to 3.4×10^{-6} M and from 3.2 to 13.1×10^{-6} M, respectively (fig. 4). Moreover, reserpinization of the guinea pig (5 mg/kg reserpine 24 h prior to the experiment) blocked completely any inotropic or chronotropic response to (+)- and (−)-cathinone.

Discussion. Taken together our results confirm the view that (−)-cathinone as a central stimulant is only slightly less potent than (+)-amphetamine (compare Kalix[1] and Kohli and Goldberg[15]). The (−)-isomer of cathinone was significantly more potent than the (+)-isomer. Half maximally stimulating doses of (+)-cathinone differed from the corresponding values for (−)-cathinone or (+)-amphetamine by factors of 5 and 8, respectively. On the other hand the two isomers have equivalent potencies in releasing NA from guinea pig cardiac tissue.

The reasons for the apparent difference in the stereospecificity of central and peripheral effects of cathinone, both involving catecholamine release, are not entirely clear. The most obvious possibility, rapid racemization of the isomers in the organ bath, was excluded by measuring circular dichroism to monitor the optical activity of enantiomer solutions as a function of time[6,17]. The rate of spontaneous racemization in Tyrode's solution was less than 8% within the 4-h period between the preparation of the experimental solution and the end of a single experiment. Differential stereoselectivity for various effects of (+)- and (−)-amphetamine have been attributed to differences in the stereoselectivity of neuronal amine uptake mechanisms in different parts of the nervous system. Taylor and Snyder[12] observed a 10-fold higher potency of (+)-over (−)-amphetamine in stimulating rat locomotor activity, but only a 2-fold difference in eliciting compulsive gnawing behavior. In keeping with these differential stereoselectivities of the motoric effects, (+)-amphetamine was much more potent than the (−)-isomer in inhibiting in vivo NA uptake into various brain regions, except for the striatum where the amine uptake mechanism showed little stereoselectivity[12].

It has also been reported that the preference for (+)-amphetamine in CNS excitatory effects is not seen in cardiovascular actions where (−)-amphetamine seemed to be somewhat more potent than its (+)-isomer[18]. The stereoselectivity of central and peripheral effects of cathinone has not been compared previously. However, it is known that peripheral NA storage sites are more sensitive than central dopamine sites to the releasing action of (−)-cathinone[16]. Moreover, interspecies variations of neuronal membrane transport[19] or of substrate stereoselectivity in metabolic reactions[20] are also known for (+)- and (−)-amphetamine and could provide alternative explanations for the differential effects of cathinone isomers in heart and brain.

1 Kalix, P., Gen. Pharmac. *15* (1984) 179.
2 Schorno, H. X., Pharmazie unserer Zeit *11* (1982) 65.
3 Rosencrans, J. A., Campbell, O. L., Dewey, W. L., and Harris, L. S., NIDA Res. Monogr. *27* (1979) 328.
4 Glennon, R., and Showalter, D., Res. Commun. Subst. Abuse *2* (1981) 186.
5 Schorno, H. X., and Steinegger, E., United Nations Document MNAR/3/1978.
6 Schorno, H. X., Diss., Bern 1979.
7 Takamatsu, H., J. pharm. Soc. Japan *76* (1956) 1219.
8 Kuschinsky, G., Lindmar, R., and Wollert, U., Kurs der allgemeinen Pharmakologie und Toxikologie. Wissenschaftliche Verlagsgesellschaft, Stuttgart 1974.
9 McIntosh, J. E. A., and McIntosh, R. P., Mathematical Modelling and Computers in Endocrinology. Springer-Verlag Berlin, Heidelberg, New York 1980.
10 Zelger, J. L., Schorno, H. X., and Carlini, E. A., Bull. Narc. *32* (1980) 67.
11 Valterio, C., and Kalix, P., Archs int. Pharmacodyn. Ther. *255* (1982) 196.
12 Taylor, K. M., and Snyder, S. H., Science *168* (1970) 1487.
13 Kalix, P., Progr. Neuro-Psychopharmac. Biol. Psychiat. *5* (1982) 43.
14 Kalix, P., Br. J. Pharmac. *68* (1980) 11.
15 Kohli, J. D., and Goldberg, L. I., J. Pharm. Pharmac. *34* (1982) 338.
16 Kalix, P., Drug Alcohol Dep. *11* (1983) 395.
17 Schorno, H. X., and Steinegger, E., Experientia *35* (1979) 572.
18 Weiner, N., in: The Pharmacological Basis of Therapeutics, 6th edn, p. 160. Eds A. G. Gilman, L. S. Goodman and A. Gilman. MacMillan, New York 1980.
19 Trendelenburg, U., in: Catecholamines. Hdbk exp. Pharmac., vol. 33, p. 348. Eds H. Blaschko and E. Muscholl. Springer-Verlag, Berlin 1972.
20 Testa, B., and Jenner, P., in: Drug Metabolism. Chemical and Biochemical Aspects, p. 238. Marcel Dekker, New York 1976.

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A possible metabolic role for *o*-diphenoloxidase in *Mycobacterium leprae*

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Summary. Among mycobacteria, *Mycobacterium leprae* is unique in its ability to oxidize a variety of diphenols to quinones in vitro. What physiologic role *o*-diphenoloxidase has in the organism remained unknown. Reducing substrates like NADPH, NADH and ascorbic acid reacted with the quinone formed from dopa (3,4-dihydroxyphenylalanine); the substrates were oxidized and the quinone was reduced back to diphenol in the process. Since the quinone undergoes reversible oxidation-reduction, diphenoloxidase might serve as an alternative respiratory mechanism in *M. leprae* for the utilization of other substrates, as has been reported in plants.
Key words. Mycobacterium leprae; o-quinone; reversible oxidation-reduction.

Mycobacterium leprae is an obligate intracellular parasite; all attempts for over a century to culture the organism in vitro have failed so far. The only specific metabolic activity detected in *M. leprae* is diphenoloxidase (EC 1.10.3.1), which converts 3,4-dihydroxyphenylalanine (dopa) and a variety of other diphenols to quinones. This activity has not been found in any other mycobacteria[1,2]. In substrate-specificity and in the effect of inhibitors, diphenoloxidase of the organisms was distinct from that in vertebrate melanocytes. It is known that in the human host *M. leprae* multiplies at sites such as the skin, peripheral nerves, adrenal medulla and the eyes where dopa or its derivatives occur.

As yet, there has been no direct experimental evidence to attribute a physiologic role for diphenoloxidase in *M. leprae*. The enzyme has been reported to function as an alternative respiratory mechanism in plants[3,4]. The quinones formed in the reaction undergo reversible oxidation-reduction and function as electron carriers; other sustrates are oxidized in the process and the quinone gets reduced back to diphenol.

$$\text{Diphenol} \xrightarrow{\text{enzymatic}} o\text{-quinone} \xrightarrow[\text{or nonenzymatic}]{\text{enzymatic}} \text{melanin}$$

o-Quinone + reducing substrates
→oxidized substrates + diphenol

Table 1. Oxidation of NADPH by *o*-quinone: absorbance 340 nm × 10^{-3}

Time (min)	*M. leprae* (10^9 bacteria)		Mushroom tyrosinase (10 µg)	
	NADPH (50 nM)	Quinone + NADPH	NADPH (100 nM)	Quinone + NADPH
0	198	198	437	437
15	198	174	437	349
30	198	152	437	286
60	198	102	437	237
120	190	67	418	155
% Decrease	4	66	4	65

Table 2. Reduction of *o*-quinone by ascorbate: absorbance 480 nm × 10^{-3}

Time (min)	Quinone + ascorbate	
	M. leprae (10^9 bacteria)	Mushroom tyrosinase (10 µg)
0	50	80
15	43	50
30	40	40
60	35	40
120	30	35
% Decrease	40	56

The reactivity of *o*-quinones is two orders higher than that of *p*-quinones[5]. The data reported in this communication show that the *o*-quinones formed from dopa oxidizes NADPH, NADH and ascorbate, while the quinone itself is reduced to diphenol.

Materials and methods. Purified suspensions of *M. leprae* were prepared as reported earlier[1]. The method essentially consists of homogenization of the infected tissue followed by differential and density-gradient centrifugations of the homogenate in solutions of sucrose and KCl. The final preparation is treated with 0.1N NaOH and 1 mg/ml trypsin to remove host-tissue materials adsorbed on the bacteria. These treatments do not impair the viability or enzyme activity of the organism[6]. The tissues (spleen or lymph nodes) used in the study were obtained from nine-banded armadillos (*Dasypus novemcinctus*) experimentally infected with *M. leprae*[7]. The material was removed aseptically at autopsy and kept at 0 °C, and the preparative procedures were done at 0–4 °C. A water extract of Harding-Passey melanoma served as a source of mammalian *o*-diphenoloxidase. Lyophilized mushroom tyrosinase was purchased from ICN, Cleveland, OH, and NADPH, NADH and ascorbate from Sigma, St.-Louis, MO.

The bacteria, melanoma extract or mushroom tyrosinase was incubated with dopa (2 µM) at 37 °C, pH 6.8 for 60 min, and the spectrum of the quinone formed was determined, as reported earlier[1]. D-dopa was used for reactions with the bacteria or mushroom tyrosinase and L-dopa for the melanoma extract. The quinone produced in the reaction (dopachrome) gives an absorbance maximum around 480 nm. The reaction mixtures containing *M. leprae* or melanoma extract were centrifuged and the supernatant fractions were used in the experiments. Oxidation of NADPH by the quinone was determined by the decrease in absorbance at 340 nm. The reduction of *o*-quinone by ascorbate was measured by the decrease in absorbance at 480 nm.

Results. NADPH was added to the reaction mixture containing *o*-quinone formed by the oxidation of dopa. The absorbance of NADPH at 340 nm gradually decreased indicating its oxidation. NADPH by itself showed no change in absorbance for up to 60 min. Intact *M. leprae* was used in the assay and it was removed by centrifugation before adding the reduced pyridine nucleotide. Apparently, the quinone present in the supernatant fraction oxidized the NADPH (table 1). NADH also reacted with *o*-quinone to give similar results.

The *o*-quinone (dopachrome) formed in the oxidation of dopa has a pink color and has an absorbance maximum near 480 nm. When the quinone is reduced to diphenol, it is decolorized and the absorbance peak disappears. This phenomenon was readily observed when ascorbate was added to the reaction mixture containing the quinone (table 2). The experiment was done using *M. leprae*, melanoma extract or mushroom tyrosinase with similar results. The data for *M. leprae* and mushroom tyrosinase are given in the table. In 3 h, the reading fell to 0.015 (which was the same as the absorbance for dopa) indicating complete reduction of the quinone. Without the added reducing agent, the quinone underwent further oxidation and polymerization to melanin pigment, giving a reading of 0.150–0.200 in 6 h.

When *M. leprae* prepared from frozen tissues of lepromatous patients was used, the results were not the same as those with the bacteria purified from fresh tissues of experimentally-infected armadillos. The former oxidized dopa to give rise to indole-5,6-quinone (sometimes called melanochrome) with an absorbance maximum at 540 nm[1]. This quinone was not reduced back to diphenol in presence of ascorbate; however, its polymerization to melanin pigment was suppressed and it also oxidized NADH and NADPH. The reason for this anomaly is not yet clear. The conversion of dopachrome to indole-5,6-quinone involves a decarboxylation step, which is probably catalyzed by a decarboxylase enzyme or by zinc ions associated with the organisms.

Discussion. In biological systems, electron transfer is mediated for four main classes of proteins: hemoproteins, flavoproteins, copper proteins, and iron-sulfur proteins. Although most oxidative enzymes are barely detectable in *M. leprae*, *o*-diphenoloxidase, which is a copper protein, is extremely active in the organism. Oxidation of dopa can be observed visually within minutes of incubation with the bacteria. The enzyme is completely inactivated by heat and by the chelator diethyldithiocarbamate[1]. Inhibitors of diphenoloxidase specifically suppressed the multiplication of *M. leprae* in mouse footpads, suggesting that the enzyme is of metabolic significance in the bacteria[8]. It is likely that the quinone formed from dopa enables the bacteria to metabolize other substrates as has been found in plants[3,4]. The presence of diphenoloxidase in the bacillus helps to explain its tissue specificity in the host. This is comparable to the exclusive multiplication of brucellae in certain animal tissues where erythritol is available[9]. Even in the armadillo i.v. inoculated with *M. leprae*, the primary site of bacterial growth is the skin; if the animal is killed during the early stages of the infection when numerous bacteria are detected in skin lesions, very few bacilli are found in the internal organs. How this parasitic organism derives energy for its survival and proliferation in the host tissues still remains obscure.

1 Prabhakaran, K., Harris, E. B., and Kirchheimer, W. F., in: Pigment Cell, vol. 3, p. 152. Ed. V. Riley. S. Karger, Basel 1976.
2 Prabhakaran, K., Harris, E. B., and Kirchheimer, W. F., Lep. Rev. *51* (1980) 341.
3 Robinson, E. S., and Nelson, J. M., Archs Biochem. *4* (1944) 111.
4 Wosilait, W. D., and Nason, A., J. biol. Chem. *206* (1954) 255.
5 Cenas, N. K., Kanapieniene, J. J., and Kulys, J. J., Biochim. biophys. Acta *767* (1984) 108.
6 Prabhakaran, K., Harris, E. B., and Kirchheimer, W. F., Int. J. Lep. *48* (1980) 330.
7 Kirchheimer, W. F., and Storrs, E. E., Int. J. Lep. *39* (1971) 693.
8 Prabhakaran, K., Harris, E. B., and Kirchheimer, W. F., Microbios *5* (1972) 273.
9 Smith, H., Bact. Rev. *32* (1968) 164.

0014-4754/85/121571-02$1.50 + 0.20/0
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