bits, and mice with cyclizine which is a compound of chemical structure very similar to meclizine hydrochloride.

Until studies on the level of histamine in the liver of the pregnant rat and of the embryo have been carried out it cannot be concluded that meclizine hydrochloride does or does not exert its teratogenic action via its antihistaminic activity (*9*).

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**References and Notes**

1. G. I. Watson, *Brit. Med. J.* No. 5317, 1446 (1962); C. G. Fagg, *ibid.* No. 5320, 1681 (1962); G. W. Mellin and M. Katzenstein, *Lancet* No. 7274, 222 (1963).
2. M. P. Carter and F. W. Wilson, *Brit. Med. J.* No. 5319, 1609 (1962); J. R. James, *ibid.* No. 5322, 59 (1963); A. David and A. H. Goodspeed, *ibid.* No. 5323, 121 (1963); L. Martin-Nahon, *ibid.* No. 5326, 331 (1963); K. D. Salzmann, *ibid.* No. 5328, 471 (1963).
3. A. Goldstein and M. H. Hazel, *Endocrinology* **56**, 215 (1955).
4. M. C. Shelesnyak and A. M. Davies, *Proc. Soc. Exptl. Biol. Med* **89**, 629 (1955).
5. H. Tuchmann-Duplessis, *Brit. Med. J.* No. 5333, 828 (1963).
6. L. E. Feild, S. J. Kreshover, J. E. Lieberman, *J. Dental Res.* **39**, 1240 (1960).
7. H. Kalter and J. Warkany, *Am. J. Pathol.* **38**, 1 (1961).
8. From experiments currently in progress in this laboratory.
9. I thank Chas. Pfizer & Co., Inc., for a supply of meclizine hydrochloride and the excipient used for the manufacture of the Bonine tablet, F. J. Kendrick for his evaluation of histologic material, and Julia Derr and Sallie Weaver for excellent technical assistance.

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## Electron Microscope Autoradiography of Bacteria Labeled with Iodine-125

Abstract. *The low-energy extranuclear electrons emitted by iodine-125 can be used for electron microscope autoradiography with results comparable to those obtained with tritium. Autoradiographs of tritium-labeled bacteria showed that 71 percent of all reacted grains localized on the cell. This figure was 86 percent when $I^{125}$-labeled bacteria were used.*

The low-energy electrons emitted by tritium contribute to accuracy of localization in autoradiography, because electrons that do not travel in paths perpendicular to the plane of the section are apt to be absorbed before they reach the photographic emulsion. Lateral scatter of electrons is reduced further in electron microscope autoradiography because the sections of cells and layers of the photographic emulsion are ultrathin (*1*). Iodine-125 has been used for autoradiography in the light microscope (*2*) and the electron microscope (*3*). The x-rays and gamma rays from $I^{125}$ do not affect the photographic emulsion significantly, and the extranuclear electrons emitted by $I^{125}$ have energies near the range of beta particles of tritium (*2*). In the present experiment, we have used bacteria as small biological objects in order to assess the accuracy of localization in autoradiography with $I^{125}$.

*Salmonella typhosa* was grown in broth, killed with formaldehyde at *p*H 7.0, and iodinated with 2 mc of $NaI^{125}$ (*4*). Uncombined $I^{125}$ was removed by washing four times in balanced salt solution. The bacteria were fixed in buffered osmium tetroxide, dehydrated through an alcohol series, and embedded in methacrylate by the usual methods. Thin sections were prepared with a Porter-Blum microtome, mounted on stainless-steel grids, coated with jelled Ilford L4 emulsion (*5*), and exposed for 5 to 21 days at 4°C. Coated grids were developed with Kodak D-19, Microdol-X, or a solution of *p*-phenylenediamine and sodium bisulfite (*5*). Most sections were treated with 0.05*N* sodium hydroxide to remove excess emulsion (*6*) and stained with 0.07*M* uranyl acetate prior to examination in an electron microscope (RCA EMU-3).

A thymine-requiring strain of *Escherichia coli* was grown in medium that contained tritiated thymidine. They were then fixed in buffered osmium tetroxide without prior treatment in formaldehyde and prepared for electron microscopy as described above.

In sections of formaldehyde-treated bacteria, large clear spaces occupied the central or nuclear areas, and the bacteria had a shell-like appearance. This aided in assessing localization, for silver grains were located on or near the cell walls and were rarely present in the clear zone (Figs. 1 and 2). Background was negligible as determined by observation of portions of the section in which there were no bacteria. Silver grains located near the bacteria but not over them were attributed to lateral scatter of electrons.

With Microdol-X or D-19 as a developer, of a total of 887 grains counted in a series of fields of $I^{125}$-labeled bacteria (Fig. 1), 768, or 86 percent, lay partially or completely over bacteria.

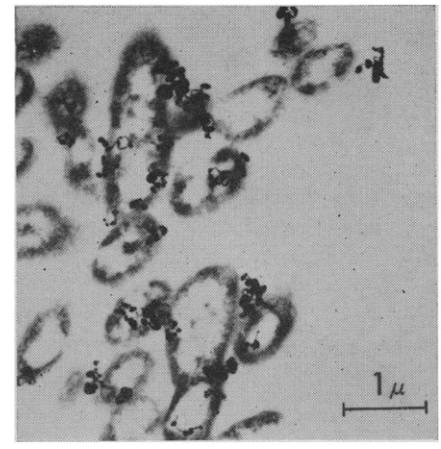


Fig. 1. Autoradiograph of formaldehyde-treated bacteria labeled with $I^{125}$ and developed in Microdol-X.

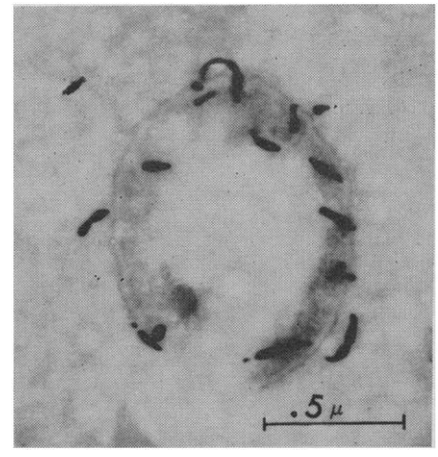


Fig. 2. Autoradiograph of formaldehyde-treated bacteria labeled with $I^{125}$ but not treated with sodium hydroxide. *P*-phenylenediamine was used as the developer.

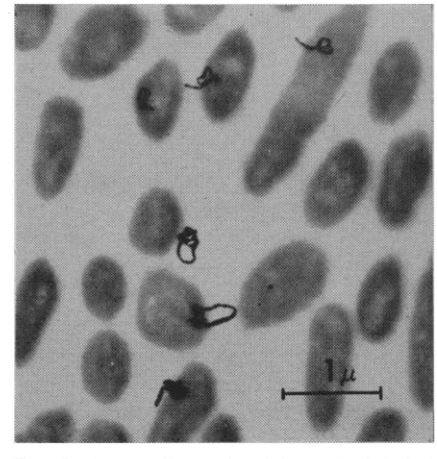


Fig. 3. Autoradiograph of bacteria labeled with tritiated thymidine and developed in Microdol-X.

When bacteria were labeled with tritiated thymidine (Fig. 3), 715 grains, or 71 percent, of a total of 1006 counted overlay bacteria.

Similar results were obtained with $I^{125}$-labeled bacteria when a physical developer consisting of *p*-phenylenediamine and sodium bisulfite in water was used (Fig. 2). This method yielded smaller grains, and of a total of 485 grains, 429, or 88 percent, overlay bacteria.

In view of this experience, $I^{125}$ appears to have excellent potential for autoradiographic localization by the electron microscope (*7*).

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### References and Notes

1. C. G. Harford and A. Hamlin, *Lab. Invest.* **10**, 627 (1961); L. G. Caro, *J. Cell Biol.* **15**, 189 (1962).
2. F. W. Fitch, J. Winebright, P. V. Harper, *Science* **135**, 1068 (1962); L.-E. Appelgren, R. Soremark, S. Ullberg, *Biochim. Biophys. Acta* **66**, 144 (1963).
3. J. Kayes, A. B. Maunsbach, S. Ullberg, *J. Ultrastruct. Res.* **7**, 339 (1962).
4. F. W. Fitch, P. Barker, K. H. Soules, R. W. Wissler, *J. Lab. Clin. Med.* **42**, 598 (1953).
5. L. G. Caro, R. P. van Tubergen, J. A. Kolb, *J. Cell Biol.* **15**, 173 (1962).
6. E. D. Hay and J. P. Revel, *ibid.* **16**, 29 (1963).
7. These investigations were carried out under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and were supported by the Office of the Surgeon General, Department of the Army, Washington, D.C. One of us (N.O.K.) is a trainee in infectious diseases, grant 2E-25 (C2), U.S. Public Health Service. We thank Alice Hamlin for valuable technical assistance and M. Ter Pogossian for suggesting the use of $I^{125}$ for this purpose. $NaI^{125}$ was obtained from the Volk Radiochemical Co.

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## Rank of Mothers and Sons in Bands of Rhesus Monkeys

Abstract. *In bands of free-ranging macaques, adolescent males typically leave their mothers in the central part of the band and assume low social rank at the periphery. But the adolescent sons of high-ranking mothers may remain central and rise to high rank without becoming peripheral.*

Macaques live in large bands which include males, females, and young. Intensive studies of Japanese monkeys (*Macaca fuscata*) in their natural habitat indicate that young females remain in the central part of the band even after they become adult (*1*). Social rank among these females follows the order of rank among their mothers. Young males, on the other hand, retire to the periphery of the band at about the age of puberty. These adolescents rank below the older central males. I observed these same tendencies in an island colony of rhesus monkeys (*M. mulatta*), but with a notable difference. Some adolescent males remained central and while still subadult gained precedence over several older and larger males. In each instance the mother of the precocious male was the highest ranking female in the band, or nearly so.

Table 1. The social rank, age, and mother of subadult and older males, in bands of rhesus monkeys, June 1962. The number of individuals in the bands were: *A*, 134; *C*, 120; *E*, 44; *F*, 31; *H*, 35; and *I*, 18. The figures or letters in parentheses beside the age of each ranking male are the identification marks of the male son and his mother respectively.

| Male rank | Age and identification of each ranking male in band | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *C* | *E* | *F* | *H* | *I* |
| 1 | 8 (14, ?) | 10+ (JF, ?) | 10+ (CL, ?) | 10+ (66, ?) | 7 (39, ?) | 8 (96, ?) |
| 2 | 5 (SO7, 119*) | 6 (ES, AT*) | 6 (95, dd) | 6 (RO8, 65*) | 4 (AG, 84*) | 7 (127, ?) |
| 3 | 4 (DW, 119*) | 10+ (103, ?) | 6 (R15, dd) | 6 (CN, 76) | 8 (DY, ?) | 7 (45, ?) |
| 4 | 4 (DV, 109) | 5 (KC, DA) | 6 (RO6, dd) | 5 (ER, dd) | 5 (S11, dd) | |
| 5 | 8 (56, ?) | 6 (132, CD) | 4 (EJ, 128) | 4 (CY, 22) | | |
| 6 | 8 (26, ?) | 8 (63, ?) | 5 (BC, ?) | | | |
| 7 | 7 (08, ?) | 8 (05, ?) | 5 (BD, ?) | | | |
| 8 | 8 (79, ?) | 8 (27, ?) | | | | |
| 9 | 7 (121, ?) | 7 (06, ?) | | | | |
| 10 | 6 (98, ?) | 7 (01, ?) | | | | |

* Highest ranking female in the band.

These studies were made on Cayo Santiago, a wooded 37-acre islet situated half a mile off the southeast coast of Puerto Rico. The social behavior and ecology of these monkeys have been studied for several years (*2, 3*). All of the present animals are descendants of stock released on the island in 1938 (*4*). Although, over the years, a few hundred animals have been removed, natural population growth has been little disturbed since mid-1956. As of mid-1962 there were 382 monkeys constituting six stable bands of from 18 to 134 members. Each band comprised sexually mature females at least 4 years old and their young, adolescent males 3 to 5 years old, and adult males. Social rank among the animals was judged by observations of exchange of threatening and submissive gestures, and by precedence at food and water. The males ranked in a definite linear dominance hierarchy. Among females differences in rank were much less pronounced, but clear enough among the few highest and few lowest ranking animals. Ages of the animals were determined from their dentition during youth.

As Kawai (*1*) has shown for male Japanese macaques, I also found that the order of rank in rhesus monkeys tended to follow the order of age (Table 1). But in three bands I found notable exceptions in which a subadult or newly adult 4- to 6-year-old male outranked older- and larger males. These exceptions were most conspicuous in the largest band, *A*. The leader, or male No. 1, was 8 years old; males No. 2 and No. 3 were 5 and 4 years old, respectively. But the next five lower ranking males were 7 or 8 years old. Males No. 2 and No. 3, which had reached high rank precociously, were the sons of the same female. One was tattooed as her infant in 1957 (*3*), while the other associated closely with her since 1 year of age. This old mother was clearly dominant over all the other females in the band. In addition, the mother of male No. 4 was among the few top females and the close companion (perhaps the daughter or sister) of female No. 1.

In the second largest band, *C*, male No. 1 was over 10 years old and male No. 2 was 6 years old. The latter was the son of the highest ranking female, as judged by his early association with her. He outranked male No. 3, which was over 10 years old. Males No. 4 and No. 5, 5 and 6 years of age, were sons of two other high-ranking females. All three of the high-ranking 5- and 6-year-old males outranked several 7- and 8-year-old monkeys. A further example of a subadult reaching second highest male rank occurred in a small band *H*. Although only 4 years old, this male outranked an 8 year old. Again, the mother of male No. 2 was the highest ranking female. In another small band, *F*, male No. 2 was also the son of female No. 1, although he outranked no older animals. Finally, a minor example of precocious high rank occurred in band *E*, where the 4-year-old son of female No. 2 outranked two 5-year-old males. Neither of the latter two, how-