growth in the body may be retarded by even small concentrations of the antibiotic. One should expect, therefore, that such resistant strains would have difficulty in maintaining themselves due to phagocytic mechanisms available *in vivo*. The impairment of phagocytosis by streptomycin should be studied in greater detail so that this important cellular factor in immunity may be more clearly defined.

Summary 1. The activity of streptomycin on a susceptible strain of *Staphylococcus aureus* was not changed in the presence of serum or whole blood. 2. The addition of fresh whole blood with or without immune serum did not augment streptomycin activity. 3. A resistant strain of *Staphylococcus aureus* was inhibited during the first 24 hours by streptomycin in low concentrations. This effect was augmented when serum was added to the medium. 4. Direct growth curves indicate that this inhibition by streptomycin of a resistant strain was attributable to interference with growth of the organism rather than inactivation of labile constituents. 5. Phagocytosis in the presence of high concentrations of streptomycin appears to be impaired. 6. The bacteriostatic activity of streptomycin was manifest on both susceptible and resistant strains. With the latter, this inhibition was transitory. The bacteriostatic mechanism and bactericidal mechanism are dissociated phenomena.

16135

Culture Procedures in Microbiologic Assays with *L. arabinosus* and *L. casei*.

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In the application of microbiological assay procedures, there have arisen questions relative to possible differences in the response of the test organisms as a result of variations in handling the stock cultures. Several investigators have mentioned the possible influence of stock culture media and conditions to which it is subjected on the subsequent response of an organism under assay environment, but few have made studies to determine the nature and extent of these influences. The most detailed work which has come to our attention has been the culture studies on *L. arabinosus* and *L. casei* by Nymon and Gortner.[1]

The present report is concerned with some notes made on frequency of transfer of stock cultures, preparation and dilution of inoculum and temperature and time of incubation of the assay tubes as these factors affect the assay.

Observations were made for a period of 6 months on the effect of frequency of transferring stock cultures of *L. arabinosus* on the subsequent growth response of this organism to graded amounts of nicotinic acid in the U.S.P.[2] assay medium. From a stab culture of *L. arabinosus* carried in agar medium (agar 1.5%, dextrose 1% and yeast 1%) 5 transfers were initially made into fresh agar medium of the same composition. After incubation at 30°C for 24 hours these cultures were stored in a refrigerator. One, designated "original", was maintained in the original stab without further transfer. The others were transferred at intervals of one, 2, 3, and 4 weeks respectively throughout the experiment. After incubation of the transplant for 24 hours it was returned to the refrigerator

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1 Nymon, M. C., and Gortner, W. A., *J. B. C.*, 1946, **163**, 277.

2 First U.S.P., XII Bound Supplement, 1943, 76.

TABLE I.
Effect of Frequency of Transfer of *L. arabinosus* on Titration Values in Nicotinic Acid Determinations After 24 Weeks.

Micrograms standard nicotinic acid	Titration values in cc 0.1N NaOH				
	0.1	0.2	0.3	0.4	0.5
Original culture	3.3	4.8	6.4	7.4	8.3
Culture transferred weekly	3.3	5.1	6.9	8.4	9.0
" " 2-week intervals	3.3	5.1	7.1	8.2	8.7
" " 3- " "	3.4	5.2	7.2	8.3	8.6
" " 4- " "	3.4	5.1	7.1	7.9	8.6

until the next transfer period. Periodic comparisons of microbiological standard response curves obtained with each of these cultures showed that there was no difference after 20 weeks. Only at the end of 24 weeks was there evidence that the "original" culture gave titration values differing from those obtained with the other 4 cultures. Results are given in Table I. Infrequent transfer over a longer period might eventually produce adverse effects, but these experiments indicate a remarkable stability of the *L. arabinosus* culture used.

The results of experiments to determine whether differences in incubation temperatures of the assay tubes caused any change in the behavior of *L. arabinosus* are presented in Table II. Two series of tubes incubated at 34°C and 35°C respectively showed identical titration values after 3 days. Two series of assay tubes incubated for periods of one, 2, and 3 days at 34°C and 37°C respectively, gave practically the same titration values after the first and after the second day but gave slightly higher values in the 34°C series after 3 days. In a third experiment incubation temperatures were held at 30°C and 33°C for periods of one to 6 days inclusively with no significant differences observed between any of the paired members. Titration values increased with increased period of incubation reaching a peak on the fifth day and remaining the same through the sixth. Duplicate titrations, the averages of which are given in the table, were more uniform after 3 days incubation than at the end of a shorter period.

TABLE II.
Effect of Temperature and Time of Incubation of *L. arabinosus* on the Titration Values in Nicotinic Acid Determinations.

Exp. No.	Temp. of incubation, °C	Time of incubation, days	Titration	
			Blank	Maximum
			cc 0.1N NaOH	
1.	34	3	1.4	9.6
	35	3	1.4	9.7
2.	34	1	0.6	5.7
	37	1	0.6	5.7
	34	2	0.7	8.3
	37	2	0.7	8.3
	34	3	0.8	9.1
	37	3	0.8	8.4
3.	30	1	1.0	6.1
	33	1	1.1	6.1
	30	2	1.2	8.3
	33	2	1.2	8.2
	30	3	1.3	9.3
	33	3	1.2	9.3
	30	4	1.2	10.4
	33	4	1.3	10.2
	30	5	1.2	11.2
	33	5	1.3	11.4
	30	6	1.3	11.1
	33	6	1.4	11.1

Inoculum is usually prepared from a broth culture after incubation for 20-24 hours in the basal medium used in the assay. It has been found, in the case of *L. arabinosus*, that subsequent storage of the incubated culture in a refrigerator for an additional 24 hours before preparation of the inoculum has no effect upon the assay results.

L. casei is known to be more susceptible to environmental influences than *L. arabinosus*. In Table III are data showing the effects of age and concentration of inocula of *L. casei* on the response of this organism to graded amounts of folic acid. A modification of the U.S.P. niacin medium[2] was used for these comparisons. Inocula were prepared from broth cultures incubated at 30°C for 16, 24, 40, and 48 hours. In addition there were included 16 hour broth cultures to which had been added a preparation containing strep-

TABLE III.
Effect of Age of Culture and Concentration of Inoculum on Titration values in Folic Acid Determination Using *L. casei*.

Micrograms standard folic acid	0	.0002	.0004	.0006	.0008	.001
	cc 0.1N NaOH					
16 hr culture—undil.	3.4	5.5	6.9	8.3	9.3	10.5
16 " " (containing strepogenin)—undil.	3.1	5.0	6.5	7.9	9.1	10.4
16 " " " " "	3.1	5.1	6.5	7.8	9.1	10.2
24 " " undil.	3.3	5.2	6.7	8.1	9.4	10.5
24 " " dil.	3.1	5.1	6.5	7.8	8.8	10.1
40 " " undil.	1.2	1.3	1.4	1.6	1.8	1.85
40 " " dil.	2.8	4.5	5.9	6.9	7.8	8.7
48 " " undil.	1.4	1.9	2.4	2.9	3.2	3.5
48 " " dil.	2.4	4.2	5.2	6.2	7.0	7.8

ogenin that was found to be active in accelerating the initial growth of *L. casei*, on purified media as originally reported by Sprince and Woolley.[3] An undiluted portion of each inoculum was tested as well as a portion diluted to the same turbidity as the 16 hour preparation without the strepogenin. There was little difference in the response to graded amounts of folic acid shown by inocula prepared from the 16 hour culture, with or without strepogenin, and the 24 hour culture. Dilution of the inoculum from either the 16 hour culture with strepogenin or the 24 hour culture did not alter the degree of this response. The concentrated inocula from the 40 and 48 hour cultures gave growth in all of the tubes insignificantly above that in the blanks. The diluted inocula for these 40 and 48 hour cultures gave a usable growth range but one that was considerably less than that produced by inocula from the younger cultures. It appears that the best results with *L. casei* can be obtained by preparing inocula from cultures not more than 24 hours old.

Discussion. Failure of the frequency of transfer of our stock culture of *L. arabinosus* to affect the nicotinic acid assay results does not agree with the findings of Nymon and Gortner. These investigators reported a gradually decreased linearity of response in their culture of *L. arabinosus* transferred every 3 or 4 weeks in yeast extract-glucose-agar medium with incubation at 37°C. This response was improved by culturing in enriched media, reducing the incubation temperature to 30°C, the optimum reported by Bergey *et al.*[4] for *L. arabinosus*, and increasing the frequency of transfer. They did not include a consideration of incubation temperatures of the assay tubes in their report.

Price and Graves[5] found, in the determination of riboflavin by *L. casei*, that incubation temperature variations of 4-5°C from the optimum of 40-42° for this organism resulted in a decrease of 25% in the titration values. In the present study, no such striking differences in the behavior of *L. arabinosus* were observed with temperature variations in the incubation of assay tubes in the nicotinic acid determination. However, it is well recognized that *L. casei* is more readily affected by environmental conditions than *L. arabinosus*. Following earlier recommendations of Snell and Strong[6] for the preparation of inocula, Bird *et al.*[7] obtained more satisfactory results when they used diluted inocula. This agrees with our findings for inocula made from older cultures of *L. casei*. On the other hand no difference could be seen in undiluted and diluted inocula from cultures not more than 24 hours old. Inocula from young cultures were superior to inocula from old cultures even though the latter had been diluted.

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[5] Price, S. A., and Graves, H. C. H., *Nature*, 1944, **153**, 461.

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Summary. The results indicate that the culture of *L. arabinosus* used in these studies for microbiological assay was not easily affected by frequency of transfer of stock cultures or by incubation of assay tubes at fixed temperatures between 30°C and 37°C. In the case of the culture of *L. casei,* both the dilution of the inoculum and the age of the culture from which it was prepared affected assay titration values.

16136

Recovery of Virus from Throats of Poliomyelitis Patients.*

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In discussions of the epidemiology of poliomyelitis much importance has been placed on the presence or absence of virus in the nose and throat of patients and of associates in so far as such findings might support theories of transmission of the disease by means of droplets. A review of literature on the subject in 1938[1] indicated that virus was reported in the nasopharynx, tonsils or trachea of patients in 15% of 105 examinations made during the first 5 days of illness and in 7% of 182 made later. Conflicting evidence has been presented during the last decade. Sabin and Ward found virus in the pharyngeal wall of 4 of 7 patients dead within 6 days of onset,[2] and Kessel *et al.* reported positive results with tonsil-adenoid tissue in 3 of 6 autopsy specimens.[3] Howe and Bodian detected virus in pooled throat swabs from at least 2 of 28 healthy children and from 1 of 6 juvenile, familial associates of cases; the positive associate may have been a nonparalytic case since the child had fever and diarrhea at the time of collection of the specimen.[4] Oropharyngeal swabs obtained by the same authors, within 3 days after onset of illness from 10 of 23 patients yielded virus, but none of 13 swabs taken between the 4th and 9th days was positive.[5] In a similar study by Horstmann, Melnick and Wenner, only one of 19 swabs and one of 15 washings obtained during the first week of illness contained virus;[6] the specimens from 10 of the 19 patients in the study were obtained within 3 days after onset of symptoms. Kramer, Hoskwith and Grossman[7] tested 18 washings of the nasopharynx and recovered virus from 2 which had been collected 5 and 9 days after onset, respectively. Ward and Walters tested material from the nose and throat of 19 patients between the second and fourth days of illness. Virus was isolated from cloth masks over the nose and mouth of two patients, from a nasal swab of one of these, from pharyngeal swabs of the second and from 5 others.[8]

This report describes attempts to recover virus from pharyngeal washings of a group of poliomyelitis patients during the outbreak

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