7. Cytronberg, S., *N.Y. State J. Med.*, 1939, v39-II, 1316.
8. Laurent, E., Laurent, G., *Bruxelles Med.*, 1932, v12, 1396.
9. Schmitz, H. W., *J. Lab. Clin. Med.*, 1922, v8, 78.
10. Sherk, G., *Klin. Wchnschr.*, 1927, v6, 2432.
11. Simmel, H., Künstecher, G., *Deutsch. Med. Wchnschr.*, 1925, v11, 1909.
12. Maupetit, J., *C. R. Soc. Biol.*, 1933, v114, 707.
13. Andresen, K. G., *Biochem. Z.*, 1921, v116, 266.
14. Kornberg, H. L., Davies, R. E., Wood, D. R., *Biochem. J.*, 1954, v56, 355.
15. Robertson, J. D., Williams, P. C., *J. Physiol.*, 1939, v95, 139.
16. Steinitz, H., *Klin. Wchnschr.*, 1928, v7, 1267; *ibid.*, 1927, v6, 949.
17. Von Korff, R. W., Ferguson, D. J., Glick, D., *Am. J. Physiol.*, 1951, v165, 694.
18. Hessel, G., Pekelis, E., Meltzer, H., *Z. f. Ges. Exp. Med.*, 1933, v91, 274.
19. Simici, D., Vladesco, R., Bibesco, Popesco, M., *Arch. Mal. App. Dig.*, 1933, v23, 88.
20. Lucke, H., *Z. f. Ges. Exp. Med.*, 1930, v70, 483.
21. Chabrol, E., Charonnat, R., Cottet, J., *Presse Med.*, 1934, v42, 412.
22. Chabrol, E., Charonnat, R., Cottet, J., Maximin, M., *C. R. Soc. Biol.*, 1933, v114, 464.
23. Fitz, R., Aldrich, M., *J.A.M.A.*, 1922, v79, 2129.
24. Cook, D. L., Lawler, C. A., Calvin, L. D., Green, D. W., *Am. J. Phys.*, 1952, v171, 62.
25. Maluf, N. S. R., *J. Urol.*, 1948, v60, 307.
26. Pendleton, W. R., West, F. E., *Am. J. Physiol.*, 1932, v101, 391.
27. Williams, J. L., Dick, G. F., *J.A.M.A.*, 1933, v100, 484.

Received October 6, 1959. P.S.E.B.M., 1960, v103.

Association of Resistance to Bile Salts and Virulence in *Salmonella typhimurium* Strains* (25492)

CONSTANCE THOMAS AND J. B. WILSON
Dept. of Bacteriology, University of Wisconsin, Madison

A number of factors have been reported to influence virulence of various *Salmonella* species. Among these are endotoxins(1), Vi antigens(2), and resistance to action of complement(3). Certain nutritionally deficient strains have also been shown to be less virulent than those from which they were derived (4,5). Results of our study with *Salmonella typhimurium* indicate that in some cases ability to tolerate high concentrations of bile salts is accompanied by increased ability of the organism to persist in tissues of mice.

Methods. S. typhimurium strains used were differentiated by ability to form colonies on SS agar (Difco) which contains 0.85% Bacto Bile Salts No. 3 (Difco). Experiments in which individual components of this medium were tested showed that bile salts were responsible for the inhibitory effect. A brief description of the *S. typhimurium* strains follows: RIA, a relatively avirulent strain unable to form colonies on SS agar, obtained from H. A. Schneider, Rockefeller Inst. for Med. Research; RIA/SS_1, RIA/SS_2, mutants of RIA able to grow on SS agar, isolated by selection on bile agar made by adding 1.5% agar to liquid bile medium described under *in vitro* tests; RIA/SS_3, mutant of RIA able to grow on SS agar, isolated from a mouse inoculated with 4 x 10^6 cells of parent strain; *rev*, SS sensitive reversion of RIA/SS_1, unable to grow on SS agar. Bacteria for use in mouse virulence tests were grown overnight in penassay broth (Difco) at 37°C with aeration and diluted in saline. Assays for viable cell count were made on nutrient agar with control platings on SS agar. Mice were inoculated intraperitoneally with 0.2 ml of various dilutions. Animals employed in all virulence tests were from a colony of *Salmonella* free white Swiss mice maintained in this laboratory. They were originally obtained from Ft. Detrick, Frederick, Md. Equal numbers of males and females 5 to 8 weeks old were used in each experiment. Animals which died were examined for necrotic foci on liver, and spleen

* This investigation supported by contract with U. S. A. Chemical Corps, Fort Detrick, Md.

TABLE I. Comparison of Virulence of *Salmonella typhimurium* Strains Inoculated Intraperitoneally into White Swiss Mice.

Strain	Cells in inoculum* $\times 10^4$	No. survivors/No. inoculated	+ isolations from spleens†	No. with necrotic foci on livers†
RIA	5	44/52	10	5
SS_1	6	43/64	41	21
SS_2	5	10/18	10	6
SS_3	8	12/20	11	9
rev	7	29/34	8	0

* Avg for all experiments included in Table.
† Figures given are for surviving mice sacrificed 28 days after inoculation.

tissue was cultured on nutrient and SS agar to determine whether salmonellae were present. Surviving mice were sacrificed at 28 days and subjected to similar examination. In some experiments measuremnts for spleen size were made on animals that survived 5 days or longer after inoculations. Prior to this time pathological changes were seldom observed although bacteria were invariably present in spleen tissue. *In vitro tests.* A liquid medium essentially like SS agar was used to determine the effect of bile salts on resistant and sensitive strains of bacteria. Its composition was as follows: proteose-peptone (Difco), 10 g; lactose, 10 g; Bacto Bile Salts No. 3, 8.5 g; sodium citrate, 8.5 g; sodium thiosulfate, 8.5 g; distilled water, 1000 ml. 0.5% sodium desoxycholate could be substituted for bile salts. Known numbers of cells from overnight aerated penassay broth cultures of strains RIA and RIA/SS_1 were added to tubes containing 10 ml of liquid bile medium and incubated with aeration at 37°C. Samples of each culture were removed at intervals up to 24 hours and assayed for viable count on nutrient agar.

Results. Virulence tests. Data from several experiments have been pooled in Table I. The enhanced virulence of bile resistant organisms was evidenced less by ability to initiate fatal infection than by prolonged persistence within the host and alteration of internal organs. Although some overlapping occurred, 3 quite distinct classes resulted when spleen measurements were tabulated. Spleens from mice receiving sterile saline only were nearly all less than 19 mm in length. From those inoculated with strain RIA, lengths to 23 mm were obtained, while in animals which received injections of bile resistant bacteria, most spleens exceeded 23 mm. In some instances adhesions, as well as extensive areas of necrotic tissue, were noted in livers of mice inoculated with bile resistant organisms, while in mice inoculated with sensitive organisms no adhesions were observed.

In vitro tests. Results obtained when strains RIA and RIA/SS_1 were inoculated into liquid bile medium (Fig. 1) indicate that the sensitive strain rapidly loses viability under these conditions. Cells of the resistant strain appeared normal when examined microscopically while the sensitive culture contained many small, spherical bodies and remnants of lysed cells. Motility had almost completely ceased after 4 hours. Loss in viability of sensitive cells could be lessened by diluting samples into peptone broth plus 20% sucrose and

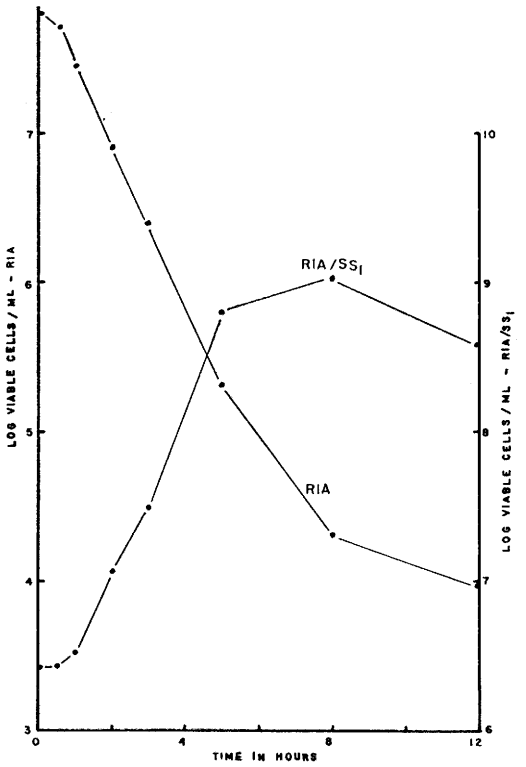

FIG. 1. Effect of liquid bile medium on viability of *Salmonella typhimurium* strains RIA and RIA/SS_1. Final counts, made at 24 hr, were as follows: RIA, 3×10^1/ml; RIA/SS_1, 2×10^8/ml.

incubating for a short time prior to plating on nutrient agar. Since the protective effect was absent when cells were diluted in peptone broth alone, presumably the high osmotic pressure of the sucrose solution was preventing lysis and allowing the cells to return to normal form(6).

Summary and conclusions. A strain of *Salmonella typhimurium,* RIA, unable to grow in the presence of high concentrations of bile salts was less virulent for mice than 3 mutants which were not inhibited under the same conditions. Two of these were isolated on inhibitory medium, the third independently from a mouse which died following infection with avirulent strain RIA. A sensitive reversion from one of the resistant mutants exhibited a virulence pattern similar to that of the sensitive parent strain. Resistant bacteria produced greater damage to internal organs and were able to persist for longer periods of time in host animals although the incidence of fatal infection was only somewhat higher than in mice infected with bile sensitive organisms. Limited studies comparing bile sensitive and resistant strains of *Salmonella paratyphi* B yielded similar but less striking results(7). These observations indicated that salmonellae able to withstand the effect of bile salts were also better able to establish chronic infection in mice leaving relatively large numbers of survivors as carriers of the organisms.

1. Morgan, H. R., *J. Immunol.,* 1948, v59, 129.
2. Felix, A., *J. Hyg.,* 1952, v50, 515.
3. Maaløe, O., *Acta Pathol. Microbiol. Scand.,* 1948, v25, 414.
4. Gowen, J. W., Stadler, Janice, Plough, H. H., Miller, Helen N., *Genetics,* 1953, v38, 531.
5. Bacon, C. A., Burrows, T. W., Yates, Margaret, *Brit. J. Exp. Pathol.,* 1950, v31, 714.
6. Lederberg, J., *Proc. Nat. Acad. Sci.,* 1956, v42, 574.
7. Thomas, Constance, Master's Thesis, Univ. of Wisconsin, 1957.

Received October 7, 1959. P.S.E.B.M., 1960, v103.

Estrogen Antagonisms: Effects of Gluco- and Mineralocorticoids on Estrone-Induced Uterine Growth.* (25493)

RICHARD A. EDGREN AND DAVID W. CALHOUN
Division of Biological Research, G. D. Searle and Co., Chicago, Ill.

The actions of estrogenic hormones are antagonized by a variety of other steroidal substances(1). We have attempted to compare the effects of various types of steroids as antagonists of estrogens. The effects of representatives of each of the 5 major classes of steroid hormones (estrogens, androgens, progestins, mineralo-, and glucocorticoids(2)), were studied in each available estrogen test. Recently we discussed the effects of progesterone, testosterone propionate and 17-ethyl-19-nortestosterone on uterine growth produced in mice by moderate dose of estrone(3). Similar studies also examined quantitative relationships among a long series of relatives of 19-nortestosterone(4). The present communication extends these observations to the effects of adrenal cortical hormones in our standard mouse uterine growth assay.

Materials and methods. The methods employed have been described(3,4,5,6). Briefly, test compound was mixed in solution with 0.3 μg of estrone; total doses were contained in 0.3 ml of corn oil. Mice received 0.1 ml of solution daily for 3 days, starting at 23 days of age, and were sacrificed on the 4th. Mean uterine wet weights from groups of 8-10 mice were used for analysis. The only deviations from these methods were in the separate administration of gluco-corticoids as microcrystalline suspensions and of the aldosterone as an alcohol-water solution.

Results and discussion. The glucocorticoids,

*We are indebted to Dr. R. T. Hill, Dept. of H.E.W., and to Ciba Corp. for 2 mg of aldosterone 21-mono-acetate. Dr. C. M. Kagawa of G. D. Searle and Co. assayed this material in his sodium-retaining test.