Extensions of these chemical and biological studies will be detailed later.

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EVIDENCE FOR THE INVOLVEMENT OF GLUTATHIONE IN THE MECHANISM OF PENICILLIN ACTION

Sir:

Several authors have suggested the involvement of —SH groups in the antibacterial action of penicillin (see review[1]). The similarity in the molecular structure of glutathione and of penicillin[2,3] suggests the possible involvement of glutathione in the antibiotic action of penicillin. The following experiments (supported partly by the Cutter Laboratories, Berkeley, California) bear on this question.

When standard penicillin assay plates are flooded with a 1% solution of 2,6-dichlorophenolindophenol in a saturated aqueous solution of sodium bicarbonate the inhibition zones promptly stain intensely blue, and are sharply delineated from the faintly bluish uninhibited background by a narrow colorless rim that locates the ring of enhanced growth that circumscribes each zone. Similar patterns obtain on plates pretreated for five minutes with acetone, which blocks —SH groups from cysteine but not those from glutathione.[4] However, if —SH groups of glutathione are blocked by flooding the plates for ten minutes with a 10% solution of formaldehyde in saturated sodium bicarbonate the 2,6-dichlorophenolindophenol is no longer reduced to the colorless form in the ring of enhanced growth, which now stains deep blue.

The reducing activity in the regions of enhanced growth may be strikingly revealed also by flooding plates with a 0.5% aqueous solution of 2,3,5-triphenyltetrazolium chloride, whereupon these regions become intensely red, while the zones of inhibition remain uncolored. Pretreatment of the plates with 10% formaldehyde blocks this reaction. When such plates are subsequently flooded with the tetrazolium reagent, the red color fails to develop, except at the extreme outer margin of the ring of enhanced growth where it is very faint.

Such simple experiments do not themselves afford unequivocal proof of the participation of glutathione in the mechanism of penicillin action. However, it is generally assumed that —SH groups are involved. Our results indicate that some of these —SH groups are less reactive than those of cysteine, and in view of the work on the role of glutamine revealed by Gale and Taylor[5,6,7] it seems reasonable to deduce the involvement of glutathione.

(1) R. Pratt and J. Dufrenoy, *Bact. Rev.*, **12**, 79 (1948).
(2) E. Fischer, *Science*, **106**, 146 (1947).
(3) R. Pratt and J. Dufrenoy, *J. Bact.*, in press (1948).
(4) L. Genevois and P. Cayrol, *Enzymol.*, **6**, 352 (1939).
(5) E. F. Gale and E. S. Taylor, *Nature*, **158**, 676 (1946).
(6) E. F. Gale and E. S. Taylor, *J. Gen. Microbiol.*, **1**, 314 (1947).
(7) E. F. Gale, *Nature*, **160**, 407 (1947).

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IMPROVED ION EXCHANGE METHOD FOR SEPARATING RARE EARTHS IN MACRO QUANTITIES[1]

Sir:

Previous communications from this laboratory[2] described ion exchange methods by which rare earths were separated from one another in kilogram quantities. The process consisted essentially of absorbing the mixed rare earths on the top of long columns of commercial IR-100 Amberlite resin, in the acid cycle, and then eluting by means of citric acid solutions whose *p*H had been adjusted to the required value by the addition of ammonium hydroxide. While these processes represented an enormous saving, in man-hours required per gram of pure rare earth produced, over the old processes of fractional crystallization, etc., they were not ideal in the sense that when a mixture of rare earths was present, shapes of the elutions bands were such that there was a slight trailing of the preceding rare earth across the main band of the following one. This cut down the amount of pure rare earth obtained from any one pass of the column and frequently resulted in the necessity of recycling considerable quantities of the material.

Considerable work has been done in this Laboratory concerning the nature of the separation process. Good spectroscopic evidence has been obtained that at least four complexes of the rare earths with citrate solution exist and that each of these in turn becomes important as the *p*H range and citric acid concentrations are changed. Recently, it has been found that separation of the rare earths in large amounts can be markedly increased by eluting with a 0.1% citric acid solution in the *p*H range between 5.0 and 5.5. Under these conditions both the front and rear edges of the elution band (amount of rare earth eluted per liter plotted against liters of the eluant passed through the column) are steep and the tops of the eluting bands are flat. Furthermore, the bands separate from each other until the front edge of the one rare earth band is riding on the rear edge of the preceding band. Increasing the length of the column beyond the limit necessary to do this does not separate the bands any further, so there is good evidence that the one rare earth is replacing the

(1) This document is based in part on work performed under Contract No. W-7405 eng-82 for the Atomic Energy Project.
(2) Spedding, *et al.*, THIS JOURNAL, **69**, 2777, 2786, 2812 (1947).