that have been fixed in alcoholic formalin. Hence staining is not limited to the cytoplasmic granules of the mast cells. Because of the low *p*H of the dye solution, however, the dye binds with only the most strongly acidic tissue components, principally the sulfonated mucopolysaccharides. The only material in the tissues we have examined other than the mast cell granules, which is deeply stained, is cartilage matrix. In some organs that have a very high content of cytoplasmic ribonucleic acid or that have numerous dense nuclei, weak staining with the toludine blue may occur. Because there is little likelihood of confusion, the method can be considered, for practical purposes, to be highly selective for mast cells. It should also be noted that an acid dye is used as a counterstain only as a matter of convenience to facilitate the identification of the various tissues and organs on the slides.

The staining schedule given was deliberately designed to overstain the mast cells in order to obtain the greatest contrast with the surrounding tissues, and thus to make counting easier. If desired, greater cytologic detail can be obtained simply by shortening the period of staining in step 3. In order to make cell counts rapidly, the low-power objective lens may be used. To prevent confusion and in the interests of accuracy, however, scrupulous cleanliness must be observed during the preparation of the slides, especially in preventing the accumulation of dust particles on newly mounted sections during drying of the slides.

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**References and Notes**

1. J. E. Devitt, W. J. Pirozynski, P. B. Samuels, *Proc. Soc. Exptl. Biol. Med.* 83, 335 (1953).
2. N. A. Michels, in *Handbook of Hematology*, H. Downey, Ed. (Hoeber, New York, 1937), vol. 1, p. 232.
3. This investigation has been supported by a research grant (C-2503) from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

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## Role of Teachers in Scholarship Programs

As a local committee, concerned with improving science teaching in the secondary schools as one means of alleviating the present critical shortage of scientists and engineers, we feel that one aspect of our findings should be brought to the attention of *Science* readers. A complaint frequently voiced by high-school science teachers concerns the burden of noninstructional duties they are expected to bear. In addition to the records and forms required by various local, state, and federal agencies, these teachers receive a multitude of questionnaires, data sheets, and various other types of requests for information from many public and private organizations. It seems unfortunate, to say the least, that many of the requests come from well-intentioned groups that are working toward improvement of our educational system.

The citation of a single example will, perhaps, serve to emphasize our point. The National Merit Scholarship Corporation, financed by grants from foundations and industry, recently initiated the largest nongovernmental scholarship program in the history of American education. In this program, about 60,000 seniors, selected from 10,000 high schools, were given a 3-hour competitive examination, after which 4000 students were selected as semifinalists and given the scholastic aptitude test of the College Entrance Examination Board. The 2000 scoring highest in the latter test will become finalists, and from this group approximately 400 will be awarded scholarships ranging from $100 to $2000 based on need. If the average cost of a 4-year scholarship including a "cost of education" grant to the college is $6000 [*Science* **122**, 508 (1955)], then the annual total value of the 400 scholarships would be $600,000. Such a contribution is truly impressive and, at first glance, seems richly rewarding for all concerned. Superior students receive recognition and financial aid, colleges fortunate enough to be selected by these superior students likewise receive recognition as well as financial assistance, while the donors receive favorable publicity not only in connection with the national interest aroused by the program but also through donor-identity which is maintained with all scholarships.

One group, however, seems to have been completely overlooked. The high-school principals and teachers who were "invited" to participate in the program undoubtedly should receive recognition for stimulating and training the superior students who are selected for scholarships. Even if this is denied on the argument that the teachers are merely carrying out their regular duties, they should, at least, receive credit for the role they play in the scholarship program. On the basis of experience in local high schools, we have attempted to calculate the number of man-hours that principals and teachers must contribute in order to participate effectively. We estimate that completion of entry forms, conferences, and record checking to select the most promising students and administration of the first screening examination required 6 hours for each of the 60,000 examinees, or a total of 360,000 man-hours. The time required to compile and submit the information required for the 4000 semifinalists and 2000 finalists is difficult to estimate but would probably bring the total close to a half-million man-hours. Assuming that the teachers' time is worth at least $1 per hour, their contribution would be nearly equal to the total value of the scholarships. Even if these estimates, which are admittedly rough, were reduced by half, the teachers' contribution would still be tremendous.

We wish to make it clear that this statement is not intended as a specific criticism of the National Merit Scholarship Program. All of the high-school principals and teachers contacted felt that the program was well worth the time required for participation. Several pointed out that less valuable programs of various types made more excessive demands on their time. We hope that the example cited may encourage administrators of such programs to examine their procedures with the view of reducing to a minimum the demands placed on secondary-school personnel. In the case cited, we should also like to suggest that some concrete form of recognition be awarded to those high schools that have scholarship winners. This might be done by awards to the teacher or teachers that the winners designate as most responsible for their success, or, if such a plan is not feasible, an unrestricted award to the school for the purpose of improving teaching might be made. We believe that such awards would serve to draw public attention to the important contributions made by the secondary-school teachers.

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## Infectivity of Tularemia Applied to Intact Skin and Ingested in Drinking Water

In his book on tularemia, Simpson described in the following words Francis' demonstration of the penetration by *Bacterium tularense* (*Pasteurella tularensis*) of the unbroken skin: "Francis dropped onto the unbroken skin of normal guinea pigs, suspensions of splenic tissue of guinea pigs recently dead of tularemia; the guinea pigs were dead in five days; at autopsy they showed the

characteristic spotted spleen and liver and the organism was recovered. . . ." (*1*). Although many investigators (*2*) have studied the infectivity of *B. tularense* and although Francis and others (*3*) have repeatedly called attention to infection by penetration, little is known quantitatively about this phenomenon. As a result, the importance of infection by penetration in the spread of tularemia has not been evaluated. Some investigators who are not familiar with *B. tularense* even question the fact of penetration. The set of data presented here is an attempt to determine quantitatively the infectivity of *B. tularense* through the unbroken skin in the albino laboratory mouse. Comparison is made to infectivity through the mucosa of the gastrointestinal tract.

For these experiments, pools of *B. tularense* suspensions were made with bacteria (*Aa strain*) (*4*) grown on blood-cystine-glucose-agar (BCGA) in petri dishes at 37°C for 3 days. The growth on each dish was suspended in 5 ml of sterile physiologic sodium chloride solution and pooled. Tenfold dilutions of these suspensions were made with tap water for testing infection by the cutaneous and oral routes. For intracutaneous infection, dilutions were made with sterile physiologic saline.

Infection by penetration was effected by rubbing 0.01 ml of *B. tularense* suspension through the fur on the abdomen with the smooth end of a glass rod. This operation was accomplished by manipulating a 4.0-mm, 10-g glass rod through a loose sleeve made of 6-mm ID tubing. The sleeve was rotated at the rate of 2 rev/sec for 15 sec, so that the rod spread the bacterial suspension over an area of from 12 to 15 mm in diameter.

In experiment B two types of oral infection were tested. In one series, mice were infected with contaminated drinking water. A bottle containing 55 ml of bacterial suspension was provided for each cage of 11 mice from which water had been withheld for the previous 18 hours. The mice in each cage consumed all of the contaminated water in 24 hours and then were given fresh bottles containing clean tap water. Another series of mice were infected by intubation; 0.2 ml of suspension was introduced into each animal's stomach through a blunt hypodermic needle sheathed with plastic tubing. For intracutaneous inoculation, 0.05 ml of suspension was injected into the right dorsal thigh of the rodent.

The mortality rate by the various routes of infection, observed in experiments A and B, are shown in Table 1. The number of viable *B. tularense* administered was calculated from BCGA plate counts. In experiment A, each intracutaneous inoculation dose of $10^0$ dilution represented 2.4 organisms; and in experiment B, 0.7 organisms. The $LD_{50}$'s were 0.96 and 0.30, respectively. Since the 95-percent confidence limits of these $LD_{50}$'s overlapped one another over a wide range (Table 2), the average value of these $LD_{50}$'s was used as a baseline for comparing results from both experiments. Thus the average lethal infective dose of *B. tularense*, by penetration through unbroken skin under 10 g of pressure, was found to be 2 to 5 times greater than *per os* and more than $10^7$ times that by intracutaneous inoculation.

These values are rough estimates, because many factors that influenced penetration have not yet been analyzed. For example, it is possible that the skins of active, mature mice have microscopic breaks caused by scratches, and so forth. Penetration would then depend on the number and extent of the breaks in the skin. Baby mice have smooth and intact skins; their skins are different from the mature mice in being tender and very thin. Also, baby mice instinctively tunnel under the mother to suck and often rub against one another during the period immediately after the application of the bacterial suspension. Thus the number of bacteria that the mice may have sucked or rubbed into membranes more susceptible than the skin, was not determined.

Table 1. Mouse mortality ratios of the different dosages of *B. tularense* administered orally, intracutaneously, and applied to the skin surface.

| Method | Number of *B. tularense* administered | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $10^{-2}$ | $10^{-1}$ | $10^0$ | $10^1$ | $10^2$ | $10^4$ | $10^5$ | $10^6$ | $10^7$ | $10^8$ |
| | *Number died/number used* | | | | | | | | | |
| Experiment A | | | | | | | | | | |
| Intracutaneous | 0/11 | 1/11 | 8/11 | 10/11 | 11/11 | 11/11 | | | | |
| Applied to the skin: 1 wk old | | | 0/ 7 | | 0/ 7 | 0/ 7 | | 2/ 7 | | 5/ 7 |
| Applied to the skin: 1 mo old | | | 0/20 | | 0/20 | 0/20 | | 1/20 | | 15/20 |
| Applied to the skin: 6 mo old | | | 0/10 | | 0/10 | 0/10 | | 0/10 | | 5/10 |
| Experiment B | | | | | | | | | | |
| Intracutaneous | 0/21 | 3/21 | 6/11 | 21/21 | | | | | | |
| Drinking water | | | | | | 0/22 | 1/22 | 1/11 | 16/22 | 22/22 |
| Intubation | | | | | | | 0/11 | 11/21 | 7/11 | 21/21 |
| Applied to skin | | | | | | 0/21 | 0/21 | 0/21 | 12/21 | |

Table 2. $LD_{50}$ doses of tularemia for oral and percutaneous experimental infection in mice.

| Method and route of the infection | Expt. | $LD_{50}$ dose | |
|---|---|---|---|
| | | Calculated | 95-percent fiduciary limits |
| Intracutaneous inoculation | A | 0.96 | 0.28–3.26 |
| Intracutaneous inoculation | B | 0.30 | 0.14–0.87 |
| Oral, intubation | B | $10^6$ | $(0.13–7.9) \times 10^6$ |
| Oral, drinking water | B | $10^7$ | $(0.36–2.8) \times 10^7$ |
| Through skin of 1 wk old | A | $2 \times 10^7$ | |
| Through skin of 1 mo old | A | $2 \times 10^7$ | |
| Through skin of 2 mo old | B | $5 \times 10^6$ | |
| Through skin of 6 mo old | A | $4 \times 10^7$ | |

Another unknown factor may be the frequent grooming reactions of older mice. Judging by the size of the dose for fatal infection through the mouth, the effect of licking the fur on the infection rate through the skin is likely to be a negligible quantity and may be disregarded. Studies are in progress to ascertain some of the more pertinent factors of skin infection with greater accuracy.

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### References and Notes

1. W. M. Simpson, *Tularemia: History, Pathology, Diagnosis and Treatment* (Hoeber, New York, 1929), p. 22.
2. G. W. McCoy and C. W. Chapin, *J. Infectious Diseases* 10, 61 (1912); C. M. Downs *et al., J. Immunol.* 56, 217 (1947); S. F. Quan, *Am. J. Hyg.* 59, 282 (1954); J. F. Bell, C. A. Owen, C. L. Larson, *J. Infectious Diseases* 97, 162 (1955).
3. E. Francis, *Medicine* 7, 411 (1928); V. E. Simpson, *Ann. Internal Med.* 2, 1092 (1929); F. Meersseman, *Rev. hyg. et méd. prévent.* 57, 591 (1935); E. Francis, *Public Health Repts. (U.S.)* 52, 103 (1937); L. Foshay, *Medicine* 19, 1 (1943); I. J. Tartakow, *N.Y. State J. Med.* 46, 1329 (1946).
4. The *Aa* strain was isolated from lone star tick from Arkansas by C. E. Hopla and C. M. Downs [*J. Kansas Entomol. Soc.* 26, 72 (1953)]. We are grateful to Cora M. Downs for supplying us with this culture.

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