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.

the superiority of the propionate, demonstrable when injected subcutaneously in oil, was lost in percutaneous administration.

An extension of these studies has appeared advisable and the observations reported herein compare the relative effectiveness of percutaneous applications of testosterone, t-propionate, and methyltestosterone when each is carried in a lanolin and in a tegin base.†

Experimental Procedure. Castrated male rats of different ages constituted 4 experimental groups. Treatment was given by daily applications of the ointment on an area of the neck and shoulders shaved cleanly of hair. Adult males were castrated 21 or 22 days prior to first treatment and young males were castrated 30 days after birth and received first treatment 12 days later. A chosen receptacle, adaptable as an accurate measure, was selected for each group and the average weight of 7 full receptacles of the base containing hormones constituted the daily dose. Animals were so caged as to avoid any contamination by contact with untreated, or differently treated ones. Twenty daily treatments were followed one day later by careful autopsy involving dissection under a binocular dissecting microscope of the ventral prostate gland, coagulating glands, seminal vesicles and periurethral tissue along with the dorsal prostate (for anatomy and terminology see figure 26, Moore '39[2]); tissues were weighed fresh on a torsion balance. Average weights of ventral prostates and seminal vesicles are recorded in the table to demonstrate the magnitude of hormonal responses.

Results. A histological study of the ventral prostate gland and seminal vesicles from this series of treated castrates reveals that in all cases the dosage employed was sufficient to maintain the normal histological secretory condition of these accessory reproductive organs, hence relative effects of the different preparations must be reflected in the weight responses of the organs in question. Ventral prostates revealed well defined light areas in the secretory cells,[3] and secretory cells of the seminal vesicles contained well defined secretion granules.[4] With responses of this intensity the histological conditions are not adequate to determine relative effects but weights of fresh tissues adequately demonstrate quantitative responses.

† These hormones in different cream bases have been graciously prepared by Dr. Erwin Schwenk of Schering Corporation; concentrations were 2 mg of hormone per gram of cream.

2 Moore, Carl R., *Sex and Internal Secretions*, Williams and Wilkins, Baltimore, 1939, Chapter 7.

3 Moore, Carl R., Price, Dorothy, and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 71.

4 Moore, Carl R., Hughes, Winifred, and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 109.

TABLE I.
Effect of Androgen-containing Ointments on Prostate and Seminal Vesicles of Castrate Rats.

	No. of animals	Body wt, g	Ventral prostate, mg		Seminal vesicles, mg	
			Actual	Per 100 g body wt	Actual	Per 100 g body wt
A. Castrated on day 30; 1st treatment 12 days later; 20 daily treatments with 102 mg ointment, containing 0.20 mg hormone.						
Castrated controls	2	167	6.9	4.1	6.5	3.8
Blank tegin	2	142	7.2	5.0	5.6	3.9
Tegin—testosterone	2	170	74.3	43.7	80.4	47.2
" —t-propionate	2	160	57.9	36.1	53.0	33.1
Blank lanol	2	150	8.1	5.4	6.6	4.4
Lanol—testosterone	2	180	70.8	39.3	49.8	27.6
" —t-propionate	2	157	31.0	19.7	19.4	12.3
B. 4½-month males, castrated 21 days prior to treatment. 20 daily treatments with 165.2 mg ointment, containing 0.33 mg hormone.						
Blank tegin	2	217	30.0	13.8	82.6	38.0
Tegin—testosterone	2	295	275.7	93.4	425.5	144.2
" —t-propionate	2	245	182.3	74.4	407.8	166.4
Blank lanol	2	255	31.4	12.3	89.5	35.0
Lanol—testosterone	2	280	203.7	72.7	449.9	160.6
" —t-propionate	2	305	102.8	33.7	111.4	36.5
C. 3½-month males, castrated 22 days prior to treatment. 20 daily treatments with 101.6 mg ointment, containing 0.203 mg hormone.						
Castrate control	1	268	26.4	9.8	89.6	33.4
Tegin—testosterone	2	299	185.2	61.9	404.3	135.2
" —methyl-test.	2	264	251.3	95.1	762.1	288.6
Lanol—testosterone	2	279	140.0	50.1	333.7	119.6
" —methyl-test.	2	257	212.4	82.6	357.0	138.9
D. 4½-month males, castrated 22 days prior to treatment. 20 daily treatments with 179.8 mg ointment, containing 0.359 mg hormone.						
Castrated controls	3	334	46.6	13.9	100.0	29.9
Tegin—testosterone	3	277	367.8	132.7	701.2	253.1
" —methyl-test.	3	266	299.0	112.4	712.7	267.9
Lanol—testosterone	3	281	249.1	88.6	478.2	170.1
" —methyl-test.	3	302	283.9	94.0	506.7	167.7

Table I reveals the actual fresh weight of ventral prostates and seminal vesicles as averages from similarly treated males, as well as the weight of these two organs per 100 g of rat.

Close attention to this table reveals several points of interest: (1) In Group A the tegin and lanol bases alone, applied daily to castrate rats as controls, failed to effect appreciably the weight of these organs, hence responses to the same bases carrying hormones clearly demonstrate the effect of the hormone. (2) Comparisons of equal weights of crystalline testosterone with t-propionate (A series 0.2 mg; B series 0.33 mg) reveal the superiority of free testosterone whether the substances are carried in a tegin or lanol base; the one exception occurs in series B in which seminal vesicle response, computed as per 100 g of rat, gives a slightly higher value for t-propionate administered in the tegin base. (3) Methyl-testosterone applications

in comparison with applications of free testosterone (C series 0.203 mg; D series 0.36 mg) produced slightly higher end organ weights in 13 cases and slightly lower weights in 3 cases. Whereas the differences are not profound in series D the trend in all compared treatments employing these 2 hormones suggests a slight advantage for methyl-testosterone. (4) A comparison of effects of the same hormone (testosterone, t-propionate, m-testosterone) carried in the 2 different bases, and in all different dosages employed, suggests that tegin provides a more effective base than lanol. It is apparent from the table that end organ weights were greater after application of hormones in the tegin base in 30 comparisons as against 2 comparisons in which hormone in the lanol base produced greater weights (Ser. B comparing free test on sem. ves. weight).

Conclusions. Based upon weight responses evoked in the end organs of castrated male rats, the results obtained with androgens, percutaneously administered, indicate the effectiveness to follow the descending order—methyl-testosterone, free testosterone and t-propionate (lowest), although the differences between the first two substances are not great. It is further suggested that androgens carried in a tegin base are more effective than when carried in a lanol base, since end organ weights were greater in 30 of the 32 compared pairs. With all dosages the histologically indicated secretory normality was apparent for both ventral prostates and seminal vesicles.

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