TABLE II. Response of Immunized Mice to Infection with *Mycobacterium tuberculosis* var. *hominis* (H37Rv).

Vaccine inj. (1 mg)	No. of mice*	% lived >29 days
H37RaN	35	82.9
H37RaN (heat killed)	20	50.0
H37Ra	40	77.5
H37Ra (" ")	39	35.9
H37Rv (" ")	38	34.2
BCG	68	82.3
BCG (" ")	71	31.0

Less than 10% of the control mice lived longer than 29 days.

* Mice which died before challenge are not included.

partic, glutamic acids and alanine.

Immunogenic studies. The results of these experiments are found in Table II. Live cells of H37RaN, H37Ra, and BCG strains immunize to a similar degree, as do the killed cells of H37RaN, H37Ra, BCG and H37Rv; however, killing the cells with heat significantly reduced the immunogenic activity of the strains. The values given in the table which indicate the number of mice which lived longer than 29 days are somewhat high. In other experiments in this laboratory, these strains have produced a lesser degree of immunity.

Summary. A variant of the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* which appears to be avirulent for mice and guinea pigs, was developed *in vitro* by continual subculture over 3 years on a nitrogen-free synthetic medium. This new strain, designated H37RaN, resembled the parent H37Rv strain both macroscopically and microscopically, but differed metabolically from the parent strain in its reduced ability to utilize for growth carbon compounds related to the tricarboxylic acid cycle. This strain immunized mice as well as the frequently used immunogenic strains, H37Ra and BCG.

1. Youmans, A. S., and Youmans, G. P., *J. Bact.*, 1954, v67, 734.

2. Youmans, G. P., and Karlson, A. G., *Am. Rev. Tuberc.*, 1947, v55, 529.

3. Youmans, G. P., and Youmans, A. S., *J. Bact.*, 1949, v58, 247.

4. Litchfield, J. T., Jr., *J. Pharmacol. and Exp. Therap.*, 1949, v97, 399.

5. Youmans, A. S., and Youmans, G. P., *J. Bact.*, 1953, v65, 96.

Received June 18, 1956. P.S.E.B.M., 1956, v92.

Notes on Impeded Estrogens. (22547)

RICHARD A. EDGREN. (Introduced by F. J. Saunders.)
Division of Biological Research, G. D. Searle and Co., Chicago, Ill.

Huggins and Jensen(1) have recently discussed a category of estrogenic substances which they have called "impeded estrogens," and defined as compounds producing dose-response curves with shallow slopes when assayed on uterine growth. The prototype of this group of substances is estriol. All of the impeded estrogens studied by Huggins and Jensen possessed either a ketone group at position 6 or a hydroxyl group at 16 leading them to conclude that an oxygenated group at 6 or 16 is necessary for impeded status. They also stated that 16-oxoestrone (16-ketoestrone) did not cause an impeded uterine growth response. Certain of these substances have been shown to have the property of inhibiting the uterine stimulating action of estrone when administered simultaneously with estrone(1,2). Observations made in this laboratory confirmed Huggins and Jensen only in part and suggested that extensions of their conclusions are warranted.

Materials and methods. The assay procedure of Rubin *et al.*(3) has been followed. Mice 23-25 days of age were used; the total dose of test material was administered in 3 divided doses in 0.1 ml corn oil each. The injections were given daily for 3 days and

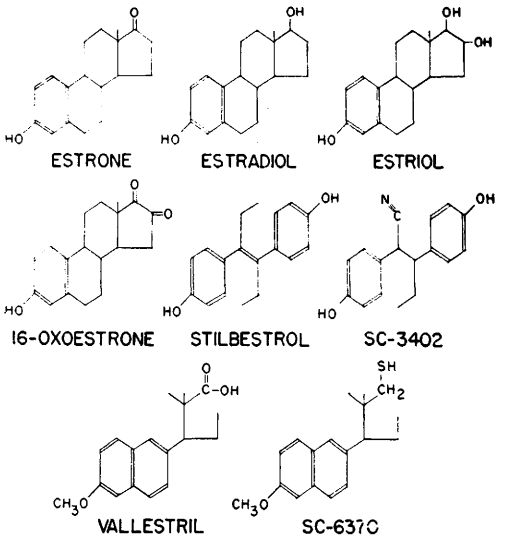

FIG. 1. Structural formulae of estrogenic substances showing widely varying dose-response curve slopes.

autopsy was performed 24 hours after the final injection when the animals were 26-28 days old.

At autopsy the uteri were removed, cleaned of adherent tissues, scored and blotted to express contained fluid, and weighed wet on a Roller-Smith torsion balance. Body weights were also obtained, but were used only as a rough index of age and health of the mice. The mean uterine weights of groups of animals (8-10 per group) at various dose levels were fitted to log-dose-response curves over the linear portions of the regressions using the method of least squares(4,5).

The compounds studied included 3 natural steroids: estrone, estriol, and estradiol 17β; 2 commercial synthetic substances: diethylstilbestrol and 2,2-dimethyl-3-(6-methoxy-2-naphthyl)-pentanoic acid (Vallestril); a steroid analog: the higher melting racemic 2, 3-bis (p-hydroxyphenyl)-valeronitrile (designated SC-3402)(6); a Vallestril derivative: 2,2-dimethyl-3-(6-methoxy-2-naphthyl)-pentanethiol (designated SC-6370; and 16-oxoestrone. Structural formulae for these compounds are presented in Fig. 1.*

* SC-3402, SC-6370 and 16-oxoestrone were synthesized by Drs. Kurt Rorig, Paul Sollman and David Tyner, respectively, of the Chemical Research Division, G. D. Searle and Co.

Results. Regression coefficients for the compounds studied are shown with their standard errors in Table I. These compounds appear to fall into 3 categories, irrespective of potency: Those with extremely steep slopes (estrone, Vallestril), those with slopes of intermediate values (estradiol, diethylstilbestrol), and the impeded estrogens (estriol, SC-3402 and 16-oxoestrone). SC-6370 had a slope which fell between the latter 2 groups and did not differ significantly from either.

Discussion. Under the condition of our test, estrogenic substances appeared to fall into 3 categories based upon slope of the dose-response curve in contrast to the 2 classes obtained by Huggins and Jensen(1). It seems not improbable that more extensive study will uncover additional classes of regression coefficients or perhaps even a spectrum of slopes, especially in view of the fact that SC-6370 had a slope which fell midway between the impeded and intermediate groups. These 3 groups are each made up of both natural estrogens and synthetic substances which can be considered steroid analogs. The compounds with shallow slopes which have been discussed here cannot be categorized on the basis of 6 or 16 oxygenation as was true of structures studied by Huggins and Jensen. The structure-activity relations of the impeded estrogens would appear somewhat more complex than was apparent previously. The impeded nature of 16-oxoestrone also differs from the statements of Huggins and Jensen. The discrepancies between our findings and theirs may result in part from species differences and from the fact that they employed hypophysectomized animals.

Summary. Using the uteri of immature

TABLE I. Regression Coefficients of Certain Estrogenic Compounds. N = No. of groups of 8-10 mice used in computation of slopes.

Compound	N	Regression coefficient
Estrone	23	43.3 ± 3.64
Vallestril	29	41.6 ± 4.13
Diethylstilbestrol	15	29.6 ± 5.98
Estradiol 17β	12	25.5 ± 5.79
SC-6370	9	18.2 ± 3.43
16-oxoestrone	8	9.4 ± 4.82
Estriol	7	9.2 ± .93
SC-3402	6	7.4 ± 1.76

mice as a test object, the slopes of the dose-response curves for several estrogens appear to fall into 3 or more categories. In addition to estriol and 16-oxoestrone, a substance related structurally to stilbestrol showed a shallow slope, and a Vallestril derivative showed a slope steeper than the impeded estrogens, but not as steep as those of certain standard substances.

1. Huggins, C., and Jensen, E. V., *J. Exp. Med.*, 1955, v102, 335.

2. Hisaw, F. L., Velardo, J. T., and Goolsby, C. M., *J. Clin. Endo.*, 1954, v10, 1134.

3. Rubin, Betty L., Dorfman, Adaline S., Black, Lila, and Dorfman, R. I., *Endocrinology*, 1951, v49, 429.

4. Snedecor, G. W., *Statistical Methods, 4th ed.*, Iowa State College Press, Ames. 1946.

5. Fisher, R. A., *Statistical Methods*, 10th ed., Hafner Pub. Co., New York, 1948.

6. Sturtevant, F. M., *Endocrinology*, 1955, v56, 256.

Received June 18, 1956. P.S.E.B.M., 1956, v92.

Determination of Respiratory LD_{50} from Number of Primary Lesions as Illustrated by Melioidosis.* (22548)

ARTHUR M. DANNENBERG, JR.† AND EVA M. SCOTT.
(Introduced by Albert P. Krueger.)
From the Naval Medical Research Unit No. 1 and the Naval Biological Laboratory, Department of Bacteriology, University of California, Berkeley.

Lurie *et al.*(1) have used the number of primary pulmonary foci as a quantitative index of resistance to experimental tuberculosis as well as an index of the virulence of the infecting bacteria. In this report it is shown that in melioidosis, where one primary pulmonary lesion is fatal to mice and hamsters, it is possible to use this lesion-count method to determine the respiratory LD_{50}. This method shows good agreement with the usual titration method and has certain advantages over the latter.

Materials and methods. Mice, Namru strain(2), and golden hamsters were exposed to aerosols of virulent *Malleomyces pseudomallei*, strain 103-67, using a Wells-type atomizer and the method described by Leif and Krueger(3). After the animals died, the lungs were removed and inflated by injecting fixative (FU 48 Technicon) intratracheally. The primary pulmonary lesions were yellow and easily distinguishable from the tan to brown background of the fixed lung parenchyma (Fig. 1).

The number of organisms in the inhaled dose was calculated from colony counts of aerosol aliquots cultured on 4% glycerine beef extract agar. The number of pulmonary lesions was determined either by dissecting the lung if the lesion count was 25 or less, or using the graph shown in Fig. 2 if the lesion count was above 25.

Determination of Respiratory LD_{50} from total number of lung lesions. A single lung lesion in experimental melioidosis in mice and hamsters is always fatal since only progressing pulmonary lesions have been found. Almost without exception, such progressing primary lesions do not occur elsewhere in the body following exposure to aerosols of the organism. It is therefore possible to determine the number of organisms necessary to generate one pulmonary melioidotic lesion, or the "ratio," by dividing the inhaled dose by the number of lung lesions. This "ratio" is an expression of virulence and the respiratory LD_{50} is equal to the "ratio" multiplied by

* This work was supported by the Office of Naval Research and the Bureau of Medicine and Surgery.

† LCDR, MC, USNR.