by inserting more than one pellet through a single incision. Smaller doses no doubt would be obtainable by using smaller pellets. No untoward symptoms or toxic effects were noted. Repeated tests of liver function, red blood cell, hemoglobin, white blood cell and blood platelet determination showed no variation from normal.

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Peptone-Dextrose Broth for Use in Studies of Antibacterial Activity.*

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A peptone-dextrose broth for use in measurements of the bactericidal activity of sulfanilamide and sulfapyridine has been described.[1, 2] This broth (PD) consisted of 0.7% Neopeptone (Difco), 0.7% Proteose peptone (Difco), and 0.7% Pfanstiehl peptones plus 0.1% dextrose and 0.5% sodium chloride buffered at pH 7.5 ± 0.1. Bactericidal action with the two compounds mentioned above, in concentrations of 10 mg% or less, was demonstrable only at incubation temperatures above 37°C. This bactericidal action was accompanied by abundant control growth in bacteria-broth mixtures containing no drug. The test culture used in these studies was Beta hemolytic streptococcus strain C 203.

Recent attempts to utilize PD broth, made from currently available batches of peptones, in continued studies of antibacterial activity, have been unsuccessful due to failure to obtain growth in broth control tubes at the elevated incubation temperatures which are required for demonstration of bactericidal action with sulfonamide type compounds. Modification of the peptone dextrose test medium thus became necessary for reproduction of previously reported results and for continuance of these studies.

Preliminary tests indicated that a pH of 7.2 ± 0.1 was optimal for growth of strain C 203 in peptone-dextrose broth. However, broth buffered at pH 7.2, and containing peptones as described above,

* This investigation has been aided by a grant from the John and Mary R. Markle Foundation.

1 White, H. J., and Parker, J. M., *J. Bact.*, 1938, **36**, 481.

2 White, H. J., *J. Bact.*, 1939, **38**, 549.

failed to support growth at 39°C even when the dextrose content was increased to 0.2%. Tests to determine the growth-supporting ability of each of the 3 peptones were then carried out. Tryptose peptone (Difco) was also included in these tests. Broths, buffered at pH 7.2, containing 0.2% dextrose and 0.5% sodium chloride, but with different combinations of the peptones, were compared in terms of their ability to support growth of an initial concentration of approximately 5000 units of strain C 203 (as determined by plate counts). Of the 4 brands of peptone tested, only Tryptose and Pfanstiehl appeared to be suitable for the growth of strain C 203 at 37°C. Neither of these 2 peptones alone supported growth at 39°C. Broths which supported growth at elevated temperatures were obtained when a concentration of 2.0% Tryptose combined with Pfanstiehl in a concentration of from 0.06% to 0.24% was used. Mixtures of 1.0% Tryptose and 1.0% Pfanstiehl, 2.0% Pfanstiehl and 0.06% Tryptose, and certain other combinations of these 2 peptones, failed to support growth at 39°C.

On the basis of these results, our peptone-dextrose medium for antibacterial activity tests was modified as follows:

Peptone-Dextrose Broth (Modified).

Distilled water	1000 cc
Tryptose peptone (Difco)	20.0 g
Pfanstiehl peptone	1.0 g
Sodium chloride	5.0 g
Phosphate buffer†	40.0 cc

Heat ingredients listed above for 10 minutes at 100° C. Add 2.0 g of dextrose, dispense and autoclave for 20 minutes at 112° C to sterilize. Final pH 7.2 ± 0.1.

This modified broth has supported growth of small inoculums of strain C 203 at temperatures above 39°C. Reproduction of the

TABLE I.

Bactericidal Activity of Sulfanilamide Against Strain C 203 at Incubation Temperatures Between 37 and 39.5°C.

Initial Bacterial Concentration	Minimal Bactericidal Concentration of Sulfanilamide in mg%			
	37°C	38°C	39°C	39.5°C
5,000,000	800	600	400	200
500,000	600	600	200	60
50,000	600	400	80	8
5,000	400	200	40	4
500	200	60	8	2
50	80	20	6	1

Initial bacterial concentrations listed above indicate the average number of bacterial units per cc of test mixture, as determined by plate counts. Minimal bactericidal concentrations of sulfanilamide represent the lowest concentrations of drug required to obtain sterilization of test mixtures during 48 hours' incubation.

† Phosphate buffer mixture containing 0.4 M $Na_2 HPO_4 \cdot 12 H_2O$ and 0.1 M $NaH_2 PO_4 \cdot H_2O$ (pH 7.2).

previously determined[2] bactericidal activity of sulfanilamide and sulfapyridine has been obtained in this broth. Its use in measurements of the *in vitro* activity of other sulfonamide-type compounds will be subsequently reported.

Table I contains data which are typical of the results obtained when the modified broth is used as a test medium in studies of the bactericidal activity of sulfanilamide. Our test procedure and criteria of bactericidal activity have been described elsewhere.[2] From this table it is evident that previous observations of a striking increase in the bactericidal power of sulfanilamide, coincident with a temperature change from 37° to slightly above 39°C., are confirmed by the present data.

Summary. (1) Peptone-Dextrose broth (PD), made with currently available samples of Neo-, Proteose and Pfanstiehl peptones, has failed to support growth of beta hemolytic streptococcus strain C 203 at the elevated incubation temperatures which are required for demonstration of bactericidal activity with low concentrations of sulfanilamide. (2) A modified PD broth containing 2.0% Tryptose and 0.1% Pfanstiehl peptones together with 0.2% dextrose and 0.5% sodium chloride buffered at pH 7.2 has been found to be satisfactory for use in studies of bactericidal activity. (3) Results obtained with this modified broth confirmed the conclusions previously drawn in regard to the critical relationship between temperature and the bactericidal activity of sulfanilamide.

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Androgenic Effects from Percutaneous Administration in Castrate Rats.*

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It has been demonstrated[1] that androgens in lanolin ointments are readily absorbed when applied on the skin. The work revealed that testosterone and t-propionate produce, when absorbed through the skin, measurable effects such as those known to result from subcutaneous injections of these hormones in oil. It was pointed out that

* This investigation has been aided by a grant from the Rockefeller Foundation to the University of Chicago.

1 Moore, Carl R., Lamar, Jule K., and Beck, Naomi, *J. A. M. A.*, 1938, **111**, 11.