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A NOTE ON THE HARVESTING OF LARGE NUMBERS OF *TRYPANOSOMA CRUZI* BLOOD FORMS IN ADULT DOGS

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SUMMARY

A mass of blood forms of *T. cruzi* large enough for immunochemical studies is not easily harvested because animals showing heavy parasitemia are small and only scanty volumes of blood can be collected from them. By giving immunosuppressive drugs to inoculated adult dogs and by bleeding these animals through arterial cannulation, we could obtain more than a liter of heavily parasitized blood per animal, containing more than 4×10^{10} trypanosomes.

INTRODUCTION

Inoculation of some strains of *Trypanosoma cruzi* leads to a fulminating acute disease with intense parasitemia and death in animals such as mice, hamsters and new-born dogs [1, 4]. Harvesting blood forms of the parasite is limited, however, by the scanty volumes of blood obtainable from such animals. Larger volumes of blood can be collected from adult dogs, but in them, the disease tends to assume a more chronic form, with very rare circulating parasites in the blood stream. It has been shown that the administration of cortisone to rats, results in an enhancement of parasitemia [5, 6]. In order to obtain a mass of blood forms of *T. cruzi* necessary for immunochemical studies of this parasite, we have submitted inoculated adult dogs to the action of immunosuppressive drugs, so that a heavy parasitemia could develop. By employing a bleeding technique through arterial cannulation plus venous administration of large volumes of isotonic solution, most of the animals' circulating blood could be collected.

MATERIAL AND METHODS

Hamsters were inoculated intraperitoneally with *T. cruzi* strain Y [7], maintained in the laboratory through mice passages, and bled by heart puncture when an intense parasitemia developed after about 3 days. Adult dogs, weighing from 10 to 20 kg were inoculated intraperitoneally with 5-10 ml of pooled hamster blood, rich in parasites. Immunosuppression was induced in the dogs by daily oral administration of azathioprine (*) (8 mg/kg) and prednisone (2 mg/kg) beginning two days before inoculation and continuing throughout the experiment. Chloramphenicol (500 mg/day) was also included.

Parasitemia in dogs was checked every 2 or 3 days in the first two weeks and then daily, in citrated blood samples collected by venous puncture or by ear prick and observed under coverslip with a 40 × dry objective. Venous blood samples were also obtained before and after inoculation, on several days, for hematological counts and serological tests. Immunofluorescence tests were done on microscope slides with cultural forms of *T.*

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(*) "Imuran", Burroughs Wellcome & Co., London

cruzi as antigen[2], employing and anti dog-globulin antiserum (**) labeled with fluorescein isothiocyanate (***) by a slow-adding dialysis technique[3] to a F/P weight ratio of about 10. Dog serums were tested at 1:20 and on, in a doubling dilution ratio. Bleeding to death of the dogs was performed according to the method used at the Harvard Medical School, U.S.A., by W. Orr, in the preparation of blood-free antigen from lymph nodes for the production of antilymphocyte globulins, as modified from A. P. MONACO (personal reference by J. E. M. CUNHA).

The dogs were anesthetized with intravenous sodium pentobarbital — 30 milligrams per kilogram — and, after tracheal intubation, placed on respirator. The femoral vessels were dissected and polyethylene catheters were inserted into them. From the femoral artery the blood was collected into a transfusion bottle containing 70 millititers of an ACDP anticoagulant solution (glucose 1.75 g, sodium citrate 1.80 g, citric acid 0.22 g, sodium phosphate 0.55 g). The femoral vein was used to administer large quantities of isotonic saline solution (until the dogs were completely "washed out") and 1 milliliter of an 1:1,000 adrenalin solution, while the blood was being collected.

RESULTS AND DISCUSSION

Trypanosoma cruzi was found in the blood of dogs receiving immunosuppressive drugs, about 12 to 15 days after inoculation (Table I). Scanty for about a week, blood forms of the parasite then suddenly increased in number to 5 to 20 or more per microscopic field (400 ×). In stained smears, about one trypanosome per 100 erythrocytes was found. Although animals appeared as normal in the first hours of heavy parasitemia, if not sacrificed they rapidly showed signs of illness and survived for no longer than 24 hours.

In animals inoculated with *T. cruzi* but not submitted to immunosuppression, parasites were never found through the microscopic scanning of fresh blood samples. However, when immunosuppression was initiated, even after a few months from the inoculation,

TABLE I

Parasitemia and fluorescence tests in dogs inoculated with *T. cruzi*, receiving or not immunosuppressive drugs

Dog	Immuno-suppression	Beginning of parasitemia *	Heavy parasitemia *	*T. cruzi* fluorescent antibody test
Am.	no	—	—	1:80 (12th day)
Cor.	no	—	—	1:320 (18th day)
Li.	no	—	—	1:80 (18th day)
Br.	yes	12th day	18th day	Negat. (18th day)
Mar.	yes	13th day	20th day	Negat. (18th day)
Cr.	yes	15th day	21th day	Negat. (20th day)
Pol.	yes	12th day	18th day	Negat. (18th day)
Cor. ***	yes	15th day **	24th day **	1:40 (20th day) **

* Days after inoculation
** Days after administration of immunosuppressive drugs
*** Same dog as above

(**) Gently furnished by Dr. Masaio Misuno, Faculdade de Medicina Veterinária, University of São Paulo, Brasil
(***) Crystalline, chromatographically pure Isomer I, B.B.L., U.S.A.

a parasitemia similar to that of dogs receiving the drugs from the beginning of experiment was observed: a few blood forms of *T. cruzi* appeared about 15 days after the beginning of drug administration, with a sudden burst to a large number of parasites about 10 days later.

Hematological counts showed sharp differences between inoculated animals receiving or not immunosuppressive drugs. While no modifications were seen in erythrocyte and hemoglobin values in dogs not submitted to these drugs, immunosuppression was followed by a sharp decrease in such values within 15 to 20 days to about a half or two thirds the initial red cell numbers and hemoglobin percentages. Dog *Cr.*, for example, with 6,300,000 red cells per cu.mm. and 15,8 grams per cent of hemoglobin on the day of inoculation, had only 3,500,000 red cells and 8.8 g of hemoglobin after 15 days.

White cell counts differed also widely in both groups with very low percentages of lymphocytes in dogs receiving immunosuppressive drugs. For example, on the 12th day of inoculation, dog *Am.*, which did not receive drugs, showed 18,400 leucocytes per cu.mm. of blood, with 72.0% neutrophils (26.4% bands and 45.6% segmented), 6,300,000 red cells per cu.mm. and 15.8 lymphocytes, 3.2% monocytes and 1.6% plasmocytes. After a similar period, dog *Cr.*, receiving immunosuppression, had 5,300 leucocytes, with 96.0% neutrophils (12.0% bands, 84.0% segm.), 0.0% eos., 0.0% bas., 2.4% lymph., 1.6% monoc.

Inoculated dogs not submitted to immunosuppression developed antibodies against *T. cruzi* as detected by the fluorescence test (Table I). Such antibodies were not found when immunosuppressive drugs were administered. However, in dog *Cor.*, which received drugs only two months after the inoculation and showed a fluorescence titer of 1/320, antibodies did not disappear, a titer of 1/40 being found even after 20 days of immunosuppressive treatment.

In this way, the use of immunosuppressive drugs in dogs, inoculated with *T. cruzi*, and the bleeding of animals through a technique which permits to collect the largest part of the circulating blood, resulted in the obtainment of large volumes of heavily parasitized blood. It could be calculated that more than 4×10^{10} trypanosomes were contained in the blood collected from only one dog. This fulfilled our needs of obtaining large numbers of blood forms of *T. cruzi* destined to immunochemical studies.

RESUMO

Nota sôbre a obtenção de grande número de formas sanguícolas de T. cruzi *em cães adultos*

A obtenção de formas sanguícolas do *T. cruzi* em quantidades suficientes para estudos imunoquímicos é difícil, porque os animais apresentando parasitemia intensa são de pequeno porte e fornecem apenas exíguas quantidades de sangue. Entretanto, pudemos obter grandes volumes de sangue altamente parasitado, em cães adultos, administrando-lhes drogas imunesupressoras e coletando o sangue por cateterização arterial, o que representou mais de 4×10^{10} tripanosomas por animal.

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