Table II. Mitotic index (MI) and phase frequencies

Time (h)	Control					10^{-6} g/ml					3×10^{-4} g/ml				
	MI	P	M	A	T	MI	P	M	A	T	MI	P	M	A	T
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.5	66.0	70.3	46.9	57.9	83.3	102.8	112.6	45.3	137.4	119.4	89.3	99.9	37.8	127.7	138.6
1	48.9	53.2	41.9	30.6	58.1	95.3	103.4	80.9	104.9	70.4	68.9	82.2	48.0	61.2	69.3
1.5	37.3	40.6	37.9	24.6	33.9	83.2	93.5	79.7	61.8	49.5	77.4	93.5	44.4	57.7	115.0
9						86.6	82.4	71.6	119.5	110.2	98.5	127.0	46.2	93.5	108.7
12						86.0	84.6	54.1	150.4	104.2	125.2	159.8	74.0	97.7	127.6

P, prophase; M, metaphase; A, anaphase; T, telophase; corresponding to the values at $t = 0$ (= 100%) during treatment with 0.1% caffeine and pemoline.

for some time nearly is total. Since gibberellic acid[10] as well as IAA[11] show antagonistic effects to colchicine, too, and have an influence on the mitotic index[10,11] similar to that of pemoline[4], the effect of pemoline on mitosis can be compared with that of growth factors.

Summary. Pemoline, the constituent of Tradon, is able to slow down the decrease of the mitotic index caused by 0.1% caffeine in roots of *Vicia faba*, and mitotic aberrations are reduced. With 0.005% colchicine and 3×10^{-4} g/ml pemoline, no metaphase-accumulation can be observed, and anaphase-disorder is delayed.

W. RÖPER[12]

Institut für Allgemeine Botanik der Universität, Arbeitsbereich Genetik, Jungiusstrasse 6–8, D–2000 Hamburg 36 (German Federal Republic, BRD), 20 May 1975.

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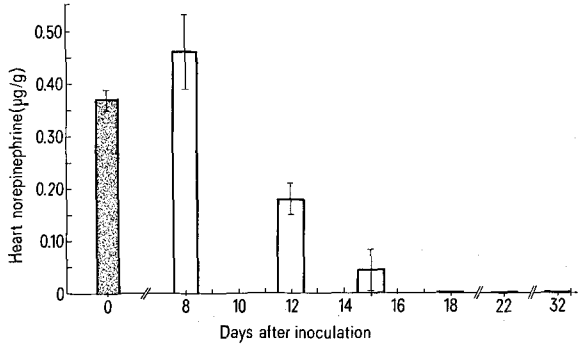
Depletion of Heart Norepinephrine in Experimental Acute Myocarditis Caused by *Trypanosoma cruzi*

Trypanosoma cruzi Chagas, 1909 is the causative agent of South American trypanosomiasis or Chagas', disease, one of the most frequent and dangerous illnesses of South America, with an estimated minimum of 7 million infected individuals[1]. Although parasitism attacks practically any organ, cardiac involvement is the most important clinical feature of Chagas' disease accounting for 87% of the deaths for which the disease is responsible[1]. It is now well established that in the course of *T. cruzi* infection there are lesions of the autonomic nervous system[2,3] resulting in extensive destruction of parasympathetic ganglion cells in human[4] and experimental[5] material. These lesions have been claimed[1] to be responsible for all the late manifestations of Chagas' disease, represented chiefly by anatomical and functional disorders of hollow muscular organs such as heart, esophagus and colon. Although the evidence for involvement of the parasympathetic nervous system is now generally accepted, the damage caused by *T. cruzi* on the sympathetic nervous system is a matter of controversy[6]. We have investigated this problem by estimating the sympathetic neurotransmitter norepinephrine (NE) in the heart of rats inoculated with *T. cruzi*.



The time course of norepinephrine depletion of the heart in rats inoculated with *Trypanosoma cruzi*. The mean values and SEM (indicated by bars) for heart NE concentrations are plotted for normal rats (12 animals) and at 8 (3 animals), 12 (3 animals), 15 (4 animals), 18 (4 animals), 22 (2 animals) and 32 days (3 animals) after inoculation.

Material and methods. Male and female Holtzman rats aged 27–30 days, and weighing 45–65 g were inoculated i.p. with blood containing 300,000 trypomastigotes of the Y-strain. Under these conditions the mortality was only 15%. At different periods after inoculation, the rats were sacrificed under ether anesthesia and their hearts were washed clean of blood by a quick saline perfusion, removed, blotted, weighed, immersed in 0.8 *N* perchloric

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acid and homogenized in a Sorvall Omni-mixer. NE was then assayed fluorometrically in an Aminco-Bowmann spectrophotofluorometer (I.P. 21) by the technique of ANTON and SAYRE[7]. The assay was performed 8, 12, 15, 18, 22 and 32 days after inoculation, running normal litter-mate controls at the same time. *T. cruzi* was detected in the blood stream of all the inoculated animals. The occurrence of congestive heart failure in these animals was ruled out by macroscopic and microscopic post-mortem examination. The course of Chagas' myocarditis was studied in histological sections of hearts from a total of 12 rats sacrificed 8, 12, 15 and 18 days after inoculation.

Results. The Figure shows the values obtained for heart NE in *T. cruzi* infected and control animals. In normal rats the mean value obtained for cardiac NE (0.37 ± 0.02 µg/g) fell within the range reported in the literature[8]. Eight days after inoculation, the NE content of the hearts (0.46 ± 0.07 µg/g) was slightly higher than that of the controls, although the difference was not significant. At day 12, however, the NE stores of the hearts (0.18 ± 0.03 µg/g) dropped to about 50% and at day 15 they were barely measurable (0.045 ± 0.04 µg/g). In the hearts of all rats sacrificed 18, 22 and 32 days after inoculation, the NE stores dropped to undetectable values.

At day 8, when the NE content of the heart was still normal, the histological study of the heart muscle cells revealed a heavy parasitism with leishmania forms of *T. cruzi*. This fact indicates that the decrease in the heart NE content was not directly related to the presence of leishmania. At day 12, however, coincident with the decrease in the NE content, the histological picture of the heart showed the inflammatory reaction found in the acute Chagas' myocarditis. This myocarditis became very intense at day 18, when the heart content of NE became undetectable. The coincidence between the decrease in the heart NE and the development of the myocarditis suggests a relationship between the two phenomena.

Discussion. Catecholamines had not yet been studied in Chagas' disease. However, a decrease in the cardiac content of these amines has been reported in some other diseases of the heart[9], and NE is markedly reduced in congestive heart failure[10]. The demonstration of NE depletion in hearts with myocarditis caused by *T. cruzi* raises the possibility that the same phenomenon might occur in myocarditis caused by other ethiological agents.

Since most NE of the mammalian heart is contained in postganglionic sympathetic fibres, our results demonstrate a massive involvement of these fibres in acute Chagas' disease. The depletion of NE might be due either to an actual destruction of adrenergic fibres or simply to an impairment of the mechanisms involved in synthesis and storage of the amine, in otherwise normal fibres. The occurrence of lesions of the cardiac nerves in Chagas' disease[3,11,12] supports the first alternative, although the techniques used to detect these lesions were not specific for adrenergic fibres.

There is evidence that the postganglionic sympathetic transmission in the heart is impaired in situations where the NE stores of the organ are markedly depleted[13]. It is therefore very probable that in acute Chagas' myocarditis, where heart NE becomes undetectable, the sympathetic regulation of cardiac function is impaired or even abolished. This would be in agreement with hemodynamic data obtained in some of the patients with Chagas' disease studied by AMORIM et al.[14], which suggest an impairment or failure of the sympathetic control of the heart rate. It is now well established that in addition to its influence on the heart rate the sympathetic nervous system has a positive inotropic effect on the myocardium[15]. An impairment of this effect, such as presumably occurs in Chagas' disease, would lead to a decline in the contractile state of the heart. This might be an important factor to explain the congestive heart failure which occurs in many patients with acute Chagas' disease and which constitutes the main cause of death at this stage of the disease[16]. The study of heart norepinephrine in chronic Chagas' disease is now in progress and will be reported elsewhere[17].

Summary. The norepinephrine content of the heart was reduced to undetectable values in rats inoculated with *Trypanosoma cruzi*. This fact indicates a massive involvement of the cardiac postganglionic sympathetic fibres in acute Chagas disease.

A. B. M. MACHADO, C. R. S. MACHADO and C. B. GOMES

Department of Morphology,
Institute of Biological Sciences,
University of Minas Gerais, C.P. 2486,
Belo Horizonte (Brazil), and
Department of Biochemistry and Pharmacology,
Escola Paulista de Medicina,
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