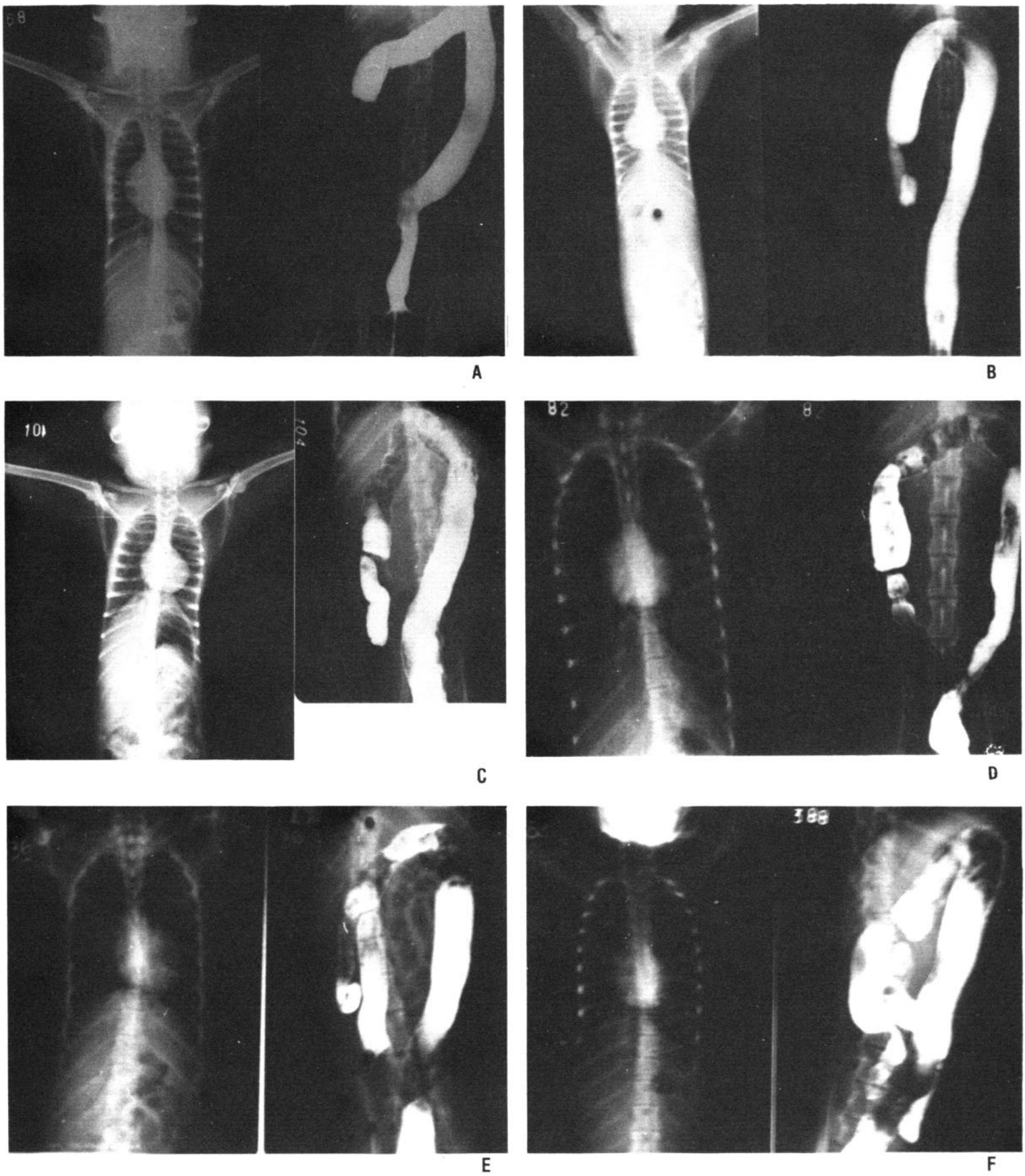
One animal inoculated with the CA1 strain that had an intermittent right bundle branch block yielded a radiologic image compatible with megacolon; and another monkey, this one inoculated with the Tulahuen strain and having a left anterior hemiblock, appeared to have dolichocolon (Photos E and F). The contrast studies of the colon did not reveal any noteworthy alterations in any of the other animals. In each case where alterations were detected radiologically, the findings were corroborated histopathologically, and lesions of the mesenteric plexus responsible for those images were found.

Electrocardiography

The observed electrocardiographic patterns were found not to be altered by administration of the drug utilized as an anesthetic. However, ECG alterations were detected in most (83.3%) of the infected monkeys. None of the control animals showed similar disturbances during the course of the experiment.

The ECG alterations observed in each group of inoculated monkeys are listed in Table 6. These alterations included intermittent right bundle branch block (IRBBB), right bundle branch block (RBBB), left ventricle overload (LVO), repolarization disturbance (RD), and left anterior hemiblock (LAH). Ajmaline did not produce any alterations in the monkeys with normal traces.

The incidences of the observed pathologies appeared influenced by the group of monkeys and inocula employed. That is, disturbances of the right bundle branch (IRBBB-RBBB) accounted for over half the pathologies detected in the adults receiving the CA1



X-ray photographs of some of the inoculated *Cebus apella* monkeys showing the chest area and large intestine. A and B are of animals with abnormal cardiothoracic indexes (0.66 and 0.64, respectively), the former with intermittent right bundle branch block and the latter with left anterior hemiblock; C and D are of animals with enlarged colon diameters; the image in E is compatible with megacolon and that in F is compatible with dolichocolon.

TABLE 6. Summary of the ECG disturbances observed in the 15 *Cebus apella* monkeys inoculated with the three *T. cruzi* strains, at periods corresponding to the chronic phase of the disease.

ECG finding	*T. cruzi* strain inoculated								
	CA1			Colombian			Tulahuen		
	Animals affected		Months elapsed since first inoculation	Animals affected		Months elapsed since first inoculation	Animals affected		Months elapsed since first inoculation
	No.	%		No.	%		No.	%	
IRBBB[a]	2	20	32–47	0	0	20	0	0	18
RBBB[a]	4[b]	40	27–47	0	0	20	0	0	18
LVO[a]	1	10	47	1	25	18	0	0	18
RD[a]	2	20	47–48	1	25	20	2	50	18
LAH[a]	1[b]	10	27	1	25	13	1	25	11
Total (all pathologic findings)	9	90	27–48	3	75	13–20	3	75	11–18

[a] IRBBB=intermittent right bundle branch block; RBBB=right bundle branch block, LVO=left ventricle overload, RD=repolarization disturbance, and LAH=left anterior hemiblock

[b] One of the animals inoculated with the CA1 strain came to exhibit both right bundle branch block and left anterior hemiblock.

strain, while the juveniles receiving the Colombian and Tulahuen strains most commonly exhibited repolarization disturbances or left anterior hemiblock. It should also be noted that the delay in the appearance of electrocardiographic pathology following the first inoculation was shorter (11 to 20 months) in the animals inoculated repeatedly i.p. with larger numbers of parasites (in those receiving the Colombian and Tulahuen strains), and was longer (27 to 47 months) in the adults receiving one conjunctival inoculation of the CA1 strain. Overall, the electrocardiographic disturbances observed in these groups of monkeys resembled those found in human Chagas' disease *(18–20)*.

Echocardiography

Nine of the fifteen inoculated monkeys that were tested showed echocardiographic disturbances. The mean values for the echocardiographic parameters exhibited by these animals, and their statistical significance relative to the same echocardiographic data for the control group, are shown in Table 7.

The frequencies of specific echocardiographic disturbances—increased right ventricle capacity (iRVC), increased left ventricle capacity (iLVC), decreased shortening fraction (dSF), decreased intraventricular septum motility (dIVSM), and increased mitral valve point E-septum distance (iE-SD)—are indicated in Table 8. In general, these are the disturbances most frequently observed in human Chagas' disease *(21)*.

Anatomopathology

Neither of the two animals sacrificed in the control group revealed any myocardial or gastrointestinal histopathologies. However, all five of the inoculated animals (three of which had received the CA1 strain and two of which had received the Colombian strain) ex-

TABLE 7. Echocardiographic parameters found for the 30 control monkeys and the 15 inoculated monkeys, showing the average value ($\bar{X}$) of each parameter, the standard error (SE), and the statistical significance of the differences between the values found for each group.

Parameters	$\bar{X} \pm$ SE for 30 controls	$\bar{X} \pm$ SE for 15 inoculated animals	t-test
End diastolic volume (EDV)	10.01 ± 0.98 cm^3	12.62 ± 2.15 cm^3	Not significant
End systolic volume (ESV)	2.65 ± 0.48 cm^3	6.02 ± 1.35 cm^3	$p < 0.05$
Systolic volume (SV)	7.35 ± 0.62 cm^3	6.60 ± 1.11 cm^3	Not significant
Ejection fraction (EF)	$77.07 \pm 0.20\%$	$54.70 \pm 5.97\%$	$p < 0.001$
Shortening fraction (SF)	$43.10 \pm 2.00\%$	$27.50 \pm 3.09\%$	$p < 0.001$

TABLE 8. Echocardiographic disturbances observed in monkeys inoculated with different *T. cruzi* strains and examined during the chronic phase of the disease.

	T. cruzi strain inoculated					
	CA1		Colombian		Tulahuen	
Disturbance	No. of animals affected	% affected	No. of animals affected	% affected	No. of animals affected	% affected
iRVC[a]	3	43	1	25	0	0
iLVC[a]	4	57	0	0	0	0
dSF[a]	2	29	0	0	0	0
dIVSM[a]	5	71	3	75	1	25
iE-SD[a]	1	14	3	75	0	0
Total	5	71	3	75	1	25

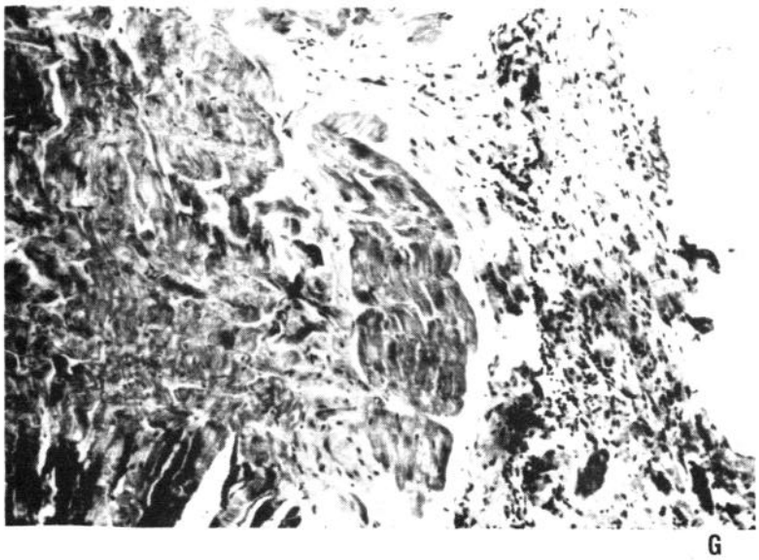
[a] iRVC = increased right ventricle capacity; iLVC = increased left ventricle capacity; dSF = decreased shortening fraction, dIVSM = decreased intraventricular septum motility, and iE-SD = increased mitral valve point E-septum distance.

hibited various histopathologic alterations. These were as follows:

All the auricular sections showed subpericardial infiltrates that were especially noteworthy in the zone adjacent to the sinus node (Photo G). One of the monkeys was found to have a large area characterized by lymphocyte-plasmocyte infiltrates arranged in a compact manner in the auricular pericardium (Photo H).

All five animals had lymphocyte-plasmocyte infiltrates in the interauricular septum. These could be observed among the muscular fibers and close to the autonomic neurons of the atrioventricular node, where they were particularly marked, surrounding and even replacing the neural elements (Photo I). This was especially evident in the two animals that had been sacrificed soonest after their inoculation with *T. cruzi*.

In addition, subpericardial and subendocardial infiltrates were observed in the ventricles of all five animals. The ventricular walls showed focal infiltrate areas that diminished as the time since the first inoculation increased,



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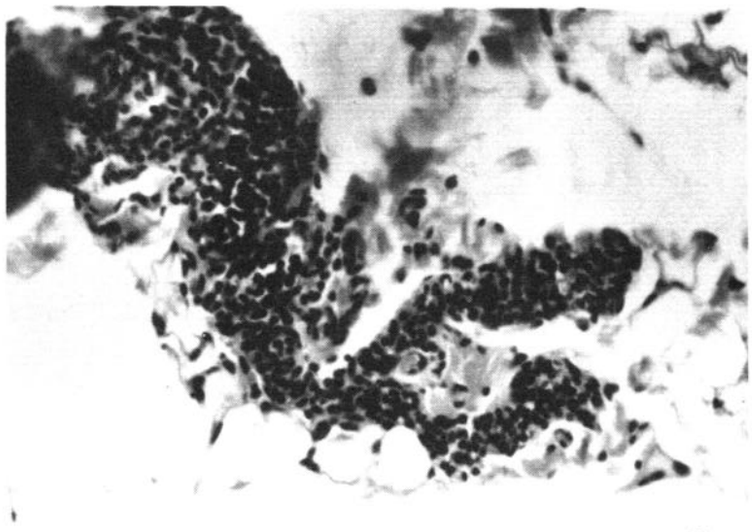

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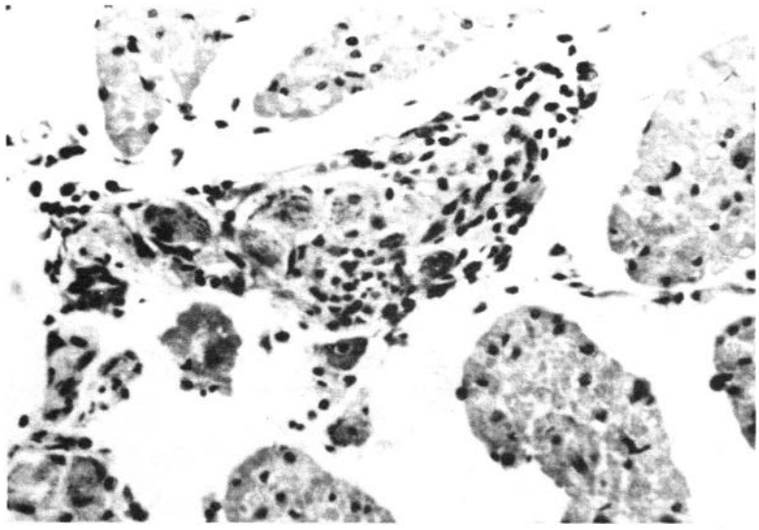

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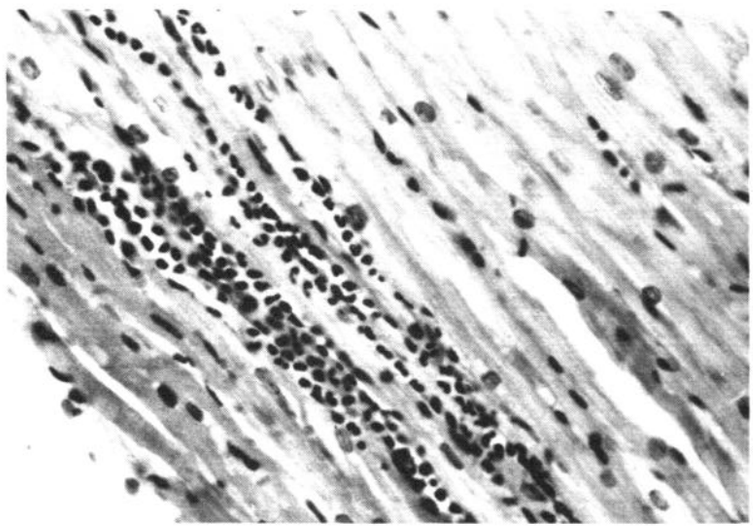

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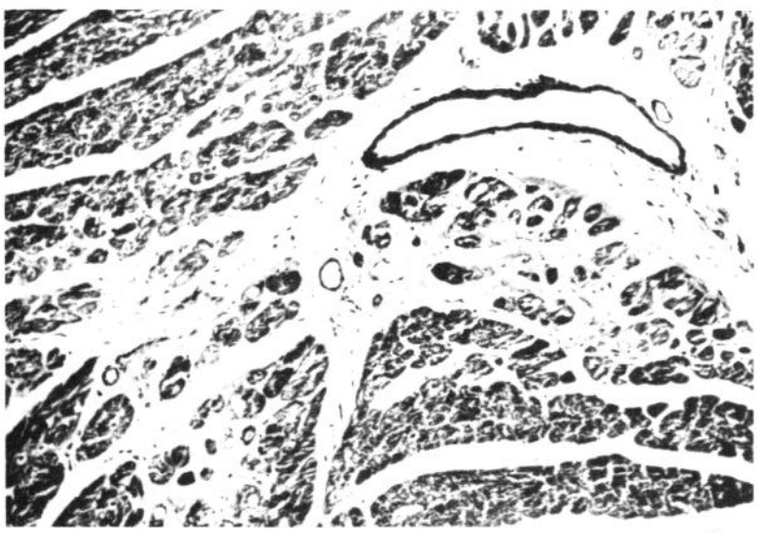

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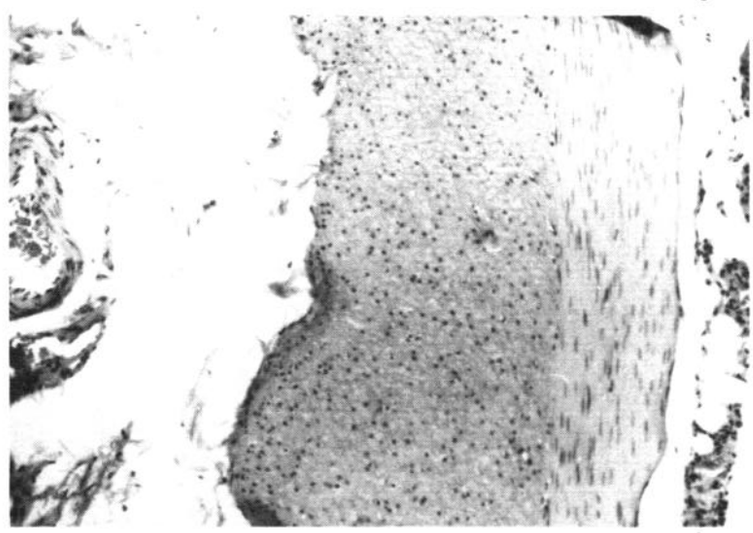

L

Photomicrographs of tissues from autopsied *Cebus apella* monkeys inoculated with the CA1 and Colombian strains of *T. cruzi*. G and H—pronounced epicardiac infiltrates in the right auricle; I—lymphocyte-plasmocyte infiltrates surrounding neurons in the interauricular septum; J—lymphocyte-plasmocyte infiltrates among the myocardial fibers of septal papillary muscle; K—fibrosis together with a near-absence of infiltrates in tissue from the free ventricular wall; L—a portion of the rectal wall showing marked diminution of the elements of the Auerbach plexus. (Hematoxylin-eosin, original magnifications: G, 40×; H through K, 100×; L, 160×.)

while fibrosis and the presence of atrophic myocardial fibers in the fibrotic tissue became more marked (Photos J and K).

Pictures similar to those found in the ventricular walls were observed in the muscular interventricular septum. Again, the animals that had been inoculated a shorter time showed only isolated lymphocyte-plasmocyte infiltration, while those that had been inoculated for over 27 months showed conspicuous fibrosis.

In all five animals the sections of the sinoauricular node showed a preserved architecture with some lymphocyte-plasmocyte elements among the conductive fibers. These elements were more prominent in the connective tissue adjacent to the node, especially in the subpericardial zone. Alterations observed in the atrioventricular node and the bundle of His were similar to those found in the sinus node.

In two of the three animals inoculated with the CA1 strain, serial sections of the colon showed an area 3 cm from the anal margin and 1 cm in length where the elements corresponding to the mesenteric plexus of Auerbach were absent or markedly diminished (Photo L). In both cases contrast X-ray of the colon showed images compatible with megaviscera.

Discussion

As previously noted, experimental *T. cruzi* infection has often been studied in the mouse *(16)* to evaluate drug and vaccine therapy; but the severe infection that it produces is usually lethal within a few weeks. Furthermore, *T. cruzi* infection of mice does not resemble human Chagas' disease, and therefore the results obtained by using mice to assess the benefits of drugs or vaccines are usually inconclusive *(22)*.

The high susceptibility of dogs to *T. cruzi* infection was established by Marsden et al. *(8)*, who infected beagle puppies with the virulent Peru strain of the parasite. Nine of the 10 infected puppies died within 35 days.

Rabbits show strong natural resistance to *T. cruzi* infection, and parasitemia is often difficult to detect by direct microscopic examination of the peripheral blood. Teixeira *(7)* studied chronic infection in these animals; he found a diffuse myocarditis without parasites, as well as megacolon in two of the test animals. Thromboembolism and congestive cardiac insufficiency seemed to be the cause of spontaneous death.

Other authors have experimentally inoculated primates of different genera (*Callithrix, Macaca, Cebus*) with different *T. cruzi* strains. In most cases a slight myocarditis was observed. In one of the experiments, however, the animals died between 95 and 243 days after inoculation, with results that varied greatly and were difficult to interpret *(11)*. These findings, taken together, led workers to theorize that primates had a natural resistance to this disease.

In the study reported here, *Cebus apella* monkeys showed serologic conversion by IHA and ELISA following inoculation with *T. cruzi*, irrespective of the *T. cruzi* strain used. In all, 83% of the inoculated monkeys yielded positive parasitologic results by the Strout test or xenodiagnosis.

It is of considerable interest that survival of the infected *Cebus apella* monkeys was related neither to the level nor to the length of patent parasitemia. Therefore, if the virulence of the parasite strain was an important determinant of

Chagas' disease pathogenicity, such virulence could not be related in our work to the levels of parasitemia attained, probably as a consequence of the host's immune response.

For example, no parasitemia was detected in three of the 10 monkeys inoculated with the CA1 strain. However, all three of these monkeys developed cardiac disturbances during the chronic phase of the disease, and one of them also exhibited histologically confirmed megaviscera. (Human patients usually survive the acute phase of infection when the parasitemia is high—*3, 5*—and die many years later when no parasitemia can be demonstrated by conventional methods.)

Regarding the electrocardiographic disturbances observed in 83% of the inoculated animals, it should be noted that the two groups receiving repeated intraperitoneal injections with 3×10^6 parasites of the Colombian or Tulahuen strains showed altered electrocardiographic parameters sooner (11 to 20 months after the first inoculation) than did the 10 monkeys inoculated once or twice by the conjunctival route with fewer parasites of the CA1 strain; the latter monkeys showed alterations 27 to 48 months after the first inoculation.

These findings suggest that variations in the numbers of parasites inoculated, the inoculation route, the frequency of the inoculations, and the virulence of the *T. cruzi* strain used could play an important role in the natural evolution of the disease, as well as in its pathogenesis and immunopathology. It should also be noted that the ECG patterns obtained with this model resemble those produced by Chagas' disease in man *(18–20)*, and that the anesthesia used in our work did not appear to produce any alterations in these patterns.

Regarding echocardiography, a noninvasive method employed in order to assess its experimental usefulness, it was found that nine of the 15 inoculated monkeys (60%) exhibited echocardiographic disturbances. This conclusion was reached after comparing these monkeys' echocardiographic patterns with those found in a control group of 30 monkeys exhibiting normal ECGs. In general, echocardiography was found to require relatively continuous and long evaluation, but the correlation found between the observed disturbances in primates and those in cases of human chagasic pathology *(21)* indicates that echocardiography could prove a very useful tool for procuring better knowledge of the pathogenesis of Chagas'-related cardiac lesions.

With respect to the contrast radiology performed, this showed neither organic dilatation nor motor disturbances of the esophagus; but it did show pictures of the colon compatible with megaviscera in three cases and with dolichocolon in one case. In three of the four animals, two inoculated with the CA1 *T. cruzi* strain and one with the Tulahuen strain, the pictures of colon dilatation and dolichocolon were corroborated by observation of pathologic lesions. Also, in the two monkeys inoculated with the CA1 strain, diminution or absence of the neural elements of the mesenteric plexus was observed, and in the monkey with dolichocolon, perineural infiltrates of the esophagus and colon were seen.

The histopathologic sections taken from the hearts of the five sacrificed animals showed scattered focal infiltrates of the lymphomonocytic type, together with foci showing atrophy and myocardial fibrosis—all of these being more often ventricular than auricular and including the bundle of His and the

interventricular septum. The pericardium showed mononuclear infiltrates. It appears that the degree of evolution of the disease could be evaluated by assessing the intensity of the infiltrating and fibrotic lesions, because the former became progressively less marked while the latter progressively increased.

Two of these monkeys, both inoculated with the CA1 strain, also showed histopathologic lesions of the mesenteric plexus approximately 3 cm from the anal margin—lesions wherein the number of neurons and structures corresponding to the Auerbach plexus were markedly diminished.

For purposes of comparison, it is worth noting that among 1,700 autopsied human patients dying from Chagas' disease, the incidence of cardiac lesions was found to be 90%, while the incidences of megacolon, megaesophagus, bronchiectasis, and other megaformations were found to be 20%, 18%, 7%, and 5%, respectively *(23)*.

Considering the results reported here, it appears reasonable to conclude that inoculation of *Cebus apella* with three *T. cruzi* strains did not produce demonstrable clinical alterations during the acute phase of the infection, but that it did lead to both cardiac and colon disturbances compatible with the chronic stage of human Chagas' disease, and that the histopathologic lesions found afterwards were similar to those observed in human chagasic pathology.

It should be emphasized that the susceptibility of the primates tested appeared independent of age and sex. However, the parasite strain inoculated, the nature of the inoculum, the number of inoculations, and the route of inoculation could have conditioned the natural evolution of the disease and could have influenced the times at which the electrocardiographic and histopathologic lesions developed.

It also appears that our test animals developed the pathology in a relatively short time, considering that humans generally do not manifest symptoms of the chronic stage until several years after natural infection. In addition, it is worth recalling that *Cebus apella* is native to zones endemic for Chagas' disease, that it has been adapted to indoor colony conditions, and that in comparison with other primates it reproduces well in captivity and can be maintained at fairly low cost.

Overall, therefore, *Cebus apella* appears to offer the most suitable available animal model for chronic Chagas' disease, one that survives the acute infection and develops the chronic cardiac and gastrointestinal form of the disease, apparently independently of age and sex.

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Summary

A principal obstacle to research on the prevention and treatment of human Chagas' disease has been lack of a good experimental animal model capable of developing symptoms of the disease's chronic stage. In seeking to develop such a model, the authors inoculated three groups of *Cebus apella* monkeys with the CA1, Colombian, and Tulahuen strains of *T. cruzi* by the conjunctival and intraperitoneal routes. They then reinoculated some of these monkeys from time to time in order to more closely approximate normal patterns of human infection with the parasite, and conducted a battery of tests designed to detect the parasite and trace the course of the disease.

Xenodiagnosis or other parasitology tests succeeded in detecting the parasite in 15 of the 18 inoculated monkeys at intervals ranging from one to 18 weeks after the last previous inoculation. Also, IHA tests indicated high levels of antibodies against the parasite lasting for about three months following infection, and ELISA tests detected elevated IgG levels in some of the infected monkeys over the course of the infection.

Radiology detected unusually large heart diameters in only two of the 18 inoculated monkeys, but electrocardiography detected ECG alterations in 15 and echocardiography detected disturbances in nine. These changes took considerable time to emerge, the ECG alterations being detected at times ranging from 11 to 48 months after the initial inoculation. Subsequently, histopathologic examinations of heart tissue from five of the 15 monkeys with ECG alterations revealed lymphocyte and plasmocyte infiltrates and fibrosis, the former appearing to diminish and the latter to increase over time.

Contrast X-rays of the inoculated monkeys also revealed four apparent cases of enlarged viscera (three of megacolon and one of dolichocolon). In two of these cases, histopathologic examination of the colon showed a marked reduction in elements pertaining to the Auerbach plexus in a part of the colon some three centimeters from the anal margin.

Overall, the results indicate that inoculation of *Cebus apella* monkeys with three *T. cruzi* strains did not produce demonstrable clinical alterations during the acute phase of the infection, but did lead to both cardiac and colon disturbances similar to those seen in chronic human cases of Chagas' disease. Histopathologic lesions found at autopsy were also similar to those observed in cases of chagasic pathology in man. These findings, together with the monkey's availability and the relatively low cost of maintaining it in captivity as compared to other monkeys, suggest that *Cebus apella* currently constitutes the most suitable available animal model for chronic Chagas' disease.

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