been found to contain synnematin(4). All the tests were made with partially purified synnematin and further purification may establish the presence of more than one chemical entity. The material investigated has certain attributes of a clinically desirable antibiotic such as: (1) It is soluble in water. (2) It is stable and active near neutrality. (3) It is active against a large number of species of bacteria. (4) The size of inoculum has little effect on the concentrations necessary to prevent growth. (5) Organisms do not appear to develop resistance rapidly. (6) It has a low toxicity for mice.

Summary. (a) A mold, a species of *Tilachlidium,* was found to produce a water soluble antibiotic, synnematin, which was partially purified. (b) Crude preparations inhibit *in vitro* certain species of *Brucella, Corynebacterium, Micrococcus, Streptococcus,* all species of *Salmonella* tested and some species of *Shigella.* Synnematin is inactive against the tested strains of *Aerobacter, Escherichia, Mycobacterium,* filamentous molds and most species of *Shigella.* (c) *In vivo* activity is demonstrated by the ability of synnematin to protect mice against infection with *D. pneumoniae* and to protect egg embryos against infection with *S. pullorum.* (d) Its low toxicity and other properties are reported.

4. Gottshall, R. Y., Roberts, J. M., Portwood, Lucile M., and Jennings, J. C., unpublished observations.

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Influence of Storage on Viability of BGG Vaccine. (18474)

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The use of BCG vaccine has grown at a tremendous rate since the war despite the unsatisfactory nature of any prophylactic that consists of living and unstable microorganisms. The lack of a wholly acceptable preparation should not prevent such improvements as are possible within the formula originally established by Calmette(1) and, with this in mind, efforts to standardize BCG vaccine have been underway in the Division during the past five years. The present report of the uniformity of 204 consecutive weekly lots of vaccine and of their viability following storage is part of those studies.

Materials and methods. The methods have been described elsewhere(2). The last 144 lots of vaccine were processed from larger amounts of semidry BCG culture and were dispersed in a modified homogenizer making 60 r.p.m. In addition, the diluent has been replaced by a glycerine-free solution consisting of one part Sauton and three parts of phosphate buffer solution, pH 7.2. It is recognized that the homogenization of semidry BCG culture is incomplete. Direct examination shows small clumps of bacilli mixed with the more uniformly dispersed ones. The number of living BCG aggregates in each weekly lot of vaccine was determined by serial tenfold dilutions each seeded on five tubes of Löwenstein's egg medium. The colony counts were made after four and eight weeks' incubation at 37.5°C. The effect of storage was determined by vigorously shaking by hand various samples of vaccine that had been stored for as long as three and one-half years at 2-4°C. The viability tests were made of serial tenfold dilutions in Dubos' medium and on Löwenstein's egg medium.

Results. For purposes of simplicity, the results of the colony counts are shown in Table I as the mean of twelve consecutive weekly tests. As may be seen from the table,

1. Calmette, A., L'Infection bacillaire et la Tuberculose. Masson et Cie, Paris, 1936, p. 912.

2. Birkhaug, K., *Am. Rev. Tuberc.,* 1949, v59, 567.

the average for the entire period was 48 ± 10.55 colonies from 10^{-6} dilution of the 1 mg/ml BCG vaccine. A significant deviation from this mean occurred only during one period.

Viability of stored vaccine is shown in Table II. Approximately 50% of the BCG elements remained viable in the 3-week-old vaccine, 30% in the vaccine 6 weeks old, and 2% survived for one year.

TABLE I. Viability of BCG Vaccine Freshly Prepared from 10-11-Day-Old Sauton Culture.

Statistical analysis of number of colonies cultured on Löwenstein's egg medium from 10^{-6} suspension of 1 mg/ml BCG vaccine diluted in physiological saline solution (120 cultures in each group).

Weekly lots of BCG vaccine, wk	No. of colonies	Probability	
		t	*P*
1 to 12	45 ± 5.39*	—	—
13 to 24	44 ± 6.25	.162	.87
25 to 36	52 ± 11.49	1.715	.10
37 to 48	47 ± 7.49	.735	.47
49 to 60	44 ± 12.09	.245	.82
61 to 72	46 ± 9.39	.294	.77
73 to 84	38 ± 7.00	2.450	.03
85 to 96	47 ± 8.78	.612	.55
97 to 108	37 ± 6.33	2.695	.02
109 to 120	38 ± 6.32	2.694	.02
121 to 132	48 ± 8.71	.906	.38
133 to 144	43 ± 3.88	.808	.42
145 to 156	63 ± 24.40	2.205	.03
157 to 168	42 ± 8.25	.612	.55
169 to 180	61 ± 23.20	2.180	.04
181 to 192	68 ± 21.03	*3.330*	*<.01*
193 to 204	57 ± 9.17	2.709	.02

* Mean ± stand. dev. of the mean.
Significant deviation italicized.

TABLE II. Viability of BCG Vaccine Stored at 2-4°C Up to 3½ Years.

Vaccine Lot No.	Storage time	Colonies per ml of dilution of 1 mg/ml vaccine	% survival rate	Growth of BCG vaccine diluted in Dubos' medium (pos. a.f.b. smear)
A-90	Fresh	49 × 10^6	—	10^{-7}
"	1 day	45 "	92	10^{-7}
"	2 days	41 "	84	10^{-7}
"	3 "	46 "	92	10^{-7}
"	4 "	37 "	76	10^{-6}
"	5 "	41 "	84	10^{-7}
"	6 "	40 "	82	10^{-6}
"	7 "	38 "	78	10^{-6}
"	2 wk	26 "	53	10^{-5}
"	3 "	24 "	49	10^{-5}
"	4 "	19 "	39	10^{-5}
B-90	6 "	15 "	31	10^{-5}
A-87	3 mo.	9 "	18	10^{-5}
A-81	6 "	6 "	12	10^{-5}
A-75	9 "	12 × 10^5	2.5	10^{-4}
A-68	1 yr	9 "	1.8	10^{-4}
A-41	2 "	4 × 10^2	0.0008	10^{-1}
A-15	3 "	0	—	0
A-2	3½ "	0	—	0

Summary. Comparison of 204 consecutive weekly lots of BCG vaccine prepared under standardized conditions were found to be satisfactorily uniform in the number of viable organisms and clumps of organisms they contained. The survival rate of BCG vaccine stored at 2-4°C has been determined.

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Oral Administration of Coxsackie Viruses to Newborn and Adult Mice.* (18475)

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Chimpanzees and cynomologus monkeys are readily infected by oral administration of Coxsackie or C virus, although with no apparent illness(1,2). In both species a virus carrier state is produced followed by the development of neutralizing antibodies to the infecting strain of virus. The present study is concerned with the susceptibility of newborn and adult mice, when tested for oral infection.

Virus strains. The following C virus strains were employed in the form of suspensions of

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1. Melnick, J. L., *Bull. N. Y. Acad. Med.*, 1950, v26, 342, and unpublished data.

2. Melnick, J. L., and Ledinko, N., *J. Immunol.*, 1949, v64, 101.