in all tumor-bearing mice. In a few mice osteosclerosis of the femor, similar to that produced by estrogenic hormones[7] is noted. In addition, most, if not all, mice with large transmitted tumors had a cavernous dilatation of the sinusoids of the liver, spleen and adrenals. The weight of the liver was approximately 3 times normal in many mice, accounted for almost exclusively by an increment of blood. Although blood volume determinations have thus far not been made, the observations made suggest a marked rise in blood volume in mice exhibiting the liver change described. The cavernous dilatation of vessels was localized to viscera of the abdomen named. The vessels leading to the grafted tumor were also tremendously distended.

The mouse in which Strain III originated received both X-rays and methylcholanthrene. Extensive experience has shown that the latter alone does not produce ovarian tumors while X-rays alone have done so in almost as high a percentage of the mice as the combined treatment with X-rays and methylcholanthrene. However, a preliminary tabulation of our data shows that in mice receiving the combined treatment the tumors were larger, appeared sooner, were more readily transmissible and more apt to metastasize than those receiving X-rays only. In the latter, most tumors were of the tubular adenoma type, presumably derived from downgrowth of the germinal epithelium, while in the former, most tumors were of the granulosa or lutein cell types.

Summary. Eleven ovarian tumors, induced by X-rays have been transmitted in successive passages. Eight were of the granulosa cell types, 2 were luteomas and one was a tubular adenoma. Histological changes indicate that cells of Strain III secrete estrogens. Mice bearing tumors of this strain also have a cavernous dilatation of the sinusoids of liver, spleen and adrenals. In mice bearing large luteoma of Strain IX there is profound atrophy of the adrenal cortex.

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7 Gardner, W. U., and Pfeiffer, C. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 678.

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A Method for Detection of Streptothricin in the Presence of Streptomycin.

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Streptomycin[1] and streptothricin,[2] two antibiotic agents having almost identical bacterial spectra,[3,4] are both produced by members of the actinomycetes, namely, *Actinomyces griseus* and *Actinomyces lavendulae* respectively. Because of this close relationship and of the still existing difficulty in the identification of the various members of the actinomycetes,[5] it is possible that strains of the organisms producing these 2 antibiotics might be confused. Such a confusion would be particularly undesirable when it is recognized that streptothricin is many times more toxic than streptomycin and in addition, has a distinct delayed toxicity.[6,7] Streptomycin,

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2 Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 207.

3 Robinson, H. J., Smith, D. G., and Graessle, O. E., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 226.

4 Robinson, H. J., Graessle, O. E., and Smith, D. G., *Science*, 1944, **99**, 540.

5 Waksman, S. A., and Henrici, A. T., *J. Bact.*, 1943, **46**, 337.

6 Rake, G., Hamre, D., Kavanagh, F., Koerber, W. L., and Donovich, R., *Am. J. Med. Sc.*, 1945, **210**, 61.

7 Robinson, H. J., Graessle, O. E., Gundel, M., and Silber, R. H., in press.

on the other hand, because of its relative non-toxicity and its active inhibition of gram-negative bacteria both *in vitro* and *in vivo*[3,8] has aroused a considerable amount of interest in the medical field. Realizing the difficulties which would be encountered if samples of streptomycin were to become contaminated with the more toxic streptothricin, it was considered essential that there be developed a means of detecting small amounts of streptothricin in the presence of large quantities of streptomycin. A method for differentiating between streptomycin and streptothricin has been announced recently.[9]

However, a more simplified microbiological test has been devised which can detect as little as 0.1% of streptothricin present as a contaminant in streptomycin. The procedure is based upon a modification of the Stebbins and Robinson[10] cup-assay for streptomycin. The organism used in the test is a strain of an unidentified gram-negative rod* which was found to be very resistant to streptomycin, but sensitive to streptothricin when tested for sensitivity by means of the agar-streak method. By this technic its growth was found to be uninhibited by 16,000 units† of streptomycin per ml, but to be inhibited by 7.5 units of streptothricin per ml. This strain is maintained by daily transfer in broth and a 6-hour culture, which has been incubated at 37°C, is used in the assay in the following manner: pour-plates are made using 10 ml of modified F.D.A. agar[10] which has been seeded while still fluid so as to contain finally a 10^{-4} dilution of the 6-hour culture. After the agar solidifies, penicylinders are set upon it, and the plates are then ready for use according to the regular cup-assay.

For the establishment of a standard curve from which to estimate the amounts of streptothricin present in the samples of streptomycin, a series of solutions are prepared as follows: (1) A stock solution of streptomycin is made in distilled water to contain 2000 units of streptomycin per ml of water. (2) A stock solution of streptothricin is prepared in distilled water to contain 40 units of streptothricin per ml of water. (3) Using solution No. 2, additional solutions are made to contain 20, 16, 8, 4 and 2 units of streptothricin per ml. (4) 0.5 ml quantities of the stock-solution of streptomycin (2000 units per ml) are then combined with 0.5 ml amounts respectively of the 6 solutions of streptothricin (40, 20, 16, 8, 4 and 2 units per ml) to give 6 standard solutions containing 1000 units of streptomycin per ml mixed with streptothricin to the extent of 20, 10, 8, 4, 2 and 1 units per ml. (5) A seventh standard solution consists of 1000 units of streptomycin per ml of water with no added streptothricin. (6) The test samples are made to correspond to the seventh standard solution; namely, to contain 1000 units of streptomycin per ml of distilled water. When prepared, the solutions of the test samples together with those of the 7 standards, which should always be run at least in duplicate with every new test, are put in the cups for assay against the streptomycin resistant strain. The plates are then incubated at 30°C for 16-18 hours, after which they are read for inhibition of growth as shown by the millimeters of zonation produced around the cups. Depending upon the degree of zonation produced, the amount of streptothricin present in the streptomycin can be detected. Table I shows the millimeters of zonation produced by the standard solutions. No sample of streptomycin, whether it was of low potency or of almost pure material, gave any zonation when used in a concentration of 1000 units

[8] Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A., *Science*, 1944, **100**, 103.

[9] Denkewalter, R. G., Cook, M. A., and Tishler, M., *Science*, 1945, **102**, 12.

[10] Stebbins, R. B., and Robinson, H. J., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 255.

* This organism was isolated by Dr. Bodenheimer from the trachea of a patient at the College of Physicians and Surgeons, Columbia University, New York City.

† One unit is that quantity of streptomycin which will just inhibit a given strain of *E. coli* (Waksman) in 1 ml of nutrient broth or agar. When measured in terms of antibacterial activity, one unit of streptothricin is equivalent to one unit of streptomycin.

TABLE I.
Diameters of Cleared Areas Produced by a Standard of Mixtures of Streptothricin and Streptomycin with Water as the Diluent.

Concentration of antibiotic agents	Zonation
1000 units streptomycin per ml only	0 mm
" " " " " plus 20 units streptothricin per ml	18.0 mm
" " " " " " 10 " " " "	16.5 "
" " " " " " 8 " " " "	15.5 "
" " " " " " 4 " " " "	13.5 "
" " " " " " 2 " " " "	12.0 "
" " " " " " 1 " " " "	8.5 "

of streptomycin per ml. However, concentrations of over 1000 units per ml produced moderate zones, for example 7.8 mm for 5000 units and 10.5 mm for 10,000 units per ml.

This culture has been maintained by daily transfer in this laboratory for a period of 8 weeks without showing any indications of changing in its response to streptomycin and streptothricin.

With this test one unit of streptothricin can be measured in the presence of 1000 units of streptomycin. Therefore it would be possible to detect as little as 0.1% of streptothricin if it were present as a contaminant in lots of streptomycin.

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Biological Conversion of n-Butyl Penicillin into a Chemotherapeutically Active Substance.

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Due to its rapid rate of absorption and excretion, it is impossible to maintain satisfactory blood levels of penicillin for prolonged periods after parenteral injection of an aqueous solution of penicillin. These characteristics necessitate either a frequent intermittent or a continuous type of injection. Attempts have been made to prolong the blood levels after parenteral injection of salts of penicillin by decreasing either their rate of absorption[1-4] or their rate of excretion.[5,6]

Since the rapid rate of elimination of penicillin following its parenteral injection is due in part to its extreme solubility in aqueous phases, it would appear advantageous to modify the penicillin molecule in such a manner as to form water insoluble derivatives. This goal has been accomplished by the production of various esters of penicillin. Meyer and his co-workers have reported the preparation of a number of such esters including the methyl, ethyl, n-butyl and benzhydryl esters.[7] All of these compounds were de-

* On leave of absence from the May Institute for Medical Research and the Department of Bacteriology of the College of Medicine, University of Cincinnati, Cincinnati, Ohio.

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2 Parkins, W. M., Wiley, M., Chandy, J., and Zintel, H. A., *Science*, 1945, **101**, 203.

3 Romansky, M. J., and Rittman, G. E., *Bull. U. S. Army Med. Dept.*, 1944, **81**, 43.

4 Trumper, M., and Hutter, A. M., *Science*, 1944, **100**, 432.

5 Beyer, K. R., Peters, L., Woodward, R., and Verway, W. F., *J. Pharmacol.*, 1944, **82**, 310.

6 Rammelkamp, C. H., and Bradley, S. E., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 30.

7 Meyer, K., Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 100.