Oxidation of 3,4-dihydroxyphenylalanine (DOPA) by *Mycobacterium leprae*

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In spite of repeated attempts for nearly a century, no generally accepted method has been described for cultivating the presumed etiologic agent of leprosy, *Mycobacterium leprae*, in vitro or transmitting it to laboratory animals, except that of Shepard (29); who showed limited multiplication of the bacilli in the foot pads of CFW mice, leading to granulomatous lesions. Because of lack of adequate material, no extended biochemical investigations have been conducted on a proven mycobacterium of human leprosy, and its metabolism has therefore remained obscure. Any study of the metabolic activities of unquestioned *M. leprae* is entirely dependent on the use of lepromatous nodules obtained from infected persons.

The present paper describes dopa oxidase activity detected in *M. leprae* (separated from human leprosy nodules), and the significance of this finding is discussed in relation to the metabolism of the organisms and to the hypopigmented skin lesions characteristic of leprosy.

It has been established that the melanin pigment of the skin is formed through the enzymatic activity of melanocytes present in the basal layer of the epidermis (17). Embryologically, the melanocytes are derived from the neural crest; they may be considered as specialized nerve cells. The neural crest also gives rise to dorsal root ganglia of spinal nerves, the adrenal medulla, and Schwann cells of peripheral nerves (20). In mammals melanocytes are present in skin, eyes and leptomeninges; they occur also in certain surface membranes, such as the oral mucosa. The bulk of the melanin pigment is contained in the dendritic melanocytes located at the dermal-epidermal junction, and in the hair bulbs (16).

The enzyme complex that catalyzes the reactions leading to formation of melanin is variously called phenolase, polyphenol oxidase, phenolase complex and dopa oxidase or tyrosinase. It occurs in a variety of organisms, including vertebrates, invertebrates, higher plants, and fungi. The only report on the possible occurrence of the enzyme in bacteria relates to the oxidation of tyrosine to pigmented products by a small vibrio *Microspira tyrosinatica* (15).

In melanogenesis, tyrosine is first converted into 3,4-dihydroxyphenylalanine (dopa) which, through a series of reactions gives rise to melanin. The chemical steps involved in the reaction were studied by Kalper in the insect *Tenebrio molitor* (24), and extended by Mason (19), using mushroom phenolase. The enzyme is assayed manometrically by measuring oxygen-uptake, or spectrophotometrically by measuring formation of dopachrome (absorption maximum 475 mp) or indole-5,6-quinone with its absorption maximum at 540 mp.

The presence of phenolase in mammalian melanocytes of skin as well as in melanomas is well established. In 1927, Bloch (16) showed that dopa, but not tyrosine, could be oxidized to melanin by frozen sections of normal human skin. Fitzpatrick et al. (14) reported the oxidation of tyrosine to melanin by sections of human skin from a person previously subjected to ultraviolet irradiation. Inhibition of tyrosine oxidation...
by extracts of human epidermis has been shown to be due to binding of Cu\textsuperscript{3+} by sulphydryl groups (4). Harper \textsuperscript{(2)} and Lerner et al. \textsuperscript{(4)} showed that dopa is the first product formed in the oxidation of tyrosine, and that both dopa and tyrosine are oxidized by the same enzyme preparations. Kelso and Mann \textsuperscript{(12)} found that crude mushroom extracts oxidized both diphenols and monophenols; on purification, the enzyme became more and more specific for diphenols. It has also been observed that monophenolase activity is less stable than that of diphenolase 2 (3).

**MATERIALS AND METHODS**

Oxidation of dopa was studied by the Warburg manometric method by measuring \textit{O}_2-uptake. The organisms were ground with sand in a chilled agate mortar, and the sand was removed by slow-speed centrifugation. The assay system consisted of 0.2 ml of 0.5 M Na\textsubscript{2}HPO\textsubscript{4}-KH\textsubscript{2}PO\textsubscript{4} buffer with a pH of 6.8, 0.5 ml of a DL-dopa 10 mg/ml suspension, and 1 ml of a bacterial suspension (protein concentration 40 mg/ml). The final volume was made up with distilled water to 3.2 ml. The temperature was 37°C and the time 120 minutes.

After incubation, the reaction mixture was centrifuged, and the spectrum of the supernatant was taken in a Beckman spectrophotometer, model DU for detection of indole-5,6-quinone, having an absorption maximum at 540 nm. Nitrogen estimation of the bacilli was made by the method of Johnson \textsuperscript{(12)}, and of the tissue extracts by the method of Folin and Wu, as described by Gradwohl \textsuperscript{(1)}.

Samples of normal human skin were obtained from amputated limbs of accident cases, and from cancer patients.

Kedrowsky's bacillus and \textit{M. phlei} were grown in glycerol broth for 21 days and \textit{M. smegmatis} in the same medium for 48 hours. \textit{M. tuberculosis} H37Ra and BCG were cultured in Dubos medium for 21 days, and \textit{M. leprae} 607 was grown in the same medium for 7 days. The organisms were separated from the medium by centrifugation at 6000 x g for 10 minutes and were washed twice with 0.85 per cent NaCl and once with distilled water.

**RESULTS**

Concentrates of mycobacteria were prepared from human leproma nodules as described previously \textsuperscript{(18)}. The details of the procedure are given in Figure 1. It is to be noted that the method entailed some loss of material. However, the final fraction \textit{J} was concentrated with respect to organisms and contained very little tissue debris, as far as could be detected by Ziehl-Neelsen staining technique (Figure 2a & b).

The preparations were found to be metabolically active, for the organisms showed consistent oxygen-uptake. Normal human skin extract prepared under identical conditions was practically inert with respect to respiration.

Figure 3 shows oxygen-uptake of \textit{M. leprae} in the presence of added dopa. It may be seen that there is considerable increase in \textit{O}_2-uptake over the endogenous uptake, when the organisms are incubated with dopa.

Dopa is known to undergo auto-oxidation to melanin under certain conditions \textsuperscript{(3)}. It was therefore essential to determine if dopa is auto-oxidized in 2 hours at the pH of 6.8 employed in these studies. For this purpose, \textit{O}_2-uptake of dopa due to auto-oxidation was measured at two different pH values. It may be seen in Figure 4 that at pH 8.5 there was considerable increase in \textit{O}_2-uptake, whereas at pH 6.8 there was no significant increase.

Indole-5,6-quinone is one of the intermediates in the enzymatic conversion of dopa to melanin. Its presence is detected by its characteristic absorption maximum at 540 nm. Curve 2 in Figure 5 shows the formation of indole-5,6-quinone in the oxidation of dopa by \textit{M. leprae}. When the reaction mixture is kept for 18 hours, the absorption maximum due to indole-5,6-quinone disappears as a result of its conversion to melanin (curve 3). Dialysis of the bacterial preparation for 4 hours against distilled water at 4°C was found to have no effect on its dopa oxidase activity. Heating at 100°C for 15 minutes completely inactivated the enzyme.

The results of comparative studies with eight other mycobacteria on the oxidation...
The fractionation scheme for preparing *M. leprae* from human leprosy nodules is shown in the figure.

From Table 1, it can be seen that none of these mycobacteria, including *M. lepraemurium* and *M. tuberculosis H37Rv*, could metabolize dopa. Thus, among nine organisms tested, only *M. leprae* was able to convert dopa to melasmin with formation of indole-5,6-quinone.

Curve 2 of Figure 6 shows the formation of indole-5,6-quinone on incubation of human skin extract (fraction D, Fig. 1) with dopa. In view of the metabolism of dopa observed with the skin extract, and since *M. leprae* is separated from skin nodules, it was necessary to examine critically to determine if apparent oxidation of dopa by the bacilli was actually due to skin particles present in *M. leprae* preparations. For this purpose, the following experiments were carried out:

1. An attempt was made to prepare a particular fraction from human skin corresponding to the *M. leprae* fraction from leprosy nodules, and test its dopa oxidase activity. It was found that no packed sediment could be obtained from the skin extract when it was centrifuged at 6,000 x g, the force used for sedimenting *M. leprae*...
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from nodule extract (Fig. 1). The skin preparation was therefore centrifuged at 18,000 x g and the various fractions were collected. Dopa oxidase activity of these fractions was compared to that of corresponding fractions from leprosy nodules. It may be noted from the results illustrated in Table 2 that the final residue from the skin (fraction J, comparable to M. leprae fraction from nodules) had no appreciable dopa oxidase activity.

2. Since the experiment described above indicated that dopa oxidase may be a characteristic property of M. leprae, the experiment was repeated in terms of specific activity of the various fractions. The results (Table 3) show that in the skin the activity falls off from 1.76 in the crude extract to 0.54 in the final residue, whereas, in the nodule, the activity increases from 3.55 in the crude extract to 7.73 in the final fraction (J) consisting of bacilli. On comparison of fraction J from the control (skin) with that from the nodules, it is clear that the bacillus-containing fraction is 14 times as active as the skin preparation.

M.607, which did not oxidize dopa, was mixed with minced human skin, kept

FIG. 3. Oxidation of dopa by M. leprae. For experimental conditions: see METHODS AND MATERIALS (1) Dopa. (2) Endogenous. (3) M. leprae + Dopa.
The incubation system consisted of: 0.2 M NaH₂PO₄·KH₂PO₄ buffer, pH 6.8 or 8.3, 0.2 ml; DL-dopa, 0.005 M; volume, 3 ml; temperature, 37°C; time, 120 minutes. (1) pH 6.8; (2) pH 8.3.

Frozen overnight, and separated from the tissue by the method used for M. lepra. The dopa oxidase of these organisms was tested to see if skin tissue enzymes were carried over in the final fraction of bacilli. The results were negative.

4. Attempts were made to determine if the dopa oxidase activity of M. lepra concentrates was due to enzymes firmly bound to the bacilli. The M. lepra fraction, as well as skin fraction F, was washed twice with distilled water and tested for dopa oxidase activity before and after washing. (Fraction F was chosen for comparison, since fraction J from skin had little activity.) Repeated washing of the M. lepra preparation produced no significant effect on its dopa oxidase activity, while the skin fraction lost over 50 per cent of its activity.

![Graph](image)

**Graph 4.** Auto-oxidation of dopa at pH 6.8 and 8.3. The incubation system consisted of: 0.2 M NaH₂PO₄·KH₂PO₄ buffer, pH 6.8 or 8.3, 0.2 ml; DL-dopa, 0.005 M; volume, 3 ml; temperature, 37°C; time, 120 minutes. (1) pH 6.8; (2) pH 8.3.

**Graph 5.** Formation of indole-5,6-quinone in the oxidation of dopa by M. lepra. For experimental conditions: see Table 2. (1) Dopa. (2) M. lepra + dopa, 2 hrs. (3) M. lepra + dopa, 18 hrs. (Dilution 3x).

![Graph](image)

**Table 1. Oxidation of dopa by various mycobacteria.**

<table>
<thead>
<tr>
<th>Bacterial preparation</th>
<th>Oxygen uptake (μl)</th>
<th>% increase</th>
<th>Formation of indole-5,6-quinone (Δ A540 μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. lepra</td>
<td>15 73</td>
<td>329 457</td>
<td></td>
</tr>
<tr>
<td>M. lepra-carrimun*</td>
<td>Not tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. tuberculosis, H37Ra</td>
<td>14 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. lepra-carrimun*</td>
<td>Not tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. tuberculosis, BCG</td>
<td>19 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. 607</td>
<td>Not tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. smegmatis</td>
<td>18 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. phlei</td>
<td>45 45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kedrosky's bacilli</td>
<td>30 29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Tested by Dr. R. J. W. Rees at the National Institute for Medical Research, London.

Auto-oxidation of dopa—10 μl.

For experimental conditions: see MATERIALS AND METHODS.

Protein concentrations: M. lepra, 40 mg/ml; other mycobacteria, 15–18 mg/ml.
TABLE 2. Dopa oxidase activity of various fractions prepared from lepromy nodules and human skin (compared on wet weight basis).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Skin</th>
<th>Nodule</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>340</td>
<td>1,250</td>
</tr>
<tr>
<td>E</td>
<td>125</td>
<td>700</td>
</tr>
<tr>
<td>G</td>
<td>55</td>
<td>140</td>
</tr>
<tr>
<td>I</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>J</td>
<td>60</td>
<td>550</td>
</tr>
</tbody>
</table>

The incubation system consisted of: 0.5 M NaH₂PO₄ - KH₂PO₄ buffer, pH 6.8; 0.1 ml. DL-dopa 0.005 M; skin and nodule extract from 0.5 gm. tissue; volume, 6 ml; temperature, 37°C; time, 120 minutes.

TABLE 3. Dopa oxidase activity of various fractions prepared from human skin and lepromy nodules (specific activity).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Skin/mg protein</th>
<th>Nodule/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>D (crude extract)</td>
<td>1.76</td>
<td>3.55</td>
</tr>
<tr>
<td>E</td>
<td>0.14</td>
<td>4.00</td>
</tr>
<tr>
<td>F</td>
<td>0.36</td>
<td>2.25</td>
</tr>
<tr>
<td>I</td>
<td>0.54</td>
<td>7.73</td>
</tr>
</tbody>
</table>

The incubation system consisted of: 0.5 M NaH₂PO₄ - KH₂PO₄ buffer, pH 6.8; 0.1 ml. skin extract from 0.4 gm. wet weight; nodule extract from 0.2 gm. wet weight; DL-dopa 0.0025 M; volume, 6 ml; temperature, 37°C; time, 120 minutes.

The washed M. leprae fraction and the skin preparation were subjected to sonic oscillation in a 9 Kc Raytheon magnetostriuctive oscillator for 10 minutes. Sonic vibration (which resulted in turbid homogeneous suspensions of fine particles) produced partial inactivation of both preparations. However, the decrease in activity was greater in the skin (24%) than in the bacilli (8%). The sonicates were centrifuged at 110,000 × g for 60 minutes in a Spinco model L ultracentrifuge. The particulate and supernatant fractions obtained were tested for dopa oxidase. In M. leprae 88% per cent of the activity (of the sonicate) was located in the particulate fraction; in the skin the activity was distributed more or less equally between the residue and the supernatant.

The results are presented in Table 4. It will be seen that dopa oxidase of M. leprae is not easily dissociated from the particulate elements, whereas in the skin the activity is more easily removed from the particles. The results suggest that the oxidation of dopa by M. leprae is due to enzymes firmly bound to the cell structure, and all evidence points to the conclusion that the activity is not an artifact due to skin particles.

**DISCUSSION**

It has thus been demonstrated that M. leprae (separated from human leprosy nodules) actively oxidizes 3,4-dihydroxyphenylalanine (dopa), giving rise to pigmented products. Indole-5,6-quinone has been identified as an intermediate of the reaction. This is in agreement with detection of this compound in the enzymatic oxidation of dopa by mushroom phenolase (19).

Dopa was not metabolized by M. leprae murium, M. tuberculosis (H37Rv and H37Ra), RCG, M.607, M. phlei, M. smegmatis, and Kedrowsky's bacilli. Among these mycobacteria, M. leprae murium and M. tuberculosis H37Rv are pathogenic organisms, and M. leprae murium, like M. leprae, is directly isolated from infected
tissues. Experiments on auto-oxidation of
dopa at different pH values have shown
that at pH 6.8, the H+ ion concentration em-
ployed for studies with M. leprae, dopa
auto-oxidation could be excluded. The ef-
fects of dialysis and heat on the dopa ox-
dase of M. leprae revealed, further, that
the activity is not catalyzed by inorganic
ions present as contaminants; heat-instabil-
ity indicates that the reaction is probably
enzymatic in character.

Since M. leprae preparations were ob-
tained from leprosy skin nodules, and since
it is known that melanocytes in skin can
convert dopa to melanin (15), control ex-
periments were carried out with the use of
normal human skin. After subjecting the
skin to the procedure employed in the pre-
paration of M. leprae from the nodules, dopa
oxidase activity was determined in all frac-
tions obtained from the skin and the
nodules. In the skin the most active frac-
tion was the starting material; the final frac-
tion (corresponding to M. leprae con-
centrates) showed little dopa oxidase.
In the case of the leprosy nodules, on the other
hand, the specific activity increased with con-
centration of organisms by the proce-
dure used in the preparation. It was ap-
parent from these observations that the
dopa oxidase detected in the bacilli could
not be an artifact caused by skin enzymes.

Further experiments by repeated wash-
ing and sonic vibration of the organisms al-
so demonstrated that in M. leprae, dopa ox-
dase is firmly bound either to the cell mem-
brane or to particulate elements in the
cytoplasm. Electron-microscopic studies by
Eihi et al. (4) and Inama and Omura (13)
of ultrathin sections of M. leprae, have in
fact revealed a system of intracytoplasmic
membranes in the bacillus, often in con-
tinuity with the cell membranes. In the
skin, the activity could be removed by
washing, and partially solubilized. It is
therefore reasonable to conclude that oxida-
tion of dopa by M. leprae is an inherent
property of the organisms. Comparative
studies with eight other mycobacteria (in-
cluding M. leprae murium and M. tubercu-
losis H37Rv) showed that none of them
could oxidize dopa, thus indicating a high
degree of specificity for dopa oxidase in
M. leprae.

The nature of the enzyme catalyzing the
oxidation of dopa in M. leprae is not clear.
The organisms fail to oxidize tyrosine to
melanin. This failure is probably due to the
presence of some inhibitor, as has been
found in human skin (3). It is known also
that monophenolase activity is less stable
than that of diphenolase (25, 30). Kertesz
and Zito (20) believe that hydroxylation
of monophenols is probably a nonenzym-
ic process and that oxidation of dipheno-
ls is the only enzyme-catalyzed reaction in
phenolase. If this is the case, dopa ox-
dase of M. leprae functions as a true pheno-
holase (Enzyme Commission 1.10.3.1, o-
diphenol oxide oxidoreductase).5

The observation that M. leprae could

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Identifications numbers assigned by Enzyme Commission approved by the International Union of
Biochemistry.
oxidize dopa, essential for formation of melanin pigment, gains added significance in light of the fact that hypopigmented skin patches are characteristic lesions of leprosy. In the human skin melanocytes located at the dermal-epidermal junction and the hair bulbs produce melanin pigment. Embryologically, as noted above, melanocytes are derived from the neural crest, which gives rise also to dorsal root ganglia of spinal nerves, the adrenal medulla, and Schwann cells of the peripheral nerves. It is interesting that M. leprae invades tissues of the body where melanocytes and other cells derived from the neural crest occur. In view of the facts that melanocytes are of neural origin and that the human leprosy bacilli have a particular affinity for nerve tissue, it appears possible that M. leprae is associated with melanocytes in skin. In this connection, the following observations are relevant: (1) Electronmicrographs of lesions produced in hamsters by inoculation of M. leprae, by Inoue et al., show the organisms to be in intimate association with cells containing pigment granules. (2) In the tissue-culture experiments by Hanks, where attempts were made to cultivate M. leprae, it was observed that the bacilli survived longest in pigment-containing cells. In view of the fact that tyrosine is converted to dopa in the melanocytes, it is conceivable that the organisms in close contact with these cells compete, through their dopa oxidase activity, for the substrate dopa, which normally would lead to pigment production in the skin. Such a deviation of dopa from normal melanin formation may be reflected finally in the hypopigmented skin patches typical of leprosy.

It should be pointed out that catecholamines are structural analogs of dopa, and may be oxidized to pigmented products. The presence of these compounds is a factor to be reckoned with in considering the invasion of peripheral nerves by M. leprae.

On the basis of the above reasoning, which attempts to explain the source of dopa available to M. leprae at the site of infection, the question arises as to the in vivo metabolism of this compound in the organisms. Boswell and Whiting were the first to suggest that phenolase could function as a respiratory enzyme. The oxidation of ascorbic acid by mushroom phenolase has been demonstrated by Kellin and Mann. Robinson and Nelson showed that, in presence of oxidizable substrates like ascorbic acid, dopa could function as a true hydrogen carrier. Wosilait and Nason have also reported that phenolase could oxidize NADH₂ or NADPH₂ through the quinones formed in the reaction:

\[ \text{Quinone} + \text{NADH}_2 \text{ or NADPH}_2 \rightarrow \text{Diphenol} + \text{NAD} \text{ or NADP} \]

Thus phenolase could find a place in the pathway of the respiratory enzymes helping to carry out oxidation of a variety of substrates. On the basis of the above mechanism, it appears that dopa oxidase does not proceed to melanin formation in the presence of other oxidizable substances, the quinones produced being reconverted to diphenol. Thus, in the in vitro state, where utilizable substrates are present, normal melanin-formation from dopa would not occur. It is most likely that in M. leprae, dopa oxidase provides an alternative mechanism by which different substrates could be oxidized effectively by the organisms.

**SUMMARY**

Human leprosy bacilli separated from lepromatous nodules actively oxidized 3,4-dihydroxyphenylalanine (dopa) to pigmented products. Indole-3,6-quinone was detected as an intermediate of the reaction.

Dopa oxidase activity showed a high degree of specificity for M. leprae. Eight other mycobacteria tested, including M. leprae, M. lepromatousum, and M. tuberculosis H37Rv, did not oxidize dopa.

Various control experiments with normal human skin revealed that dopa oxidase of M. leprae was a characteristic property of the bacilli, and not due to accompanying skin tissue enzymes. Heat-inactivation and

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NAD = Nicotinamide-adenine dinucleotide, NADP = Nicotinamide-adenine dinucleotide phosphate.
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dialysis of the bacterial preparations demonstrated that the activity was not catalyzed by inorganic ions.

Sonic vibration of *M. leprae* suspension and ultracentrifugation of the product showed that dopa oxidase activity of the organisms was firmly bound to particulate elements in the cell structure.

It is suggested that dopa oxidase provides an alternative mechanism by which a variety of substrates could be oxidized by the bacilli.

**RESUMEN**

Reales leprosos humanos separados de nódulos lepromatosos activamente oxidan 3,4-dihydroxyphenylalanina (dopa) a productos pigmentados. Indol-5,6-quinone fue revelado como efecto intermedio de la reacción.

La actividad de oxidación de dopa mostró un alto grado de especificidad para *M. leprae*. Otros micobacterias experimentados, incluyendo *M. leprae nudum* y *M. tuberculosis* H37Rv, no oxidan dopa.

Varios experimentos de control con piel humana normal revelaron que dopa oxidasa de *M. leprae* era una característica propia de los bacilos, y no una consecuencia de las enzimas que acompañan los tejidos de la piel. Inactivación por el calor y la dialisis de las preparaciones de las bacterias demostró que la actividad no era catalizada por iones inorganicos.

La detección sónica de una suspensión de *M. leprae* y la ultracentrifugación del sedimento demostró que la actividad dopa oxidasa de los organismos estaba firmemente relacionada con elementos particulares en la estructura celular.

Se sugiere que dopa oxidasa constituye un mecanismo alternativo por el cual una variedad de sustancias podrían ser oxidadas por los bacilos.

**RESUMÉ**

Ajouté à des composés pigmentés, des bacilles de la lèpre humaine séparés de nodules lepromateux ont oxydé activement la 3,4-dihydroxyphenylalanine (dopa). L’indol-5,6-quinone a été décélée comme intermédiaire de la réaction.

L’activité de la dopa-oxidase a montré un haut degré de spécificité pour *M. leprae*. Il n’y a pas eu d’oxydation de la dopa chez huit autres micobacteries qui ont été étudiées, parmi lesquelles *M. leprae nudum* et *M. tuberculosis* H37Rv.

Diverses expériences témoignent de la présence d’une dopa-oxidase dans le *M. leprae* qui est une propriété caractéristique des bacilles et n’est pas due aux enzymes présents dans le tissu cutané. L’inactivation par la chaleur et la dialyse des préparations bacillaires a montré que l’activité n’est pas catalyzzée par des ions inorganiques.

La mise en évidence par ultrasons d’une suspension de *M. leprae* et l’ultracentrifugation du précipité passé aux ultrasons a montré que l’activité dopa-oxidase des organismes était firmement liée aux éléments particuliers de la structure cellulaire.

On émet la suggestion que la dopa-oxidase fournit un mécanisme de remplacement grâce auquel nombre de substrats peuvent être oxydés par les bacilles.

**Acknowledgments**

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