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TRYPANOSOMA CRUZI INFECTIONS IN *RHODNIUS PROLIXUS* REFED ON DIFFERENT HOSTS

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SUMMARY

250 third-stage larvae of *Rhodnius prolixus*, fed on mice highly parasitized with the "Bertoldo" strain of *Trypanosoma cruzi*, were subsequently given two additional blood meals on one of the following hosts: "exposed" chickens, "fresh" chickens, mice, rabbits, and lizards. Lots of 10 insects from each host group were examined individually by dissection on 5 different occasions after the last blood meal. Results showed that the levels of infection in each host group were quite low and similar (averaging 26-32%), and that the parasitaemia of the initial host bore no relation to the level of the infection established in the insects. There is no suggestion of the presence of any trypanolytic factor in the blood of the secondary hosts. It is concluded that the limiting factor for the development of *T. cruzi* in the intestine of *R. prolixus* is inherent in the insect itself, and not dependent on the type of blood supplied in the refeedings.

INTRODUCTION

Although it is well known that the reduviid bugs of the subfamily Triatominae are the natural vectors of *Trypanosoma cruzi*, the factors governing the establishment and multiplication of this flagellate in these hosts need further study.

MAEKELT & ALCAÑIZ [6] have enumerated and discussed these factors. WOOD [12] demonstrated that the rate of multiplication of *T. cruzi* in *Triatoma protracta* was affected by the environmental temperature.

The intestinal symbionts of *Rhodnius prolixus* have an effect on the parasites; MÜHLPFORDT [8] reported that *T. cruzi* multiplied more actively in bugs deprived of their symbionts.

PIFANO [10] observed that on some occasions, certain insects did not become infected, although fed on animals with high parasitaemias of *T. cruzi*.

PHILLIPS & BERTRAM [9], working with three strains of *T. cruzi* and four species of triatomids, demonstrated that there was no significant variation of susceptibility between recently established strains of insects and those inbred for many years. Additionally, they supposed that the geographic origin of the insects was not of major importance, thus contradicting DIAS [1], who counselled employment of the most important local vector in xenodiagnosis.

The present work is concerned with the possibility that the type of blood ingested by *R. prolixus* subsequent to the infective blood meal might influence the development of *T. cruzi* in the gut of the insect. Thus, *R. prolixus* fed on infected mice were refed on various hosts — avian, mammalian, and poikilothermic reptile.

MATERIAL AND METHODS

250 hungry, laboratory-reared, third-stage *R. prolixus* larvae were allowed to feed to repletion on mice with high parasitaemias

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(averaging seven flagellates/microscope field 400 × in caudal blood) of the "Bertoldo" strain of *T. cruzi*. The insects were then arbitrarily divided into five groups of 50, and each group allowed to feed to repletion, on two subsequent occasions, on one of the five following hosts: a) chickens utilized frequently during 10-12 months to feed *R. prolixus*, infected or not — "exposed" chickens; b) chickens never before used to feed *R. prolixus* — "fresh" chickens; c) healthy mice; d) healthy rabbits; e) healthy lizards (*Ameiva ameiva*, Teiidae). With the exception of the exposed chickens, a fresh animal was used for each refeeding.

Insects were refed on lizards by the technique of SCORZA [11], the lizard being immobilized with masking tape, warmed briefly under a 100-watt incandescent bulb, and placed immediately into a 24 x 12 x 6 cm plastic container, within which the insects were allowed to run freely. Insects were refed on the other hosts in the normal manner.

All five groups of engorged insects were maintained on paper in standard baby-food jars covered with gauze, in a climatized room at 28°C and 70% relative humidity, following GOMEZ-NUÑEZ & FERNANDEZ [4].

From each group, ten insects were examined individually on each occasion — 22, 29, 36, 43, and 50 days after the last blood meal — by dissecting the entire intestine in 0.05 ml of 0.85% saline, according to the technique of GUEDES [5]. Then, the flagellates in the total intestinal content of each individual insect were counted in a haemocytometer, to provide the basic data of the investigation.

RESULTS

Table I gives the results obtained by refeeding on five different hosts.

It can be seen that all five types of blood ingested by *R. prolixus* permitted development of metacyclic flagellates in the intestine, and that there are no statistically significant differences in the averages of the flagellates seen in the five groups.

The number of flagellate forms observed in each individual dissection also varied widely from group to group and from time to time.

DISCUSSION

The fact that percentages of infection were very similar (26-32%) in the groups of insects fed on five different hosts (birds, mammals, reptile) appears to indicate that the type of blood supplied in the refeedings of bugs already exposed to infection is not a limiting factor for the development of *T. cruzi* in the intestine of *R. prolixus*.

In spite of the high parasitaemias in the mice that served as infective hosts, the incidence of infection in the insects was very

TABLE I

Trypanosoma cruzi infections of third-stage larvae of *Rhodnius prolixus* fed on five different hosts after the initial infective blood meal on mice with high parasitaemias

Refeedings upon:	"Exposed" chickens	"Fresh" chickens	Healthy mice	Healthy rabbits	Healthy lizards
Number of insects surviving until examination	42	45	41	35	37
% of infected insects	26	29	32	31	32
Average number of flagellates per infected insect	74,32	196,54	139,79	109,77	178,54
Range in number of flagellates per insect examined	0-232	0-845	0-872	0-377	0-880

low (average 30%), and the absolute numbers of flagellated forms in the infected insects were also low. This finding cannot be explained by the conclusions of FISTEIN & CHOWDHURY [2], that the number of parasites obtained in *R. prolixus* is proportional to the parasitaemia of the infective host.

The number of flagellates in each insect examined was very variable, both among the five groups and among the individual insects in each group. The range was very great, from zero in the great majority of insects examined to a maximum of 880. The present investigation, wherein all insects were initially fed on very highly parasitized animals and maintained under identical conditions, has yielded a low incidence of infection and a very wide range in numbers of metacyclic flagellates in the feces of the insects exposed to infection. This may be taken as indicating that the type of blood employed in reefeding cannot explain this variation by itself. Other factors, possibly those inherent in the individual insects, remain to be investigated. It appears improbable, from these results, that a trypanolytic factor, as postulated by MAEKELT [7] and PIFANO [10] may be invoked to explain the variation in infection and in number of parasites.

Undoubtedly, the results given above show the great difference in susceptibility of individual *R. prolixus* to infection by *T. cruzi*, differences in susceptibility which are maintained whether the insects are refed on the blood of birds, mammals, or reptiles. These differences would be still more critical when the infected donor animal was in the chronic stage of the disease. Thus, as a practical consideration, xenodiagnosis in the chronic stage of the illness ought to be carried out with the largest possible number of refeedings on the same host, in order to overcome the effect of the individual resistance demonstrated by the majority of the insects to infection from a single blood meal on the infected host. MAEKELT [7] has emphasized the necessity, in xenodiagnosis, of employing the largest number of insects possible and of giving them as many blood feeds as possible on the infected host.

Since it is known that the greatest susceptibility to *T. cruzi* is achieved when using local strains of the parasite, together with those species of triatomids that are the principal vectors of the region, as mentioned by DIAS [1], these experiments were carried out with the Venezuelan "Bertoldo" strain of *T. cruzi* and with *R. prolixus*, the chief vector of Chagas' disease in Venezuela — GAMBOA [3], giving a particular significance to the above results.

RESUMEN

Infecciones por Trypanosoma cruzi *en* Rhodnius prolixus *re-alimentados en diferentes huéspedes*

250 ninfas de III estadio de *Rhodnius prolixus* experimentalmente infectadas con *Trypanosoma cruzi*, cepa "Bertoldo", recibieron dos comidas adicionales en uno de los siguientes animales: pollos "expuestos", pollos "frescos", ratones, conejos y lagartos sanos; lotes de 10 chipos se examinaron individualmente por disección en 5 oportunidades después de la última ingesta sanguínea.

Los datos obtenidos revelan que los índices de infección en los triatominos fueron bajos y muy similares (26-32%) a pesar de emplearse 5 tipos de comidas sanguíneas y que, la parasitemia no está directamente relacionada con los niveles de infección alcanzados en los insectos.

No se obtuvieron resultados que indiquen la presencia de un factor tripanolítico en la sangre de ninguno de los hospedadores empleados.

Se concluye que la susceptibilidad individual de los chipos a la infección por *T. cruzi* es un factor limitante y que, la calidad de la sangre ingerida por los insectos no influye en la evolución y multiplicación del *T. cruzi* en el intestino de este vector.

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REFERENCES

1. DIAS, E. — Técnica do xenodiagnóstico na Moléstia de Chagas. *Mem. Inst. Oswaldo Cruz* **35**:335-342, 1940.

2. FISTEIN, B. & CHOWDHURY, M. N. H. — The uptake of *Trypanosoma cruzi* by *Rhodnius prolixus* and its later excretion. *Trans. Roy. Soc. Trop. Med. & Hyg.* **63**:883-884, 1969.

3. GAMBOA, J. — Función de los triatominos como vectores de la Enfermedad de Chagas. *Rev. Ven. San. Asist. Soc.* **31**:183-191, 1966.

4. GOMEZ-NUÑEZ, J. C. & FERNÁNDEZ, M. J — La Colonia de *Rhodnius prolixus* en el Instituto Venezolano de Investigaciones Científicas. *Bol. Inform. Direc. Malariol. San. Amb.* **3**:132-137, 1963.

5. GUEDES, A. D. — Determinação do índice de infecção de Triatomíneos por *Schizotrypanum cruzi* pelo exame simples de fezes obtidas por expressão e por disecção do intestino posterior do inseto. *Rev. Brasil. Malariol. Doenças Trop.* **4**:433-436, 1952.

6. MAEKELT, G. A. & ALCAÑIZ, M. P. — Contribución para el estudio de la parasitemia del *Schizotrypanum cruzi* en el huesped mamífero. *Acta Cient. Venez.* **11**:137-142, 1960.

7. MAEKELT, G. A. — *El Diagnóstico Parasito-inmunológico de la Infección chagásica.* Caracas, Universidad Central de Venezuela, 1965, p. 32.

8. MÜHLPFORDT, H. — Influence of intestinal symbionts of *Rhodnius prolixus* upon *Trypanosoma cruzi*. *Trop. Dis. Bull.* **57**:798, 1960.

9. PHILLIPS, N. R. & BERTRAM, D. S. — Laboratory studies of *Trypanosoma cruzi* infections. In: *Rhodnius prolixus* — larvae and adults. In: *Triatoma infestans, T. protracta,* and *T. maculata* — adults. *J. Med. Ent.* **4**:168-174, 1967.

10. PIFANO, F. — Evaluación de los procedimientos de laboratorio empleados en el diagnóstico de la Enfermedad de Chagas. *Arch. Venez. Med. Trop. & Parasit. Méd.* **5**:171-187, 1965.

11. SCORZA, J. V. — Personal communication, 1969.

12. WOOD, S. F. — Environmental temperature as a factor in development of *Trypanosoma cruzi* in *Triatoma protracta*. *Exp. Parasit.* **3**:227-233, 1953.

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EVIDENCE OF PROTEIN-LOSING ENTEROPATHY IN STRONGYLOIDIASIS

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SUMMARY

The Authors present a case of Strongyloidiasis with intestinal loss of proteins and malabsorption. The patient's rapid recovery and the return of serum albumin to normal values, shortly after the treatment for parasitosis, led us to believe that the cause of the loss of proteins was due to infestation by *Strongyloides stercoralis*. During the 90 days treatment period, the excretion of ^{51}Cr tagged albumin, varied from 5.3 to 0.9% per day, while albuminemia went from 1.5 g% to 4.1 g%. The relative nutritional importance of this parasite in endemic areas was discussed.

INTRODUCTION

A massive infestation by *Strongyloides stercoralis* is sometimes accompanied by malabsorption or may lead to death [2, 3, 4, 7].

Hypoalbuminemia was also recorded in severe strongyloidiasis leading to suspect the possible existence of serum protein loss through the digestive tract invaded by the parasite [1, 13]. However, there is still no Laboratory evidence to prove this hypothesis.

This paper provides this evidence by reporting a case of severe strongyloidiasis with hypoproteinemia and loss of albumin through the digestive tract. The latter, quickly returned to normal after treating the parasitic disease with thiabendazole.

CASE REPORT

C.F.B., a 32 years old unmarried male, farm hand, from the State of Paraná was admitted to the Hospital das Clinicas on April 26, 1971. For sixteen months prior to admission, the patient had been having bouts of diarrhea, with four to five daily bowel movements. He noticed progressive weakness and a month later edema of the legs. The frequency of bowel movements varied up to ten times per day. He now noticed facial edema.

Past History — His family and past history were unremarkable.

Systems Review — Essentially negative.

Physical Examination — Poor general condition with pale mucous membranes, no cyanosis or jaundice. Edema of the face and legs (++). Weight 60 kg, height 1.72 m. Blood pressure 120/80 mm of Hg, pulse 100/minute, temperature 37°C. Thorax negative. Abdomen slightly protuberant with slight ascitis (+).

Laboratory Findings — The results of additional examinations before and after treatment are shown in Table I.

The variations in serum albumin during the period studied are found in Table II.

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TABLE I

Laboratory findings before and after treatment

	Before treatment	After treatment
R.B.C.	3.400.000 mm³	4.200.000 mm³
Hemoglobin	9.5 g/100 ml 59%	13.4 g/100 ml 84%
W.B.C.	8.000 mm³ Eos (10%)	8.600 mm³ Eos (3%)
Albumin	1.9 g/100 ml	4.1 g/100 ml
Globulins	2.3 g/100 ml	2.7 g/100 ml
Fat excretion (Kamer)	29.3 g/day	5.1 g/day
Stool examination for parasites (Baermann)	Numerous larvae of *S. stercoralis*	negative
Biopsy of jejunum	No specific jejunitis	normal
X Rays	Thickening and irregular pattern of the mucous membrane of the small bowel	normal
Albumin ^{51}Cr	5.3% of the dose per day	0.9% of the dose per day

TABLE II

Variations in serum protein during the 90 days period of treatment

	Albumin g/100 ml	Globulins g/100 ml	Total g/100 ml
Before treatment	1.5	2.3	3.8
15 days after	1.9	2.3	4.2
30 days after	1.8	2.2	4.0
50 days after	1.9	2.3	4.2
65 days after	2.6	2.4	5.0
95 days after	4.1	2.7	6.8

The fecal excretion of Albumin ^{51}Cr was estimated as plasma cleared through the intestinal wall (Fig. 1) the technique being already related [5, 8, 9, 10, 11, 12]. Normal values are always on the range of 1% of the administered dose per day.

DISCUSSION

The reasons we considered the parasite as the cause of serum protein loss from the digestive tract were: the massive infestation by strongyloides with hypoproteinemia, absen-

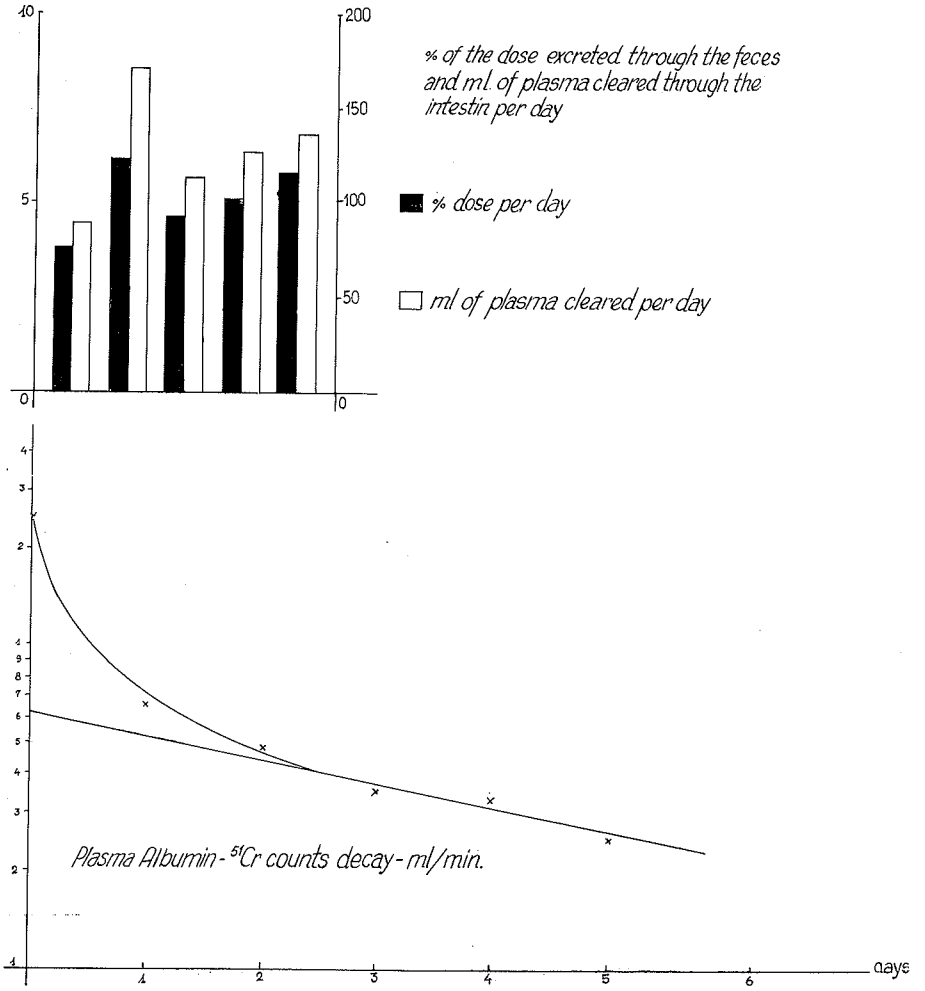

Fig. 1

ce of any other symptoms and pertinent data suggestive of other causes, and the finding of intestinal excretion of albumin reaching average daily values of 5.3% of Chromium 51 tagged albumin with return to normal within 90 days of the beginning of treatment of strongyloidiasis (Table II).

The prompt reversal of the serum protein values to normal (Table I) and the total normalization of the digestive excretion of albumin (^{51}Cr), emphasize the importance of this parasitic disease in the decreased availability of protein where the infestation is endemic [1, 13]. In endemic areas strongyloidiasis may be an additional factor preventing the utilization of proteins obtained in the diet. The protein diet in these areas is already poor.

Considering the severe loss of serum protein in this patient associated with malabsorption syndrome refered in the literature, it is easy to understand the nutritional importance of this parasitic disease in some regions.

Even though evidence is lacking, we believe that the loss of serum protein through the digestive tract, must be connected with characteristics of parasitic enteritis [6]. The X-rays and biopsy of the small intestine support this evidence (Fig. 2).

The role and the quantitative significance of enteric protein loss, and its implications

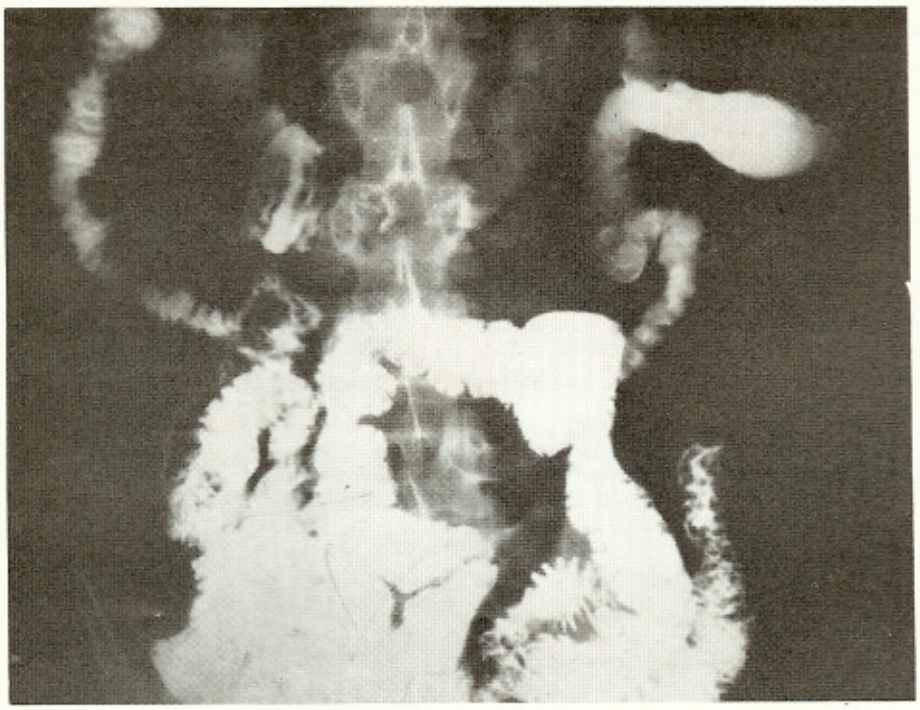

Fig. 2 — X Ray — Mucous coarsining and irregularity

with nutritional aspects specific in those regions where strongyloidiasis is common, deserve further study.

RESUMO

Perda intestinal protêica na estrongiloidíase

Os Autores apresentam um caso de estrongiloidíase com perda intestinal de proteínas e má absorção. A rápida recuperação do paciente e a elevação da albumina sérica a valores normais, em prazo curto após o tratamento da parasitose, levaram à convicção de que a perda de proteínas tivesse como causa a infestação por *Strongyloides stercoralis*. No prazo de 90 dias, desde o início do tratamento, a excreção de albumina marcada pelo Cromo 51 variou de 5,3% para 0,9% por dia da dose administrada, enquanto que a albuminemia passou de 1,5 g/% para 4,1 g/%. Os aspectos relativos à importância nutricional dessa parasitose nas áreas endêmicas foram comentados.

REFERENCES

1. AMIR-AHMADI, H.; BRAUM, P.; NEVA, F. A.; GOTTLIEB, L. S. & ZAMCHECK, N. — Strongyloidiasis at the Boston Hospital. *Amer. J. Dig. Dis.* **13**:959-973, 1968.

2. BRAS, G.; IRVINE, R. A.; BARTON, C. J.; BRAS, G. & RICHARDS, R. — Intestinal malabsorption in *Strongyloides stercoralis* infection. *Gut* **6**:574-576, 1965.

3. CRUZ, T. G.; REBOUÇAS, G. & ROCHA, H. — Fatal *Strongyloides* in patients receaving corticosteroids. *New Engl. J. Med.* **275**:1093-1096, 1966.

4. DE PAOLA, D.; DIAS, L. B. & DA SILVA, J. R. — Enterites due to *Strongyloides stercoralis*. A report of 5 fatal cases. *Amer. J. Dig. Dis.* **7**:1086-1098, 1962.

5. HEINRICH, H. C.; GABBE, E. E.; MEINEKE, G. & BECKER, M. — Metabolic behavior of the ^{51}Cr-labelling substance in the human body after intravenous injection. *Med. Welt.* **16**:835-839, 1965.

6. STEMMERMANN, G. N. — Strongyloidiasis in migrants. Pathological and clinical considerations. *Gastroenterology* **53**:59-70, 1967.

7. STEMMERMANN, G. N. & NAKASONE, N. — *Strongyloides stercoralis* infestation. Malabsorption defect with reaction to dithiazanine iodide. *JAMA* **174**:1250-1253, 1960.

8. TENGSTRÖM, B. — Critical evaluation of ^{51}Cr labelled serum albumin in the study of gastrointestinal protein loss. *Scand. J. Clin. Lab. Invest.* **17**:299-304, 1965.

9. TONGEREN, van J. H. M. & MAJOOR, C. L. H. — Demonstration of protein losing gastroenteropathy. The disappearance rate of ^{51}Cr from plasma and the binding of ^{51}Cr to different serum proteins. *Clin. Chim. Acta* **14**:31-41, 1965.

10. TONGEREN, van J .H. M. & MAJOOR, C. L. H. — Demonstration of protein losing gastroenteropathy. The quantitative estimation of gastrointestinal protein-loss, using ^{51}Cr labelled plasma proteins. *Clin. Chim. Acta* **14**:42-49, 1966.

11. WALDMANN, T. A. — Protein-losing enteropathy. *Gastroenterology* **50**:422-443, 1966.

12. WALKER-SMITH, J. A.; SKYRING, A. P. & MISTILIS, S. P. — Use of ^{51}Cr C13 in the diagnosis of protein-losing enteropathy. *Gut* **8**:166-168, 1967.

13. YOELI, M.; MOST, H.; BERMANN, H. & TESSE, B. — The problem of strongyloidiasis among the mentally retarded in institutions. *Trans. Roy. Soc. Trop. Med. Hyg.* **57**:336-345, 1963.

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