Regional distribution of AChE activity found in this study follows the regional distribution of ACh in the rat brain[18]; the structures with high AChE activity and ACh content are assumed to represent cholinergic structures[19]. We have found a slight discrepancy between the degree of AChE and $(Na^+ + K^+)$-ATPase inhibition by means of physostigmine in cerebral cortex and caudate; this is presumably due to the fact that the former brain structure represents one of the least cholinergic structures of the brain, and vice versa. Namely, $(Na^+ + K^+)$-ATPase inhibition occurred to a greater degree in the structures with high amounts of AChE activity.

There are some similarities between the effect of ACh application to the neuron membrane and the effect of the specific $(Na^+ + K^+)$-ATPase inhibitor ouabain[20] with respect to membrane permeability and sodium flux. Both ACh and ouabain application on the neuron surface resulted in increased membrane permeability, increased intracellular sodium, and depolarization[21-23]. Increased intracellular sodium concentration, caused by ACh, stimulates the sodium pump, which can be inhibited by addition of ouabain[21]. We assume that ACh, increased after inhibition of AChE, inhibits $(Na^+ + K^+)$-ATPase activity; this is followed by increased intracellular sodium, due to stimulation of passive transport, and depolarization of the postsynaptic membrane. It is worthwhile mentioning that the inhibitory action of ACh on $(Na^+ + K^+)$-ATPase in vitro is specific and observed only with synaptosomal membranes, not with other membranes of any subcellular structures[5]. Hence, having in mind the above mentioned data and the results of this study, we suggest the hypothesis that the physiological action of ACh is mediated through its inhibitory influence on postsynaptic $(Na^+ + K^+)$-ATPase.

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A possible method for improving the efficacy of dapsone

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Summary. The antileprosy drug dapsone is unable to penetrate intact *Mycobacterium leprae* in vitro, as determined by its effect on o-diphenoloxidase in the bacilli. When combined with the peptide polylysine, the sulfone drug passes through the bacterial cell membranes, and penetrates the enzyme protein, resulting in a 100% inhibition of its activity.

Dapsone, 4-4′diaminodiphenyl sulfone (DDS) is the most widely used drug in the treatment of leprosy. Even after years of treatment with dapsone, viable leprosy bacilli persist in the tissues of lepromatous cases. DDS has been reported to inhibit folic acid synthesis in other bacteria, but the mechanism of action of dapsone in leprosy remains unknown. Our studies show that the drug does not penetrate intact *M. leprae* in vitro. Making an antibacterial agent to permeate its target organisms should enhance its effectiveness. o-Diphenoloxidase is the only enzyme proven to be present in the leprosy organisms[1-3]. We have reported earlier that diethyldithiocarbamate (DDC), which penetrates the bacilli and inhibits the enzyme, is bactericidal to the leprosy organism[4-6]. However, the compound is unstable under acid conditions. In this communication, we present evidence to show that, when combined with the peptide polylysine, dapsone penetrates *M. leprae* and produces complete inhibition of its o-diphenoloxidase.

Materials and methods. There are as yet no authenticated procedures for culturing *M. leprae* in vitro. Purified suspensions of the organisms were prepared from the spleen tissue of experimentally infected armadillos[7], as described before[4]. If precautions are not taken to prevent enzyme denaturation and if the bacterial preparations are contaminated with host-tissue elements, little o-diphenoloxidase activity would be detected in them[8]. The bacilli were disrupted by ultrasonic oscillation in a Sonifier-Cell Disruptor, coupled with a Time-Temperature Control Module, which prevents heat build-up and denaturation of proteins. DDS powder was purchased from Sigma Chemical Co., polylysine HCl (mol.wt 27,000) from Miles Laboratories, and D-DOPA from ICN Nutritional Biochemicals.

DDS was suspended in water, or dissolved in 50% ethanol at a concentration equivalent to 0.02 M. Polylysine was added to the DDS suspension or solution at 5 mg/ml. o-Diphenoloxidase was assayed spectrophotometrically, as reported earlier[2,4]. The reaction mixture contained: D-DOPA (final concentration), 0.02M; DDS (with or without polylysine), 0.04M; and bacilli, 5×10^9. The volume was 3 ml, pH 6.8, temperature 37 °C and incubation time 60 min. After centrifugation of the reaction mixtures, absorbance maximum of the quinone (dopachrome) formed from DOPA was determined in the supernatant fraction. The readings were corrected for any absorbance

Effect of DDS on o-diphenoloxidase of *Mycobacterium leprae:* Absorbance 480 nm ($\times 10^{-3}$)

Inhibitor	Intact bacilli			Disrupted bacilli		
	Bacilli + DOPA	Bacilli + DOPA + inhibitor	% inhibition	Bacilli + DOPA	Bacilli + DOPA + inhibitor	% inhibition
DDS suspension in water	58	52	11	50	36	28
DDS solution in ethanol	60	54	10	50	38	24
DDS suspension + polylysine	60	0	100	50	0	100
DDS solution + polylysine	60	0	100	50	0	100
Ethanol	70	72	-	50	51	-
Polylysine	64	65	-	50	50	-

due to the bacilli or the reagents. Each experiment was done at least 3 times; values given are for representative experiments, since no significant variations were observed between results of different experiments. Heating the bacilli at 100 °C for 3 min inactivated the enzyme.

Results and discussion. The results are presented in the table. DDS in suspension or solution showed very little inhibitory effect on the oxidation of DOPA by intact *M. leprae;* in the disrupted bacilli, the inhibition was greater, but it was still less than 30%. However, when mixed with polylysine, DDS produced 100% inhibition of the enzyme in both intact and disrupted bacilli. o-Diphenoloxidase is a copper protein. The sulfur atoms of DDS might bind the copper and inactivate the enzyme. We tested ethanol and polylysine separately for their effect on the oxidation of DOPA by *M. leprae.* Both reagents showed neither inhibition nor stimulation of the activity at the concentrations used.

The results reported here demonstrate that polylysine enables dapsone not only to pass through the bacterial cell membranes, but also to penetrate the enzyme molecules readily. Further studies have to be done with lower concentrations of DDS-polylysine combination to determine its effect on the growth of drug-resistant *M. leprae* in animals and to assess any toxicity it might have. It has been shown that polysine can interact with lipids[9]. The peptide, which remains stable over a wide range of conditions, can pass through the lipid bilayers of the cell membranes easily. This observation is consistent with our earlier data on the complete inhibition of o-diphenoloxidase of *M. leprae* by DDC, which contains lipid-soluble ethyl groups[4]. Rifampin which is bactericidal to the organism is a lipid-soluble drug. The leprosy bacilli have been shown to become resistant to both dapsone and rifampin[10-12]. Developing permeability barrier is one of the mechanisms by which bacteria become drug-resistant. In such cases, dapsone combined with polylysine or other similar compounds would be more effective than DDS alone. Rifampin-resistance in bacteria is mediated by substitution of one amino acid in the enzyme RNA polymerase. Since dapsone binds the copper moiety of o-diphenoloxidase and not the enzyme protein itself, mutations altering the protein structure may not affect its inhibitory action.

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Vitamin A antibodies: application to radioimmunoassay

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Summary. A radioimmunoassay for serum vitamin A is described which can detect as little as 1 ng of retinol. The statistical characteristics of this assay are presented and its use in a nutritional experiment is discussed.

Conrad and Wirtz[2] were able to produce antibodies to vitamin A by injecting into rabbits a retinoic-acid-albumin conjugate. The antiserum reacted equally well with retinal, retinol and retinoic acid and very poorly with retinyl palmitate, beta carotene and beta ionone. In this paper we describe a radioimmunoassay (RIA) for retinol in serum and plasma.

Materials and methods. Crystalline retinol was obtained from Eastman Organic chemicals. Tritiated-retinol and Aquasol-2 counting cocktail were obtained from New England Nuclear. Norit-A carbon came from Fisher Scientific Co. and dextran (clinical grade, 200,000-300,000) from Nutritional Biochemicals Corp. The vial of ^{3}H-retinol received from the supplier (0.25 mCi, 0.029 mg in 0.25 ml ethanol) was diluted with 62.5 ml absolute ethanol and stored under nitrogen at −20 °C. The buffer used in the radioimmunoassay was 0.05 M phosphate, 0.1 M NaCl, pH 6.8. Antiserums against vitamin A were produced in rabbits according to the method of Conrad and Wirtz[2]; the antiserum used in this work displayed an affinity constant of 2×10^8 l/M as determined by a Scatchard plot of RIA data. The antiserum was prepared for RIA by diluting 1/805 with buffer and adding 0.05 ml of diluted ^{3}H-retinol solution (see above) per 25 ml of diluted antiserum. The