limbs are similar to those obtained after administration of pitressin, and substantiate the explanation given for the effect of pitressin.

It is known that an immediate but short-lived increase in the oxygen consumption follows the administration of epinephrine. The mechanism of this action is not known. Epinephrine, by causing increased cell activity (oxygen consumption), brings about an increased utilization of ATP leading to a lowering of the "labile phosphate" content. The increase in the oxygen consumption following epinephrine administration and the vasoconstrictive action of epinephrine, decreasing the tissue supply of oxygen, may lead to a temporary tissue anoxia.

It is generally assumed that many of the biological effects of x-irradiation can be attributed to the action of the decomposition products of water which result from the irradiation. It is also becoming increasingly evident that the degree of x-radiation effects is, to an important extent, dependent on the oxygen content in the cellular fluids. Dowdy, Bennett and Chastain(5) exposed rats in very low oxygen tension (5%) and found that the acute effects of radiation are only half as severe. Remarkable decreases in many x-ray induced cellular phenomena by reduction of oxygen concentration during irradiation have been reported(6,7).

Summary. 1. The survival rate of rats, exposed to lethal x-ray dosage, was found to be significantly increased after pretreatment with pitressin or epinephrine. 2. The protective effect of these principles may be due to their property of producing a temporary tissue anoxia.

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Comparative Antigenic Potency of Killed Irradiated Tubercle Bacilli and Living BCG Vaccine. (19389)

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Criticism is raised against artificial immunization of man with living BCG vaccine on the assumption that the organisms may survive for years and gradually regain virulence(1). The U. S. Public Health Service(2) and the American Trudeau Society(3) recommend further research to develop a tuberculosis vaccine containing only dead organisms. It is well known from the experimental and clinical studies(4) that such vaccines hitherto have yielded less effective protection against a challenge virulent infection than living BCG vaccine. The question has arisen whether or not the prolonged heating required to kill tubercle bacilli vitiates against their antigenic potency. In order to eliminate this factor, vaccine has recently been prepared by killing virulent human tubercle bacilli by brief ultraviolet irradiation(5-7). Such vaccines are reported to be equal or superior to living BCG vaccine on the basis of guinea pig and mouse protection tests. The advantages of a killed tuberculosis vaccine are twofold since it would

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be absolutely safe and remain relatively stable upward to one year while the optimal usefulness of living BCG vaccine is approximately 10 days and one fears that it may regain virulence. For the purpose of evaluating the comparative antigenic potencies of killed irradiated and living BCG vaccines we undertook the following study.

Methods. Two lots of killed irradiated tuberculosis vaccine were kindly supplied us by R. W. Sarber(6) and Dr. A. Milzer(7). These were prepared 15 and 3 months previously with human type tubercle bacilli, strains H37Rv and 199RB and contained 1 mg of organisms per ml. A 1:10,000 dilution of merthiolate was added to each lot as preservative. The vaccines had been cleared for sterility and safety in guinea pigs and mice. Following the procedure detailed by Sarber (6), the H37Rv irradiated vaccine was administered intraperitoneally in three 5 ml (5 mg of organisms) doses at weekly intervals to 24 normal and tuberculin-negative guinea pigs weighing 421 ± 27.61 g.† Similar inoculations with freshly prepared(8) living BCG vaccine (Lots B-110, A-111 and B-111 currently distributed for use in man in New York) were made in another lot of 24 normal and tuberculin-negative guinea pigs weighing 431 ± 35.99 g. One mg of these respective lots of BCG vaccine was found to contain 46, 56, and 54 million living elements (colonies) in Löwenstein cultures. Two similar groups of guinea pigs weighing respectively 436 ± 25.75 and 426 ± 25.68 g were given identical inoculations with 199RB irradiated vaccine and living BCG vaccine (Lots A-115, B-115, and A-116), the latter containing 58, 65, and 51 million living elements (colonies) per mg of organisms. The first series of 48 animals was allowed an immunization period of 3 months, and the second series of 1 month after the last intraperitoneal inoculation. At this time, both series of immunized animals, together with 18 normal and tuberculin-negative control guinea pigs weighing 620 ± 15.17 g, were given 0.005 mg of a 3-week-old Löwenstein culture of H37Rv (of established virulence) by the intramuscular route in the right hind leg. This dose gave rise to 2,862 eugonic colonies on a series of Löwenstein cultures after 12 weeks' incubation. At the time of the challenge virulent inoculation, no significant difference in the body weight obtained between the first series of immunized animals and the controls while a difference of approximately 100 g existed between the second series of immunized animals and the controls.

† ± means stand. dev. of weights of animals.

Results. All the animals inoculated intraperitoneally with either lot of irradiated vaccine experienced extreme irritability and distress immediately following the inoculations, a phenomenon not observed in the animals similarly inoculated with BCG vaccine. We attributed this occurrence to the presence of merthiolate in the irradiated vaccine while BCG vaccine contained no preservative. The post-immunization mortality rate was greater in the animals given the irradiated vaccine than in the BCG vaccine group. Within 4 weeks after the last immunizing dose, 7 and 6 animals (27.1%) died respectively in the first and second series of irradiated vaccine groups and 2 in each of the BCG vaccine groups (8.3%).

Post-immunization tuberculin reactions 48 hours after the intradermal inoculation with 0.0001 mg PPD showed greater induration volumes in the animals inoculated with BCG vaccine than with irradiated vaccine in tests performed at bi-weekly intervals after immunization (Table I presents data on tests done 1 month after immunization). After the challenge virulent inoculation, we observed in both series of animals a more retarded increment in tuberculin hypersensitiveness in the animals immunized with BCG vaccine than with either lot of irradiated vaccine. Table I gives data on tests done 3 months after the challenge inoculation. We also ascertained by palpation a much slower development of lymphonodular hypertrophy in the vicinity of the virulent inoculation in the animals immunized with BCG than with either lot of irradiated vaccine.

Approximately 3 months after the challenge virulent inoculation, the body weight of the BCG immunized animals was 780 ± 90.22 g and 716 ± 78.10 g respectively in the first

TABLE I. Post-Immunization and Post-Challenge Findings in Guinea Pigs Inoculated with Killed Irradiated Tubercle Bacilli and Living BCG Vaccine.

Material	First series		Second series		Controls
	Irrad. tbc. 1	BCG	Irrad. tbc. 2	BCG	
No. animals at start	24	24	24	24	18
Post-immunization deaths	7	2	6	2	—
Post-challenge deaths	5	3	6	4	8
Induration (mm^2) with .0001 mg PPP after 48 hr					
1 mo after immunization	426 ± 109	760 ± 403*	422 ± 121	572 ± 198*	—
3 " " challenge	2953 ± 1598	1910 ± 319*†	2184 ± 1143*	1085 ± 298*†	2850 ± 1511
No. animals autopsied	17	22	18	22	18
Wt at autopsy, g	698 ± 117	789 ± 90*	624 ± 94	716 ± 78*	617 ± 64
Completely protected	0	13	0	12	0
Localized tuberculosis	9	4	10	4	2
Generalized "	8	5	8	6	16
Rt. sup. inguinal glands, g	.89	.32*	.54*	.24*	1.41
" deep " " "	.30*	.16*	.32	.10*†	.59
" iliac " " "	1.46	.42*†	1.17	.34*†	2.00
Pooled 17 sets " "	9.45	3.10*†	8.29*	2.14*†	13.47
Spleen "	2.50	1.00*†	1.78*	1.20*	3.22
Liver "	37.32	34.30*	36.33	32.50*	41.39
Lungs "	7.86*	5.94*†	6.37	6.13*	9.58

* Difference between control and these figures
† " " BCG and irrad. tbc. figures
} significant by: $\underline{t} = \frac{x - x'}{s}\sqrt{\frac{(n_1+1)(n_2+1)}{n_1+n_2+2}}$ at the $\underline{P} = .\underline{01}$ level.

± is stand. dev. by $\sqrt{\frac{S(d^2)}{n-1}}$.

and second series of BCG immunized animals, 698 ± 117.13 g and 624 ± 94.87 g in the irradiated vaccine groups while the controls weighed 617 ± 63.90 g. Death from generalized and advanced tuberculosis occurred in 8 (44%) control animals between 65 and 102 days after the virulent inoculation, in 3 and 4 animals in the 2 series of BCG immunized animals (15.9%) and in 5 and 6 animals in the series of irradiated vaccine animals (31.4%). In order to assess the tissue resistance against the challenge inoculation in the variously immunized animals before progression of the infection obliterated it, it was decided to kill the surviving animals after approximately half of the non-immunized control animals had died spontaneously with generalized tuberculosis. This was done between 102 and 105 days after the challenge virulent inoculation.

The same autopsy procedure was adhered to as on previous occasions(9) and the completed data were tested for significant differences by Fisher's method(10) for comparison of 2 means. Autopsies revealed that all the animals having received 3 intraperitoneal inoculations with H37Rv and 199RB irradiated vaccines presented an advanced form of dry tuberculous peritonitis with little or no fluid in the peritoneal cavity, but a dense plastic exudate gluing the intestines together by firm adhesions which separated with difficulty. These findings were in striking contrast to the nearly complete absence of fibrous adhesions in the peritoneal cavity of the BCG immunized animals in which the omentum was regularly thickened and contracted into a sausage-like band containing numerous caseous nodules from which typical BCG virulent organisms were cultured on Löwenstein's medium. The gross pathological picture of the non-immunized control animals showed localized tuberculous lesions in only 2 animals (11%) while the remaining 16 animals presented various degrees of advanced and fulminating tuberculosis. We were greatly surprised to find 13 and 12 animals in the 2 series of BCG immunized groups (56.8%) com-

pletely protected against the spread of the challenge inoculation, and 4 in each group (18.2%) presenting only localized tuberculous lesions and the remaining 5 and 6 animals (25%) showing advanced and generalized tuberculous lesions. Table I shows that neither group of animals immunized with irradiated vaccine showed an animal completely protected against the challenge inoculation, that 9 and 10 animals (54.3%) presented localized tuberculous lesions and the remaining 8 animals in each group (45.7%) showing advanced and generalized tuberculosis.

The quantitative data on enlarged regional lymph glands, the pooled total 17 sets of routinely dissected lymph nodes, and on the spleen, liver and lungs from animals immunized with 2 types of irradiated vaccine and BCG vaccine, together with those from the control animals, are given in Table I. These data bear out the gross pathological findings mentioned above, namely that irradiated killed tuberculosis vaccine is surely capable of producing specific resistance against a challenge virulent tuberculous infection. But in comparison with either series of BCG immunized animals, the protective value of living BCG vaccine is significantly greater than that of killed irradiated tuberculosis vaccine. In view of the incomplete immunity produced in animals and in man with the relatively avirulent BCG living organisms, our study suggests that it would be premature to substitute for BCG a vaccine consisting of killed tubercle bacilli.

Summary. Living BCG vaccine has shown superior antigenic potency to killed irradiated tuberculosis vaccines by producing significantly greater tuberculin hypersensitiveness and specific resistance against a challenge virulent inoculation in guinea pigs.

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Ionization and Distribution Between Aqueous and Non-Aqueous Solvents of Dyes in Relation to Gastric Secretion.* (19390)

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Recently(1) a formula was devised in this laboratory that correlated the pK_B of certain basic dyes with their tendency to be secreted by the stomach. While successful in leading to the discovery of new compounds that were secreted in substantial amounts, certain discrepancies were evident(2). In the new series only compounds with a pK_B over 10 were secreted while in the original series the optimum was 6.5 to 9. These discrepancies might be caused by the fact that highly purified dyes were used in the original(1) pK_B determinations while Ingraham and Visscher

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