produced by lead poisoning in guinea pigs. J. Neuropathol. Exp. Neurol. **25** (1966) 214–236.
6. KAHN, P. and SCOTT, T. The pathology of a radial nerve biopsy in leprosy: Light and electron microscopy. J. Pathol. **114** (1974) 97–100.
7. LEVY, L. Studies of the mouse foot pad technic for cultivation of *Mycobacterium leprae*. III. Doubling time during logarithmic multiplication. Lepr. Rev. **47** (1976) 103–106.
8. LINOIS, H. and SEBILLE, A. Electrophysiological evidence for motor unit impairment during the treatment of leprosy. J. Neurol. Sci. **45** (1980) 57–63.
9. SEBILLE, A., TABTI, N., GUELPA, C. C. and GIROIR, A. M. Electrophysiological studies of the sciatic nerves in *Mycobacterium leprae* foot pad-injected rats. Int. J. Lepr. **52** (1984) 365–370.
10. SHEPARD, C. C. Acid-fast bacteria in nasal excretion in leprosy and results of inoculation of mice. Am. J. Hyg. **71** (1960) 147–157.

NADH-Methemoglobin Reductase and Reticulocytosis

TO THE EDITOR:

Magna and Beiguelman ([5]) have shown that the activity of NADH-methemoglobin reductase varies widely among leprosy patients under dapsone treatment and is negatively correlated to their hemoglobin level. Since dapsone has a hemolytic effect ([1]), while NADH-methemoglobin reductase is more active in younger than in older erythrocytes ([3, 4]), it was supposed that this correlation might be due to an increase in the reticulocyte rate in leprosy patients.

This hypothesis was tested by studying venous blood samples of leprosy patients (30 males and 30 females) submitted to chronic sulfone therapy. The activity of NADH-methemoglobin reductase was determined according to Scott's method ([6]) slightly modified, and the hemoglobin level was determined following Benesch, *et al.*'s method ([2]). The reticulocyte rate was obtained as usual. The mean and standard deviation of the variables studied are given in The Table.

THE TABLE. *Mean values ($\bar{x}$) and standard deviations (s) of NADH-methemoglobin reductase activity ($10^4 \cdot A_{600}$/min), hemoglobin level (g%) and reticulocyte rate (%) of 60 adult leprosy patients under sulfone therapy.*

Variable	Sex	$\bar{x}$	s
NADH-methemoglobin reductase	M	43.54	10.76
	F	61.73	14.14
	M + F	52.63	15.48
Hemoglobin	M	13.16	1.52
	F	11.05	1.95
Reticulocytes rate	M	2.34	1.41
	F	2.41	1.63
	M + F	2.38	1.51

In the present study, no significant correlation was found between the NADH-methemoglobin reductase activity and the hemoglobin level ($r = -0.05$ in males and $r = 0.20$ in females) or between the former and the reticulocyte rate ($r = 0.04$ in males and $r = -0.23$ in females).

It also seems important to point out that in the samples analyzed in the present study the NADH-methemoglobin reductase activity among females was significantly higher as compared to males.

—Omar Sergio Caticha-Alfonso

Fellowship No. 83/1779-4
Fundação de Amparo à Pesquisa do Estado de São Paulo
Departmento de Genética Médica
UNICAMP
Campinas 13.100, SP, Brasil

REFERENCES

1. BEIGUELMAN, B., PINTO, W., JR., EL-GUINDY, M. and KRIEGER, H. Factors influencing the level of dapsone in blood. Bull. WHO **51** (1974) 467–471.
2. BENESCH, R. E., BENESCH, R. and YUNG, S. Equations for the spectrophotometric analysis of hemoglobin mixtures. Anal. Biochem. **55** (1973) 245–248.
3. GONZÁLEZ, R., ESTRADA, M., WADE, M., TORRE, E., SVARCH, E., FERNANDEZ, O., ORTIZ, R., GUZMAN, E. and COLOMBO, B. Heterogeneity of hereditary methaemoglobinemia: a study of 4 Cuban families with NADH-methaemoglobin reductase deficiency including a new variant (Santiago de Cuba variant). Scand. J. Haemotol. **20** (1978) 385–393.
4. JAFFÉ, E. R. Hereditary methemoglobinemia associated with abnormalities in the metabolism of erythrocytes. Am. J. Med. **41** (1966) 385–393.

5. MAGNA, L. A. and BEIGUELMAN, B. NADH-methemoglobin reductase and methemoglobinemia among leprosy patients. Int. J. Lepr. **52** (1984) 475–481.

6. SCOTT, E. M. The relation of diaphorase of human erythrocytes to inheritance of methemoglobinemia. J. Clin. Invest. **39** (1960) 1176–1179.

Purification of Phenolic Glycolipid I from Armadillo and Human Sources

TO THE EDITOR:

Phenolic glycolipid I ([1-3]) has become an important tool for the specific serodiagnosis of leprosy ([4-9]) and is capable of inducing suppression of mitogenic responses of lepromatous leprosy patient lymphocytes ([10]) and thus may be implicated in the aberrant cell-mediated immune response in lepromatous leprosy. Phenolic glycolipids and the related nonantigenic and nonspecific dimycocerosylphthiocerol ([11, 12]) may also be implicated in the intracellular persistence of the leprosy bacillus ([12]). The original protocols for the purification of phenolic glycolipid I (PGL-I) from infected armadillo tisues ([2, 3, 13]) resulted in preparations that were of the order of 90% pure. Because of the importance of the glycolipid and the need for large amounts of material of optimal purity, we have further modified the protocol and now present it in the form of 22 detailed steps, with three alternative strategies for final purification.

Although there is immunological evidence for the presence of PGL-I, or more precisely, its antigen determinant, 3,6-di-*O*-methyl-β-D-glycopyranoside, in human lepromatous leprosy tissue ([14]), direct chemical evidence is scant ([15]). We now also describe in an accompanying Letter to the Editor the preparation of pure PGL-I from human lepromatous nodules and provide chemical evidence for its complete structural concordance with the product from the armadillo source.

Purification of PGL-I from infected armadillo tissue

1. *Mycobacterium leprae*-infected armadillo livers and spleens are obtainable from Dr. Eleanor Storrs, Division of Comparative Mammalogy and Biochemistry, Medical Research Institute, Florida Institute of Technology, 3325 West New Haven Avenue, Melbourne, Florida 32901, through Dr. Darrel D. Gwinn, Leprosy Program Officer, Bacteriology and Virology Branch, NIAID, Westwood Building—Room 738, 9000 Rockville Pike, Bethesda, Maryland 20205. Homogenize the tissue in 25 g lots according to the procedure of Draper ([16]), but without detergent ([3]), and in the presence of 1 mM benzamidine ([14]). Centrifuge at 10,000 × *g*. The pellet is used as a source of *M. leprae*. The supernatant is lyophilized and weighed and used as a source of glycolipid.

2. Extract with 40 ml $CHCl_3$:CH_3OH (2:1) per g of material for 18 hr in a 50°C water bath in a large Erlenmeyer flask. Keep flask covered but not sealed, since $CHCl_3$ is volatile. Usually 80 g of lyophilized supernatant is processed at one time.

3. Remove flask from water bath. Filter extract through two Eaton-Dikeman, grade 515, 32 cm, fluted filter papers (VWR Scientific, P.O. Box 7900, San Francisco, California 94120) into an appropriate size vessel. Return the filter papers and residue to the original flask and re-extract with the same amount of $CHCl_3$:CH_3OH (2:1), for 18 hr at 50°C. Filter the second extract as before, combine the extracts, dry on a rotary evaporator, and weigh (typically 20 g).

4. Perform a biphasic wash using 400 ml $CHCl_3$, 200 ml CH_3OH, and 100 ml H_2O per 20 g of extract. Mix the dried extract with ½ volume of $CHCl_3$ and CH_3OH; pour into an appropriate size separatory funnel. Rinse the flask with the remaining volume of $CHCl_3$ and CH_3OH and add to the solution in the separatory funnel. Add the requisite amount of H_2O. Shake gently, and allow the mixture to separate into two distinct phases. Collect the lower organic phase, dry, and weigh (typically 8 g). Remove the interfacial residue and aqueous phase for discarding or storage.

5. Build a Florisil column using 20 g Florisil (Fisher Scientific, 711 Forbes Ave., Pittsburgh, Pennsylvania 15219) per g of organic phase crude lipid. Weigh Florisil in beaker. Use a 5 × 50 cm glass column (Chromaflex; Kontes of California, 3045 Teagarden Street, San Leandro, California 94577). The volume of Florisil will be approximately 2 cm^3/g. Fit with a stopcock, end piece, clamp, filter disk, and gasket. Set the column perpendicular with a spirit level. Pour some $CHCl_3$ into the column to check for leaks around the gasket and stopcock.

6. Add enough $CHCl_3$ (e.g., 2 ml/g) to the Florisil in a beaker to make a thin slurry. Pour the slurry into the column. Open the stopcock allowing the $CHCl_3$ to exit. Tapping the column with a cork ring helps pack Florisil as the $CHCl_3$ exits. Record the final Florisil height (e.g., 22 cm). Rinse the beaker and upper column with more $CHCl_3$. Wash the Florisil with one bed volume of $CHCl_3$ [bed volume = column radius^2 × 3.14 × Florisil height; e.g., (2.5 cm)^2 × 3.14 × 22 cm = 341 ml]. Close the stopcock when the $CHCl_3$ meniscus reaches the top of the Florisil.

7. Dissolve the dried crude lipid sample in a few ml (<10 ml) of $CHCl_3$ (warming helps to dissolve the sample). Using a Pasteur pipette, apply the sample down the sides of the column so as not to disturb the Florisil top. Rinse the flask with $CHCl_3$ (1–2 ml) to allow ap-