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RADIOAUTOGRAPHIC STUDY OF THE PROTEIN SYNTHESIS IN THE PURKINJE CELLS AT THE ACUTE PHASE OF THE EXPERIMENTAL TRYPANOSOMIASIS *CRUZI* IN RATS

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SUMMARY

The Author has studied the rate of protein synthesis in the Purkinje's cell of the cerebellum by the radioautographic technique at different times during the acute phase of the experimental infestation by the *T. cruzi* in rats. The leucine H^3 was used as a precursor of protein and the results of the grain counting in the radioautographies showed a sudden lowering in the rate of protein synthesis which was proportional to the intensity of the local parasitism and occurred in the absence of inflammatory infiltration and of direct parasitism of the nerve cell itself.

The Author suggests that this disturbance might be explained by the glial parasitism reflecting secondarily on the metabolism of the nerve cells assisted by this glia.

INTRODUCTION

The mechanism of *Trypanosoma cruzi*'s pathogenicity in Chagas' disease is still a subject in discussion. However one fundamental fact in the development of the lesions in this disease is the nerve cell destruction of the intra-mural plexuses of the hollow organs including the cardiac ganglia. This denervation was by the first time quantitatively demonstrated by KÖBERLE [11, 12, 13, 16] and confirmed by many Authors [1, 2, 3, 7], mainly in the chronic phase of Chagas' disease. Quantitative studies of nerve cells made in the acute phase of the experimental infection by the *T. cruzi* yielded to different results. There are many works stating the neuronal destruction in areas of the central and peripheral nervous system [4, 10, 22, 25] but there are some that deny this statement [6]. Thus there is not agreement as the time when the nerve cell destruction in Chagas' disease occurs. Opinions vary according to the ideas of each Author about the mechanism of the pathogenic action of the *T. cruzi*. To those believing in the deleterious action of products liberated by immature parasites by the time of the pseudocysts rupture in the tissues [14, 15] and to those who admit the role of the direct parasitism of the neurons as a factor of prime importance in its destruction [21], the denervation would occur in the early evolutive phase of the disease when the tissular parasitism is high. To those believing in a hypersensibility mechanism [20, 21], and in the noxious effect of the inflammatory reaction in the nerve cell lesion [23, 24] the neuronal destruction could happen throughout the course of the disease, even in the presence of a mild tissular parasitism.

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The denervation being the factor of prime importance in the pathogenesis of the late manifestations of Chagas' disease (cardiopathy and "megas") and considering that these are the most important clinical manifestation of the disease, it is unnecessary to stress the necessity of determining how and when the nerve cells lesion happens. The present work is an attempt to approach these difficult questions.

In the hope of finding an early functional disturbance in the neurons and correlate it with the evolutive phase of the disease, an estimation was made of the rate of protein synthesis in the Purkinje's cells of the cerebellum at different times in the acute phase of experimental Chagas' disease. This was done through the study of the incorporation, in the cytoplasm of these cells, of the DL Leucine 4,5 T (leucine H^3).

The protein synthesis in nerve cells is great and though its exact role in the cell function is not yet known, it is most probable that this intense synthesis must be related to the production of enzimatic proteins [9]. According to HYDÉN [9] the neuron under this aspect could be compared to a big secreting cell. So we thought that an assesment to the rate of protein synthesis might be a good indication of the functional state of the nerve cell.

The intensity and localization of the protein synthesis, approached by the radioautographic technique, at a cytological level in neurons and using leucine H^3 as a precursor have been studied in many works. According to LEBLOND & AMANO [18] the cell protein synthetized during the time elapsed between the injection of the tritiated aminoacid and the sacrifice of the animal incorporate this aminoacid and can be detected in tissue sections by the radioautographic technique.

DROZ & WARSHAWSKY [8] studying the use of tritiated leucine and its incorporation in tissue proteins showed, by chemical analysis of a pool of sections of different tissues, that 97% of the radioactivity was strongly bounded to the protein fraction. The same Authors demonstrated a little loss of radioactivity in the fixer and alcohols used in the tissues processing.

LEBLOND & AMANO [18] in the referred paper state that the protein synthesis in the Purkinje's cell of the cerebellum is continuous and that after a single injection of leucine H^3 the silver grains concentration in the radioautographies heightens progressively, atainning a maximum 4 hours after the injection.

By the above listed facts we think worse to consider that in the radioautographies studied the value obtained by counting the silver grains in the cytoplasm of the Purkinje cells give the rate of protein synthesis in the time elapsed between the administration of the aminoacid and the killing of the animal.

MATERIAL AND METHOD

Three experiences were made in which the number and age of the animals and the doses of the labelled aminoacid varied. These variations were due to problems of disponibility of animals and of radioactive material by the time the experiences were made.

In each of them, however, there is a control group of animals. In this way the experiences represent, when individually considered, the events related to the rate of protein synthesis occurring at different times of the acute phase of experimental infection by the *T. cruzi* in the nerve cells studied.

Experience no. 1 — Fifteen Wistar newborn rats received intraperitoneally the Y strain of the *T. cruzi*. On the 8th day of the infection when the parasitemia was high, four of the survivors animals received one dose of 20 mC of leucine H^3/g of body weight and were killed 90 minutes after by bleeding under slight ether anesthesia. The CNS was immediately removed and fixed in alcohol-formol-acetic-acid. Four healthy animals of the same age received the same treatment with the labelled aminoacid and were used as witnesses. The cerebellums were included in paraffin and sections of 5 μ were obtained. The sections were put in the slides in such a way that each slide contained the sections of all experimental and witnesses animals.

Experience no. 2 — Thirty Wistar rats with age between 18 and 21 days were injected intraperitoneally with the Y strain of the *T. cruzi*. On the 14th day of the infection 3 animals, selected by the high parasitemic level determined on the 8th day of the infection received intraperitoneally one dose of 5 μC/g of body weight of leucine H^3 and were killed 90 minutes after the injection. Three healthy animals of the same age received the same treatment with the aminoacid and were used as witnesses. The cerebellum of these animals were taken off immediately after death and treated as in experience no. 1.

Experience no. 3 — On the 19th day of infestation, 3 animals of the same group used in experience no. 2, selected by showing symptoms of central nervous system lesion (paralysis of the hind legs, ataxy and urinary incontinence) received the same treatment with leucine H^3 as those used in experience no. 2 and were killed at the same time interval after injection. Here too 3 healthy animals of the same age received the same treatment. The cerebellums were processed in the same way as previously described for experience no. 1.

In the three experiments the animals were kept at fast since the day before receiving the aminoacid.

The slides containing the sections of both infected and witnesses animals of each experience were submitted to the radioautographic technique according to KOPRIWA & LEBLOND [17].

The Ilford K5 liquid emulsion was used. After an exposure time varying according to the used dose of the aminoacid, the slides were processed and stained with hematoxilin-eosin. The silver grains were counted in the cytoplasm of the Purkinje's cells morphologically preserved in all the sections in each experience. The counting was made using a reticulum of known dimensions in the ocular lens of the microscope (Fig. 1). 120 areas of the same size in each section were counted. This represents a total area of 8.266, 2 μ^2 in each animal. The results were expressed in number of grains per area of cytoplasm.

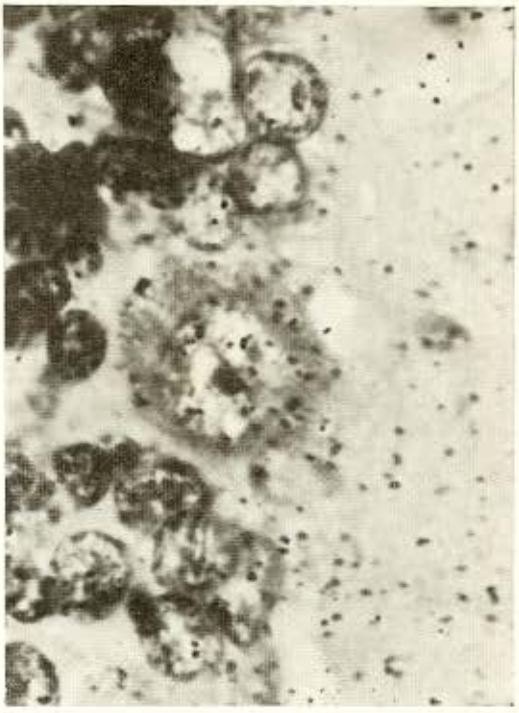

Fig. 1 — Radioautography of an animal sacrificed in the 14th day to show the disposition of the grains in the cytoplasm and nucleus. They were counted only in the cytoplasm with the aid of a reticulum.

In the infested animals the number of parasitary pseudocysts was estimated and the results expressed in plus (+) from one to five.

RESULTS

The tissular parasitism was slight in the animals killed on the 8th (+) and 14th (++) days of infestation. However, those killed on the 19th day showed a high concentration of parasitary pseudocysts (+++++). In these animals the parasites were frequently found in glial cells between the Purkinje's neurons (Fig. 2). Parasites were not found in the nerve cell itself and infiltration of inflammatory cells in this area of Purkinje's cells was not observed.

The results of the silver grains counting in the three experiences are summarized in the Graphs I to VI (Figs. 3, 4 and 5). These graphs show in abscissae the num-

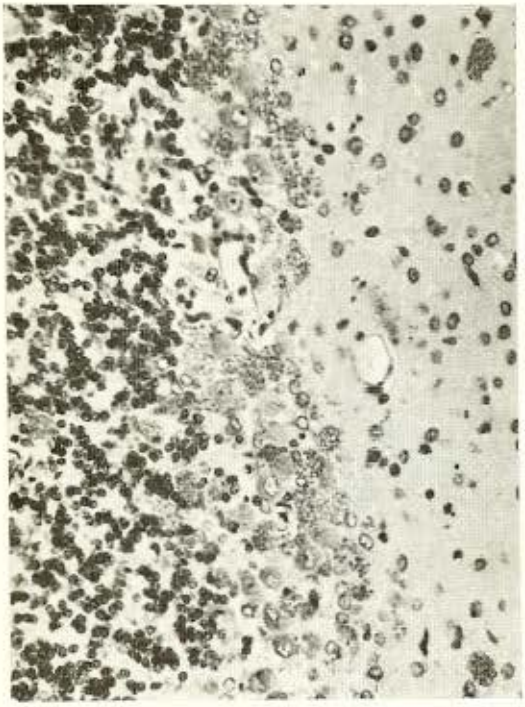

Fig. 2 — Section of the cerebellum of a rat killed in the 19th day. It can be seen the concentration of parasites in the glial cells around the Purkinje's cells and the absence of inflammatory infiltration.

ber of grains in each examined area and in ordinates the number of areas with the same number of grains. These results refer to groups of animals infected and witnesses in each experience.

DISCUSSION

The Graphs I to VI show that the frequency of areas containing the same number of silver grains is not significantly different in the nerve cells of infected and witnesses animals in experience number one (8th day). In experience number two (14th day) there is a clear tendency in the infected group to a lowering in the number of areas with more than eleven grains though the highest frequency of areas containing the same number of grains is the same in infected and control groups. In experience number three (19th day) it is manifest the greater frequency of areas containing a lower number of grains in infected animal with reference to those of the control group.

On the other side our results show a greater concentration of parasites in animals killed on the 19th day of the infection (exp. no. 3) than in those killed on the 8th and 14th (exp. no. 1 and 2 respectively). Thus there is an inverse relationship between the degree of tissular parasitism and the rate of incorporation of the leucine H^3 in the cytoplasm of the Purkinje's cells.

Another fact to be stressed is the non existence of lympho-plasmocitic infiltration near the studied cells. There are some papers published in which the Authors working with other organs — heart, intestines and peripheral nervous system — emphasize the importance of the inflammatory process in the mechanism of the nerve cell lesion, though they do not specify exactly how this happens [6, 23, 24]. In the cerebellum only sparse glial granulomas were found in perivascular situation as it is already described by others [5]. Thus the functional disturbance found in the neurons cannot be related to this "inflammatory mechanism".

To the parasitism of the nerve cell itself — claimed by some people as the fact responsible for the neuronal destruction in Chagas' disease — any role can be given in the nerve cell disfunction observed in this work, because this direct parasitism was not found even in animals with a high concentration of parasites in the cerebellum. With respect to the parasitism of the nerve cell we would like to emphasize the close anatomic relation of the nerve cells and the glia which envelop them almost completely, making it difficult to state, if you have thick sections, that the parasites are in the nerve cell and not in the glia.

The sudden appearence of the nerve cell disfunction during the acute phase of the experimental infection by the *T. cruzi*, showed in this paper by the lowering in the rate of protein synthesis in Purkinje's cells, proportional to the local tissular parasitism and without inflammatory infiltration and parasitism of the nerve cell itself suggests as an explanation to it: 1) a nerve cell lesion determined by some kind of substance liberated by the parasite but still

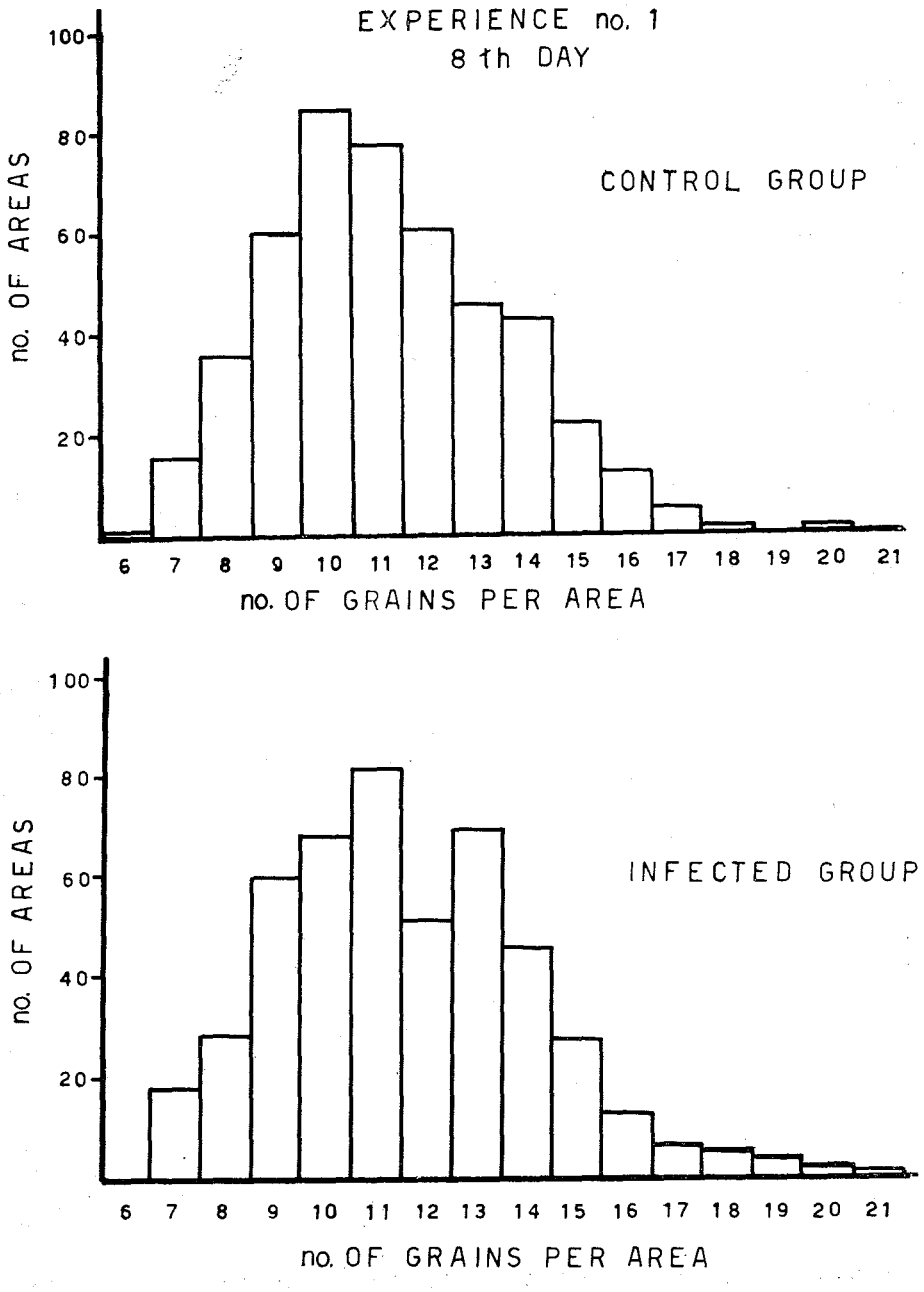

Fig. 3 — These graphs show that at the 8th day, when it was very low the local parasitism in the cerebellum, there is not a significant difference between control and infected groups of animals with reference to the rate of the labelled amino-acid incorporation.

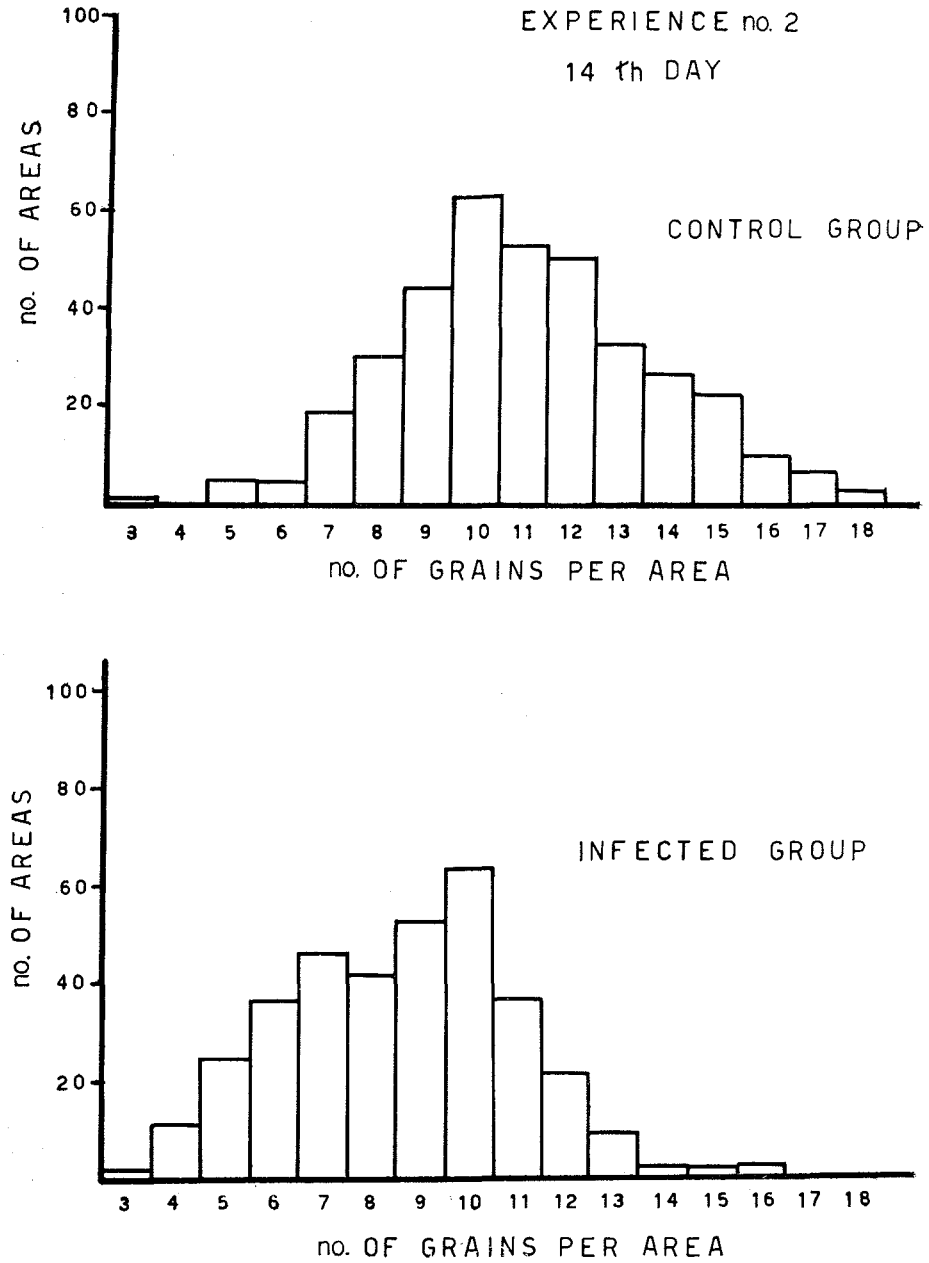

Fig. 4 — Here it can be seen that the highest frequency of areas contains the same number of grains in the control and infected groups but in the infected group there is a tendency for the disappearance of the areas with more than eleven grains. Here the parasitism in the cerebellum was still low.

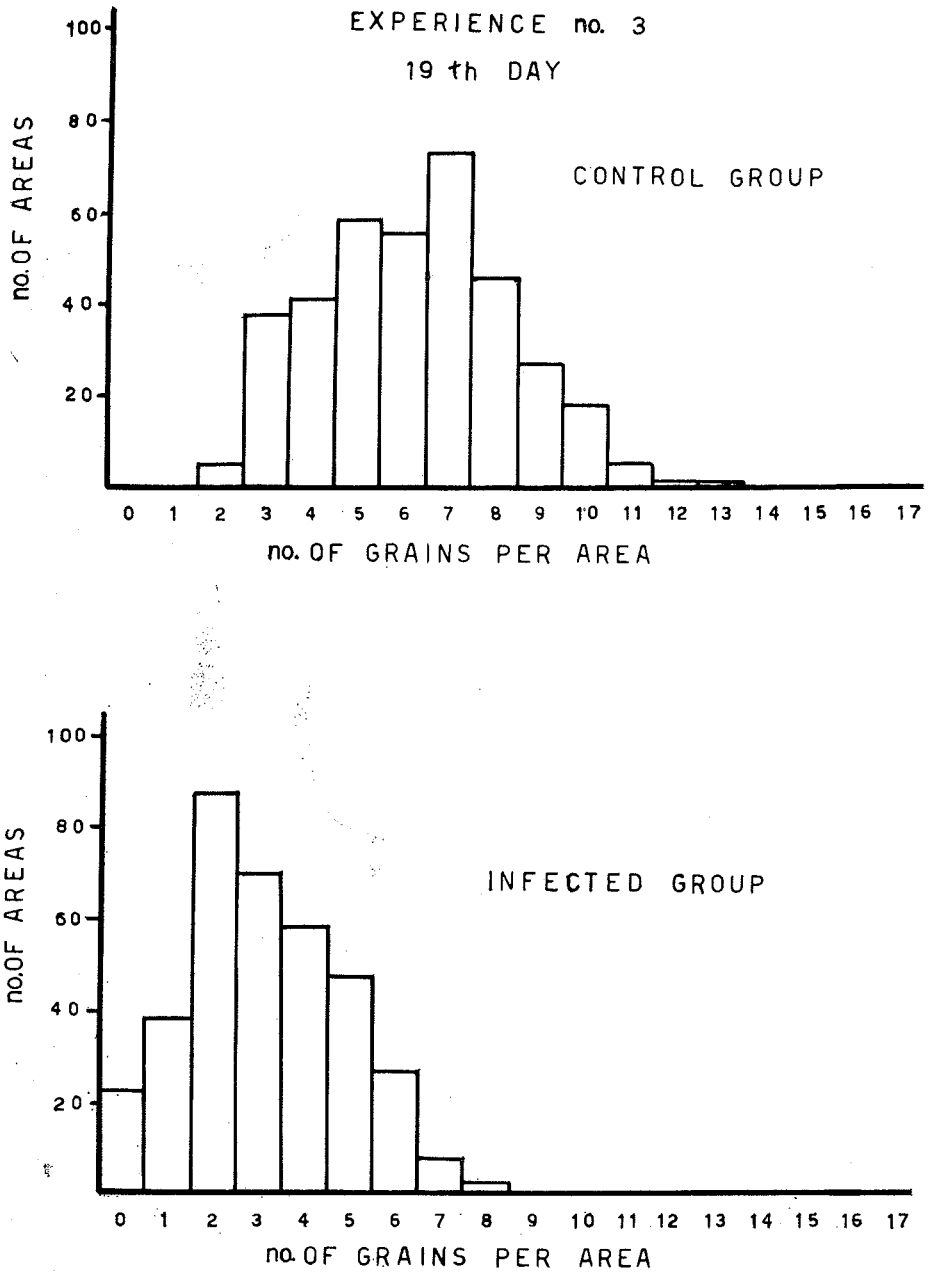

Fig. 5 — At the 19th when it was the highest the cerebellar parasitism. It is clear the great lowering in the aminoacid incorporation in the infected group with reference to the control one.

not demonstrated and 2) a lesion of the parasitized glial cell reflecting in the neuron assisted by this glia, considering that "the neuron and its glia represent a biochemical and functional unit" as it was well demonstrated by HYDÉN [9].

At last it can be stated that in the acute phase of the experimental infection by the *T. cruzi*, in the rat, there is a metabolic disfunction of the nerve cell represented in this work by a reduction in the rate of protein synthesis in the areas sampled, in the cytoplasm of Purkinje's cells of the cerebellum and this disfunction is concomitant with the raising of the local parasitism of glial cells and occurs in the absence of inflammatory infiltration and of direct parasitism of the nerve cell itself.

RESUMO

Estudo autorradiográfico da síntese de proteínas nas células de Purkinje do cerebelo na fase aguda da tripanosomíase cruzi experimental

O Autor estudou a taxa de síntese protéica nas células de Purkinje do cerebelo pela técnica radioautográfica em épocas evolutivas diferentes, na fase aguda da infestação experimental pelo *T. cruzi* em ratos. Foi utilizada a leucina H^3 como precursor de proteínas e os resultados obtidos evidenciaram uma nítida diminuição na taxa da síntese protéica no citoplasma daquelas células — que foi proporcional ao grau do parasitismo local do tecido e ocorreu na ausência de infiltrado inflamatório e de parasitismo — direto da célula nervosa.

O Autor sugere que êste distúrbio tenha ocorrido por parasitismo da glia, refletindo secundàriamente sôbre o metabolismo dos neurônios por ela assistidos.

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