tamine sulfate, convulsant doses of diisopropylfluorophosphate and pyrogenic doses of piromen produced hyperlipemia in intact animals. 3. The same doses of these substances injected into adrenalectomized and hypophysectomized rats did not produce hyperlipemia. 4. The hyperlipemic action of protamine or DFP is not attributable to direct inhibition of lipoprotein lipase but to a secondary effect mediated through the adrenals and pituitary. 5. The hyperlipemic action of protamine, DFP, pyrogens and possibly other stressors is best explained by release of LM.

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Evaluation of Germicidal Efficiencies of a Group of Antibiotics Tested by Tissue Culture Technic. (22665)

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An improved one-step tissue toxicity technic was proposed recently(1) for evaluation of germicides intended for clinical application.

The germicides were tested for their effect on living embryonic chick heart tissue fragments as well as for their ability to kill bacteria. A number known as the Toxicity Index was calculated from the results, which was defined as the ratio of highest dilution of germicide required to prevent growth of the tissue fragments in 10 minutes to highest dilution required to kill the test bacteria in the same period and under similar conditions. Theoretically an index less than one means that the germicide is more toxic to the test organism than to the embryonic tissue fragments; an index greater than one means that the germicide is more toxic to the tissue fragments than to the bacteria. The smaller the index the more nearly perfect the chemotherapeutic agent. There is no relation whatsoever between a phenol coefficient and a toxicity index.

The same procedure was followed for evaluation of the germicidal efficiencies of a group of antibiotics including: Bacitracin, Chloramphenicol (Chloromycetin) Penicillin G sodium, Polymyxin B sulphate, Streptomycin sulfate, and Terramycin HCl.

Aureomycin (Chlortetracycline HCl) and Achromycin (Tetracycline HCl) precipitated from saline shortly after being prepared and could not be evaluated. Likewise, Erythromycin failed to dissolve in saline.

Methods and materials. Embryonic chick heart tissue fragments from embryos 12 days old were used. Hearts were selected because of the ease by which a constant source of tissue could be obtained. The hearts were removed from the embryos and minced into fragments 0.5 to 1.0 mm in diameter by means of a sharp knife. The fragments were

washed twice in normal saline, then resuspended in saline in the proportion of one heart per ml. This suspension of tissue fragments was handled like a broth culture of bacteria.

Micrococcus pyogenes var. *aureus* is generally used as the organism in germicide testing but since it liquefied plasma it could not be used here; *M. pyogenes* var. *albus* was used instead. Unless the fragments are embedded in coagulated plasma, microscopic observations of Carrel flasks for tissue proliferation could not be made.

The culture was grown on FDA nutrient agar slants incubated at 37°C and transferred daily. The organisms were washed from a 24-hour culture with saline and the suspension standardized in a photometer to correspond to the same turbidity as a No. 1 McFarland nephelometer standard.

For each test, a series of 8 dilutions of antibiotic was prepared in screw cap test tubes measuring 20 x 150 mm. The dilutions were prepared in normal saline according to the following scale of dilutions: Dilutions from 10 to 100 at intervals of 10; from 100 to 200 at intervals of 20; from 200 to 500 at intervals of 50; from 500 to 1000 at intervals of 100; from 1000 to 50,000 at intervals of 1000.

In another series, each screw cap test tube contained a mixture of 1.5 ml saline, 0.5 ml bacterial suspension, and 0.5 ml tissue suspension, making a total volume of 2.5 ml in each tube.

All tubes were placed in a 37°C water bath containing a shaking device and kept in constant agitation for 30 minutes. At the end of 30 minutes, 2.5 ml of the first antibiotic dilution was pipetted into one of the tubes containing the mixture of saline, bacteria, and tissue fragments, making a final volume of 5 ml. The cap was screwed firmly in place and the tube shaken vigorously to wet completely the inner surface before returning it to the water bath. This same procedure was continued at 1-minute intervals for the remaining dilutions in the series. The tubes were kept in agitation in the water bath throughout the test period.

At the end of 9½ minutes, the first tube of the series was removed from the bath and the supernatant aspirated from the fragments. At the end of exactly 10 minutes, the tube was filled with about 25 ml saline to dilute the small amount of antibiotic still remaining in the tube, then set aside. The remaining tubes were treated at 1-minute intervals in the same manner. The fragments were washed 2 more times, then embedded in plasma in Carrel flasks.

The embedding medium consisted of 0.3 ml heparinized rabbit plasma, 0.5 ml Tyrode's solution, and 0.4 ml embryonic extract.* After coagulation of the plasma medium, 1 ml embryonic extract was added as nutrient. Flasks were plugged with paraffined corks and incubated at 37°C for 120 hours before making final readings.

Each flask contained a total of approximately 35 pieces of tissue measuring 0.5 to 1.0 mm in diameter. After all ingredients were added, the Carrel flasks were gently rotated to insure uniform distribution of the tissue fragments before coagulation of the plasma occurred.

Results. The highest dilution of antibiotic that showed no growth of bacteria in the Carrel flask was taken as the killing dilution. The highest dilution of antibiotic that showed no growth of tissue in the Carrel flask was taken as the killing dilution. Several series generally were necessary to determine the killing dilution for both bacteria and tissue. The toxicity index was calculated by dividing the highest dilution of antibiotic required to kill the tissue fragments by the highest dilution required to kill the test organism. Results of tests on a number of well-known germicides (1) have shown iodine to exhibit the greatest degree of germicidal efficiency with a toxicity index of 0.10. This figure indicates that under the conditions of the test iodine is 10 times more toxic to the bacteria than to the chick heart tissue fragments, a remarkable figure for a germicide. Since iodine exhibited the greatest degree of efficiency of the germicides tested, it was used as the basis of com-

* The extract was prepared by mixing 10-day-old minced chick embryos with 5 times its volume of Tyrode's solution and centrifugated at 3000 rpm for 30 minutes. The clear supernatant is the embryonic extract.

TABLE I. Highest Killing Dilution of Antibiotic for Embryonic Chick Heart Tissue and *Micrococcus pyogenes* var. *albus* and Corresponding Toxicity Index.

Antibiotic	Tissue (A)	Bacteria (B)	Toxicity index (A/B)
Iodine	1:2000	1:20,000	.10
Penicillin G sodium	1:12	1:5000	.0024
Streptomycin sulfate	1:20 growth	1:1000	<.02
Polymyxin B sulfate	1:50 growth	1:80	<.63
Terramycin	1:600	1:900	.67
Bacitracin	1:10 growth	1:10 growth	—
Chloramphenicol	1:100 growth	1:100 growth	—

parison for a number of well-known antibiotics. Of the antibiotics tested (Table I) penicillin G sodium exhibited the greatest degree of germicidal efficiency, combining high potency with low tissue toxicity. A toxicity index of 0.0024 indicated that penicillin was more than 400 times more toxic to the test bacteria than to the tissue cells, and approximately 42 times more efficient than iodine. Terramycin with an index of 0.67 was inferior to iodine as a germicide. Streptomycin sulfate was nontoxic to tissue in the highest concentration used and gave an index less than 0.02. A more concentrated solution could not be prepared without sacrificing accuracy. Likewise, Polymyxin B sulfate was nontoxic to tissue in the highest concentration used but killed the test organism in a dilution of 1:80 giving an index of less than 0.63. Chloromycetin and Bacitracin had no effect on tissue or bacteria in the concentrations used which would indicate that their actions were bacteriostatic rather than bactericidal.

Summary. A new method was proposed recently for the evaluation of germicides intended for clinical application. Iodine, with a toxicity index of 0.10 exhibited the greatest degree of germicidal efficiency. The same procedure was followed for the evaluation of a group of antibiotics. Penicillin G sodium gave an index of 0.0024 which indicated that it was more than 400 times more toxic to the test organism, *Micrococcus pyogenes* var. *albus*, than to the tissue cells, and approximately 42 times more efficient than iodine. Results on other antibiotics in order of decreasing efficiency are: Streptomycin sulfate, 0.02—; Polymyxin B sulfate, 0.63—, Terramycin, 0.67. Bacitracin and Chloramphenicol were bacteriostatic and could not be evaluated. Likewise, Aureomycin HCl, Achromycin HCl, and Erythromycin could not be evaluated; the former two precipitated from solution shortly after being prepared, and the latter failed to dissolve in saline.

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Quantitative Determination of C-Reactive Protein by Complement Fixation.*†‡ (22666)

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Interest has been growing in the measurement of serum levels of C-reactive protein as an indicator of inflammatory processes, in particular, those associated with rheumatic fever, myocardial infarction, and cancer. The original determination of this substance(1) was based on the precipitate it forms with pneumococcal C-polysaccharide. Lack of sensitivity led to an immunochemical method,

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