and having a purity of 2,050 Lf./mgm. nitrogen, were added and the pH adjusted to 7.2-7.4. The final concentrations of formaldehyde and glycine were thus 0.05 M and 0.1 M respectively, the concentration of toxin being 500 Lf./ml. The mixture was kept at room temperature for four days and then at 35° C. for twenty-five days. The pH was checked daily during the first week and adjusted when necessary. After twenty-five days at 35° C. the preparation was non-toxic, the activity being 480 Lf./ml. (that is, 4 per cent loss). The flocculation time was twice that of the purified toxin.

For control a mixture of the same composition but without glycine was kept at room temperature for four days and then at 35° C. After four days at 35° C., the activity was completely destroyed.

The toxoid was precipitated with sodium trichloroacetate at pH 4.2², dissolved in water, dialysed, sterilized by filtration and adsorbed on aluminium phosphate.

Antigenicity. Half a human dose (that is, 25 Lf.) was injected subcutaneously into guinea pigs of 500 gm. weight. The animals were bled after four weeks. A pool of sera from seven animals contained 2.8 A.U./ml.

This experiment indicates that diphtheria toxin reacts only with free formaldehyde but not with the amino-acid-formaldehyde complex. The antigenic activity of the toxoid obtained was in excess of standard requirements.

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¹ French, D., and Edsall, J. T., "Adv. in Protein Chem.", **2**, 278 (1945).

² Ericsson, H., and Neumüller, C., *Acta Path. et Microbiol. Scand.*, **31**, 124 (1952).

Bacterial Antagonism by *Serratia marcescens*

ARISING out of class-work associated with the separation of mixed cultures of *Serratia marcescens* and *Staphylococcus aureus* (*M. pyogenes* v. *aureus*), an inhibitory effect of the *Serratia marcescens* on the *Staph. aureus* has been observed.

The antibiotic responsible differs from that described by Fuller and Horton¹ in several respects. For example, its presence has not been demonstrated in shallow broth cultures of either the usual peptone-'Lemco'-broth or the medium used by Fuller and

Horton. Again, using a streak-plate method on peptone-'Lemco'-agar, we have been able to show that the inhibiting zone is a maximum after two days incubation at 30° or five days incubation at 22° (Fig. 1).

Organisms sensitive to this antibiotic included *Bacillus megatherium*, *B. subtilis*, *B. mycoides*, *Corynebacterium hoagii*, *Mycobacterium phlei*, *Micrococcus flavus*, *M. roseus*, *M. viscosus*, *Staph. aureus*, *Staph. albus*, *Sarcina lutea*, *Streptococcus lactis* and *Str. faecalis*.

Organisms insensitive to the antibiotic action included *Alcaligenes faecalis*, *A. viscosus*, *Aerobacter aerogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas fluorescens*, *Micrococcus tetragenus* and *Streptococcus cremoris*.

Owing to the lack of suitable facilities, it is not proposed to carry the work any further in these laboratories. The strain of *Serratia marcescens* is, however, available to anyone interested in following up the work.

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¹ Fuller, A. T., and Horton, J. M., *J. Gen. Microbiol.*, **41**, 417 (1950).

Progesterone in Human Blood and Tissues

THE problem as to whether progesterone is present in human peripheral blood, and in what concentrations, has not yet been solved satisfactorily.

Investigations on the content of progesterone in human blood, made with bioassay methods, have yielded contradictory results. Findings obtained by different methods showed enormous differences of concentration. Hoffmann and von Läm¹ found a maximum of 0.004 γ per c.c. of blood in the luteal phase and a maximum of 0.009 γ per c.c. of blood in pregnancy. Forbes² found much higher levels, up to 5.2 γ per c.c. of plasma in the luteal phase and up to 2 γ per c.c. of plasma in pregnancy. On the other hand, hitherto no progesterone has been isolated from peripheral animal³ or human⁴ blood by chemical methods. This led to the conclusion that biological tests are probably lacking in specificity, so that they react upon other substances as well as progesterone.

Considering the great importance of progesterone in the therapy of endocrine disturbances, the solution of this problem seemed indispensable. The development of very sensitive micro-methods permitted an approach to the problem of chemical measurement of progesterone from the experimental side^{5,6}.

Our method consists of the following steps⁶: extraction of the progesterone and partition in different solvents, isolation by paper chromatography and quantitative determination by ultra-violet spectrophotometric means; definitive identification of these final products with colour-tests, ultra-violet spectra with sulphuric acid and infra-red absorption spectrum. By this method we estimate a maximal loss of 20 per cent. The smallest amount of progesterone which can be detected is 0.05 γ per c.c. blood.

The elimination of the pregnanediol complex has been considered as an indirect criterion of the amount of progesterone produced in the organism. For this reason we considered that the best time for finding

Fig. 1. Inhibition of *Staphylococcus aureus* by *Serratia marcescens*