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EXPERIMENTAL INFECTION OF THE GUINEA PIG WITH CHAGAS' DISEASE AND SUPERIMPOSED LEISHMANIASIS, AND ELECTROPHORECTIC ANALYSIS OF THE SERUM

Rodrigo ZELEDÓN (1) and Cecilia LIZANO (2)

SUMMARY

A total of 9 male guinea pigs were inoculated with *Schizotrypanum cruzi* and 60 days later with tissue forms of *Leishmania enriettii*. Another group of 9 male guinea pigs received only the second inoculum and were used as control. Both groups of animals contracted leishmaniasis but the initial and metastasic lesions appeared much later in the chagasic group. The average values of total serum proteins showed a tendency to decrease in the group with trypanosomiasis and leishmaniasis and practically no change in the group with only leishmaniasis. The electrophoretic patterns of the latter group showed and increase of β and γ globulins at the expense of albumin. In the former group, *Schizotrypanum* infection produced an increase only in the γ globulin and the superimposed leishmaniasis brought on an increase of the β fraction.

INTRODUCTION

The relative resistance of the guinea pig to infection with Costa Rican strains of *Schizotrypanum cruzi*, and its great susceptibility to *Leishmania enriettii*, gave us the idea of studying the possible effect that the former infection could have in the course of the latter, when both types of protozoosis are present in the same animal within a convenient interval of time. The experiments were also used to observe the changes that occurred in the serum of animals with single or double infections.

Electrophoretic studies of the proteins in infections by hemoflagellates are relatively few and there seem to be none dealing with cutaneous leishmaniasis, either in man or in experimental animals (see literature up to 1954 in STAUBER [16]). In human kala-azar there is commonly found an increase of globulin at the expense of albumin, which can be very low (JENKINS *et al.*[7], KUMAR *et al.*[8], PIMENTA & BRENER [10], SHANKER [15]). Also, in patients with kala-azar, a slow-moving γ-globulin fraction has been observed (cf. STAUBER [16]).

In experimental infections with *L. donovani*, qualitative and quantitative differences have been observed in the electrophoretogram according to the animal employed (cf. STAUBER [16]). In the hamster an increase in the γ fraction and also in the β and α-globulins can occur (ROSSAN [12], SENECA *et al.*[14], STAUBER *et al.*[17]). ROSSAN [12] found that various species of susceptible rodents respond to the infection with hypoalbuminemia and hyper-γ-globulinemia while both the rabbit and the guinea pig, more resistant to infection, showed perceptible increase of α-globulin; furthermore, the rabbit showed an increase of the β fraction. ROSSAN [12] observed lipemic sera in some of his injected animals, and he thinks that this could ex-

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(1) Departamento de Parasitología, Universidad de Costa Rica.

(2) Sección de Pediatría, Hospital San Juan de Dios, San José, C.R.

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plain, in part, the increase of the β fraction. In human and experimental trypanosomiasis, besides an increase in γ-globulin, a perceptible increase of α-globulin is often observed (CHATTÁS *et al.*[2], FERREIRA & ELEJALDE [4], GAZIN *et al.*[6], PINTO & FALCÃO [11], SALUM *et al.*[13], SÉNECA *et al.*[14]).

In the present paper our experiences, which were the subject, of a preliminary note, are reported in detail (ZELEDÓN & LIZANO [19]).

MATERIAL AND METHODS

A total of 18 male guinea pig weighing from 300 to 500 gm were divided into two groups for the experiment. The animals were fed a comercial concentrate supplemented with fresh vegetables. Cardiac blood from an infected (*S. cruzi*, P.E. strain) guinea pig was diluted with Alsever's solution and 1 ml, containing 5×10^5 trypanosomes, was inoculated intraperitoneally into each of 9 animals. During the infection weekly counts of the trypanosomes, from three animals taken at random, were made with a hemocytometer. After 60 days, both the experimental and the control groups were inoculated intraperitoneally with 1 ml of a suspension, containing 500 U of penicillin and 0.625 mg of streptomycin per ml and 12×10^6 leishmania forms, derived from a triturated nasal nodule from an animal infected with *L. enriettii*. All animals were bled from the heart before inoculation and during the course of infections. The sera, kept in a deep-freezer, were used for the determination of total proteins by the method of LOWRY *et al.*[9], and the fractions studied by paper electrophoresis using a Beckman-Spinco apparatus, with veronal buffer at pH 8.6 and 0.075 ionic strength. The strips were colored with bromophenol blue and analysed in the "Analytrol" according to the manufacturer's instructions.

RESULTS

The guinea pigs of the first group reached a peak parasitemia around the 34th day (average number: 3.9×10^6 trypanosomes per ml of blood). At the time of the second bleeding (on the 48th day) the trypanosomes were few in direct fresh blood examinations and none were found at the moment of the inoculation with *L. enriettii* (on the 60th day). A month after the leishmaniasis infection, some of the guinea pigs from the first group showed a slight and soft testicular inflammation, whereas the greater part of the animals from the control group showed firm nodular periorchitic lesions. After 35 days, nodular testicular lesions began to become evident in the first group whilst in some animals of the control group external scrotal ulcers and metastasis to nose and feet were present, without evidence of secondary infection. From this moment on, the disease followed a normal course in both groups, until the chagasic guinea pigs showed the same extensive testicular and metastatic lesions of the control group. Deaths began to occur earlier in the control group than in the chagasic group, due to secondary infections.

The average values of total proteins showed a tendency to decrease in the group with trypanosomiasis and leishmaniasis and practically no change in the group with only leishmaniasis.

The electrophorectic patterns revealed an increase of both β and γ-globulins in the latter group. In the period in which some animals were infected only with trypanosomiasis, an increase was produced in the γ fraction, and the superimposed infection with *L. enriettii* showed an increment in the β fraction that was not as pronunced as in the animals with only leishmaniasis (Tables I and II). In Figures 1 and 2, typical patterns before and after infections are presented. In every case the mean A/G ratio was greatly decreased.

DISCUSSION AND CONCLUSIONS

COUTINHO [3] found that one injection of killed leptomonas of *L. enriettii* or *L. braziliensis* gives guinea pigs complete or partial immunity to the former species. A greater number of spontaneous cures occur in vaccinated animals than in the non-immune groups. In the present case, a previous chagasic infection did not prevent leishmaniasis in the test animals, but delayed its develop-

TABLE I

Protein electrophoretic values of guinea pigs sera before and after infection with Chagas' disease and leishmaniasis.

Guinea pig No.	Protein fractions (gm%)																	
	Serum 1						Serum 2						Serum 3					
	T.P.	Alb.	α_1	α_2	β	γ	T.P.	Alb.	α_1	α_2	β	γ	T.P.	Alb.	α_1	α_2	β	γ
1	7.9	4.42	0.31	1.31	0.61	1.25	7.8	4.02	0.21	1.33	0.58	1.66	5.8	2.55	0.23	0.97	0.70	1.35
2	6.4	3.46	0.35	1.42	0.56	0.61	7.4	4.21	0.29	1.26	0.46	1.18	7.8	3.46	0.40	1.48	1.02	1.44
3	6.9	3.19	0.34	1.51	0.91	0.95	6.2	2.39	0.29	1.35	0.74	1.43	6.6	2.59	0.46	1.26	0.91	1.38
4	7.6	3.23	0.33	1.75	1.04	1.25	6.6	2.54	0.34	1.01	0.81	1.90	7.8	2.84	0.37	1.77	1.32	1.50
5	7.0	4.19	0.30	1.20	0.53	0.78	6.3	2.27	0.38	1.12	0.95	1.57	5.6	2.21	0.52	0.99	0.89	0.99
7	7.0	3.72	0.41	1.67	0.81	0.39	6.6	2.39	0.40	1.84	0.58	1.39	—	—	—	—	—	—
8	7.6	4.45	0.55	1.53	0.55	0.52	7.2	3.07	0.50	1.09	0.53	2.01	6.2	2.67	0.36	1.31	0.77	1.09
9	7.9	4.09	0.21	1.95	0.76	0.89	6.8	2.52	0.39	1.36	0.98	1.55	6.6	2.78	0.33	1.34	0.96	1.19
Mean	7.3	3.84	0.35	1.54	0.72	0.83	6.8	2.93	0.35	1.30	0.70	1.59	6.6	2.73	0.38	1.30	0.94	1.28
Mean A/G	1.12						0.74						0.70					

Serum 1: Before infection.

Serum 2: After infection with *S. cruzi*.

Serum 3: After infection with *S. cruzi* and *L. enriettii*.

Guinea pig No. 6 was not included due to accidental loss of the sera; guinea pig No. 7 died before the 3rd bleeding.

TABLE II

Protein electrophoretic values of guinea pigs sera before and after infection with leishmaniasis.

Guinea pig No.	Protein fractions (gm%)											
	Serum 1						Serum 2					
	T.P.	Alb.	α_1	α_2	β	γ	T.P.	Alb.	α_1	α_2	β	γ
10	5.9	3.12	0.25	0.91	0.66	0.96	6.6	2.46	0.32	1.05	1.76	1.01
11	8.4	4.70	0.31	1.71	0.69	0.99	7.8	3.11	0.38	1.92	1.37	1.02
12	6.6	3.26	0.34	1.59	0.75	0.66	7.8	3.24	0.35	1.70	1.22	1.29
14	6.0	2.73	0.45	1.17	0.72	0.93	6.6	2.24	0.43	1.33	1.21	1.39
15	5.8	2.88	0.40	1.07	0.66	0.78	5.2	2.14	0.35	1.35	0.72	0.64
16	7.0	3.75	0.54	1.25	0.79	0.67	6.6	2.65	0.30	1.15	0.96	1.54
17	6.4	3.47	0.24	1.29	0.68	0.72	7.4	2.64	0.34	1.63	1.10	1.69
18	5.8	2.67	0.37	1.34	0.49	0.93	5.6	2.63	0.32	0.74	0.44	1.47
Mean	6.5	3.32	0.36	1.29	0.68	0.83	6.7	2.64	0.35	1.36	1.10	1.26
Mean A/G	1.05						0.65					

Serum 1: Before infection.

Serum 2: After infection with *L. enrietti*.

Guinea pig No. 13 died before the second bleeding.

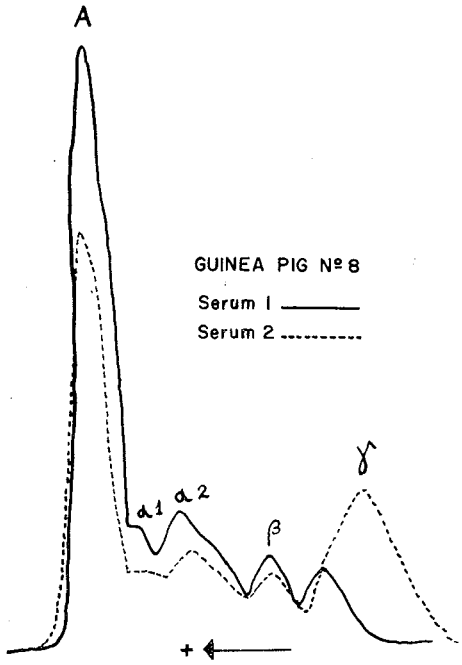

Fig. 1 — Representative electrophoretic patterns of a guinea pig serum before and after infection with *S. cruzi*.

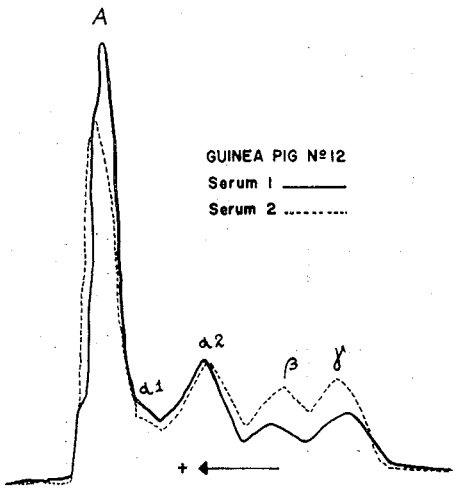

Fig. 2 — Representative electrophoretic patterns of a guinea pig serum before and after infection with *L. enriettii*.

ment, perhaps in a nonspecific manner due to elevated γ-globulin levels resulting from the trypanosome infection.

Our experiments also revealed that in the case of leishmania infected guinea pigs, there is an increase in the γ and β fractions. This could be explained either by an increase of associated serological substances to the latter fraction or by the formation of at least two types of antibodies, in contrast to only one in the chagasic infection of this animal.

D'ALESSANDRO [1] found, by electrophorectic and ultracentrifugal studies, that in the infection of the rat by *T. lewisi* there are at least two types of antibodies which migrate between the slow moving β-globulins and the fast moving α-globulins. This area corresponds to the T component of the serum, even though no new protein peak in this zone was found.

In sleeping sickness, GALL [5] also found a globulinic fraction between β and γ, clearly present in the electrophorectic diagram. TERRY [13] found that the natural resistance of cotton rats to *T. vivax* is explained by the presence in the serum of these animals of a protein fraction associated with the β and fast moving γ-globulins.

In our experiment, only one animal failed to show an increase in the β and γ fractions as a consequence of leishmaniasis infection (No. 15). This could be explained by the relatively low concentration of total proteins of this animal, which did not permit an adequate response to the infection. This seems to be true in view of the sudden and early death of the animal a few days after the second bleeding. The increase of the β fraction in the group with the double infection was not as pronounced as in the control group, possibly because the animals were less able to respond to the infection, mainly due to the decrease, already evident, of the albumin.

SUMARIO

Infecção experimental do cobaio com moléstia de Chagas e leishmaniose superposta, com análise electrofrética do sôro.

Um lote de 9 cobaios machos foi inoculado com *Schizotrypanum cruzi* e, 60 dias após, com formas tissulares de *Leishmania enriettii*. Como contrôles usaram-se 9 cobaios machos que receberam apenas o segundo inóculo. Ambos os grupos de animais contraí-

ram a leishmaniose; entretanto, as lesões iniciais e metastáticas apareceram mais tardiamente no grupo chagásico. Os valores médios de proteínas séricas totais demonstraram tendência a diminuir no grupo com tripanossomíase e leishmaniose, sendo que não apresentaram alterações apreciáveis no grupo leishmaniótico apenas. Os diagramas electroforéticos dêste último grupo apresentaram aumento de β e γ-globulinas às expensas da albumina. No outro grupo, durante a infecção chagásica pura, o aumento verificou-se apenas na γ-globulina; a infecção superposta de leishmaniose trouxe consigo, também, o aumento da fração β.

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