TABLE II.—COMPARISON OF TACKINESS OF SEVERAL CREAM PREPARATIONS RUBBED IN THE SKIN FOR 20 SECONDS

Sample	Withdrawal Wt., Gm.	Time for Withdrawal, sec.
1	4	129, 165, 234, 212
2	4	101, 65, 71
3	2	6, 2, 2

was not too shear sensitive. Alternately, a weighed geometric spot was applied to the glass. In either case, the finger was brought to bear in a similar manner.

For actual measurement on human skin, the sample was applied in the appropriate manner and the finger applied directly with the 500-Gm. load. The cup was rotated back and clear for this. In general, the finger was contacted for 20 seconds before withdrawal; voluntary motion or quivers often induce the break-away after longer periods of contact. Typical data are shown in Table II.

DISCUSSION

The fundamental equations covering the use of the tackmeter were derived by Green (1), and the rheology of tack has been discussed by Bikerman (2).

The time required for break is inversely proportional to the viscosity function of the sample, the weight used for drawing, and the square of the sample film thickness, but is proportional to the fourth power of the radius of the finger. Thus, it is possible to derive a viscosity term from the tackmeter to define the sample film. In this work, it has not been necessary, or even desirable, to do this, since all desired comparisons can be readily made in units of withdrawal time and pulling weight. If, however, some knowledge of absolute viscosities of dried films is desirable, then the instrument may be calibrated by standards and used as a rheometer.

Obviously, the choice of finger dimensions and film thickness permit an extremely wide range of study. In the study on human skin, the bearing weight and duration of contact could conceivably be of different magnitudes in some applications.

Since the primary value of this instrument is for empirical testing, the finger surface may readily be modified to permit the attachment of dressings if it is felt to be important.

SUMMARY

The tackmeter described permits the measure of tack of lotions, creams, and ointments. It may be utilized on human skin and as a laboratory test.

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Assay Methods for Total Neomycins B and C

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Data from assays of neomycin C sulfate and mixtures of neomycin C sulfate and neomycin B sulfate were obtained using six cylinder-plate methods. Statistical analyses show that with three commonly used methods, neomycin C responses are only 35 to 50 per cent of neomycin B responses. With three modified methods, the response of neomycins B and C are approximately equal.

NEOMYCIN FERMENTATIONS produce two major antibacterial components—neomycin B and neomycin C. Commercial preparations are composed mostly of B, but may contain as much as 50% C (1). Kaiser (2) found that the U.S.P. neomycin standard contained about 15% C. The present assay systems do not give equivalent responses for B and C and with significant differences in C content between the test and standard preparations, the assay results are not in terms of total neomycin relative to the standard.

Some problems in the assay of neomycin were discussed in a previous paper (3). The purpose of this study was to compare responses of neomycin B and neomycin C obtained by three assay methods commonly used and by three methods which were designed to and did reduce differences between the responses of neomycins B and C.

EXPERIMENTAL

Neomycin Preparations.—The physical and chemical properties of the neomycin B preparation and the neomycin C preparation were described by Ford, *et al.* (4). With a radioisotopic assay procedure (2), the neomycin B preparation was found to contain 11% C, while the neomycin C preparation was found to be free (<1%) of neomycin B. Both preparations were sulfate salts, and potency values for Tables I–III were recorded as neomycin sulfate. The potency values for Table IV are recorded in terms of neomycin base equivalent.

The Upjohn Co. control standard, potency 742 mcg./mg., was used to determine the potencies of commercial neomycin sulfate lots (Table IV).

Preparation of Neomycin Solutions.—The neomycin powders were weighed after drying in a vacuum oven at 60° for 3 hours. One milligram per milliliter stock solutions were made with distilled water. Stock mixtures in ratios of 1:1 and 9:1 (9 neomycin B + 1 neomycin C) were prepared from these solutions. Working solutions were prepared by diluting the stock solutions with the buffer appropriate to the method.

Assay Methodology.—The cylinder-plate method was used for all assays. Twenty by 100-mm. plastic Petri dishes (Plastomatic Corp., Malvern, Pa., cat. No. 94), were used with glazed porcelain tops. Six stainless steel cylinders (inside dimensions 6 × 10 mm.), were placed on each plate with a Shaw

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TABLE I.—AVERAGE POTENCIES OF NEOMYCIN C AND NEOMYCIN C:B MIXTURES

Assay Method	C: No. Assays	C: Av.[b] Potency	C: 95% C.L.	1C:1B[a]: No. Assays	1C:1B[a]: Av.[b] Potency	1C:1B[a]: 95% C.L.	1C:9B[a]: No. Assays	1C:9B[a]: Av.[b] Potency	1C:9B[a]: 95% C.L.
1	10	452	409–499	5	666	588–756	11	865	789–948
2	9	379	354–406	11	678	626–734	11	898	848–950
3	10	373	347–402	6	660	620–702	6	877	854–900
4	8	1077	982–1132	13	1054	982–1132	13	975	927–1028
5	10	1153	1064–1249	7	1009	926–1100	5	967	778–1200
6	12	1108	1047–1171	11	961	894–1033	9	927	872–985

[a] Ratios of the neomycins C and B preparations; the neomycin B preparation contained 11% C. [b] Micrograms of neomycin B sulfate per milligram.

TABLE II.—STANDARD ERRORS ESTIMATED FROM AMONG ASSAY VARIATION

Assay Method	S.E. Of Log Potency	S.E. As % of Potency
1	0.0506	11.7
2	0.0430	9.9
3	0.0337	7.8
4	0.0441	10.2
5	0.0534	12.3
6	0.0405	9.3
Av.	0.0445	10.2

dispenser. Using a 3 × 3 assay design (5), three concentrations of neomycin B were applied to each plate alternately with three corresponding concentrations of (*a*) neomycin C, (*b*) 1:1 mixture, or (*c*) 9:1 mixture. There were nine replicate plates in each assay. The assays were run by two technicians over a period of 6 months. Individual assay results and weighted mean averages were calculated on the IBM 1620 computer.

Media.—All media ingredients were purchased from Baltimore Biological Laboratories (BBL) or Difco Laboratories (Bacto).

Preparation of Inoculum.—The inoculum for the assay plates in all but *Method 4* was a suspension of *Staphylococcus aureus* FDA 209P prepared as follows. Two BBL trypticase soy seed agar (TSA) slants, cultured overnight, were washed with BBL antibiotic assay broth (AAB) into a Roux bottle containing 300 ml. TSA. The Roux bottle was incubated for 18 hours at 32°, after which the growth was suspended in 40 ml. AAB. The suspension was adjusted so that a 1:10 (1 + 9) dilution with distilled water gave a 65% light transmission at 650 mμ in an 18 × 150-mm. test tube with a Lumetron model 401 spectrophotometer. The undiluted suspension was stored at 4° for as long as 2 weeks. The inoculum rate in *Methods 1, 2, 5,* and *6* was 0.3 ml. suspension per 100 ml. agar at 48°. The inoculum rate in *Method 3* was 0.05%. The inoculum in *Method 4* was 0.3% of a *Bacillus subtilis* UC 564 aqueous spore suspension (6) containing 2 × 10^9 spores/ml.

Procedures Currently Used for Neomycin Determinations.—Dilutions of 10, 20, and 40 mcg./ml. were made from the neomycin B stock solution and the 1:1 and 9:1 stock mixtures with 0.1 *M* pH 7.9 potassium phosphate buffer. However, because neomycin C was expected to have a smaller response than neomycin B, the neomycin C stock solution was diluted to 20, 40, and 80 mcg./ml.

Method 1.—Assay plates were prepared with 10 ml. base layers and 5 ml. *S. aureus* seed layers of BBL neomycin assay agar.

Method 2.—Assay plates were the same as in *Method 1*, except that 0.5% sodium chloride was added to the neomycin assay agar before autoclaving.

Method 3.—Assay plates were prepared with 15 ml. trypticase soy base layers and 4 ml. TSA seed layers inoculated with 0.05% *S. aureus*.

Methods for Total Neomycins B and C.—Neomycin C working solutions were equal in concentration to working solutions of neomycin B and B:C mixtures. The concentrations used in each assay are given in Table III.

The medium designated yeast beef agar (YBA)

TABLE IV.—POTENCIES OF COMMERCIAL NEOMYCIN SULFATE AS BASE EQUIVALENT

Neomycin Sulfate Lots	Neomycin C Sulfate[a], %	Potency, mcg./mg.[b] *Method 3*	Potency, mcg./mg.[b] *Method 6*	Corrected for C Bias from *Method 3*[c]
B	11	731	...	791
C	100	241	...	791 (assumed)
1	21	590	691	685
2	19	588	738	669
3	27	549	651	663
4	12	639	688	693
5	10	642	647	687

[a] Determined by radioisotopic assays. [b] Micrograms of neomycin standard per milligram. [c] Estimated by using the 241 value for 100% C and the 791 value for total neomycin.

TABLE III.—DOSES AND AVERAGE ZONES FOR NEOMYCIN C *vs.* NEOMYCIN B

Assay Methods	No. Assays	Neomycin B[a] d	Neomycin B[a] z	Neomycin B[a] d	Neomycin B[a] z	Neomycin B[a] d	Neomycin B[a] z	Neomycin C[a] d	Neomycin C[a] z	Neomycin C[a] d	Neomycin C[a] z	Neomycin C[a] d	Neomycin C[a] z
1	10	10	16.2	20	17.6	40	18.4	20	16.6	40	17.6[b]	80	18.6
2	9	10	15.9	20	17.6	40	19.2	20	15.2	40	17.2[b]	80	18.5
3	10	10	12.8	20	14.4	40	15.9	20	12.2	40	13.8	80	15.2
4	4	5/7[c]	13.5	10/14	15.0	20/28	16.1	5/7	13.9	10/14	15.1	20/28	16.3
	4	10	14.6	20	15.6	40	16.7	10	14.5	20	15.8	40	16.8
5	5	20	16.4	40	17.9	80	19.3	20	16.7	40	18.4[b]	80	19.7
	3	7	14.4	14	16.2	28	17.3	7	14.9	14	16.4[b]	28	17.4
6	12	10	16.6	20	18.0	40	19.1	10	16.8	20	18.2[b]	40	19.3

[a] d = Dose, mcg./ml.; z = average zone diameter, mm. [b] Significant curvature on C. [c] Two assays 5, 10, 20; two were 7, 14, 28.

was prepared as follows. Bacto peptone (0.6%), 0.3% Bacto yeast extract, and 0.15% Bacto beef extract were dissolved in distilled water, and the solution was adjusted to pH 7.9 with 1 *N* sodium hydroxide. One and one-half per cent Bacto Noble agar was added, and the medium was autoclaved.

Method 4.—Plates were prepared with 20 ml. base layers of BBL streptomycin assay agar with yeast extract and 4 ml. YBA layers inoculated with 0.3% *B. subtilis*. Working solutions were prepared by diluting the stock solutions with 0.1 *M* pH 7.9 tris-hydroxymethyl aminomethane (tris) buffer.

Method 5.—The medium was YBA with 0.2% sodium chloride added before autoclaving. Assay plates were prepared with 10 ml. base layers and 5 ml. *S. aureus* seed layers. Neomycin stock solutions were diluted with 0.1 *M* pH 7.9 tris buffer as in *Method 4*.

Method 6.—Assay plates were the same as in *Method 5*, except that 0.2% glucose was added to the seed agar before autoclaving. Working solutions were prepared by diluting neomycin stock solutions with 0.25 *M* pH 7.9 tris buffer.

RESULTS AND DISCUSSION

Methods 1, 2, and *3* (with some variation in the preparation of inoculum and in volume of agar medium) are commonly used for neomycin determinations. Table I indicates that these methods have a 50 to 65% negative bias in the response of the neomycin C preparation relative to the neomycin B preparation. This bias is also evident in the assays of B:C mixtures.

With *Methods 4, 5,* and *6*, the neomycin C responses were 98 to 125% of the responses of the neomycin B preparation. It is not certain, however, that the high responses reflect a positive neomycin C bias because the actual potencies of these preparations are unknown. In fact, potency values of neomycin sulfate preparations as free based equivalent in general are relative to standards of uncertain purity. The present U.S.P. standard with an assigned value of 700 mcg. neomycin base equivalent per milligram was reported to contain 13.7 to 18.6% neomycin C (2). Potency estimates as now obtained are dependent on the extent of neomycin C bias in assay method and the B-C content in the test and standard preparations.

The standard errors of assay potency (Table II) did not differ significantly among the six methods; the average standard error of potency was 10%. A considerable number of individual assays failed the usual validity tests specified in U.S.P. XVI (7). However, all assays were included in the analyses for Tables I, II, and III.

The average zone diameters for neomycin C compared to neomycin B in all six methods are given in Table III. The neomycin C responses showed significant curvature with all but *Methods 3* and *4* and *Method 5* at high doses. Among assays that did not pass validity tests, curvature was the main cause of failure. This may reflect a basic difficulty in comparing preparations of high neomycin C content with preparations of high neomycin B content.

Some commercial lots of neomycin sulfate varying from 10 to 27% neomycin C were assayed by *Methods 3* and *6* against The Upjohn Co. (TUC) control neomycin standard which contained only 4% neomycin C and had an assumed value of 742 mcg. base equivalent per milligram. These two assays were chosen for comparison because both use *S. aureus* with salt-free media. As expected, the assay results with *Method 3* were consistently lower than with *Method 6* due to the neomycin C bias. An approximation of the extent of bias was made by using the *Method 3* potency results on the B and C preparations which were 731 and 241 mcg. base equivalent per milligram, respectively. Knowing the response of C alone (no B present in the C preparation), the following determinations were made: 0.11C + 0.89B = 731 (*Method 3* value, Table IV) 1.0C = 241, 0.11 (241) + 0.89B = 731 = total neomycin response, and total neomycin base = 791.

Using the same method for calculation, corrections were made for C bias on *Method 3* results for commercial neomycin sulfate lots (Table IV). The corrected values are rough estimates and should not be regarded as absolute. Exact measurements would consider the 4% C in the TUC standard and the sulfate content of each preparation as well as the neomycin base content for which a method is not available. It is obvious that the corrected values are relatively close to the values obtained with total neomycin *Method 6*.

Of the three total neomycin methods, the *B. subtilis* method (*Method 4*) is the most applicable in routine operation for three reasons: (*a*) the media are commercially available; (*b*) the test organism as a spore suspension is stable for long periods; (*c*) the inhibition zone edges are sharp and easily read.

In the development of total neomycin assay methods, one of the major considerations was the effect of the ionic strength of the assay medium on the response of neomycin. Salt increases the slope of the dose-response curve (6), [as does Noble agar (3)], but salt also adversely affects the activities of the neomycins (3, 8, 9). Neomycin C is affected more than neomycin B so that by decreasing the ionic strength of the assay medium, the response of neomycin C relative to neomycin B is increased. In a medium such as the YBA medium used in this study, the neomycin C response can be made to approximate the neomycin B response by adjustment of ionic strength.

SUMMARY

The commonly used assay procedures for neomycin have a negative bias for neomycin C. The response of neomycin C in agar-diffusion assays is affected by the ionic concentration of the medium, more so than is B. A total of six assay methods is described which include three presently used procedures with a neomycin C bias and three procedures which additively assay the B and C components in neomycin.

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