cells, however, could be readily demonstrated: the penicillin-treated cells showed increased ability (in fresh medium) to use lactose as a sole carbon source for growth and division, indicating a decrease in crypticity (*10*).

As Fig. 1 shows, this treatment with penicillin hastened the onset of killing by streptomycin (40 μg/ml) in fresh medium. In addition, it lowered the threshold concentration of streptomycin: 10 μg/ml was sufficient to kill penicillin-treated cells, whereas this low concentration did not affect untreated cells during several generations of growth. Chloramphenicol, which protects normal cells from the lethal action of streptomycin (*6*), also protected penicillin-damaged cells.

In the reverse sequence, brief treatment with streptomycin failed to hasten the subsequent killing by penicillin. Indeed, longer treatment, into the phase of exponential decline of viability, even slowed the killing of survivors by penicillin.

Even after penicillin-treated cells were allowed to grow for one generation without the drug, they were killed faster than untreated cells on exposure to streptomycin. It therefore appears that the damage caused by penicillin is not repaired immediately but persists through further growth and division.

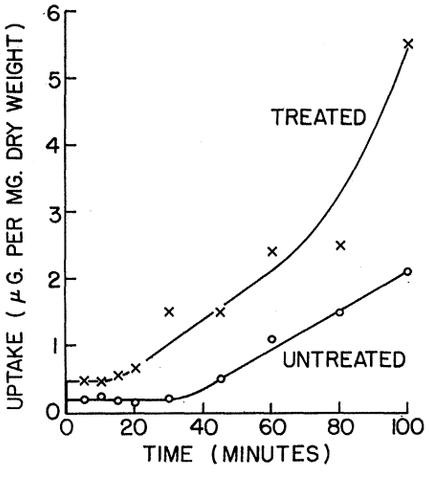

Fig. 2. Effect of penicillin treatment on uptake of streptomycin-C^{14}. The results are from expt. *B* of Fig. 1. Streptomycin-C^{14} (40 μg/ml; 240 counts per minute per microgram) was added. At appropriate intervals 1-ml samples were pipetted onto a membrane filter (Millipore HA), washed twice with 5 ml of water (*12*), and dried. Radioactivity was determined on a thin-window gas-flow counter (Nuclear-Chicago Corp.). The 5- to 20-minute values are based on duplicate samples. All samples had at least 35 counts per minute. The data are corrected for the blank values (about 20 counts per minute) obtained by filtering medium containing streptomycin-C^{14} but no cells. The primary uptake with *E. coli* strain ML-35 is considerably lower, and the secondary uptake is somewhat later, than the values previously found (*12*) with strain W.

To relate the synergism to possible changes in access to binding sites, we observed the effects of penicillin treatment on the uptake of streptomycin-C^{14} (*11*). As noted above, the kinetics of streptomycin uptake follow a biphasic curve: an immediate uptake occurs with both sensitive and resistant cells; and after a lag it is followed, with sensitive cells only, by a gradually increasing secondary uptake (*8*). As Fig. 2 shows, penicillin treatment hastened the onset of the secondary uptake of streptomycin, which represents (*8, 12*) an increase in the number of freely accessible binding sites.

Figure 2 shows that the level of the primary uptake also was increased by penicillin treatment. This effect, however, may involve sites irrelevant to streptomycin action since it occurred to an equal extent with resistant cells, which exhibited (see below) no response to streptomycin.

Our results suggested that penicillin treatment might also increase the sensitivity of streptomycin-resistant cells, if the resistance were associated with a change in the membrane rather than in an intracellular site. Indeed, Lederberg and St. Clair (*13*) reported that with protoplasts of streptomycin-resistant *Escherichia coli* K-12, prepared by penicillin treatment or by diaminopimelic-acid starvation in hypertonic medium, growth on solid medium was inhibited by low levels of streptomycin. They considered this response to be due to "negation of the S^r effect when the wall is stripped." We found, however, that a streptomycin-resistant mutant of *E. coli* ML-35, treated with penicillin as described above, immediately resumed exponential growth in fresh medium A whether or not streptomycin (1000 μg/ml) was present. Furthermore, the presence of streptomycin (1000 μg/ml) did not accelerate the killing during longer exposure to 60 units of penicillin per milliliter. The site of streptomycin resistance thus appears uncertain, and the effect of streptomycin on the growth of resistant protoplasts requires further investigation.

Our findings provide evidence that the damaging effect of penicillin on the growing membrane is responsible for the synergism of this drug with streptomycin. While these findings suggest that membrane damage by either drug may be only a precursor to lethal action of streptomycin at an intracellular site, the possibility has not been excluded that the membrane is itself the site of streptomycin's lethal action (*14*).

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9. During this exposure there was no change in viable number in the pencillin-treated culture, while the untreated culture continued to grow exponentially.
10. Membrane damage was also shown by a decrease in crypticity to *o*-nitrophenyl-β-D-galactopyranoside. This index, however, is not suitable for present purposes since most of the damage could be shown to reside in cells that had been rendered nonviable (to be published).
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Iodine-125 as a Protein Label in Immunology

Abstract. Iodine-125 has the relatively long half-life of 60 days, and emits 27.3- to 35.5-kev photon radiation, permitting counting in an ordinary well crystal scintillation counter with 30 to 40 percent efficiency. The emission of low-energy electrons having the same general energy range as tritium beta radiation electrons allows high-resolution autoradiography. These properties, together with the other advantages of radioactive iodine, make this nuclide a particularly satisfactory label for immunologically active proteins.

There is need for more adequate methods to study tissue and cellular localization and metabolic fate of antigens. Various radionuclides have been used for this purpose. Carbon-14, tritium, and sulfur-35 have the disad-

vantage of being recycled into many tissue components, and of these, only H^3 gives high-resolution autoradiographs. Radioactive iodine has a number of advantages as such a label. It can be coupled readily with proteins through a stable chemical bond, and the metabolic degradation products are rapidly excreted with virtually no recycling of the label (*1*). There is little change in immunologic activity of labeled proteins if only a few iodine atoms are introduced into the protein molecule (*2*). Iodine-131 has been used in most studies, although I^{130} and I^{133} have also been employed (*3*). These isotopes emit rather high energy β-particles and γ-rays, and although they have been used in autoradiographic studies, the high energy of the β-particles does not allow precise localization. Iodine-125, which is a nuclide which may be prepared by thermal neutron activation of xenon (*4*), decays by electron capture followed by a 0.035-kev γ transition. The principal photon radiations, 27.3 to 35.5 kev, may be detected in tissue and serum samples with 30 to 40 percent efficiency with an ordinary well crystal scintillation counter. Accompanying the photon radiation are low energy conversion and Auger electrons which have the same general energy range as the tritium beta radiation (*5*) that permits precise autoradiographic localization. The half-life of 60 days provides long shelf-life for labeled materials, and the low energy dissipation rate, approximately 15 percent that of I^{131}, greatly reduces radiation damage to tagged compounds. Iodine-125 is available commercially, carrier-free in high purity at reasonable cost (*6*).

Formalin-killed *Salmonella typhosa* bacilli were iodinated with I^{125} by the general method previously described (*7*). In the final vaccine suspension, 98 percent of the radioactivity was protein-bound, and there were 3.4×10^6 count/min and 1×10^9 organisms per milliliter. Five rats were given single intravenous injections of 1 ml of the labeled vaccine. Two were killed 15 minutes after injection, and tissues were sampled for measurement of radioactivity and for autoradiography. An average of 77 percent of the injected radioactivity was present in the liver and 2.5 percent in the spleen. The level in the liver was somewhat higher, and that in the spleen was somewhat lower, than the levels found previously with I^{131}-labeled typhoid vaccine (*7*). The remaining three animals were bled on the 6th and 8th days after injection of labeled vaccine, and agglutinin titers were compared with those found in normal rats injected with unlabeled vaccine. Agglutinins were determined with doubled dilution of serum and unlabeled typhoid vaccine as antigen. The agglutinin level for the two groups differed by less than one tube dilution at both intervals after immunization. Autoradiographs were prepared from 6-μ paraffin sections of liver and spleen, with Kodak fine-grain autoradiography stripping plate, AR 10, and an exposure time of 1 week. Sections were lightly stained with hematoxylin and eosin after the emulsion was developed. The results are compared in Fig. 1 with the results of previous studies in which I^{131}-labeled typhoid vaccine and Eastman nuclear track plates type NTB were used for autoradiography (*7*). The clusters of silver grains are smaller and more discrete in the autoradiographs prepared after injection of the I^{125}-labeled vaccine, and in the liver of these animals (Fig. 1*B*), the clusters are definitely localized over single Kupffer cells. With shorter exposure time, grain counts can provide quantitative data on the amount of antigen present. Although differences in the emulsions may contribute to some of the differences noted, this does not appear to be a major factor. Studies are now in progress using I^{125}-tagged flagella to determine tissue and cellular localization and catabolism of these antigens (*8*).

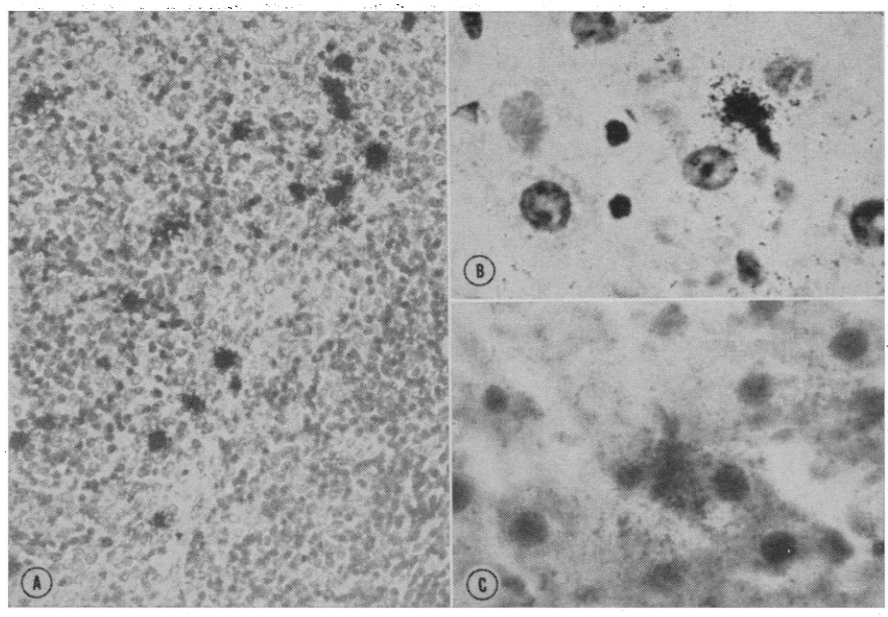

Fig. 1. *A*, Microscopic section of rat spleen 15 minutes after intravenous injection of I^{125}-tagged typhoid bacilli. Autoradiograph prepared with Kodak fine-grain autoradiography stripping plate, AR 10, with 1 week exposure. Section stained with hematoxylin and eosin (× 290). A lymphoid follicle is in the lower right hand corner. Radioactivity is located mainly in the marginal zone. Grain clusters are small and discrete. *B*, Microscopic section of liver from same rat as in *A*; autoradiograph prepared in the same manner (× 830). A discrete cluster of silver grains is located over a Kuppfer cell. *C*, Microscopic section of liver from rat 15 minutes after intravenous injection of I^{131}-tagged typhoid bacilli. Autoradiograph prepared with Kodak NTB nuclear track plate (*7*). Section stained with hematoxylin and eosin (× 830). The cluster of silver grains is apparently located over a Kuppfer cell, although the scatter is too great to permit accurate localization.

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