TABLE I. Results of Drug Sensitivity Tests on the Luna and 200 Strains of *E. histolytica* Transplanted in Presence of Low Concentrations of Aureomycin, Terramycin, and Emetine HCl Compared with Sensitivity of Unexposed Amoebae of the Same Strains.

	Sensitivity											
	Luna strain						200 strain					
Transplant	Aureomycin* (mg/ml)		Terramycin (mg/ml)		Emetine HCl (mg/ml)		Aureomycin (mg/ml)		Terramycin (mg/ml)		Emetine HCl (mg/ml)	
	N.†	E.‡	N.	E.	N.	E.	N.	E.	N.	E.	N.	E.
10	.0332	.0332	.1329	.1329	.0216	.0216	.0332	.0332	.1329	.1329	.0216	.0432
20	.0332	.0166	.2658	.2658	.0432	.0432	.0166	.0332	.1329	.1329	.0216	.0216
30	.0664	.0332	.1329	.1329	.0108	.0108	.0332	.0166	.0664	.0329	.0108	.0216
38	.0332	.0332	.2658	.2658	.0216	.0216	.0166	.0166	.1329	.1329	.0216	.0432

* Concentrations in these columns resulted in a decrease of at least 75% in the number of amoebae seen in cultures after 48 hr.

† "Normal" *E. histolytica* not exposed to the drugs.

‡ Experimental *E. histolytica* transplanted in the presence of partially effective concentrations of the drugs.

in no case was there more than a 2-fold difference in the concentration of a compound found capable of destroying 75% or more of the "normal" or experimental cultures. In 5 instances the concentration found effective in the experimental cultures was twice that found effective in the "normal" cultures, in 3 instances the reverse was true, and in 15 instances the concentrations were identical. Thus, the differences observed were probably due to experimental error and no evidence of the development of resistance by either strain of *E. histolytica* was observed. The inability to maintain transplants in the presence of drug concentrations somewhat above those routinely used, as mentioned previously, is also interpreted as evidence of failure to develop resistance.

Discussion. The method used in these experiments for testing the drug sensitivity of *E. histolytica* has the disadvantage that there are still a certain number of viable bacterial cells in the cultures at the time the compounds are added(2). However, evidence has been obtained(3) which indicates that the effects observed using this technic are the result of activity of the drugs on the amoebae rather than the bacteria. If this be true, then the results shown in the table are suggestive that *E. histolytica* does not readily develop resistance to aureomycin, terramycin, or emetine HCl *in vitro*.

Summary. Two strains of *E. histolytica* failed to show evidence of the development of resistance to aureomycin, terramycin or emetine HCl after 38 serial transfers in S-F medium containing partially effective concentrations of the agents.

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Viability and Dispersion of BCG Inoculated Subcutaneously in Guinea Pigs. (19524)

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Studies on the fate of BCG in guinea pigs inoculated by the intradermal route(1), multiple puncture(2,3), and by scarification(4,5) have shown that BCG were dispersed to regional lymph nodes within one week and to the spleen and distant lymph nodes in 2 to 4 weeks and that they may remain viable in the latter organs up to 6 months. Van Deinse(6)

obtained viable BCG from a suppurating lymph node in a child vaccinated by scarification 11 months earlier. The subject of BCG's viability and dispersibility in experimental animals and in man was recently reviewed by Fenner(7), and it appears that our finding viable BCG in the mesenteric lymph nodes 577 days after the intraperitoneal inoculation of 1 mg BCG in the guinea pig is the longest survival hitherto recorded without the organisms producing generalized tuberculosis(8). But these observations mostly dealt with sporadic findings and no long-term study is available on the viability and dispersion of BCG in animals inoculated with a fixed dosage of vaccine. The present investigation attempts to clarify this subject.

Experimental. In a recent work on uniform methods for continuous weekly production of BCG vaccine for use in man(9) we assessed 60 consecutive weekly lots of vaccine quantitatively for BCG's invasiveness and production of tissue hyperplasia in guinea pigs killed 3 and 6 months, respectively, after the subcutaneous inoculation of 10 mg BCG in the left hind leg. The inoculum abscess, regional and distant lymph nodes, the spleen, liver, and the lungs were removed and weighed to determine the degree of hypertrophy caused by a fixed amount of BCG. In the present study, the same procedure was followed, using 20 guinea pigs sacrificed at 3 months and another group of 20 killed 6 months after the subcutaneous inoculation in the left hind leg of 10 mg semi-dry weight of freshly prepared vaccine (Lots B92-B112: July 1950-April 1951). The average number of viable BCG (colonies) in these 20 weekly lots of vaccine was 43868000 ± 8603000/mg BCG. Careful *aseptic technic* was employed throughout. Separate instruments were used for the removal of individual organs. The entire lymph node, spleen, and inoculum abscess and approximately 2 g of the liver and lungs were finely partitioned with scissors and the macerated tissue placed individually in 1-ounce sputum jars containing approximately 20 g glass beads and supplied with a metal screw-top. The organs in the sputum jars were *homogenized* in a paint-shaking machine for 10 minutes. Ten ml of 4% NaOH were then added and the shaking repeated for 10 minutes. The homogenized material was centrifuged for 20 minutes at 3000 r.p.m. The supernatant was decanted and the sediment neutralized by 1.0, 0.1 and 0.01 normal HCl in the presence of 2 drops of phenol red indicator. After adding 200 units penicillin, the sediment was seeded in 0.2 ml amounts on 5 large tubes of Löwenstein-Jensen egg medium. The plugged tubes were left at 37°C in the horizontal position until excess liquid had evaporated (approximately 24 hours) when they were sealed with rubber stoppers. The tubes were left in the horizontal position for 1 week and in the vertical position for 7 weeks. The cultures were examined weekly for growth, the final reading being made after 8 weeks.

TABLE I. Comparative Wt of Organs in Normal and BCG Inoculated Guinea Pigs 3 and 6 Months, Respectively, after Inoculation. 20 animals in each group.

	3 months		6 months	
Organs	Normal, $\bar{x}$	BCG inoculated, g	Normal, $\bar{x}$	BCG inoculated, g
Body wt	674	654	826	796
BCG abscess	—	3.38	—	1.25
Lf. sup. inguinals	.07	.20*	.07	.23*
" deep "	<.01	.05*	<.01	.05*
" iliacs	.02	.11*	.03	.13*
Trach. bronchials	.08	.13*	.08	.15*
Cervicals	.06	.09	.07	.09
Axillaries	.05	.05	.05	.06
Spleen	.67	.79*	.69	.74
Liver	35.77	35.02	35.84	41.75
Lungs	4.73	5.64	5.04	6.52

* Significantly different from normal group at the $P \leq .01$ level.

Results. Table I presents data on weight of animals and 10 different organs removed from each of the BCG inoculated guinea pigs and also from a similar group of uninoculated animals. It is apparent that the weight of individual lymph nodes, especially those adjacent to the site of inoculation, was significantly greater in both groups of inoculated animals than in the controls. It is noteworthy that the weight of the spleen was significantly greater in the animals inoculated with BCG 3 months earlier than in the controls. These data confirm the relative but not absolute avirulence of BCG.

Table II presents data on the dispersion of

TABLE II. Survival of BCG in Organs of Guinea Pigs Inoculated Subcutaneously with 10 mg BCG 3 and 6 Months Earlier. 20 animals in each group.

Organs	Positive smears	Positive cultures	No. of colonies
	3 months animals		
BCG abscess	12/20	18/20	236 ± 167*
Lf. sup. inguinals	1/20	14/20	196 ± 152
" deep "	1/20	10/20	175 ± 196
" iliacs	0/20	9/20	171 ± 172
Trach. bronchials	0/20	13/20	141 ± 137
Cervicals	0/20	2/20	55 ± 50
Axillaries	0/20	3/20	70 ± 38
Spleen	1/20	7/20	316 ± 186
Liver	0/20	6/20	122 ± 74
Lungs	0/20	3/20	134 ± 107
	6 months animals		
BCG abscess	13/19	9/19	344 ± 130
Lf. sup. inguinals	1/20	6/20	203 ± 180
" deep "	0/20	5/20	264 ± 220
" iliacs	1/20	6/20	170 ± 176
Trach. bronchials	0/20	13/20	158 ± 191
Cervicals	0/20	5/20	166 ± 194
Axillaries	0/20	3/20	40 ± 33
Spleen	0/20	6/20	285 ± 209
Liver	0/20	3/20	40 ± 31
Lungs	0/20	3/20	211 ± 203

* Symbol ± stand. dev.

BCG from the inoculated area to regional and distant lymph nodes, to the spleen, liver, and lungs, as well as the rate of survival of BCG in these foci. It may be noted that the inoculum abscess yielded 60 and 68% positive smears of acid-fast organisms in animals killed 3 or 6 months after inoculation while positive smears were obtained irregularly from regional and distant organs. Positive cultures of BCG, on the other hand, were obtained from the inoculum abscess and regional lymph nodes twice as frequently in animals killed 3 months than 6 months after inoculation. However, the dispersion and survival of BCG in distant lymph nodes, the spleen, liver, and the lungs were approximately the same in both groups of inoculated animals. It is interesting that BCG survived more abundantly in the tracheo-bronchial lymph nodes in both groups of inoculated animals (65%) than in other distantly located lymph nodes since the hypertrophy of the tracheo-bronchial lymph nodes is also greater than that of other groups.

The average macroscopic growth of BCG colonies on the Löwenstein-Jensen egg medium was approximately the same in cultures of organs located regionally or distantly from the inoculum site. In spite of the generalized dispersion and long survival of BCG no macroscopic tuberculous lesions were abscessed except at the site of the inoculation and occasionally in the regional lymph nodes.

Summary. Twenty weekly lots of BCG inoculated subcutaneously in normal guinea pigs were widely dispersed and remained viable at site of inoculation and in distant lymph nodes, the spleen, liver and lungs up to 6 months without producing macroscopic tuberculous lesions except in the site of inoculation.

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