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An Attempt to Stimulate and Depress the Functional Activity of the Inflammatory Cells from Lesions Experimentally Induced by *M. Leprae* and *M. Lepraemurium*

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The tissue macrophages of not previously sensitisized guinea pigs are able to lyse *M. leprae* and *M. lepraermurium* already phagocytised by them (Hadler, 1953a); after the lysis takes place they give origin to epithelioid cells. The rate of mycobacterial lysis performed by the tissue macrophages increases as a consequence of previous sensitization (Hadler, 1953b, 1955). The rat tissue macrophages, on the other hand, are unable to lyse the phagocytised mycobacteria either previously or after sensitization (Hadler, 1953a; Hadler and Ziti, 1955). They store the bacilli within the cytoplasm and transform themselves into lepra cells.

It was stated (Hadler, 1959) that the injection of electro-negative colloidal particles together with either *M. leprae* or *M. lepraemurium* stimulates some functional activities of the macrophages from rat leprosy lesions. There was, as a consequence of the additional effect of the colloidal particles, much evidence supporting the view that the mycobacteria could be readily lysed by the rat tissue macrophages, which further undergo transformation into epithelioid-like cells. Regarding the guinea pig and the rabbit lesions, as a result of the additional effect of the electro-negative colloidal particles, there is a slight decrease of the mycobacterial, rate of lysis, accomplished by the macrophages, and therefore a slower rate of evolution of the leprosy lesions takes place.

These findings are interpreted as a stimulant effect of the colloidal particles on the rat macrophage and an opposite effect on the guinea pig and rabbit macrophages. They suggest, otherwise the possibility that the functional activity of the inflammatory macrophage might be experimentally changed.

On the basis of these statements, an attempt was made to stimulate and depress the inflammatory cells experimentally produced by mycobacterial inoculation, with the aid of substances already proved to have successful action on the inflammatory reaction. Two kinds of substances have been used for this purpose: corticoid hormones and antihistaminic drugs.

Several steroid hormones from the adre cortex are able to influence the inflammatory reaction (Menkin, 1940, 1942, 1951, 1953; Selye, 1949a; Kass and Finland, 1953). Some of them, such as cortisone, exert an inhibitory effect (Hench and col., 1949; Thorn and col., 1950; Woods and Wood, 1950, 1952; Osgood and Favour, 1951; Michael and Whorthon, 1951; Spain and col., 1952; Taubenhaus, 1953), whereas another one, namely desoxycorticosterone, produces an opposite effect, increasing the

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inflammatory reaction (Selye, 1949; Taubenhaus and col., 1951; Rindal, 1953; Taubenhaus, 1953). Hormones from the former group were effective in decreasing the rate of evolution of lesions induced by the *M. lepraemurium* (Nagib and Robinson, 1956; Takayama, 1957; Buttle and col., 1958).

On the other hand, antihistaminic drugs, besides the inhibitory effect on the acute inflammatory reaction (Halpern, 1953), decrease the functional activity accomplished by the inflammatory cells (Jancso, 1947; Kátó, 1956; Kátó and Gözsy, 1956a; Gözsy and Kátó, 1956). As a consequence, they enhance the rate of evolution of experimental tuberculosis (Gözsy and Kátó, 1955) and inhibit the natural defence mechanism of guinea pigs against *M. lepraemurium* (Kátó, 1957).

TABLE I

Experimental animals and treatments. The animals were inoculated with *M. leprae* and *M. lepraemurium* at a same time, except those concerning with the groups 10, 11, 12 and 13, which have received only the latter mycobacterial species

			INOCULUM		TREATMENT OF THE ANIMALS AFTER INOCULATION				
Group	*Animal species*	*No. of animals*	*Dose (mg)*	*Route*	*Treatment previous to inoculation*	*Drug*	*Dose (mg) (*)*	*Route*	*No. of injections throughout the experiment*
1	rat	20	2.0	SC (**)	98°C–1h	DCA	1.0	site	at the 1st day
2	rat	20	2.0	SC (**)	98°C–1h	DCA	1.0	site	at the 1st, 12th and the 24th day
3	rat	20	2.0	SC (**)	98°C–1h	DCA	1.2	p	daily
4	rat	20	2.0	SC (**)	98°C–1h	–	–	–	– (control)
5	rat	20	2.0	SC (**)	98°C–1h	peanut	1 ml	site	at the 1st, 12th and the 24th day
6	rat	15	1.0	ic	4°C–6mon	DCA	1.0	site	at the 1st day
7	rat	15	1.0	ic	4°C–6mon	DCA	1.0	site	daily from the 7th until the 37th day
8	rat	15	1.0	ic	4°C–6mon	DCA	1.2	p	daily
9	rat	15	1.0	ic	4°C–6mon	–	–	–	– (control)
10	rat	30	5.0	p	–	DCA	2.0	SC	each two days
11	rat	30	5.0	p	–	CORT	2.0	SC	each two days
12	rat	30	5.0	p	–	–	–	–	– (control)
13	rat	30	5.0	p	–	DDS	40.0	oral	daily
14	rat	15	2.0	SC	98°C–1h	CORT	2.0	site	each two days
15	guinea pig	15	2.0	SC (**)	98°C–1h	DCA	1.0	site	at the 1st, 12th and 24th day
16	guinea pig	15	2.0	SC (**)	98°C–1h	CORT	1.0	site	at the 1st day
17	guinea pig	15	2.0	SC (**)	98°C–1h	CORT	1.0	site	at the 1st, 12th and the 24th day
18	guinea pig	15	2.0	SC (**)	98°C–1h	–	–	–	– (control)
19	rat	10	2.0	SC	98°C–1h	–	–	–	– (control)
20	rat	10	2.0	SC	98°C–1h	Cl+Pr	2.0	p	daily
21	guinea pig	15	2.0	SC	98°C–1h	Cl+Pr	2.0	p	daily
22	guinea pig	15	2.0	SC	98°C–1h	Cl	2.0	p	daily
23	guinea pig	15	2.0	SC	98°C–1h	Pr	2.0	p	daily
24	guinea pig	15	2.0	SC	98°C–1h	–	–	–	– (control)

(**) The inoculation was carried on 'granuloma pouch' performed by the SELYE (1953) technique.

(*) Dose for each 100g of body weight.
SC: subcutaneous.
ic: intracutaneous.
p: peritoneal.
site: injection performed on the site of inoculation.

DCA: desoxycorticosterone acetate (peanut oil solution).
CORT: cortisone (water solution).
Cl: chlorpromazine: 10–(–dimethylaminopropyl) 2–chlorophenotiazine.
Pr: prometazine: dimethylamino–2–propil–N–thiodiphenylamine hydrochloride.
DDS: 4, 4′ –diaminediphenysulphone.
peanut: peanut oil.

MATERIAL AND METHODS

Adult guinea pigs and rats of both sexes, weighing respectively 300–350g and 130–200g, were inoculated with *M. leprae* and *M. lepraemurium* suspensions, and prepared by the usual techniques, and rendered free of tissue particles with the aid of the Hanks (1951) technique slightly modified. The route of inoculation, the dose of inoculum and the treatment performed on the bacilli previously to inoculation, are given in Table 1. The dose of the inoculum was determined from the weight of already dried bacilli, derived from a suspension sample. Either killed (98 °C for 1 h and 4 °C for 6 months) or living bacilli were used. The animals injected subcutaneously and intracutaneously have received *M. leprae* at one side and *M. lepraemurium* at the other side.

After inoculation the animals were treated as shown in Table 1. Some of them have received corticosteroid hormones, whereas others have been injected with an antihistaminic drug (prometazine) alone or mixed with a hypometabolic drug (chlorpromazine).

Every two days one or two animals of each group were killed by ether inhalation and the site of inoculation was excised for histological examination. From the animals peritoneally inoculated pieces of liver, spleen, lymph nodes and lungs were excised for histological studies. The histological study was carried out on material fixed in Bouin's fluid, embedded in paraffin and stained by HE., Masson's trichromic, azur II – eosin and the Faraco (1938) modification of Ziehl-Neelsen technique.

From the group 10, 11, 12 and 13, 20 animals were kept until natural death, to provide data for the study of the effect of treatment on the animal survival.

RESULTS

The results were based on histological comparison of the inflammatory cells or the bacillary amount, as between treated and control animals. The control animal lesions will not be described, since they were carefully studied elsewhere (Hadler, 1953; Hadler and Ziti, 1955).

DCA effect – the DCA injection performed into the lesion increases the acute inflammatory reaction induced by *M. leprae* and *M. lepraemurium* either on guinea pigs or on rats. The lesions become larger and always display a central abscess (Figs. 1 and 2), surrounded by the inflammatory tissue, where further a greater connective fibrocytic reaction takes place. The development and the rate of evolution of the lesions are shorter in treated rather than in control animals.

Regarding the guinea pig lesions they remain tuberculoid in type, where the macrophages are able to lyse the phagocytised mycobacteria and to develop into epithelioid cells.

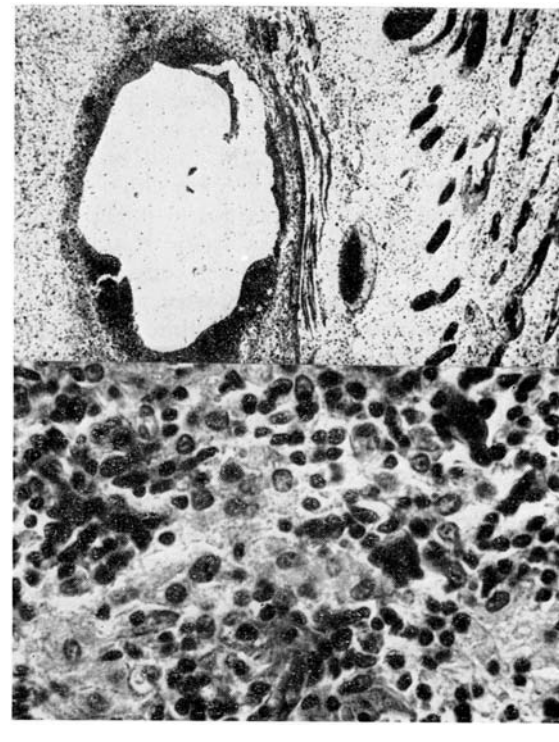


FIG 1 Rat subcutaneously inoculated with *M. lepraemurium* and topically treated with desoxycorticosterone acetate; HE, 50X. Central abcess surrounded by the inflammatory tissue; two days after mycobacterial inoculation.

FIG 2 Rat subcutaneously inoculated with *M. leprae* and topically treated with desoxycorticosterone acetate, Masson's trichromic, 500X. Macrophages showing signs of damage to cytoplasm and nuclei; 10 days after inoculation.

The lesions of treated rats, on the other hand, display some histological changes compared to the control animals. Although most of the lesions are lepromatous in histology, where the macrophages are unable to lyse the phagocytised bacilli and therefore they transform themselves into lepra cells, there are some areas inside the lesions where the structure becomes altered. In these areas the macrophages (Fig. 2) display evidence of cell damage (striking cytoplasmic vacuolization; nuclear pyknosis), and contain only a few bacilli within the cytoplasm. The phagocytised bacilli show striking morphological alterations; they lose their alcohol-acid resistance and become progressively less numerous. Simultaneously, the macrophages transform themselves into epithelioid-like cells, without bacilli within the cytoplasm, suggesting that they are able to lyse the phagocytised bacilli.

The described histological changes only occur in well limited areas of the lesions near the place where DCA was injected and could be better seen when DCA was injected many times on the site of inoculation. The peritoneal injection of DCA was ineffective so far as the histological changes of cutaneous lesions are concerned.

As the DCA treatment was started 7 days after the bacillary inoculation, at a time when the control rat lesions already displayed some lepra cells, an acute inflammatory reaction took place into the lesions. At the same time some lepra cells and macrophages showed signs of cytoplasmic and nuclear damage. Four days later some epithelioid-cells without bacilli inside appeared in the lesion.

The injection of peanut oil alone, does not produce any change concerning the histological structure either of rat or of guinea pig lesions.

Table 2 shows the results concerning the survival of rats inoculated with living *M. lepraemurium* and treated with DCA (group 10 in Table 1). The comparison between the mean of survival of these animals and that concerning the untreated control group, carried on through the analysis of variance, shows no significant differences. Histologically there are no changes in the lesions of treated animals in comparison to the control ones; both show a large amount of active lesions, very rich in bacilli, six months after inoculation.

Cortisone effect – The injection of cortisone either together with the bacilli suspension or into the lesion throughout its development, inhibits the acute inflammatory reaction induced by *M. leprae* and *M. lepraemurium* and decreases the rate of the evolution of the lesion. Such cortisone effect is better seen in the guinea pig lesions.

The lesions of cortisone treated rats are smaller than those of untreated controls and formed by lepra cells, containing a large amount of bacilli within the cytoplasm.

The guinea pig lesions, however, besides longer development and evolution, show some histological changes as an effect of cortisone treatment. The mycobacteria are soon phagocytized by blood leucocytes and tissue macrophages suggesting an increase in the phagocytic activity of these cells. The phagocytized bacilli remain stored within the macrophage cytoplasm for a much longer time than they do in the homo-

TABLE 2

Mean of survival of rats inoculated with *M. lepraemurium*. Effect of cortisone, desoxycorticosterone (DCA) and DDS (4, 4′–diaminodiphenylsulfone) treatment. The DDS treated group provides data concerning the effect of an active drug on the animal survival

	Treatments			*Control*
	Cortisone (*)	DCA (*)	DDS (**)	
Number of animals	20	20	20	20
Mean of survival (days)	228.3±3.5	229.5±4.0	383.0±9.2	237.4±3.9

(*) Treatment started at the first day after inoculation.

(**) Treatment started at the 7th day after inoculation.

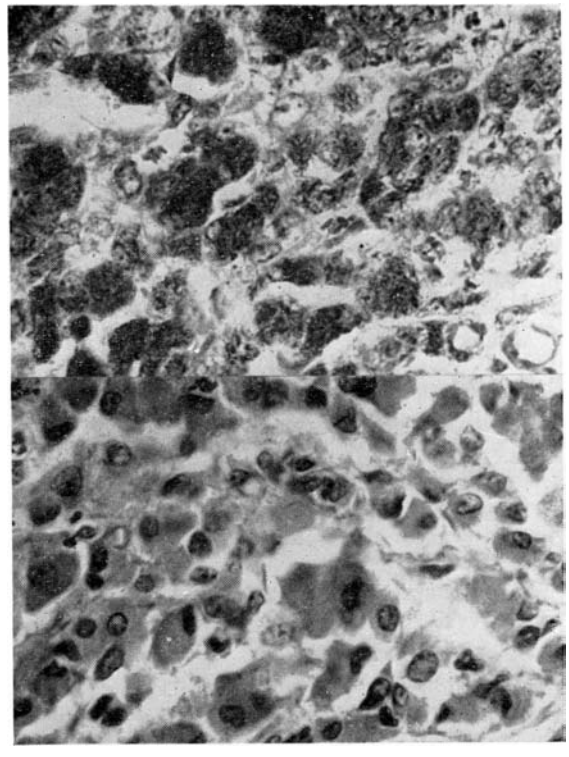
logous cells of control lesions. On the other hand, there is no macrophage damage in the greater part of treated lesions, in spite of the large amount of bacilli within the cytoplasm. Further, two different modes of behaviour of the containing bacillary macrophage could be seen: (a) the bacilli remain stored within the cytoplasm of the macrophage and the cell becomes morphologically similar to the lepra cell, containing a large amount of bacilli and arranged as in leprosy histology; (b) the bacilli are lysed by the macrophage that further undergoes transformation into the epithelioid cell, without bacilli within its cytoplasm. The former macrophage behaviour is more frequently found near the central abcess, at a place where cortisone was injected; the latter were seen in lesion areas far from the abscess, suggesting some correlation between concentration and cortisone effect.

In the lesion areas where the bacillary lysis seems to occur there is cytological evidence of macrophage damage and the mycobacteria show deep morphological alterations.

The cortisone treatment performed subcutaneously on rats peritoneally inoculated with living *M. lepraemurium* does not show any effect on animal survival (Table 2). Furthermore histologically the leprosy lesions of treated animals do not differ from those of control ones.

Chlorpromazine and prometazine effect – This effect is similar to that of cortisone. It appears as an inhibition of the acute inflammatory reaction mainly found in guinea pig lesions. Moreover, the rat treated lesions are also smaller than the control. They are formed by lepra cells, arranged as a lepromatous structure, like the control lesions.

The rate of evolution of treated guinea pig lesions is slower than those of the controls. In the former lesions, the inoculated mycobacteria are sooner phagocytised by macrophages, but remain stored within the cytoplasm, without any evidence of bacillary lysis and of cell damage, in the greater part of the lesion. The bacilli-laden macrophages transform themselves into lepra-like cells (Figs. 3 and 4), containing a large amount of well preserved bacilli. These cells are arranged as a lepromatous-like structure. Only in very limited lesion areas are there morphologi-



FIGS. 3 and 4 Guinea pig subcutaneously inoculated with *M. lepraemurium* treated by chlorpromazine-prometazine; 560X. Lepra-like cells stained by HE and by Ziehl-Neelsen, showing a great amount of bacilli within its cytoplasm; 20 days after inoculation.

cal evidences of mycobacterial lyses and of macrophage damage, until the 30th day of the lesion evolution, in contrast with what happens in the lesions of control animals, where evidences of bacillary lysis can be seen sooner.

The findings concerning the effect of either chlorpromazine or prometazine injected alone support the view that both are active. Nevertheless, it appears that these two substances used together seem to be more effective at an equal dose (interaction).

DISCUSSION

Two distinct and opposite effects could be established with the substances used by us: (1) an increase of the acute inflammatory reaction followed by a functional stimulation of the tissue

macrophages, elicited by desoxycorticosterone acetate; (2) a decrease of the acute inflammatory reaction followed by a partial inhibition of the activity of the tissue macrophages, as a consequence of cortisone and chlorpromazine plus prometazine treatment.

The first effect could be well observed in rat lesions, where some macrophages seems to acquire, as a consequence of the treatment, the ability to lyse the phagocytised mycobacteria. As a result, the histological structure of the lesions is affected and some tuberculoid-like areas containing epithelioid-like cells do occur. The lysis of bacilli seems to act in parallel with the macrophage damage, supporting the view that DCA treatment enhances the bacilli-macrophage interaction.

On the other hand, the second effect was better seen in the guinea pig lesions, where a partial inhibition of the macrophage activity is responsible for the decrease of the mycobacterial rate of lysis. As a consequence the macrophages store a great amount of bacilli and become morphologically similar to the lepra cells.

Both effects show that the two main structural types of leprosy lesions might be affected by drugs.

Several results concerned with the effect of corticosteroid hormones on inflammatory lesions support our interpretation. Cortisone appears to accomplish their effect on the inflammatory cells by inhibiting its physiological activity (Menkin, 1953a; 1953b). As a consequence, although the actual rate at which the *M. tuberculosis* is phagocytosed by the macrophages remains normal after treatment, the number of mycobacteria within each cell is greater than normal, since the digestive capacity of the macrophages decreases (Kass and col. 1953; Lurie, 1955; Lurie and Zapparodi, 1955). This effect is responsible for the latter appearance of the epitheliod cell, as we have observed in the lesions of guinea pigs inoculated with *M. leprae* and *M. lepraemurium* and treated by cortisone. This effect seems to depend upon the action of cortisone that decreases the cellular metabolism and further the cellular activities (Kass and Finland, 1953).

Nevertheless, cortisone acts by increasing the rate of evolution of the rat tuberculosis, the disease becoming therefore able to kill the animals so treated (Michael and col., 1950). Concerning the murine leprosy, our findings show that cortisone does not modify the rate of evolution of the disease. This result suggests that the general defence mechanism against *M. lepraemurium* is not effected by cortisone treatment, in contrast to what happens with *M. tuberculosis*.

It was pointed out that the phagocytoses of *M. lepraemurium* by tissue macrophages either of rats or of guinea pigs is not influenced by antihistaminic drugs (Kátó, 1956). This statement is in agreement with our results concerning chlorpromazine-prometazine treatment. Antihistaminic treated guinea pigs display lesions whose histological structure is similar to those of rats (Kátó, 1957); that is also confirmed by our findings. Nevertheless, either the antihistaminic drug prometazine or the chlorpromazine, which is admitted to be effective as if it decreases the cellular metabolism (Laborit and Huquenard, 1952; Decourt, 1955), produce a similar effect. Both seem to be able to inhibit the mycobacterial lysis accomplished by the macrophages, but they would act by different mechanisms.

The cortisone and antihistaminic effects may be correlated, since some adrenal corticoids inhibited the histaminic liberation by the tissues (Ungar, 1944; Halpern, 1953; Ashi, Funaki and Ono, 1955), increasing the amount of antifibrinolysine (Ungar, Damgaard and Hummel, 1951). Such effect would depend upon the decreasing of mycobacteria-host cell interaction which could be considered as very important concerning the enzyme biosynthesis. Some of these enzymes would be responsible for the mycobacterial lyses. We admit that cortisone and chlorpromazine-prometazine treatment inhibits the synthesis of lytic enzymes by the macrophages. Desoxycorticosterone acetate would show an opposite effect, since it seems to increase the mycobacteria-host cell interaction which stimulates the synthesis of lytic enzymes by the macrophages.

Our findings show, otherwise, that the functional activity of some leprosy lesion cells could be experimentally altered, which produces a striking change in the histological structure of the lesions.

SUMMARY

The attempt to produce an experimental change in the functional activity of the macrophages from guinea pig and rat lesions induced by

M. leprae and *M. lepraemurium* was made with the aid of adrenal corticoid hormones, an antihistaminic drug and a substance that is admitted to depress the cell metabolism.

The findings showed that two opposite effects could be traced, such as:

(1) Cortisone and chlorpromazine-prometazine treatment besides a decrease of the acute inflammatory reaction, exert an inhibitory effect upon the activity of the tissue macrophages. As a consequence there is a decrease of mycobacterial rate of lysis by the macrophages which readily become able to store a great amount of bacilli inside their cytoplasm. This effect could be well seen in guinea pig lesions, where cells similar to the lepra cells do appear after treatment, allowing a marked alteration of the histological structure of the lesion. A lepromatous-like structure emerges in guinea pig treated lesions, whereas a tuberculoid structure arises in untreated ones.

(2) Desoxycorticosterone treatment in contrast increases the acute inflammatory reaction and stimulates the tissue macrophages, as could be established in the rat lesions. As a consequence, some rat macrophages become able to lyse the phagocytized mycobacteria, which allows of the development of some areas containing epithelioid-like cells, without bacilli within the cytoplasm.

Both effects show that the two structural kinds of lesions induced by *M. leprae* and *M. lepraemurium* might be affected by treatment that influences the mycobacteria-host cell interaction. This interaction would be related to the biosynthesis of enzymes, some of which have lytic properties concerning the phagocytised mycobacteria.

RESUMO

Tentou-se modificar experimentalmente a atividade funcional dos macrófagos de lesões produzidas em cobaios e ratos, pelo *M. leprae* e pelo *M. lepraemurium*, mediante o emprêgo de corticóides da adrenal, de um antihistamínico e de uma substância que se admite deprimir o metabolismo celular. Os resultados mostraram que dois efeitos opostos foram obtidos:

(1) O tratamento efetuado com cortisona ou com a mistura clorpromazina-prometazina, além de diminuir a reação inflamatória aguda apresenta ação inibidora sôbre a atividade dos macrófagos dos tecidos lesados. Consequentemente, diminui a velocidade de lise das micobactérias pelos macrófagos, os quais tornam-se capazes de armazenar grande número de bacilos no citoplasma. Ésse efeito é melhor observado nas lesões de cobaios, nas quais surgem células semelhantes à célula leprosa, responsáveis por intensas alterações estruturais das lesões. Ocorre estrutura de tipo lepromatoso nas lesões submetidas a tratamento, enquanto que nos animais não tratados as lesões são de tipo tuberculóide.

(2) O tratamento pela desoxicorticosterona, ao contrário, intensifica a reação inflamatória aguda e estimula o macrófago tissural – conforme foi verificado em lesões do rato. Como decorrência, alguns macrófagos do rato tornam-se aptos em lisar as micobactérias fagocitadas, provocando o aprecimento de algumas áreas contendo células semelhantes às epitelióides, isentas de bacilos.

Êsses efeitos mostram que os dois tipos estruturais encontrados nas lesões provocadas pelo *M. leprae* e pelo *M. lepraemurium* podem ser alterados por substâncias que atuam na interação entre as micobactérias e as células do hospedeiro. A referida interação parece responsável pela biosíntese de enzimas, algumas das quais seriam líticas para as micobactérias fagocitadas.

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